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# Trait trade-offs in phagotrophic microalgae: the mixoplankton conundrum

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**ABSTRACT**

Analysis of trait trade-offs, through which physiological traits requiring common resources are 'traded' to optimize competitive advantage, provides a route to simplify and more readily understand the complexities of ecology. The concept of trait trade-offs has found favour in plankton research, especially directed at phytoplankton, defined here as phototrophs incapable of phagotrophy. Mixoplankton, defined as protists that combine phototrophy and phagotrophy, are now recognized as being widespread and significant members of the protist plankton community; many photoflagellate 'phytoplankton' are actually mixoplankton, as are many '(microbial) zooplankton'. Mixoplankton might be expected to be dominant, being able to exploit different trophic strategies while simultaneously eliminating competitors. That mixoplankton are not dominant suggests that physiological trait trade-offs erode their apparent competitive edge. We present a systematic analysis of potential trait trade-offs in phototrophic protists focused on mixoplankton. We find no clear evidence to support trait trade-off arguments in plankton research, except perhaps for acquired phototrophy in mixoplanktonic ciliates versus zooplanktonic ciliates. Our findings suggest that the presence of various mixoplankton throughout the surface ocean waters is most likely explained by factors other than trait trade-offs. Diversities in mixoplankton form and function thus reflect that evolution of these organisms from very different lineages, provide them with advantages to function competitively in mature ecosystems with complex trophic interplay. Indeed, the complexity of those lineages is inconsistent with core trait trade-off definitions; there is no single ancestral mixoplankton nor a common environment supporting trait-trade-off-directed evolution.

**HIGHLIGHTS**

- Trait trade-offs do not explain the breadth of mixoplankton ecophysiological capabilities.
- Diversity of mixoplankton form and function reflects phylogenetic diversity.
- Only one potential trait trade-off was identified which was for ciliates that steal chloroplasts.

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**KEYWORDS** Mixoplankton; mixotroph; phagotrophy; phototrophy; phytoplankton; protist plankton; trait trade-off; zooplankton

## Introduction

The concept of trait trade-offs in biology probably emerged from Charles Darwin's theory of variation (Garland, 2014) and has traditionally focused on terrestrial plants or animals for which trade-offs are defined as 'costs paid in the currency of fitness when a beneficial change in one trait [within a given organism] is linked to a detrimental change in another' (Stearns, 1989). Identification of trait trade-offs is typically supported by an empirical analysis of the co-occurrence of physiological traits. Negative relationships are looked for that may signal mutual exclusivity between those traits which require common resources; these traits are argued to have been 'traded' to optimize competitive advantage in a given environmental setting. The environment in this context applies to common spatial and temporal settings subjected to constant change (Snell-Rood *et al.*, 2015). Accordingly, trait trade-off analyses should only be made in reference to organisms from the same ecological setting (Litchman & Klausmeier, 2008), and between organisms with sufficient similarity in evolutionary lineage that trade-offs could provide a plausible mechanism (Garland, 2014).

The trait trade-off concept has proven to be a rich research strand in plankton research (e.g. Dolan & Pérez, 2000; Finkel *et al.*, 2010; Kiørboe *et al.*, 2018; Serra-Pompei *et al.*, 2020; Litchman *et al.*, 2021). Assumptions from such analyses have then been employed to inform configuration of global plankton models to predict oceanic carbon fixation (e.g. Ward & Follows, 2016). These efforts have typically been directed at phytoplankton (e.g. Litchman & Klausmeier, 2008; Finkel *et al.*, 2010), with less emphasis on zooplankton (e.g. Kiørboe, 2011). This emphasis on phytoplankton aligns with the dichotomy of plankton between phototrophic phytoplankton and heterotrophic zooplankton that forms the bedrock of traditional marine ecology and biological oceanography.

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The last decade has seen a growing appreciation that this perceived plant-animal dichotomy within the plankton community is at least overly simplistic, if not flawed (Flynn *et al.*, 2013; Stoecker *et al.*, 2017; Glibert & Mitra, 2022). It transpires that the marine protistan plankton community is not dominated by just 'plant-like' phytoplankton and 'animal-like' zooplankton but also includes organisms that engage in both phototrophy *and* phagotrophy (Mitra *et al.*, 2016). These organisms have been termed 'mixoplankton' (Flynn *et al.*, 2019; Fig. 1), and their members include many organisms referred to, or ecologically considered, as 'microalgae', such as phototrophic members of the dinoflagellate genus *Dinophysis*, and the ciliate genus *Myrionecta*/*Mesodinium*. Indeed, various protist species traditionally labelled as 'phytoplankton' or 'zooplankton' are actually mixoplankton (Leles *et al.*, 2017, 2019), including such iconic 'phytoplankton' as *Tripos furca* (Smalley & Coats, 2002), *Emiliania huxleyii* (Avrahami & Frada, 2020) and *Phaeocystis globosa* (Koppelle *et al.*, 2022).

The term mixoplankton was coined specifically to delineate phagotrophic-microalgae from the other microalgae, namely phytoplankton, which cannot eat but are nonetheless mixotrophic, by virtue of the well-known coupling of phototrophy with osmotrophy (an ancestral feeding mode – Bremer *et al.*, 2022 – exploiting dissolved organic resources; e.g. Lewin & Hellebust, 1970; Antia *et al.*, 1981; Flynn & Butler, 1986; Burkholder *et al.*, 2008; Meyer *et al.*, 2022). This capability of microalgae for photo-osmo-mixotrophy has been exploited in biotechnology to boost algal production at high organic substrate levels under light-limitation (e.g. Cupo *et al.*, 2021). Following the emergence of the mixoplankton paradigm, here we undertake an assessment of trait trade-offs that may have been employed by the different protist plankton functional groups – phytoplankton, mixoplankton and zooplankton – to succeed in a given environment.

## *Mixoplankton functional types and physiologies*

Collectively, mixoplankton have a global significance (Leles *et al.*, 2017, 2019; Faure *et al.*, 2019) and contribute in various ways to ecosystem functioning (Mitra *et al.*, 2014; Leles *et al.*, 2021; Li *et al.*, 2022). Mixoplankton include representatives across a diverse range of protists and a schematic showing core trait differences between different protist plankton functional groups is given in Fig. 1. Mixoplankton can be divided broadly into two groups based on (i) whether they possess an innate, constitutive ability to perform photosynthesis (constitutive mixoplankton; CM), or (ii) whether they acquire phototrophy from their prey or from symbionts (non-constitutive mixoplankton; NCM). The NCM can be further divided into (a) generalist non-constitutive mixoplankton (GNCM, e.g.

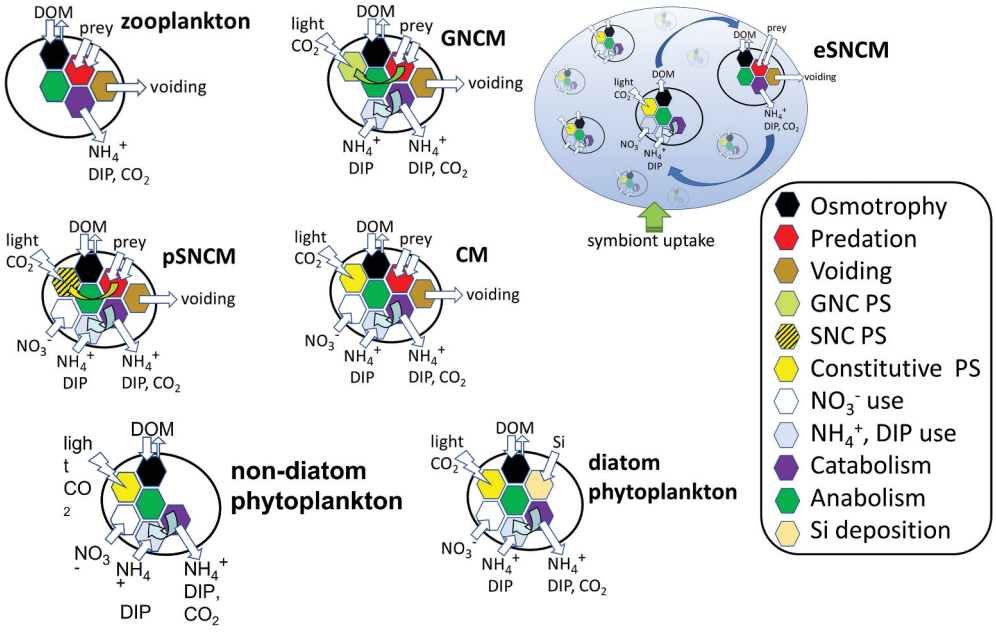


**Fig. 1.** Schematic of protist plankton functional types. Shown here are schematics for protozoan zooplankton (with no phototrophy), the generalist, plastidic-specialist and endosymbiotic-specialist non-constitutive mixoplankton (GNCM, pSNCM, eSNCM, respectively; note their acquired phototrophy), constitutive mixoplankton (CM), non-diatom and diatom protist phytoplankton (with no phagotrophy). The schematic for the eSNCM (such as the Rhizaria) shows the interplay between the phytoplankton-like symbionts (of which there may be hundreds or thousands of cells) growing within the zooplankton-like host cell. All protist types can use dissolved organic matter (DOM); phytoplankton (including diatoms) are thus mixotrophs by combining phototrophy with osmotrophy. Schematics are not to scale; eSNCM can be as large as mm to cm in cell size while all the other functional groups are typically in the size range of c. 3–200 µm (Mitra *et al.*, 2023).

*Strombidium rassoulzadegani, Laboea strobila*) that acquire phototrophy from a range of different prey items, (b) plastidic specialist NCM (pSNCM, e.g. *Mesodinium rubrum, Dinophysis acuta*) that can acquire phototrophy only from specific prey taxonomic groups. and (c) endosymbiotic specialist NCM (eSNCM, e.g. green *Noctiluca scintillans, Globigerinoides sacculifer*) that maintain prey symbionts for acquired phototrophy (Mitra *et al.*, 2016; Flynn *et al.*, 2019).

While CM appear simply as 'phytoplankton that eat', and NCM as '(microbial) zooplankton that photosynthesize', the contributions of photosynthesis and eating for growth are very variable within members of both groups (Caron, 2000; Stoecker *et al.*, 2009, 2017; Jeong *et al.*, 2010; Gomes *et al.*, 2018; Wilken *et al.*, 2020). Furthermore, while photosynthesis is inevitably associated with provision of carbon (C) and energy, eating may additionally or perhaps primarily be associated with the acquisition of nitrogen (N), phosphorus (P) or other nutrients. Further, feeding in mixoplankton may not necessarily align with strict interpretations of 'phagotrophy' which would require a significant size difference between consumer and the engulfed prey. Rather, feeding may involve, after initial capture, engulfment (Tillmann, 1998; Jeong *et al.*, 2005), semi-extracellular phagocytosis (Kamennaya *et al.*, 2018), use of a peduncle (akin to a feeding straw inserted into the prey to suck out material; Larsen, 1988; Nagai *et al.*, 2008) or the use of mucus nets to entrap potential prey (e.g. Blossom *et al.*, 2017; Larsson *et al.*, 2022). Alternatively, mixoplankton may release toxins that lyse the prey (and, also potentially other non-prey organisms), releasing particulate and dissolved organics which can then be consumed through a combination of phagotrophy and osmotrophy (Tillmann, 2003; Granéli *et al.*, 2012).

## *Trait trade-offs in context*

The origins of protist plankton saw repeated cycles of gains and losses of functionality traits (de Castro *et al.*, 2009; Keeling *et al.*, 2014; Bremer *et al.*, 2022; Fig. 2). Many organisms appearing to be closely related are actually products of different evolutionary paths played out in different ecological settings at different times (Mansour & Anestis, 2021; Bremer *et al.*, 2022). Put simply, extant protist microalgae did not live and compete together such that evolution could select for different traits by 'trading options' against each other. While it may be tempting to consider the oceans as one environment, for microbes that is certainly not so (Zehr *et al.*, 2017) and there are many additional drivers that select for competitive advantage other than resource demand and allocation (notably resilience against disease and predation). Application of trait trade-offs for plankton are, therefore, problematic (Flynn *et al.*, 2015). Even ignoring the 'same-environment' and the 'evolution from same lineage' caveats for trait trade-offs (cf. Litchman & Klausmeier, 2008; Garland, 2014), there is also the question of significance in resource costs to which the trade-offs may be applied.

A trait trade-off must be associated with a significant saving in energy and/or material resources. The question arises as to how can significance be judged. One of the most expensive biochemical processes for phototrophic plankton is the assimilation of N supplied as nitrate versus that as ammonium. Using nitrate costs c. 20% more than when using ammonium in total photo-reductant production, with additional significant costs in iron (Fe) allocation (Flynn & Hipkin, 1999). Despite this very significant overhead cost, phototrophic plankton grown on nitrate typically grow at rates similar to those using ammonium (e.g. Thompson *et al.*, 1989; Wood & Flynn, 1995). This is perhaps a salutary note of warning with respect to the level of cost differential needed to mark a putative trait trade-off as being of significance. We must also be careful not to confuse changes in trait expression within a species, in response to changes in environmental conditions (e.g. Blossom & Hansen, 2021), as evidence of metabolic trait trade-offs rather than as simply the consequence of a series of (de)repression feedback processes (Flynn *et al.*, 2015).

## *Evaluation of trait trade-offs within protist plankton*

A key driver for a need to reappraise plankton trait trade-offs, in consequence of the mixoplankton paradigm (Glibert & Mitra, 2022), is the formulation of plankton functional type models. Describing a modelled plankton as mixotrophic just by combining phototrophic and heterotrophic features could produce an all-conquering configuration (e.g. Thingstad *et al.*, 1996; Hammer & Pitchford, 2005; Troost *et al.*, 2005). To prevent mixoplankton from always dominating, modellers have applied assumed trait trade-offs, such that the phototrophic and phagotrophic activities in the mixoplankton are configured as individually less competitive than those exhibited by the 'pure' phytoplankton or the 'pure' zooplankton (e.g. Ward *et al.*, 2011). The motivation for this current work grew from investigating and questioning approaches where trait trade-offs for photo-phagotrophs have been configured and implemented for applications from theoretical biology through to considering global plankton productivity studies (e.g. Ward *et al.*, 2011; Andersen *et al.*, 2016; Ward & Follows, 2016; Cadier *et al.*, 2020). Here, we expand on such suggestions, to present an extensive critique of possible trait trade-offs that could affect competitiveness of mixoplankton versus their non-phagotrophic phytoplankton and non-phototrophic zooplankton counterparts.

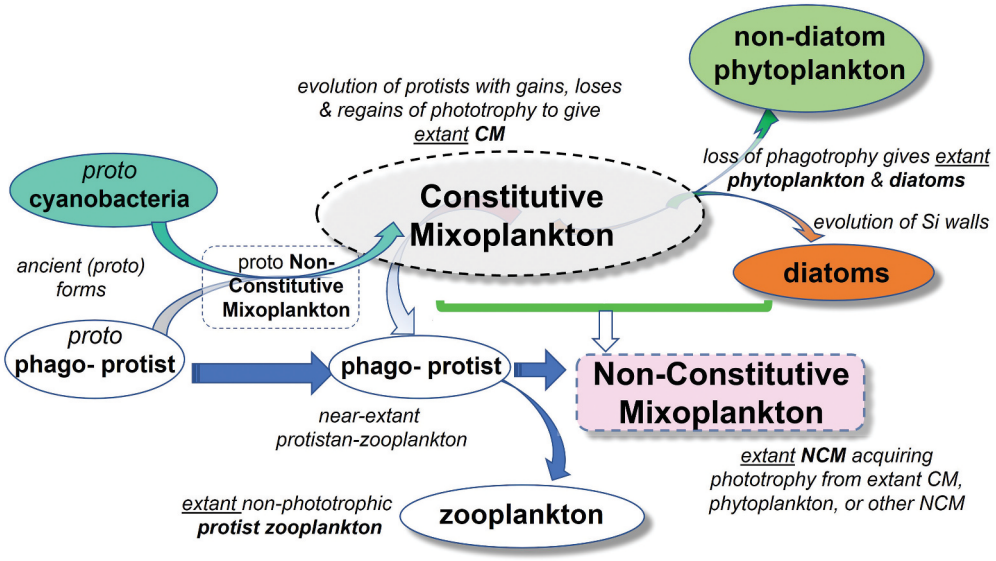


Fig. 2. An interpretation of the evolution of protist functional types. Multiple gains and losses of phototrophy have occurred within what we now term constitutive mixoplankton. Some of these have resulted in extant zooplankton and extant phytoplankton groups. See Fig. 1 for schematics of the physiological traits of these organism types.

Phagotrophic protists were the ancestral form from whence all protists evolved, while phytoplankton evolved from mixoplanktonic lineages (Fig. 2; Raven, 1997; Raven *et al.*, 2009; Ponce-Toledo *et al.*, 2017; Sánchez-Baracaldo *et al.*, 2017; Bremer *et al.*, 2022; Mitra *et al.*, 2023). Thus, we first examine the different traits and potential trait trade-offs in mixoplankton versus zooplankton (Table 1), and then in mixoplankton versus phytoplankton (Table 2). As there are several fundamentally different mixoplankton functional groups, each of which contain organisms of very different evolutionary lineages (Mansour & Anestis, 2021), we have also undertaken an evaluation of advantages and disadvantages of traits within the mixoplankton themselves (Table 3). Following from Flynn *et al.* (2019), we reserve the term 'phytoplankton' specifically for phototrophic protists that cannot feed, 'zooplankton' for protists that have no ability for phototrophy, and 'mixoplankton' for protists that engage in photosynthesis and phagotrophy (Fig. 1). As all phytoplankton are assumed mixotrophs by virtue of their capability for photo-osmo-mixotrophy (Flynn *et al.*, 2019), we explore putative trait trade-offs in mixoplankton and not trait trade-offs for mixotrophy in phytoplankton (e.g. Litchman & Klausmeier, 2008) or zooplankton (e.g. Litchman *et al.*, 2013).

## Mixoplankton versus protist zooplankton

### *Allometry*

Hypotheses H1.1 and H1.2 in Table 1 consider trait trade-offs due to perceived conflicts when housing two contrasting nutritional strategies within the mixoplankton cell (Figs 1, 3). There is no evidence of competition at the cell surface for nutrient uptake required for phototrophy versus that required for phagotrophy to underpin a trait trade-off between nutrient uptake to support phototrophy and prey ingestion (H1.1; Fig. 3; Li *et al.*, 1999; Hausmann, 2002; Gavelis *et al.*, 2017). Analysis of experimental data did not provide any clear evidence of a relationship between surface area or cell size with growth rate potential amongst mixoplanktonic versus zooplanktonic dinoflagellates (Fig. 4). Cell size variations of the scale in question, to accommodate both feeding vacuoles and chloroplasts, are common features of protist plankton (Flynn *et al.*, 1996; Li *et al.*, 1999; Lee *et al.*, 2014). The trait trade-off concept of space sharing (H1.2; Fig. 3) also does not take into account that mixoplankton do not necessarily ingest whole prey items (Tillmann, 2003; Park *et al.*, 2006); rather they can use a peduncle (feeding tube), a mucus trap, etc. and therefore, do not need to allocate significant cell volume to digestive vacuoles. We thus find no evidence to support the hypotheses H1.1 or H1.2, that mixoplankton are compromised relative to zooplankton due to their need for more space to maintain two trophic modalities.

### *Physiology*

Hypotheses H1.3 and H1.4 (Table 1) consider potential conflicts in resource allocation and in light dependency, respectively. The primary drivers for mixoplankton evolution from zooplankton are likely to have been:

(i) a mechanism provided by phototrophy as a back-up supply of C and energy, and
(ii) the retention of nutrients through phototrophy that would otherwise be lost during

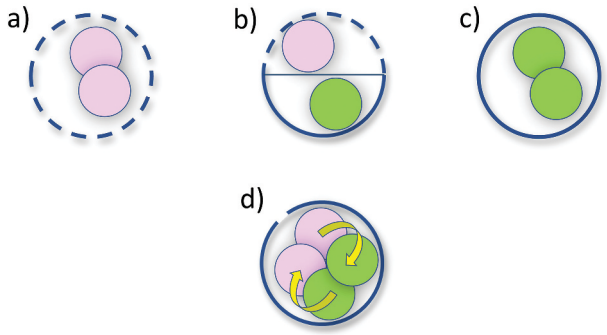


**Fig. 3.** Example putative trait trade-offs for mixoplankton versus zooplankton and phytoplankton. The diagram shows different protist cell configurations with cell-surface allocations to nutrient transport (solid line) or ingestion (gaps), and resource allocation within the cell for prey digestion (pink) or photosynthesis (green). **a**, zooplankton cell; **b** & **d**, mixoplankton cell; **c**, phytoplankton cell. **b**, shows presumptive trait trade-offs for mixoplankton due to the need to house two nutritional pathways. **d**, portrays the reality where ingestion occurs over a very small proportion of the cell surface, and cell volume and physiology are not constrained by space; it also shows the synergism between the phagotrophic and photosynthetic processes (yellow arrows). See also Tables 1 and 2.

digestion and assimilation. This can comprise as much as 30% of prey being assimilated into new biomass through specific dynamic action (SDA; McCue, 2006).

Aspect (ii) alone would cover the nutrient resource demands of phototrophy, dismissing H1.3; in reality, additional nutrients would likely be taken up as well. There is an additional line of evidence indicating that resources for phototrophy are not restrictive. Both GNCM and SNCM may void, rather than digest, failing chloroplasts (Stoecker & Silver, 1990; Schoener & McManus, 2012; Kim *et al.*, 2016), although there are examples of SNCM digesting sequestered chloroplasts (e.g. *Elphidium crispum*, Lee *et al.*, 1988) similar to events observed in zooplankton (e.g. *Oxyrrhis marina*, Öpik & Flynn, 1989).

**Table 1.** Trait trade-off hypotheses for mixoplankton compared with protist zooplankton. SDA, specific dynamic action; GNCM, generalist non-constitutive mixoplankton; SNCM, specialist non-constitutive mixoplankton.

| Trait context | Trait trade-off hypothesis Mixoplankton vs zooplankton | Counter argument |
|---|---|---|
| Allometry | H1.1 Cell surface area sharing in mixoplankton for nutrient transport to support photosynthesis represents a trait trade-off against the surface area needed for prey ingestion. | Feeding typically occurs rapidly over a small and confined area of the cell surface.[1] Therefore, there is no need to sacrifice surface area for feeding versus for nutrient transport. Feeding demands for C and energy are also lessened with C-fixation in phototrophy.[2] |
| Allometry | H1.2 The presence of chloroplasts in mixoplankton represents a trait trade-off against space required for digestive vacuoles. | Assumes, erroneously, that the cell volume is tightly constrained,[3] or that ingestion is always of whole prey.[4] Further, C-fixation through photosynthesis would lessen the need for C acquisition through feeding.[2] |
| Physiology | H1.3 Synthesis and maintenance costs for phototrophy in mixoplankton represents a trait trade-off in resource allocation. | GNCM and some SNCM avoid this cost by a use-and-throw approach.[2] CM and SNCM at the least exploit nutrients otherwise lost from SDA, coupled with light; the costs are clearly more than self-sustaining for phototrophy in the euphotic zone.[5] |
| Physiology | H1.4 Dependence on coupled photo-phagotrophy places an absolute need for light,[6] representing a trait trade-off restricting mixoplankton active growth to the euphotic zone. | For those feeding on prey in the photic zone, where most production occurs, this is not a compromise, especially set against SDA recovery enabled by phototrophy. Some mixoplankton can grow heterotrophically in darkness.[7] |
| Physiology | H1.5 NCM species are hindered by the need to acquire phototrophy from specific prey[8] creating a trait trade-off where enhanced growth through compensating for SDA requires dependence on acquired phototrophy. | For GNCM species, acquired phototrophy is supported by a wide range of prey, which would be grazed on in any case. For SNCM species, the acquired phototrophy is relatively well maintained over weeks and months in various instances. Phototrophy, even if only to balance basal respiration, provides a buffer against starvation.[2,5] |
| Physiology | H1.6 Conflicts between phototrophy and phagotrophy resulting in down-regulations of physiology represents a trait trade-off for growth rates. | Synergies in recycling wastes and co-operativity between phototrophy and heterotrophy provide a better homeostatic cellular environment for growth.[9] |
| Ecology | H1.7 Phototrophy generates a trait trade-off between growth enhancement vs enhanced losses caused by attraction of predators to leaked products of C-fixation.[10] | Zooplankton grazers also release organics due to partial or inefficient digestion.[11] Provision of additional N, P, Fe nutrients from feeding would support better use of newly fixed C and thus less may be leaked. |

[1]Li *et al.* (1999), Stoecker (1999), Hausmann (2002), Gavelis *et al.* (2017). [2]Schoener and McManus (2017), Johnson (2011), Stoecker *et al.* (2017). [3]Li *et al.* (1999), Lee *et al.* (2014), [4]Tillmann (2003); Park *et al.* (2006), [5]Gomes *et al.* (2018), [6]Adolf *et al.* (2003), Stoecker *et al.* (2017), [7]Caron *et al.* (1990), Rottberger *et al.* (2013), McKie-Krisberg *et al.* (2015), McManus *et al.* (2018), Millette *et al.* (2017), [8]Dolan & Pérez (2000), Johnson (2011), Decelle *et al.* (2012), Kim *et al.* (2012), Gomes *et al.* (2014); Moeller and Johnson (2017), [9]Jeong *et al.* (2010); Mitra & Flynn (2010). [10]Verity (1991), [11]Flynn & Davidson (1993).

We must assume that the net gain over the period of operating the acquired chloroplasts, and then voiding defunct plastids, exceeds the nutritional gain in simply directly digesting the chloroplasts else the trait of non-constitutive mixoplanktonic activity would not have survived in evolution.

Both the above-mentioned drivers require light, and this could be seen as a potential trait trade-off for those species that have an obligatory requirement for photosynthate (H1.4). However, while there are indeed examples where predation is coupled with phototrophy in mixoplankton (Adolf *et al.*, 2003; Stoecker *et al.*, 2017), there are examples where that coupling is not strong, or indeed where mixoplankton growth may continue in darkness (Caron *et al.*, 1990; Hansen *et al.*, 2000: McManus *et al.*, 2012, 2018; Rottberger *et al.*, 2013; McKie-Krisberg *et al.*, 2015; Millette *et al.*, 2017). Thus, there is no overwhelming evidence to support the absolute need for light as a generic trait trade-off (H1.4) for mixoplankton versus zooplankton.

Hypothesis H1.5 suggests a trait trade-off restricting the success of NCM to situations where their prey from whence they acquire phototrophy are available. It could be argued that the non-constitutive mixoplankton (NCM; e.g. plastidic ciliates, HAB forming *Dinophysis* sp., bloom forming green *Noctiluca scintillans*) which depend on coupled photo-phago-trophy for their nutritional needs and thence have to acquire their phototrophic potential from prey organisms are at a disadvantage compared with zooplankton (H1.5). The plausibility of such a trait trade-off especially between GNCM ciliates and their zooplanktonic counterparts (Fig. 1) is raised not least because these organisms can inhabit the same environment, thus meeting the critical criterion for considerations of trait trade-offs (Litchman & Klausmeier, 2008), but also because, as ciliates, they share similar evolutionary lineages (Mansour & Anestis, 2021). As many as 50% of ciliates in the euphotic zone may be GNCM (Stoecker *et al.*, 2017) and their presence could be attributed to their ability to negate SDA loss through phototrophy compared with their zooplankton counterparts (Anschütz & Flynn, 2020). It is, however, worth noting that a trait trade-off relating consumer success to the presence of appropriate food applies to all consumers, and not just to NCM.

It could also be argued that a physiological conflict may occur between phototrophy and digestion (H1.6), leading to down-regulations of these individual processes resulting in a decrease in growth rate. However, that would only be a real conflict if the physiologies were viewed as combative rather than providing synergy in support of growth; it is difficult to see why such a conflicting trait combination would be to competitive advantage under any circumstance. The data of Jeong *et al.* (2010) indicate that mixoplanktonic dinoflagellates grow at rates similar to their zooplanktonic counterparts (Fig. 4).

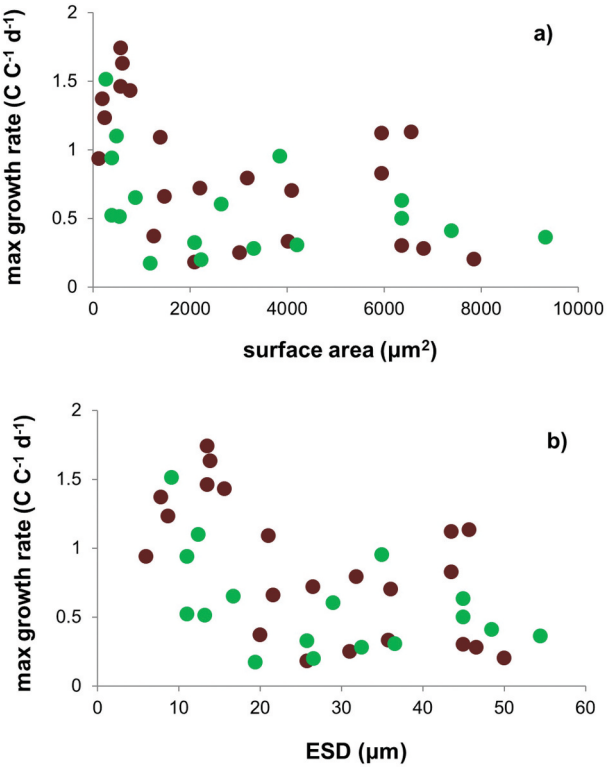


Fig. 4. Comparisons between maximum growth rates for mixoplanktonic dinoflagellates (green) and zooplanktonic dinoflagellates (brown) for different protist cell surface areas (**a**) and equivalent spherical diameter (ESD, **b**). Source data from Jeong *et al.* (2010).

## *Ecology*

Hypothesis H1.7 (Table 1) presents an argument that leakage of organics from phototrophic processes of a mixoplankton would attract predators employing chemo-receptors (Verity, 1991); a photo-pigmented mixoplankton could also be more obvious to visual predators. These two factors could lead to a greater level of loss of mixoplankton to predation as a trait trade-off. However, a zooplankton containing phytoplankton prey would also be pigmented, and they also leak organics (Flynn & Davidson, 1993) which would leave a scent trail for other protist grazers (Spero, 1985; Martel, 2006).

In total, while we see some support for H1.5, applied for ciliate zooplankton versus ciliate GNCM, there are no generic grounds to support trait trade-offs between zooplankton and mixoplankton.

## Mixoplankton versus phytoplankton

For a comparison of mixoplankton versus phytoplankton, we turn to the argument that the demands for the support of phagotrophy may compromise the demands for phototrophy (Table 2). Many of the hypotheses have parallels with those in Table 1, though they are now viewed from a different perspective, i.e. addition of phagotrophy to a phototroph, rather than phototrophy added to a phagotroph, noting that while the latter occurred through evolution, the former did not.

### *Allometry*

As in Table 1 (H1.1, H1.2), the hypotheses here (Table 2, H2.1–H2.4) are based on assumptions of trait trade-offs due to sharing of cell surface area and volume between phototrophy and phagotrophy (Fig. 3). Housing two sets of trophic machinery may be expected to demand a larger cell size which, by biophysical arguments (Andersen *et al.*, 2016), may suggest that mixoplankton fulfil an intermediate point on the allometric scale between smaller phytoplankton and larger zooplankton (H2.1). In reality, the smallest protist plankton size class spectrum

**Table 2.** Trait trade-off hypotheses for mixoplankton compared with protist phytoplankton. SA, surface area; SDA, specific dynamic action.

| Trait context | Trait trade-off hypothesis Mixoplankton vs phytoplankton | Counter argument |
|---|---|---|
| Allometry | H2.1 A mixoplanktonic cell, having to house two nutritional systems, will be larger and thence have a trait trade-off in size such that they are larger than optimal for phototrophy, consistent with biophysics theory.[1] | Mixoplankton of very different sizes (3–>1000 μm diameter), with very different extremes of phototrophy vs phagotrophy, are seen across the oceans.[2] There is no evidence yet that mixoplankton have lower photosynthetic efficiency (e.g. $CO_2$ assimilation per chlorophyll as a surrogate for the rest of the photosynthetic machinery) compared with phytoplankton of the same size from the same environment. |
| Allometry | H2.2 A mixoplankton containing feeding vacuoles will be larger, hence having a thicker larger boundary layer,[3] representing a trait trade-off for nutrient transport, and/or leakage of organics. | Additional nutrient from feeding, and recovery from SDA, would compensate for any loss of nutrient transport capability. Any loss of organics (e.g. leakage of photosynthates) could attract potential prey and thence be an advantage. Uptake (and thence recovery) of organics is common in mixoplankton as well as phytoplankton.[4] See also H2.1; H2.7. |
| Allometry | H2.3 Cell surface area sharing in mixoplankton for nutrient transport to support photosynthesis represents a trait trade-off against the area needed for prey ingestion. | Digestive vacuoles in mixoplankton decrease the effective C-cell density so the demand for transport site area is diluted per SA of cell. A level of counter-provision is also provided by recovery of nutrients from digestion (e.g. $NH_4^+$ from SDA – see Table 3a); phytoplankton are fully dependent on external nutrient source and thus have to compete with other phytoplankton, bacterioplankton and mixoplankton.[1] See also Table 1, H1.1. |
| Allometry | H2.4 Cell volume sharing in mixoplankton for digestive vacuoles represents a trait trade-off against space required for chloroplasts. | The cell volume for a mixoplankton is not tightly constrained.[5] See also Table 1, H1.2. |
| Physiology | H2.5 Space restrictions lead to a trait trade-off in the size of the phototrophic capacity, resulting in mixoplankton having a lower Chl:C. | Feeding in mixoplankton could alleviate the need for C-fixation and thus for high Chl:C. In mature ecosystems where mixoplankton grow,[6] a high Chl:C could potentially be a dangerous trait (photo-oxidation in high light, low nutrient systems where D1 damage-repair may be slower).[7] |
| Physiology | H2.6 Resource expenditure for prey capture and processing in mixoplankton, such as membranes, peduncles and traps, presents a trait trade-off for resource allocation. | Phototrophs regularly over-produce organics, which are leaked;[8] there is no evidence yet that feeding costs would be limiting for mixoplankton. Phagotrophic membranes are very rapidly (tens of minutes) synthesised, deployed and recovered.[9] Phytoplankton expend energy in making cell walls of various rigidity and costs (e.g. Si for diatoms, coccospheres in coccolithophorids).[10] |
| Physiology | H2.7 Conflict between phototrophy and phagotrophy resulting in down-regulation of physiology represents a trait trade-off for growth rates. | Synergies in recycling wastes and co-operativity between phototrophy and heterotrophy provide a better homeostatic cellular environment for growth.[11] |
| Ecology | H2.8 A mixoplanktonic cell, having to house two nutritional systems and thence being larger, will have a trait trade-off as it is more likely to encounter its predator compared with a phytoplankton cell. | No evidence that mixoplankton are indeed larger than comparators.[12] See also H2.1. |
| Ecology | H2.9 Hunting requires motility, presenting a trait trade-off against increasing the likelihood of encountering a predator. Motility also costs energy.[13] | Encounters are also increased by sinking and turbulence, which in diatoms gives similar nutritional advantages to motility.[14] Diel vertical migrating (DVM) 'phytoplankton' are also motile. Motility also enables escape from predators.[15] The energetic cost is minor,[16] especially set against the increased likelihood of using $NH_4^+$ rather than $NO_3^-$ **17** through motility-enhanced nutrition. |

[1]Andersen *et al.* (2016), [2]Leles *et al.* (2017, 2019), [3]Flynn *et al.* (2018), [4]Antia *et al.* (1981); Meyer *et al.* (2022), [5]Flynn *et al.* (1996); John & Flynn (2002), [6]Mitra *et al.* (2014), [7]Li *et al.* (2021), [8]Biddanda & Benner (1997); Wetz & Wheeler (2007); Flynn *et al.* (2008), [9]Li *et al.* (1999), [10]Sikes & Wilbur (1982), [11]Jeong *et al.* (2010); Mitra & Flynn (2010), [12]Flynn *et al.* (2019), [13]Fenchel & Finlay (1983), [14]Ross (2006), [15]Caron & Swanberg (1990); Anderson (1993); Caron (2016), [16]Fenchel & Finlay (1983), [17]Anschütz & Flynn, (2020).

includes mixoplankton, as well as similar sized phytoplankton (Finkel *et al.*, 2010; Unrein *et al.*, 2014; Flynn *et al.*, 2019; Leles *et al.*, 2019; Visintini *et al.*, 2021). As there is evidence that viruses can be ingested by protist plankton (González & Suttle, 1993), it is possible that even the very smallest phototrophic protist could eat. There are thus no grounds to support H2.1.

Larger cells have thicker boundary layers and if mixoplankton were larger as a consequence of containing food vacuoles then this could be argued as deleterious for nutrient uptake (H2.2). However, mixoplankton would be acquiring nutrients from other sources (their prey), and this may be expected to mitigate against any shortfall in nutrient uptake (Tittel *et al.*, 2003), including from thickening of the boundary layer. In addition, any increase in cell size due to the presence of food vacuoles would also increase the effective ratio of surface area (SA) to growing cell biomass (SA:biomass; i.e. ignoring digestive vacuoles containing ingested prey). It is this SA:biomass ratio, and not the SA:volume ratio, which is important in this regard as seen in competitive advantages shown by larger and more vacuolated diatom species against smaller diatom species (Flynn *et al.*, 2018). Net leakage of metabolites (loss exceeding recovery) per cell may also be expected to be greater from larger cells (Flynn & Berry, 1999), though there is no simple size-relationship for the leakage or uptake of dissolved free amino acids (Flynn, 1990). However, even if the net leakage from mixoplankton was greater compared with similar-sized phytoplankton, such a loss of organics could well be advantageous for mixoplankton as these would attract microbial 'prey' (Martel, 2006; Wilken *et al.*, 2014; Smriga *et al.*, 2016). Through feeding on bacteria and cyanobacteria (e.g. Yoo *et al.*, 2017), which are well adapted to acquire N, P and Fe from extremely low concentrations and from recalcitrant forms (Zehr *et al.*, 2017), mixoplankton can access nutrients that are limiting for the growth of other planktonic primary producers (Zubkov & Tarran, 2008; Hartmann *et al.*, 2012; Mitra & Flynn, 2023).

If prey ingestion required a significant part of the surface area of the mixoplankton cell, then the absence of that area for locating nutrient transporters could be expected to decrease scope for nutrient uptake (H2.3, Fig. 3b; Ward *et al.*, 2011). We have already considered this above (Table 1, H1.1); processes associated with feeding do not occupy much surface area (c. <5%, Fig. 3d). On the plus side, a feeding mixoplankton will acquire nutrients (N, P, Fe, etc.) from its prey, with further saving of energy through the internal production of ammonium during prey digestion negating the need and use of nitrate (Anschütz & Flynn, 2020) with its allied costs in terms of Fe and reductant (see also section on 'Trait Trade-Offs in context', above). We have also considered whether the presence of food vacuoles could be argued to compete with space for chloroplasts within the mixoplankton cell (H2.4, cf. Table 1, H1.2); there is no evidence to support this hypothesis as protist cell volume for a given species is highly variable in response to nutrient status (Flynn *et al.*, 1996; John & Flynn, 2002) as well as during the halving and doubling of cell volume over the cell cycle (e.g. for the mixoplankton *Chattonella*, Demura *et al.*, 2009).

### *Physiology*

Photoacclimation is required to maximize productivity without risking photodamage (Richardson *et al.*, 1983). The situation is complicated by the high variability of light over the day which generates a trait trade-off, especially for those phototrophs growing in high-light summer waters, i.e. too little Chl:C and the cell is outcompeted in low light, while too much Chl:C with too much light causes photodamage. This modulation is more problematic if nutrient supply fluctuates and then becomes sub-optimal as this restricts the D1 repair cycle (Li *et al.*, 2021). From hypothesis H2.4, H2.5 suggests a trait trade-off with the phototrophic potential of mixoplankton, as reflected by their low Chl:C in comparison with diatoms (Leles *et al.*, 2021). Aside from the rejection of H2.4 which is required for H2.5, a low Chl:C itself does not evidence a trait trade-off. Mixoplankton can obtain C and energy from sources other than phototrophy, often growing in environments with high surface light in which they can migrate to optimize light incident on the cell surface. In consequence they do not require a high photopigment content (i.e. large chloroplast content). Mixoplankton thus have lowered risks of producing damaging oxidizing radicals. In mono-species blooms, a low Chl:C is advantageous to the collective (Flynn & Hansen, 2013). Phototrophic energetic costs for N assimilation are also lowered in mixoplankton through the previously noted decreased need for nitrate enabled by directly assimilating reduced prey-N (amino acids, nucleic acids), and through the internal recycling of ammonium released during anabolic prey assimilation. Mixoplankton exploiting those N-sources will save the 20% extra photoreductant cost for nitrate assimilation (Flynn & Hipkin, 1999) and, all else being equal, could have pro rata decreased Chl:C ratio in comparison to phytoplankton using nitrate-N. In contrast, diatoms in high light conditions with high Chl:C may need to vent excess photoreductant by superfluous nitrate reduction (Glibert *et al.*, 2016), an opportunity unavailable in low-nitrate waters.

To add to the structural demands required with phototrophy, mixoplankton also have to resource

the means to kill prey (e.g. with toxins), capture, ingest and then digest them. This could be argued as a trait trade-off for resource allocation and in physiology (Table 2, H2.6). The main expenses for capture and ingestion are for energy and especially for C. Phytoplankton release a large proportion of C-fixation as dissolved organic carbon (c. 10%; Biddanda & Benner, 1997; Wetz & Wheeler, 2007; Flynn *et al.*, 2008) as do mixoplankton (Aaronson *et al.*, 1971) and heterotrophic protists (Pelegri *et al.*, 1999; Strom *et al.*, 2003). Such releases are indicative of over-production (phototrophy), voiding (incomplete digestion with phagotrophy) and/or a lack of demand to recover losses. There is no specific reason to suspect that a re-direction of C and energy towards the synthesis of prey capture and processing apparatus should present a physiological challenge (Tillmann, 2003; Lee *et al.*, 2014; Larsson *et al.*, 2022) such that it would comprise a trait trade-off in mixoplankton. In addition, the time scales for synthesis and dissolution (recycling) of membranes for prey capture is in the scale of tens of minutes (Li *et al.*, 1999). All protist plankton autodigest and recycle cell components; the physiological machinery exists in them all. The (re)direction of resources for toxin production in protist plankton appears to be minor (John & Flynn, 2002), and there is no evidence that the toxin production represents a trade-off (Pančić & Kiørboe, 2018). In addition, phototrophy in mixoplankton supports additional routes for production of secondary metabolites providing offensive, defensive or allelopathic capabilities (Granéli & Flynn, 2006).

Physiological conflicts could be envisaged through resources flowing from phototrophy and phagotrophy that lead to down-regulations of these processes (H2.7; cf. Table 1, H1.6), resulting in a decrease in growth rate. The coupling of phototrophy and phagotrophy in the light phase of the diel cycle, although not ubiquitous (Caron *et al.*, 1990; Rottberger *et al.*, 2013; McKie-Krisberg *et al.*, 2015; cf. Table 1, H1.4), may be seen as particularly likely to promote conflicts. However, in reality, these processes are more likely complementary as they use waste products produced by each other; most obviously the nutrients lost with phagotrophy-related SDA are recycled with phototrophy, and excess organics from phototrophy also counters other catabolic and anabolic demands (including those associated with motility, prey capture and digestion). Where it could possibly be argued for a trait trade-off is that the growth rate of the mixoplankton is not a sum of phagotrophy +phototrophy but is capped at the whole-cell level; this may give the impression that the individual processes are incapable of functioning at the rates seen in the zooplankton and phytoplankton comparators and hence for trait trade-off between these different organism types. This is addressed further below.

### *Ecology*

Ecological-facing trait trade-offs of being a mixoplankton rather than a phytoplankton include the size of the cell (Table 2, H2.8) and motility required to enhance predation (H2.9), and therefore the possibility of increased encounter rate with their own predators. We have already shown that there is no evidence for the former (i.e. H2.8; cf. H2.1, H2.2). Motility is common across protist plankton including phytoplankton, either self-propelled or through a combination of buoyancy and turbulence; all of these increase encounter rates. For example, non-phagotrophic phytoplankton flagellates are self-propelled, and the turbulence required to maintain phytoplanktonic diatoms in suspension (Raven & Beardall, 2022) equally brings them into contact with predators. Further, diatoms *Ethmodiscus* and *Rhizosolenium* exhibit vertical migration within a cell division cycle by changing their cell density relative to seawater, thus enhancing nutrient acquisition at the nutricline and photon acquisition nearer the surface (Kemp & Villareal, 2013, 2018). Large eSNCM Rhizaria (Acantharia, Polystine Radiolaria) do not swim (they float) and capture motile and non-motile prey in webs of pseudopodia (Caron & Swanberg, 1990; Anderson, 1993; Caron, 2016). Rapid jumping motions in ciliates, including mixoplanktonic species, helps them to escape predation (Jonsson & Tiselius, 1990; Jiang & Johnson, 2017; Jiang *et al.*, 2018).

In summary, there is little if any evidence to support generic trait trade-off arguments for mixoplankton versus phytoplankton (Table 2). For most putative aspects, much of the biochemical machinery is common between mixoplankton and phytoplankton. In mixoplankton, phototrophy enables the retention of nutrients that are otherwise lost during phagotrophy. While there is a possible trade-off if a potential synchronized linkage is considered between photosynthesis and phagotrophy (as that may restrict feeding to the light phase; H2.7), there are also sufficient exceptions to detract from this being a trait trade-off rule (Caron *et al.*, 1990; Rottberger *et al.*, 2013; McKie-Krisberg *et al.*, 2015).

## Potential advantages and trait trade-offs in mixoplankton physiology

We now review mixoplankton traits that could be considered to be of advantage for these organisms compared with the phytoplankton or zooplankton (Table 3).

**Table 3.** Mixoplankton traits that could be considered as providing competitive advantages over protist zooplankton and/or phytoplankton, and possible trait trade-offs. SDA, specific dynamic action.

| | Mixoplankton trait | Potential advantage over zooplankton and phytoplankton | Observations |
|---|---|---|---|
| (a) | Energy and resource saving from synergism | Mixoplankton can save potentially 30% of ingested resources by countering SDA losses. The N thus recovered, as $NH_4^+$, saves an additional c. 20% in photo-reductant versus uptake and assimilation of external $NO_3^-$.[1] | This trait will be of especial advantage under nutrient-limiting conditions. Assumes that nutrients lost with SDA are indeed reassimilated in mixoplankton, and that products from photosynthesis do not otherwise hinder prey digestion. |
| (b) | Balance of DIC and $O_2$ production/demand | In mixoplankton, the coupling of phagotrophy with phototrophy provides $CO_2$ for C-fixation and $O_2$ for phagotrophy, thus mitigating DIC limitation and $O_2$ toxicity/inhibition of photosynthesis. | Requires synchrony of feeding and photosynthesis. See also Table 1, H1.4. |
| (c) | More constant cellular stoichiometry | In mixoplankton, coupled nutrition enables a better stoichiometric balance, less stress with better growth.[2] Phototrophy also aids essential lipid synthesis.[3] | Nutrient limitation promotes secondary metabolite production for toxins,[4] conflicting as a trait trade-off with the potential gain of having a more constant stoichiometry. Optimal stoichiometry likely equates to good quality food for predators which could result in more predation on the mixotroph. |
| (d) | Removal of competitors and predators through death and ingestion | Removal of competitors, and potentially of predators, also supplying nutrients to the mixoplankton. | Most likely only of advantage once mixoplankton are locally numerically dense. Death of other organisms need not be linked to ingestion by the mixoplankton (e.g. *Prymnesium* toxins).[5] |
| (e) | Niche specialism | Mixoplankton exploit traits to competitive advantage in niche environments (Table 3a–d). Mixoplankton may be slow growing to optimize growth potential in K-select, mature ecosystems with close interconnectivity between organisms.[6] | Optimizing growth rate potential is a suggested universal trait trade-off.[6] There is also great variability in non-mixoplankton growth rate potential.[7] Some mixoplankton species can grow rapidly[8] enhanced by coupling trophic modes. Organisms from immature ecosystems inevitably have more simple nutrient acquisition mechanisms. |

[1]Anschütz & Flynn (2020), [2]Stoecker *et al.* (1988, 2017); Adolf *et al.* (2006); Flynn & Mitra (2009),[3]Wickham & Wimmer (2019); Sato (2020).[4]John & Flynn (2002); Granéli & Flynn (2006),[5]Thingstad *et al.* (1996); Olli & Teeveer (2007),[6]Flynn & Skibinski (2020),[7]Finkel *et al.* (2010),[8]Jeong *et al.* (2010).

Zooplankton, and indeed all consumers, inevitably lose a significant proportion of assimilated resources through biochemical conversions and the synthesis of their own biomass. This loss associated with anabolic respiration, as specific dynamic action (SDA), can cost a consumer c. 30% of nutrients. A mixoplankton, through photosynthesis, has scope to directly recover this loss of N, P and Fe (Table 3a). In the case of N, there is an ancillary advantage (over phytoplankton) in that the internally recycled nitrogen as $NH_4^+$ is far cheaper than using externally sourced $NO_3^-$ in terms of both photo-reductant and Fe (both required for nitrate reduction; Flynn & Hipkin, 1999). While, as noted above, the theoretical significant saving in resources does not often equate to differences in growth rate between ammonium versus nitrate grown phytoplankton, this trait would be of especial advantage under nutrient-limiting conditions. There is no evidence for how interactions between phagotrophy and ammonium versus nitrate consumption may affect mixoplankton growth at low light; for phytoplankton, there is, counterintuitively, no difference in growth using these DIN sources at low light, but there can be at high light (Thompson *et al.*, 1989). The only caveat to the perceived advantage of internal nutrient recycling with photo-phagosynergism in mixoplankton, and one that is important in modelling, is that just because mixoplankton are able to be more efficient at retaining ingested prey-nutrient, they are not necessarily more efficient all the time; all consumers exhibit lower efficiency when resources are in abundance (Mitra & Flynn, 2007) and high growth efficiencies when prey abundance is limiting (Schoener & McManus, 2017).

Concurrent phototrophy and phagotrophy in mixoplankton provides scope for optimizing both physiologies (e.g. Wilken *et al.*, 2014). There will be benefits through internal production and consumptions of $O_2$ and $CO_2$ (Table 3b) and perhaps the stabilization of cell surface pH. Consumption of $O_2$ is of especial importance in optimizing C-fixation through RuBisCO (as $O_2$ uptake competes with $CO_2$ uptake). This also alleviates the need for carbon-concentrating mechanisms (CCMs; most dissolved inorganic C is as bicarbonate, while $CO_2$ is the substrate for RuBisCO), though a role for CCMs in mixoplankton is unclear (Raven *et al.*, 2009, 2020). During phototrophy pH increases and conversely during heterotrophy pH falls; at extremes, these changes can be deleterious and even lethal (Hansen, 2002), a situation that is more problematic with ocean acidification as the buffering capacity of seawater is weakened (Hofmann *et al.*, 2010). Mixoplankton have scope to modulate near-cell and thence bulk-water pH levels, in the same way that calcification in coccolithophorids may stabilize external pH (Flynn *et al.*, 2016). Caveats include that other plankton will also benefit from any such modulation in bulk water pH, and we do not know how

temperature changes may affect the balance of phototrophy and phagotrophy (Ferreira *et al.*, 2022).

Organism growth can only be maximal when internal nutrient conditions are optimal. This state of optimal stoichiometry may be considered to be more likely in a mixoplankton than in a phytoplankton or a zooplankton (Table 3c; Stoecker *et al.*, 1988; Adolf *et al.*, 2006; Flynn & Mitra, 2009). The need for a balanced diet, for provision of different lipids for example (Wickham & Wimmer, 2019; Sato, 2020), is also more easily met with an internal phototrophic potential. The caveat in the advantage of this healthier cell status is that the organisms may also then provide a good (perhaps more attractive) food source for predators. The production of toxins and allelopathic compounds are also often associated with low nutrient stress (John & Flynn, 2002; Granéli & Flynn, 2006), and may thus be expected to be depressed in mixoplankton rather than in phytoplankton if the former are less stressed.

Of critical ecological importance, and a factor that will be missing from any autecological analysis of trait trade-offs focused on phytoplankton versus mixoplankton, is the role of predation. Predation offers mixoplankton scope to remove their competitors and even to kill potential grazers (Table 3d; Thingstad *et al.*, 1996; Tillmann, 2003). That activity is of value even though death of other organisms need not be directly linked to phagotrophy by the mixoplankton (Olli & Teeveer, 2007). Killing and exploiting competitors can occur at low mixoplankton abundances, but to control a much faster growing competitor this action requires high mixoplankton numbers as this is a density-dependent process. Likewise, the collective action of many mixoplankton cells against larger competitors or predators is dependent on high cell abundances. The complexities of interactions between organisms which display allelopathic and toxic potential makes predicting the winner extremely difficult (Flynn, 2008). Further, identifying generic trait trade-offs becomes even more problematic as the winner may not be the organism that we may think it is from autecological considerations. There are reasons to suspect that mixoplankton could ingest viruses, given evidence that viruses can be ingested by protist plankton (González & Suttle, 1993). There is, however, no reason to also suspect that such phagotrophy in mixoplankton would make them any more prone to viral infections than are zooplankton. If phagotrophy did provide a route for entry of viruses into protists resulting in infections, then this would represent a trait trade-off to the advantage of phytoplankton. There are many factors that impact on the success of viruses that would impact the assessment of such a putative trait trade-off (Flynn *et al.*, 2022).

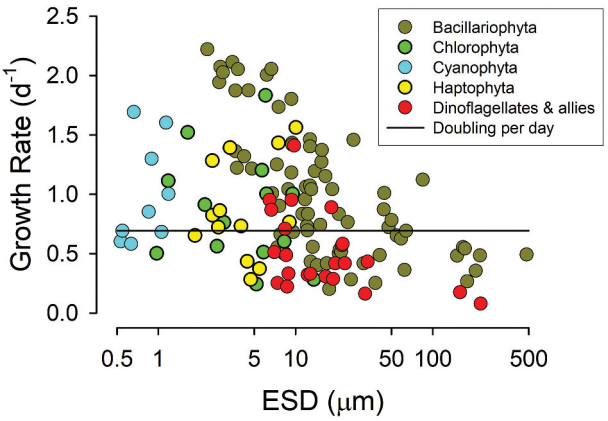


**Fig. 5.** Maximum growth rates for phototrophic plankton of different sizes. Many of the dinoflagellates and haptophytes are known, or are prospective, mixoplankton (cf. the mixoplankton database, Mitra *et al.*, 2023). Note the great variety in growth rate potential within a given size range. The horizontal line indicates growth at 0.693 day$^{-1}$ (a doubling per day) as an exemplar of rates expected of cells with division cycles synchronized to the diel light-dark cycle (Nelson & Brand, 1979). Source data from Finkel *et al.* (2010).

Research on mixoplankton has been complicated by the sensitivity of these organisms to conditions and difficulties in maintaining them in culture, especially in axenic cultures. In large measure such problems probably reflect ignorance over the need for specific abiotic and biotic growth conditions for the growth of these niche specialists (Table 3e). Some CM can be grown as de facto 'phytoplankton' (as in most plankton culture collections), while some may be grown heterotrophically in the dark (Lie *et al.*, 2018; Abreu *et al.*, 2022), and others require feeding with specific prey (notably pSNCM). This cultivation problem itself flags how little we know of the ecophysiology of these organisms (e.g. Blossom *et al.*, 2017; Larsson *et al.*, 2022). Indeed, there are suspicions that the ability to feed is lost on prolonged maintenance of cultures without prey (Blossom & Hansen, 2021). Unless we understand the required conditions for optimal growth, we cannot formulate meaningful trait trade-off tests of general applicability. Perhaps the breadth of mixoplankton functionality prevents such a formulation.

Finally, we return to the issue of additive growth support from phagotrophy+phototrophy (associated with Table 2, H2.7) and the perception that mixoplankton are slow growers. Growth rate is the most readily used benchmark of trait trade-off discussions, making the (not necessarily correct) assumption that this relates to fitness. If a mixoplankton has a lower maximum growth rate than a similar sized (purely phototrophic) phytoplankton or (purely phagotrophic) zooplankton, then this may be argued to evidence a trait trade-off in being mixoplanktonic. However, the great variation in growth rate potential

within a given phototrophic plankton size (Fig. 5, with $r^2$ = 0.15, see Finkel *et al.*, 2010) clearly flags that a simple trait trade-off is not at play. Similarly, we see such variation within dinoflagellates, be they mixoplankton or heterotrophic zooplankton (Fig. 4). Furthermore, while many mixoplankton are slow growing (Table 3e), there are exceptions (Dolan & Pérez, 2000; Adolf *et al.*, 2006), suggesting that being mixoplanktonic per se is not the explanation. Combining niche specialization (Table 3e) with the proposed universal trait trade-off of matching the maximum growth rate potential to the environmental conditions (Flynn & Skibinski, 2020) provides an explanation; this is revisited in detail below. Also important is that mixoplanktonic activity is synergistic or cooperative (Mitra & Flynn, 2010, 2023; Wilken *et al.*, 2014). Only in some instances does the activity appear to be additive (e.g. Jeong *et al.*, 2010). Any perceived weakness in a particular physiological trait could reflect a trait trade-off but it could equally, if not more likely, reflect a balance between supply and demand for resource acquisition and handling through different complementary mechanisms.

## Discussion

### *Why are mixoplankton not all-conquering?*

Our analysis finds no compelling evidence for trait trade-offs between mixoplankton and their non-mixoplankton competitors except for one instance, associated with GNCM versus non-mixoplanktonic ciliates (Table 1, H1.5). The balance of advantage and disadvantage for GNCM ciliates is consistent with the repeated gain and loss of photosynthesis by protists over evolution (Fig. 2; Raven *et al.*, 2009). The assumptions required to support the generality of the other potential trait trade-off arguments (Tables 1, 2) have too many exceptions and too little (if any) lines of evidence. Considerations of trait trade-offs within mixoplankton functional types are greatly tempered by the great taxonomic range across these organisms. The analysis in Mitra *et al.* (2023; their Fig. 4) shows that while GNCM are confined to Ciliophora, pSNCM are in Foraminifera, Ciliophora and Dinoflagellata, eSNCM are in Radiolaria, Foraminifera, Ciliophora and Dinoflagellata, while CM are found in Cercozoa, Dinoflagellate, Ochrophyta, Haptophyta, Cryptophyta and Chlorophyta. Taken with other views that ecological factors can readily overturn autecology arguments (Sommer *et al.*, 2017), we conclude that the complexity of the ecosystems in which different mixoplankton live identifies the trophic interactions that are key to the success or failure of different members of each functional type in any given time and space. This raises the question as to why mixoplankton are not dominant everywhere.

Mixoplankton proliferate in mature (K-selecting) ecosystems, which are characterized neither by non-limiting inorganic nutrient concentrations, nor by abundant prey species supportive of the growth of specialist phytoplankton and zooplankton, respectively. Phytoplankton and protistan zooplankton dominate as r-select species in immature ecosystems (Mitra *et al.*, 2014). Flynn & Skibinski (2020) suggest that the maximum growth rate evolves to match the potential of the environmental conditions to support that growth rate (consistent with Droop, 1974); a high growth rate potential leads to deleterious stresses in an organism growing in an environment that can only support low growth rates. On the contrary, growth in optimal conditions selects for faster growth rates in microbes (Lenski *et al.*, 1998). Such a concept helps explain the variety of growth rates for a given size group of organisms, as seen in Figs 4 and 5, when we consider that the organisms tested were isolated from very different environments. A key emergent trait, the potential maximum growth rate, then becomes unavailable for mixoplankton trait trade-off arguments.

Phytoplankton evolved from mixoplanktonic lineages (Fig. 2); the more appropriate trade-off question concerns the loss of phagotrophy in phytoplankton. In this context the diatoms stand out as being the primary comparator against which to consider mixoplankton. Diatoms are an extremely successful and relatively recently evolved group (Behrenfeld *et al.*, 2021); they are not known to be capable of phagotrophy (akin to feeding), though mixotrophy via osmotrophy is well documented (see Introduction). There is, however, evidence in diatoms of: endocytosis in vegetative diatom cells in the form of siderophore uptake (Kazania *et al.*, 2018); intracellular bacteria in diatoms within chloroplast invaginations of *Pinnularia* (Schmid, 2003*a*, 2003*b*); and symbiotic diazotrophic cyanobacterium *Richelia intracellularis* within the diatoms *Hemiaulus* and *Rhizosolenia* (Tuo *et al.*, 2021). Presumably, phagocytosis was involved in (cyano)bacteria entering the diatom protoplast; in view of the much greater size of these (cyano)bacteria than of pores in the diatom frustules, (cyano)bacterial entry to the protoplast of *Hemiaulus* and *Pinnularia*, and association with the plasmalemma surface in *Rhizosolenia*, entry is most likely during sexual reproduction when cell walls are temporarily absent.

While it is tempting to try and consolidate different plankton species into a few simple groups, in reality, the variation between organisms reflects selective pressures for evolution in (especially considered on a microbial scale) very different environments. By comparing the schematics in Fig. 1, it can be readily

appreciated that the variety amongst mixoplankton functional types exceeds that within the non-diatom and diatom phytoplankton. The mixoplankton are far from a single functional group that could be amenable to a single set of trait trade-off arguments, or to a sliding scale of physiological constraints (e.g. Ward & Follows, 2016). The model analysis by Anschütz & Flynn (2020) shows how physiological differences may affect the success of protist plankton groups but there are clearly many avenues that remain to be explored. For example, the sensitivity of success for each organism to ecosystem nutrient loading affects the balance of competitors, predators and potential prey (Anschütz *et al.*, 2022). The varieties in mixoplankton ecophysiology are consequences of the food web structure in which these organisms live (Leles *et al.*, 2021) and ultimately evolve. It is important to note that net growth rate set against gains and losses is the critical issue in evolution (Flynn & Skibinski, 2020), and not the gross growth rate of the individual which most readily forms the base of trait trade-off considerations. The high connectivity between organisms and resources in mature systems is probably one of the factors affecting the sensitivity of mixoplankton to specific conditions (Table 3e).

Mixoplankton rarely thrive in the high nutrient and turbulent conditions that favour most diatoms, fully consistent with Margalef's mandala relating these conditions to phytoplankton succession (Margalef, 1978; Glibert, 2016). Whether that is typically a consequence of direct physical damage to the mixoplankton cells, or acts through disruption of their food web, is unclear. The diatoms *Azpeltia, Coscinodiscus, Rhizosolenia, Stephanopyxis* and *Thalassiosira* occur at the deep chlorophyll maximum in stratified ocean waters (Kemp & Villareal, 2013, 2018) where the nutrient concentrations are significantly higher than near the surface waters. These are environments where mixoplankton may also dominate, and it is within such a restricted context that trait trade-offs may perhaps be sought. The bottom line, however, is that considerations of generic trait trade-offs between diatoms and mixoplankton are fraught with problems (Table 2), and conflict with the prime trait trade-off caveats to consider organisms from the same environment and of a similar evolutionary lineage that could have traded traits during their evolution. Furthermore, just as some diatoms thrive in calm conditions, so some mixoplankton thrive in turbulent conditions (e.g. coccolithophorids; Avrahami & Frada, 2020). Reports of bacterivory in the 'phytoplankton' *Emiliania huxleyii* (Avrahami & Frada, 2020) and *Phaeocystis globosa* (Koppelle *et al.*, 2022) will surely not be the last revelations. The organisms that break the rules may be the winners in a given situation.

### *When "trait trade-offs" are not trade-offs*

We must be careful not to align what could be a series of disparate evolutionary events (Mansour & Anestis, 2021; Bremer *et al.*, 2022) in organisms far separated in spatial and temporal scales, to generic trait trade-offs. An organism with a coupled phagotrophic and phototrophic metabolism may not have been subjected to evolutionary pressures to develop high affinity acquisition mechanisms as may organisms with only one of these trophic routes. At the extreme, prolonged lack of a need to express a particular trait probably results in (deleterious) mutations that are not selected against; this is seen in cultures of CM maintained solely as phytoplankton which lose the ability to eat (Blossom & Hansen, 2021). Such situations are indicative of the outcome of different evolutionary lineages developed in different environments and are not evidence of the existence of a trait trade-off. While some may argue that this is a matter of semantics, the net result being the same (mixoplankton perhaps being less well equipped than the 'pure' functional types for each resource route when grown in nutrient or prey replete conditions), we must only apply the trait trade-off label to an event that is indeed a plausible scientifically established trait trade-off. For other instances, we must use appropriate concepts and terminologies.

For organisms dominant in immature systems, from whence coincidentally most cultured plankton are isolated, considerations of trait trade-offs through primarily autecological aspects may possibly be appropriate. The mature ecosystem in which mixoplankton are more common, typified by the temperate summer, is inherently more complex than the immature temperate spring. We suggest that the exploitation of trait trade-offs as a meaningful route to drive research, is not applicable for mixoplankton. We need to search elsewhere for reasons as to why different mixoplankton succeed where and when they do. More appropriate marine plankton ecology models are required that are specifically developed to reflect diversity in physiological functionality, rather than the biogeochemical models which employ gross simplifications built around perceived generic rules, such as trait trade-offs (Flynn *et al.*, 2015). After all, biogeochemistry is ultimately an emergent function of ecology.

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## Data availability statement

All data relating to this work are presented herein or referenced.

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