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Competition between vacuolated and mixotrophic unicellular plankton

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Abstract
Trait-based ecology allows much of the complexity of ecosystems to be projected onto a low dimensional trait space. We conjecture that three key traits capture the main aspects of diversity of unicellular planktonic organisms: cell size, trophic strategies (relative investment in photosynthesis and phagotrophy), and vacuolation. The three selected traits are representative of two groups: mixotrophic protists, which in addition to phototrophy use phagotrophic grazing as a food source and diatoms that use fluid-filled vacuoles to increase their physical size relative to carbon biomass. We construct a trait-based model related to the three traits and determine the optimal trait values of cells of a given carbon size in a given environment. We also perform fully dynamic simulations to study the self-assembled trait distribution at steady state and throughout a seasonal cycle. Diatoms’ strategy is shown to be advantageous among small cells and under early spring conditions, in which highly vacuolated diatoms are simulated, while mixotrophy is dominant among larger cells when nutrients are limiting (summer conditions). The novelty of this approach lies in the mechanistic understanding of plankton physiology that is successfully used to capture how community trait structure emerges from trade-offs that constrain the different strategy among unicellular plankton.
Introduction

Unicellular marine plankton are taxonomically and functionally diverse, characterized by a wide range of traits specialized to face numerous abiotic and biotic pressures, the most significant of which are nutrient limitation, light limitation, competition for resources (Tilman et al., 1982) and predation (Paine, 1966). Their interactions with each other (competition, predator-prey interactions) and with their environment form complex microbial ecosystems with interwoven pathways through which energy and matter are channelled. The coexistence and high diversity of planktonic organisms found in the ocean can therefore be explained by their broad distribution over a multidimensional trait space, resulting in different strategies to deal with ever changing environmental constraints (Barton et al., 2013, a and b). In this context, the complexity of planktonic communities challenges traditional taxonomically oriented modelling approaches that are based on species or functional groups (Thingstad et al., 2010). For this reason, the use of a trait-based approach (McGill et al., 2006) that projects the diversity onto an appropriate low-dimensional trait space is well suited to address the competitive and trophic mechanisms that structure unicellular plankton communities across environmental gradients (Merico et al., 2009; Edwards et al., 2013; Kenitz et al., 2018; Kjørboe et al., 2018). The question then arises as to which traits to use to best characterize the diversity (Litchman et al., 2008, 2010 and 2013).

Cell size is a key trait of unicellular plankton (Finkel et al 2009; Andersen et al., 2016). Overall, the large size range of unicellular plankton reflects a competition-defense trade-off, the outcome of which depends of prevailing conditions. Specifically, due to a high surface/volume ratio, small cells are better competitors when resources are limiting (Litchman et al., 2007) while large cells are less vulnerable to predation (Hirst and Kjørboe, 2002). A large proportion of planktonic protists have been recognized as being capable of combining photo-autotrophy and phago-heterotrophy (Mitra et al., 2016). This prevalent mixotrophic strategy is advantageous to deal with nutrient or carbon limitation and is a dominant component of planktonic assemblages in oligotrophic waters (Hartmann et al., 2012; Stoecker et al., 2017). Cell size is a strong determinant of trophic strategy, with small cells being autotrophic, large being heterotrophic, and a wide spectrum of mixotrophic strategies for intermediately sized cells (Andersen et al., 2016; Chakraborty et al., 2017).
Besides mixotrophs, diatoms represent another important component of unicellular plankton contributing to ~20% of the total primary production on earth (Falkowski et al., 1998; Field et al., 1998). Diatoms are conspicuous being encased in a silica shell and typically with a large central fluid filled vacuole that can occupy up to 80% of the cell volume (Raven, 1995). While the shell-vacuole structure confers several properties to diatoms, two to note here are (1) the large vacuole allows diatoms to have a larger physical size for a given carbon content than non-diatoms and (2) the shell prevents them from performing phagotrophy. Diatoms are therefore obligate phototrophs. In terms of traits, the main characteristic of diatoms is in this context their vacuole.

Among unicellular plankton, mixotrophs and diatoms constitute two important groups of unicellular plankton that have taken distinctly different approaches to contend with the growth-competition-defense trade-offs along a range of cell sizes. The competition between diatoms and mixotrophs has been previously investigated using empirical data (Barton et al., 2013a) and modelling (Leles et al., 2018). These studies show the strong dominance of pure phototrophs such as diatoms during the spring bloom and under nutrient-replete conditions while oligotrophic conditions sees an increase in the proportion of mixotrophs, outcompeting strict autotrophs and strict heterotrophs. Leles et al., 2018 show that the presence of mixotrophs increases trophic transfer efficiency and that the size of mixotrophs is reduced in nutrient depleted conditions compared to light limited, nutrient replete conditions. Overall, diatoms tend to dominate over other groups of protists, including mixotrophs in a wide range of environmental conditions, including both nutrient-depleted (Werner, 1977; Malviya et al., 2016) and nutrient-replete waters (Werner, 1977; Armbrust, 2009; Barton et al., 2013a).

Although it is now well recognized that diatoms exhibit higher growth rate compared to other groups (Edwards et al., 2012), the exact mechanisms behind the success of diatoms and why they tend to dominate production are still unclear (Hansen and Visser, 2019). Among the most widely accepted views are the resistance to predators provided by the silica shell protection (Smetacek, 1998; Hamm et al., 2003) and the high specific nutrient affinity provided by vacuolation (Raven, 1997).

In this paper, we use the mechanistic trait-based approach (Kiørboe et al., 2018) applied to unicellular protists. This approach relies on the mechanistic understanding of the trade-offs between the fundamental traits, based on benefits and costs that are purely related to physical and allocation constraints. We use our description to explore competition and co-existence
between two major groups of unicellular plankton, namely those organisms with varied degrees of investment along the photo-phagotrophy axis (Andersen et al., 2015; Chakraborty et al., 2017), versus those organisms that are obligate phototrophs, but with varied vacuole size and shell requirement (Hansen and Visser, 2019). That is, between generalist auto-mixo-heterotrophic unicellular plankton, and diatoms.

As phagotrophy and vacuolation/silica shell are mutually exclusive strategies, the generalist (mixotrophic) and diatoms’ strategies are distinctively and respectively represented by two continuous trait axes: the trophic strategy for generalists and degree of vacuolation for diatoms. The investment in phagotrophy describes the continuum of mixotrophic strategies, from purely phototrophic to purely heterotrophic cells. Indeed, this investment represents the central trade-off for generalists in that resources that are invested in phagotrophy cannot be invested in light harvesting and vice versa. For diatoms, vacuolation increases the cell’s physical size, providing the potential for increased specific take-up of inorganic nutrients and light affinity (Raven, 1997; Smetacek, 1999) - the so-called Winnie-the-Pooh strategy (Thingstad et al., 2005) while also affording a measure of defense against predation (Smetacek et al., 2004). It is generally understood that vacuolation and a silica shell are attendant features of diatoms, where the shell serves as an exoskeleton providing resistance to turgor pressure (Raven and Waite, 2004), while the vacuole can serve in providing buoyancy to off-set the ballasting of the dense shell material (Gross and Zeuthen, 1948; Boyd and Gradmann, 2002). In this, the central trade-off for diatoms is a higher specific uptake of resources (Raven, 1997; Smetacek, 1999) and a lower predation risk (Hamm et al., 2003; Liu et al., 2016, Pančić et al., 2019) balanced against the need for silicon and a greater investment in cell membranes.

Beyond the differences in phagotrophy and shell/vacuolation between generalists and diatoms, all other formulations of the cells’ physiology are kept identical in our model. For instance, in contrast to previous studies, we do not prescribe the maximal growth rate of diatoms to be higher than for mixotrophs (Bopp et al., 2005; Follows et al., 2007; Aumont et al., 2015). Likewise, we do not (and cannot) include all of the strategies expressed in nature by unicellular plankton such as vacuolation in non-diatom protists or colony formation diatoms, or vertical migration and luxury uptake of nutrients a behaviour that is seen some diatoms as well as dinoflagellates. Our aim is to focus solely on the two principal trait axes characterizing generalists and diatoms and how these shape their competition and coexistence. In our model, we describe the individual cell by its size and its investment in
either vacuolation or trophic strategy. First, the model is used for optimization, i.e., determining which strategies lead to the highest population net growth rates in various environments. Second, to explicitly describe competition, we study a full dynamic model in static and seasonal environments. We show how vacuolation gives a competitive advantage in low and intermediate size classes over a large range of environmental conditions whereas mixotrophic strategy is more advantageous (higher net growth rate) among larger cells with the use of phagotrophy when nutrients are limiting (summer). Vacuolation provides a protection against grazers in addition to the silica shell due to increased physical size and silica limitation constraints the maximum vacuole size, due to a high silica to carbon requirement in highly vacuolated cells.

**Methods**

**Physical attributes of plankton cells**

We consider individual cells (Fig. 1a) characterized by their size, the size of their inner vacuole or their trophic strategy (i.e., investment in light harvesting vs. phagotrophy). Cells are composed of carbon and nitrogen in stoichiometric proportions (we assume a constant C:N ratio, denoted $c_{CN}$) contained in the cytoplasm and in the membranes while silicon (Si) is also taken up and stored by vacuolated cells in the silica frustule. Size is expressed as the carbon mass excluding membranes and denoted $x$ ($\mu$gC). The total carbon ($x_{tot}$ in $\mu$gC) includes the membrane mass: $x_{tot} = x + x_{MI}H(v) + x_{MO}$ where $x_{MI}$ and $x_{MO}$ are the carbon contents of inner (vacuole) and outer (plasma) membranes (see Fig. 1a). $H(v)$ is a Heaviside function of $v$, the fraction of radius occupied by the vacuole, which is used to indicate that only cells with a vacuole ($v > 0$) have an inner membrane. Membranes are modelled explicitly, as they are carbon rich (Raven, 1987), a property that becomes important for small cells as well as cells with large vacuoles. The relations between carbon mass $x$, radius $r$, volume, vacuolation factor $v$, the thickness of the shell and the C:S ratio ($c_{CS}$) are described in details in Appendix A. The principle effect of vacuolation is to increase the physical size (radius $r$) of the cell without increasing its carbon mass $x$. A larger radius has the advantage of allowing higher carbon-specific diffusive fluxes of nutrients (Munk and Riley, 1952), increased capacity to harvest light due to lower self-shading (Morel and Bricaud, 1981), and reduced risk of predation mortality (Hirst and Kiørboe, 2002). The costs of a vacuole include the need of a
silica shell for support, together with the extra (inner) membrane surrounding the vacuole, and the inaccessibility of phagotrophy as a trophic strategy.

The total amount of carbon apportioned to different functions of biosynthesis (structural mass; $\phi_{struct}$, membrane mass ($\phi_M$), light harvesting ($\phi_L$) and phagotrophic feeding($\phi_F$) is (see Table 1):

$$\phi_{struct} + \phi_M(x, v) + \phi_L + \phi_F = 1 \quad (1)$$

The fraction of total carbon invested in structure $\phi_{struct}$ encompasses machinery for biomass synthesis (ribosomes etc.) and nutrient uptakes (nitrogen and silicon in the case of vacuolated cells). For the sake of simplicity, this investment is kept constant for all cells and sizes and does not change with the cell’s environment: $\phi_{struct} = 0.3$.

Membranes are assumed to be of uniform thickness 8 nm. Vacuolated cells, $v > 0$, contain two membranes as the vacuole is surrounded by an inner carbon membrane in addition to the outer plasma membrane (Fig. 1a). Therefore, the total investment in membranes $\phi_M$ varies as a function of both carbon mass $x$ and vacuolation factor $v$. The calculation of $\phi_M(x, v)$ is given in Appendix B.

The incompatibility between vacuolation and phagotrophy means that we can rewrite (1) as:

$$\phi_L(x) = 1 - \phi_{struct} - \phi_M(x, 0) - \phi_F \quad \text{for non-vacuolated cells (} v = 0 \text{)} \quad (2a)$$

$$\phi_L(x, v) = 1 - \phi_{struct} - \phi_M(x, v) \quad \text{for vacuolated cell (} v > 0 \text{)} \quad (2b)$$

In this way $\phi_F$, is completely determined by the investment in $\phi_L$ for generalist cells, and $\phi_L$ is determined by vacuole size for diatoms. The trait space thus becomes two dimensional for each of the two types with the dimensions being carbon size $x$ and $\phi_L$ for the generalists and $x$ and $v$ for the diatoms.

**Nutrients, light and prey affinities**

Affinities for light, nutrients, and prey are determined by the investments ($\phi_L$ and $\phi_F$ in concert with limitations set by cell size.

The affinity for nutrients $A_R$ with $R$ being nitrogen or silicon has the form:

$$A_R(x, v) = c_R r(x, v), \quad (3)$$

where $c_R$ is the maximum affinity for resource $R$ (L d$^{-1}$ µm$^{-1}$). The proportionality with radius reflects the diffusion limited uptake of any dissolved material to a spherical absorbing sphere
as being linearly dependent on the radius of the sphere (Crank, 1979). For a given $x$, increased vacuolation $v$ increases cell radius $r$, thus providing higher carbon-specific nutrient fluxes.

The affinity for light $A_L$ scales with the surface of an equivalent spherical cell with the form (Appendix C):

$$A_L(x, v, \phi_L) = c_L r(x, v)^2 \left[ 1 - \exp(-\theta \phi_L r_0(x)(1 - v^3)^{-2/3}) \right],$$  \hspace{1cm} (4)

where $\theta$ is the absorption coefficient ($\theta = 0.2 \text{ m}^{-1}$, Table 1; Morel and Bricaud, 1981; Raven, 1984) and $\phi_L$ is given by Eq. 2b if $v > 0$. Here, $c_L = \pi q$ where $q$ is the quantum yield of photosynthesis (Appendix C) and $r_0$ is the equivalent carbon mass radius, i.e. without vacuole (Appendix A). Overall, Eq. (4) represents self-shading of affinity for light. When the product of radius and investment in light is small, the affinity scales linearly with $\phi_L x$ while larger cells with high investment are limited by the self-shading and affinity scales with $r^2$.

Last, non-vacuolated cells have an affinity for prey (phagotrophy) that scales with the cell volume (Kiørboe, 2011):

$$A_F(x, \phi_F) = c_F r(x, 0)^3 \frac{a_F \phi_F}{a_F \phi_F + c_F},$$  \hspace{1cm} (5)

where $a_F$ is the prey affinity per investment in phagotrophy and $c_F$ the maximum prey affinity. The functional dependence of $A_F(x, \phi_F)$ reflects the reasonable assumption that as investment $\phi_F \to 0$, so too does affinity, while for full investment $\phi_F \to 1$ affinity approaches its maximum.

### Uptake rates and cell division rate

The rates at which cells take up resources $J_R$ are assumed to be linearly dependent on resource’ abundance in the environment (units of carbon, nutrient or silicon per time):

$$J_R = A_R R,$$  \hspace{1cm} (6)

where $R$ represents environmental resources: $L$ light, $N$ nitrogen, $S$ silicon or $F$ food.
Taking up nitrogen, silicon and photosynthesizing carbon each have a metabolic cost, noted \( \beta_R J_R \) (\( \mu g \) C (\( \mu g R \))\(^{-1} \)) quantified by the constants \( \beta_R \) that represent the amount of carbon respired per unit of resource taken up \( J_R \).

In addition to the cost paid for resource’ uptake, the basal metabolism of the cell, \( J_{\text{resp}} \) (\( \mu g C d^{-1} \)) is proportional to the carbon mass \( x \):

\[
J_{\text{resp}} = \beta_0 x,
\]

where \( \beta_0 \) is the basal respiration cost (d\(^{-1} \)).

The amount of resource available for biomass synthesis is determined by Liebig’s law of the minimum, stating that growth will occur according to the supply rate of the limiting resource. As the required resources are different for vacuolated and non-vacuolated cells, this amount is found separately for each type.

Vacuolated cells take up silicon, but do not ingest prey. To prevent the cell taking up (and incurring the cost of) excess nitrogen and silicon in case of light limitation, both uptakes and costs are down-regulated to take into account co-limitation of the three resources (Appendix D). For vacuolated cells the effective uptake \( J_{\text{eff,vac}} \) thus becomes:

\[
J_{\text{eff,vac}} = \min \left\{ J_L (1 - \beta_L) - J_{\text{resp}} - \sum_{R \in \{N,L,S\}} \epsilon_R \beta_R J_R , c_{C:N} \epsilon N J_N, c_{C:S} \epsilon S J_S \right\},
\]

where \( \epsilon_R \) is a reduction factor for resource \( R \) computed by applying the Liebig’s law to the uptake of and \( C, N \) and \( Si \) to maintain \( c_{C:N} \) and \( c_{C:S} \) ratio in the cell.

For the non-vacuolated cells, both the cells and the ingested prey have a fixed C:N ratio (\( c_{C:N} \)). However, the C:N ratio of the effective flux into the cell \( J_{\text{eff}} \) depends on the relative magnitudes of carbon uptake from photosynthesis and the uptake of inorganic nutrients. In the case of light limitation, the nitrogen uptake is reduced by a factor \( \epsilon \) (Appendix D):

\[
J_{\text{eff,non-vac}} = \min \{ J_F + J_L (1 - \beta_L) - J_{\text{resp}} - \beta_F J_F - \beta_N \epsilon N J_N, c_{C:N} \epsilon N J_N + J_F \}
\]

The division rate \( g \) (d\(^{-1} \)) is limited by the maximum synthesis capacity of the cell \( g_{\text{max}} \) (d\(^{-1} \)) with a type II functional response:
\[ g(x, v, \phi_L) = g_{\text{max}} \frac{J_{\text{eff}}}{J_{\text{eff}} + g_{\text{max}}(x_{\text{tot}})} \]  

(10)

**Grazing by metazoan predators**

Plankton cells are subject to predation mortality, \( m_p \), by larger organisms. Vacuolated cells are bigger and hence have a lower specific mortality risk than non-vacuolated cells. Moreover, the silica shell protects the cell from predators by making it difficult to crack, handle and ingest (Hamm et al., 2003), hence decreasing its profitability (Visser and Fiksen, 2013) to a predator. We introduce a vulnerability factor \( p \) to represent the reduced mortality risk of vacuolated silicified cells compared to non-vacuolated cells of the same size. The mortality by metazoan predators can be written:

\[ m_p(x, v) = m_{p0}(1 - H(v)p)Zr(x, v)^{-3/4}, \]  

(11)

where \( m_{p0} \) is a grazing constant, \( Z \) is the abundance of copepods grazers in the environment, \( H \) is the Heaviside function, and the term \( r^{-3/4} \) represents the decreasing predation risk with size (Kiørboe and Hirst, 2014).

**Optimization of the net growth rate and implementation of the size based-model**

The net growth of a cell \( g_{\text{net}} \) (d\(^{-1}\)) is the division rate minus the grazing mortality:

\[ g_{\text{net}}(x, v, \phi_L) = g - m_p \]  

(12)

For different environmental conditions (nutrient, light, predators and food), we assess the optimal trait values by finding the traits \( (v, \phi_L) \) that maximizes the net growth rate \( g_{\text{net}} \) for cells of a given carbon mass \( x \) for generalist mixotrophs and diatoms respectively:

\[ \phi_L^*(x) = \arg\max_{\phi_L} \{ g_{\text{net}}(x, 0, \phi_L) \} \text{ for generalist mixotrophs} \]  

(13a)

\[ \nu^*(x) = \arg\max_{\nu} \{ g_{\text{net}}(x, \nu, \phi_L(x, \nu)) \} \text{ for diatoms} \]  

(13b)

where \( \phi_L(x, \nu) \) is determined by Eq. 2.
Dynamic unicellular plankton community model

The cell model explained above is embedded in a dynamical model that represents the photic zone modelled as a chemostat (Evans and Parslow, 1986) as shown on Fig. 1b. The total biomass of plankton \( P \) (\( \mu \text{g C L}^{-1} \)) is distributed between 9 discrete trait classes \( i \), each representing a unique combination of cell mass \( x_i \), vacuolation \( v_i \) and investment in photoharvesting \( \phi_{L.i} \). Carbon mass \( x \) ranges from \( 10^{-7} \) to \( 10 \) \( \mu \text{g C} \) using a \( \log_{10} \) distribution. Trophic strategies and vacuolation traits are further discretized in ten classes with \( v = 0 \) to \( 1 \) for vacuolated cells and investment in phototrophy \( \phi_L = 0 \) to \( 1 \) for generalist mixotrophs.

The unicellular plankton compete for nutrients and the mixotrophs predate on smaller cells. The model simulates two potentially limiting inorganic nutrients: nitrogen (\( N; \mu \text{M N} \)) and silicon (\( S; \mu \text{M Si} \)). The model is forced using a seasonally varying light typical of temperate shelf seas, a vertical exchange rate to mimic the seasonal stratification and empirically derived abundance of copepod grazers (Fig. 1c). As a diagnostic we also implement the dynamic model under steady conditions for three sets of environmental conditions corresponding to a gradient from winter-like (low light, low copepod abundance, high nutrient and high dilution rate) to summer-like conditions (the reverse) through spring (intermediate) conditions.

The net growth rate of class \( i \) (Eq. 14) is the difference between the gains from biomass synthesis (division rate) \( g_i \) minus the losses through predation (\( m_i \) and \( m_{p.i} \)) and a background quadratic loss term \( m_{2.i} \) due to viral lysis. The physical dynamics of the upper layer is represented by a time varying dilution rate \( D \) with \( P_0 \) being the ‘background’ small plankton concentration in the deep layer, constant over time (Table 1):

\[
\frac{dP_i}{dt} = (g_i - m_{p.i} - m_i - m_{2.i} P_i) P_i + D(t)(P_0 - P_i). \tag{14}
\]

The division rate \( g(x_i, v_i, \phi_{L.i}) \) is given by Eq. 10. Grazing occurs from two sides: from the larger cells that are present in the model \( m_i(x_i, v_i) \) and from higher trophic levels, e.g., copepods, \( m_{p.i}(x_i, v_i) \) that are not explicitly represented in the model. The latter term is given by Eq. 11, but only for the three largest size classes (\( x > 10^{-2} \) \( \mu \text{g C} \)) that are not subject to
internal predation in the model. In the following, the dependencies on the traits \((x, \phi \text{ and } v)\) are suppressed to simplify the notation.

The total food available for a non-vacuolated cell is the sum of all other cells, both vacuolated and non-vacuolated, multiplied by a size preference \(\theta_{i,j}\) for all prey \(j\):

\[
F_i = \sum_j H(v)p_j \theta_{i,j} P_j, \tag{15}
\]

where \(p_j\) again represents the reduced vulnerability of vacuolated cells due to the silica shell.

The size preference \(\theta_{i,j}\) for smaller cells is represented by a log-normal function:

\[
\theta_{i,j} = \exp \left[ \left( \frac{\ln \left( \frac{r_i}{r_j} \right)}{2\sigma^2} \right) \right], \tag{16}
\]

where \(r_i\) and \(r_j\) represent the radius of predator and prey. \(\kappa\) is the preferred predator-prey size ratio and \(\sigma\) is the width of the size preference.

The mortality due to predation by larger cells \(m_i \, (\text{d}^{-1})\) is the sum of the grazing by all predators \(j\):

\[
m_i = \sum_j A_{F,j} H(v) p_j \theta_{i,j} (1 - f_j) \frac{P_j}{x_{\text{tot},j}}, \tag{17}
\]

where the feeding level \(f_j\) follows from Eq. 10 and the fraction \(P_j/x_{\text{tot},j}\) is the abundance of predator cells of size \(x_j\).

The temporal dynamics of dissolved nitrogen \(N \, (\mu M)\) and silicon \(S \, (\mu M Si)\) are:

\[
\frac{dN}{dt} = D(N_0 - N) \tag{18} \\
+ \sum_i \left( \epsilon_{\text{remin,N}} \left( m_{2,i} p_i + m_{p,i} p_i / c_{\text{C,N}} - \mu_{N,i} \right) \right)
\]

\[
\frac{dS}{dt} = D(S_0 - S) + \sum_i \left( \epsilon_{\text{remin,S}} \left( m_{2,i} p_i + m_{p,i} p_i / c_{\text{C,S}} - \mu_{S,i} \right) \right) \tag{19}
\]

where \(N_0\) and \(S_0\) are the deep layer nitrogen and silicon concentrations (\(\mu M \, N/S\)). \(\epsilon_{\text{remin,N}}\) and \(\epsilon_{\text{remin,S}}\) are the remineralization rates at which nitrogen and silicon released through
mortality by viral lysis $m_2$ and higher trophic levels mortality $m_{p,i}$ are remineralized into the dissolved pools.

$\mu_{N,i}$ and $\mu_{S,i}$ are the effective uptake fluxes of nitrogen and silicon by plankton $i$ (Eq. 20).

These differ from the potential uptake rates $J_R$ (Eq. 6) by the amount $\eta_{R,i}$ representing the flux of unrequired nutrients and by scaling using the functional response $f_i$ (Eq. 10):

$$\mu_{R,i} = (J_{R,i} - \eta_{X,i})(1 - f_i) \frac{P_i}{x_{total,i}}.$$  \hspace{1cm} (20)

That is $\mu_{N,i}$ and $\mu_{S,i}$ are the stoichiometrically balanced fluxes constrained by Liebig’s law of the minimum. Details of how these fluxes are calculated are given in in appendix D.

Results

Trait optimization

We examine the predicted optimal trait values (i.e. those that maximize the net growth rate) for both non-vacuolated and vacuolated cells for a range of environmental conditions (Fig. 2a). The smallest generalist cells ($x < 10^{-5}$ µgC) predominantly invest in light harvesting over phagotrophy (Fig. 2b). Under summer oligotrophic conditions (towards the top of the panel), they are unable to achieve positive growth rate conditions due to the lack of nutrients for autotrophic growth. Conversely, large generalist cells are pure heterotrophs in all environmental conditions. For sizes between $10^{-5}$ and $10^{-1}$ µgC, mixotrophy is a prevalent strategy with larger mixotrophs under eutrophic (winter) compared to oligotrophic (summer) conditions. In the size range $10^{-4}$-$10^{-2}$ µgC, cells shift in their optimal trophic strategy from autotrophy/mixotrophy under eutrophic conditions towards heterotrophy under oligotrophic conditions. This change is a result of the lower nutrient levels and higher food availability.

For diatoms cells, the optimal vacuole size also exhibits a contrasting pattern with regards size (Fig. 2c). Small cells ($x < 10^{-5}$ µgC) have highest growth rate when the vacuole is absent ($v \approx 0$, blue area on Fig. 2c) due to the relatively high carbon cost of the inner membrane, while a high degree of vacuolation ($v > 80\%$) is optimal for large cells ($x > 10^{-3}$ µgC). Only in summer conditions when nitrogen and silicon depletion, high light and high grazer abundance, do mid-range cells ($10^{-3} < x < 10^{-4}$ µgC) benefit from large vacuoles ($v > 70\%$).

Comparing diatoms and generalists (Fig. 3), it appears that small diatom cells have higher growth rates than similar sized generalist cells, while mixotrophic cells have highest growth
rates among larger unicellular plankton. Vacuolation is a superior strategy over a larger size range in eutrophic conditions (e.g. winter to early spring) than in oligotrophic (e.g. late spring to summer) conditions. The highest growth rates among small diatoms occur even when vacuolation is negligible, $v = 0$ (blue area in Fig. 2c): beyond the benefits of vacuolation (higher nutrient affinity, higher light affinity, and lower predation risk), the silica shell protect vacuolated cells against grazers. For small cells ($x < 10^{-5}$ µgC; Fig. 2c), the advantage of diatoms on other phototrophs is thus given by the protection provided by their silica shell.

Small cells with carbon mass of $10^{-5}$-$10^{-4}$ µgC large vacuole size is optimal to counteract an increase in grazer abundance (Fig. 4). For these cells, an increase in light intensity also results in an increase of optimal vacuole size (green line on Fig. 4). Indeed, very low light levels no vacuolation is optimal since the requirement for inner membrane carbon is too high and cannot be covered by photosynthesis. Conversely, lower vacuole size is optimal when nitrogen concentration increases, showing the role of vacuolation in coping with low nutrient conditions. In larger cells ($x > 10^{-4}$ µgC), vacuolation is largely limited by available silicon, and an increasing silicon availability increases vacuolation, especially in the intermediate size class. Overall, vacuolation is a complex response to both top-down and bottom-up forcing, for most cell sizes except the picoplanktonic cells where the demand for inner membrane carbon is too high to allow the presence of a vacuole.

**Dynamic unicellular plankton community model**

**Steady state**

Figure 5 shows the structure of the unicellular plankton community at steady state under three different light levels and varying external concentrations of nitrogen $N_0$, silicon $S_0$ and copepods $Z$: (1) low light, high nutrient availability, low grazer abundance and high dilution rate (winter), (2) no limitation of light and nutrients together with moderate grazing and intermediate dilution rate (spring), and (3) nutrient limitation, high copepod grazing pressure and low dilution rate (summer).

The total biomass varies strongly between the three environments, with low unicellular plankton concentration in winter, intermediate concentration during summer and high concentration in spring conditions (Fig 5a-c). The high spring concentration is a clear response to the unlimited light and elevated nutrient conditions.
The size distribution of biomass is fairly flat for the non-vacuolated cells but dominated by small- to intermediate-sized cells among the vacuolated cells (10^{-6} to 10^{-3} µgC) under winter and spring conditions. The reason for the wide size range of the non-vacuolated cells is their phagotrophic ability. Indeed, there is a shift from phototrophy over mixotrophy to pure heterotrophy with size, as evidenced by higher biomass investing in light-harvesting) for small cells (10^{-4} µgC) compared to larger cells being present in lower abundance and primarily heterotrophs (Fig 5d-e). The predation pressure exerted by the phagotrophic cells and by the external mortality by higher trophic levels, induces a trophic cascade which is seen as an undulating biomass size distribution under summer conditions (Fig. 5c).

Vacuolated cells dominate the small to intermediate size range (Fig. 5a-b), except in summer conditions where they are outcompeted by mixotrophs (Fig. 5c). Large vacuole sizes are dominant among the largest vacuolated cells (10^{-4} µgC; Fig. 5g-i). In this size range the vacuole facilitates higher uptakes of nutrients and light than for non-vacuolated cells of the same carbon mass. Non-vacuolated cells are not completely outcompeted, though, because they switch to a mixotrophic strategy. Consequently, the two strategies of vacuolated phototrophic specialists and mixotrophic cells coexist over a wide size range (Fig. 5a-b).

Between winter (low light, low grazing) and spring conditions (high light, high grazer abundance), the maximum size of vacuolated cells is reduced as large vacuolated cells (10^{-3} µgC) are outcompeted by large heterotrophic protists (Fig. 5b).

Among the smallest vacuolated cells, vacuoles are generally small (and the maximum size of the vacuole increases with the carbon size of the cell; Fig. 5, g-h). This begs the question as to why they are not outcompeted by the non-vacuolated cells, as the vacuolated cells also need to invest in the inner membrane. Here, the benefit offered by the silica shell provides the vacuolated cells an additional predation protection. We have explored the role of the predation protection by removing the shell (dashed lines in Fig. 5a-c) and without the shell, the vacuolated cells generally do worse due to higher predation. In winter conditions (low light, high nutrients, low predation; Fig. 5a) the reduction in biomass is relatively small, indicating that under these conditions the main role of vacuolation, even if small, is to cope with low light intensity a by increasing the surface:volume ratio of the cell. In summer conditions, where the biomass and therefore the predation pressure is higher, protection is more important, and without the shell the vacuolated cells no longer dominate in the small-
intermediate size range (Fig. 5b). Overall, the three advantages of the vacuole: (1) increased nutrient and (2) light affinities and (3) lower predation risk play together, though with varying importances through the seasonal cycle.

Finally, among the smallest cells, vacuolated cells are outcompeted by the non-vacuolated cells. In this size range the costs of the inner membrane becomes so high that it offsets the advantages of the vacuole and the silica shell. Overall, the three scenarios highlight a strong coexistence of vacuolated and non-vacuolated cells, though vacuolated cells dominate the small- to intermediate size range due to increased competitive abilities and lowered predation.

**Seasonal cycle**

The model is forced with a typical seasonal cycle of light intensity, dilution rate and mesozooplankton grazer abundance to represent a seasonally stratified system (upper layer) as may be found in temperate shelf seas. This forcing results in a classical seasonal cycle with high nutrient levels and low unicellular plankton biomass during winter, a spring bloom in May with a concurrent draw down of nutrients (N and S) and a second autumn bloom in September (Fig. 6).

Vacuolated and mixotrophic cells exhibit distinct seasonal successions. Vacuolated cells dominate in the early phase of the spring bloom and during the autumn bloom, while during the summer, non-vacuolated, mixotrophic cells dominate with the presence of large heterotrophic cells (Fig. 6 and 7a-b). The maximum size of vacuolated cells is reduced during the summer compared to spring ($<10^{-4}$ µgC; Fig. 7a).

Consistent with steady state simulations, the trait distributions (Fig. 7c-d) of diatoms show increasing vacuolation with cell carbon size with the very smallest cells being essentially non-vacuolated. Similarly, the generalist mixotroph cells again show a shift from phototrophy among small cells to mixo- and heterotrophy among larger cells. For both vacuolated and mixotrophic strategies, a temporal change in the dominant trait value is simulated for the cells with intermediate carbon mass ($10^{-4}$ to $10^{-2}$ µgC). Therefore, among diatoms, cells without vacuole dominate during spring and autumn whereas cells having a big vacuole are dominant during the summer (Fig. 7c). Among mixotrophs, the dominant strategy switches from autotrophy to heterotrophy between spring/autumn and summer (Fig. 7d). The coexistence
between different strategies is also maximum in these size classes during spring and autumn periods (Fig. 7e-f).

**Discussion**

We have analyzed the competition between the two main strategies of unicellular plankton: mixotrophic and vacuolated cells as a function of cell size. Vacuolated cells represent the diverse group of diatoms. In this context, “mixotrophs” is interpreted very broadly to represent all unicellular plankton without silica shell and vacuole (apart from a feeding vacuole) along the entire phototrophic-heterotrophic continuum (Andersen *et al.*, 2015), from pure phototrophs to pure heterotrophs. This mixotrophic strategy is shared by flagellates, dinoflagellates, green algae, ciliates, coccolithophores, and non-diazotrophic cyanobacteria, among others (Mitra *et al.*, 2016). However, our model is limited to phagotrophy both in pure heterotrophs and mixotrophs. Specifically we do not include bacteria or other small mixotrophs with the ability of osmotrophy (uptake of dissolved organic compounds) which can probably explain the absence of very small mixotrophs in our results and the small occurrence of mixotrophy during winter. We also restrict vacuolation here to be associated with a silica shell, as is found in diatoms. Food vacuoles occur in a wide range of protists. Further, vacuolation not associated with particle ingestion can occur in non-diatom protists, sometimes occupying a relatively large part of the cell volume, (e.g. dinofagelllate pusule). It has been posited that these vacuoles act in an osmoregulation/floatation/storage capacity, but otherwise their function is unclear.

The competition between generalist mixotrophs and diatoms in our model reproduces the seasonal successions of size and strategies among unicellular plankton. Diatoms are known to be particularly successful in eutrophic environments and less so in oligotrophic environments (Smetacek, 1999), as reproduced in Fig. 5a-c. In a typical seasonal succession, diatoms precede mixotrophic dinoflagellates followed by obligate heterotrophic protists (Barton *et al.* 2013a). Our model reproduces this pattern as a dominance (albeit small) of vacuolated cells in spring followed by a dominance of mixotrophic cells in summer (Fig. 6b). Furthermore, the mean size of diatoms decreases from spring to summer (Barton *et al.* 2013a), also in agreement with the model simulations (Fig. 7a). The model fails, however, to predict the
overwhelming presence of diatoms in early spring bloom generally seen in temperate and sub-polar latitudes. On a cellular level, our model makes predictions of the optimal investment schedules amongst unicellular organisms, and how these vary over an annual cycle. Ostensibly, these investment would impact the stoichiometric requirements of the cell (Finkel et al 2009), providing a possible avenue for a cellular level validation of the model.

The success of diatoms over other groups of phototrophic organisms is commonly attributed to their high division rates, about a factor two higher than other cells (Geider et al., 1997; Davidson et al., 1999). These high division rates are widely believed to be responsible for their fast blooms and dominance in spring and are therefore commonly explicitly parameterized in biogeochemical PFT models (Le Quéré et al., 2005). This aspect is not well represented by our mechanistic model: even though vacuolated cells in our model dominate over mixotrophs in spring, they do not produce the strong blooms observed. The specific characteristics of diatoms that are accounted for in our model (namely vacuole and silica shell) with their associated cost/benefit do not seem sufficient to reproduce higher emergent growth rates of diatoms compared to mixotrophs. Therefore, the reason for the higher maximum growth rates must be sought elsewhere. One possibility is that maximum growth rate is related to investment in biosynthetic machinery (ribosomes). The costs of ribosomes could be offset by lower needs for chloroplast due to a higher surface area and a smaller self-shading in vacuolated cells. We have experimented with introducing investments in biosynthesis as an extra trait (not documented here), and we found that the lower chloroplast needs were insufficient to lead to higher emergent maximum growth rates. Introducing a difference in maximum growth rates would require the knowledge of the trade-off associated with that trait, which currently does not exist.

A central aim of this work has been to understand which factors are responsible for the remarkable success of diatoms in competition with the other unicellular plankton groups. To facilitate insight into the ultimate causes, the model of vacuolated cells did not rely on empirical parameterizations of mixotrophs and diatoms but relied only on a purely mechanistic description of the constraints and trade-offs of mixotrophy and vacuoles. Specifically, the description is based on trade-offs between phototrophy and phagotrophy (for mixotrophs), the costs of membranes, and constraints associated by cell size rooted in physics of diffusion and self-shading by the chloroplast. The only empirical relations used in the model are constants that quantify functions of individual cells, such as the self-shading,
specific uptake efficiency of nutrients, clearance rate, maximum synthesis rate, mortality rate through viral lysis and metabolic costs. In this manner the description is based on fundamental physical or physiological relations, founded in physics, chemistry and conservation laws, at the level of individual cells. These fundamental mechanistic relations demarcate the constraints for evolutionary creation of taxonomic diversity. The agreement with general observed patterns is a remarkable testament to the power of evolution to fill all allowed niches within those fundamental constraints. Further, the agreement demonstrates the universality of the fundamental constraints in shaping the emergent structure and function of aquatic pelagic ecosystems.

The model provides the scaffolding for general size- and trait-based models of pelagic ecosystems. As it is, the model resolves the mixotrophic continuum and the role of vacuoles. Two major strategies are not resolved: heterotrophic bacteria feeding on dissolved organic matter and diazotrophs that are able to fix dissolved nitrogen gas (Mulholland, 2007; Monteiro et al., 2010). These two strategies are key for recycling primary production and for boosting new primary production in oligotrophic areas. Further complications that are not resolved are the chain formation in diatoms, the effect of which is still subject to debate and remains somewhat unclear but would certainly affect the grazing vulnerability (Bergkvist et al., 2012; Bjærke et al., 2015) and nutrient diffusive uptake properties (Pahlow et al., 1997). As such the mechanistic trait-based approach (Kiørboe et al., 2018) risks falling in the same complexity trap that threatens classic functional type approaches. Nevertheless, the strength of the mechanistic approach is that it is rooted in hard constraints that can be considered universal, also in a situation of fundamental change of environmental conditions or changes in species composition. In this way mechanistic basis provides a solid foundation for robust predictions of ecosystem structure and function on a global scale under environmental change.

**Conclusions**

The aim of this study was to investigate the trait distribution within unicellular plankton in a range of environmental conditions. To do so, we have selected three key traits of marine protists: size, vacuolation and trophic strategy. Using those three traits, we have designed a model resolving two opposite, mutually exclusive strategies (mixotrophy and vacuolation) accounting for the major groups of unicellular plankton. The analysis was rooted in
mechanistic descriptions of costs and benefits related to investments in phototrophy (chloroplast), membranes and the shell, and well-established physical constraints of cell size. The major finding is a size-dependent dominance of the different strategies: pure autotrophs without a vacuole dominate among the smallest picoplanktonic cells; vacuolated cells dominate among nanoplankton, with vacuole fraction increasing with size; and mixotrophs dominate among microplankton. Despite this pattern of dominance, the two strategies of vacuolation and mixotrophy generally coexist apart from among the largest plankton (radius > 100 µm) where vacuolated cells disappear completely.

References


**Acknowledgements**

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Table and Figure legends:

Figure 1: (a) Schematic view of the modelled cells (a): generalist mixotrophs (upper panel) and diatoms (lower panel), with $r$ the radius of the cell ($\mu$m) and $v$ the proportion of the radius occupied by the vacuole. $J_L$, $J_N$, $J_F$ and $J_{Si}$ are the fluxes of light, nitrogen, carbon from the food and silicon, respectively, to the cell. (b) Schematic of the dynamic unicellular plankton community model. (c) Seasonal variation of forcing environmental parameters: light (green, $\mu$E m$^{-2}$ s$^{-1}$), dilution rate (grey, d$^{-1}$) and grazer abundance (red, mgC m$^{-3}$).

Figure 2: Optimal unicellular plankton traits at steady state as a function of environmental conditions (a) and cell carbon mass $x$ for (b) generalist mixotrophs and (c) diatoms. Panel (b) shows the heterotrophic fraction (%) of total resources investment for a generalist mixotrophs:

$$\Delta_F = \frac{\phi_F}{\phi_F + \phi_L}$$

and panel (c) shows the vacuole size (% of radius) for a vacuolated cell. Blue areas represents the absence of vacuole ($v = 0$), environmental conditions range from winter-like (bottom; high N and Si, low light, low food and low predation risk) to summer-like (top; low N and Si, high light, high food and, high predation risk) conditions. White regions indicate that the optimal growth rate is negative.

Figure 3: Net difference in growth rates (d$^{-1}$) between optimized diatom and generalist trait configurations as a function of carbon size over a winter-summer transition of environmental parameters (same as Figure 2). Red color highlights diatoms having a larger optimal net growth rate and blue color shows larger optimal net growth rate for generalist mixotrophs.

Figure 4: Maximal change in optimal vacuole size (%) following a change in environmental parameters within the range shown on Fig. 2a for light: green, nitrogen: blue, silicon: cyan and grazer abundance: red and as a function of carbon size. For each tested parameter, all other environmental conditions are kept constant at concentrations that allow for growth saturation (unlimited levels). The absence of lines highlights the absence of any change in the
optimal vacuole size following a change of the corresponding environmental parameter (the
difference is equal to 0).

Figure 5: Size distribution of total biomass (gC m⁻³) at steady state in winter eutrophic
conditions (L=46 μEin m⁻² s⁻¹, N= 12 mmolN m⁻³ Si = 7 mmolSi m⁻³, Z = 5 mgC m⁻³ and D=
0.5 d⁻¹) (left column), spring conditions (L=230 μEin m⁻² s⁻¹, N= 12 mmolN m⁻³ Si = 7
mmolSi m⁻³, Z = 10 mgC m⁻³ and D= 0.2 d⁻¹) (middle column) and summer conditions (230
μEin m⁻² s⁻¹ m⁻², N= 2 mmolN m⁻³ Si = 2 mmolSi m⁻³, Z = 20 mgC m⁻³ and D= 0.1 d⁻¹) (right
column). (a-c) total biomass of generalist mixotrophs (full line, blue) diatoms (full line,
green), hypothetical vacuolated cells without a silica shell (dotted line, green) and non-
vacuolated cells in the presence of vacuolated, non-silicified cells (dotted line, blue). (d-f)
biomass distribution along the size/trophic strategy trait space for non-vacuolated cells. (g-i)
biomass distribution along the size/vacuole size trait space for vacuolated cells.

Figure 6: Seasonal variation of nitrogen (blue, µmolN L⁻¹), silicon (cyan µmolSi L⁻¹) and
plankton biomass (gC L⁻¹; black full line: diatoms and dotted line: generalist mixotrophs).

Figure 7: Biomass distribution (gC m⁻³) (a,b), dominant trait values (vacuole size v for
vacuolated cells and heterotrophic fraction (%) of total investment investment for non-
vacuolated cells) (c,d) and standard deviation (STD) of traits values (e,f) in each size class
(y-axis) over a seasonal cycle (x-axis) for vacuolated cells (left column) and non-vacuolated
cells (right column).

Table 1: Model parameters: symbols, description, value, unit and references
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<td>(\beta_N)</td>
<td>Cost of N uptake and synthesis</td>
<td>0.4544</td>
<td>(\mu gC (\mu gN)^{-1})</td>
<td>Appendix D</td>
<td></td>
</tr>
<tr>
<td>(\beta_S)</td>
<td>Cost of Si uptake</td>
<td>0.45</td>
<td>(\mu gC (\mu gSi)^{-1})</td>
<td>Appendix D</td>
<td></td>
</tr>
<tr>
<td>(\beta_F)</td>
<td>Cost of food uptake</td>
<td>0.4544</td>
<td>(\mu gC (\mu gC)^{-1})</td>
<td>Appendix D</td>
<td></td>
</tr>
<tr>
<td>(\varepsilon_R)</td>
<td>Reduction factors for vacuolated cells, (L, N and Si)</td>
<td>-</td>
<td></td>
<td>Appendix D</td>
<td></td>
</tr>
<tr>
<td>(\varepsilon)</td>
<td>Reduction factor for non-vacuolated cell (N)</td>
<td>-</td>
<td></td>
<td>Appendix D</td>
<td></td>
</tr>
</tbody>
</table>

**Growth and mortality**

| \(g_{net}\) | Net growth rate | \(d^{-1}\) |  | Equation 13 |
| \(g\) | Division rate | \(d^{-1}\) |  | Equation 11 |
| \(g_{max}\) | Maximum synthesis rate | 1 | \(d^{-1}\) | Equation 11 |
| \(f\) | Limitation of biosynthesis | - |  | Equation 11 |
| \(m_p\) | Predation mortality rate (copepods) | \(d^{-1}\) |  | Equation 12 |
| \(m_{p0}\) | Grazing constant | 0.1 |  |  |
| \(p\) | Palatability factor | 0.6 |  |  |

**Dynamic set-up**

| \(P\) | Total plankton biomass | \(\mu gC L^{-1}\) |  | Equation 15 |
| \(i\) | Trait index | - |  |  |
| \(D\) | Dilution rate | \(d^{-1}\) |  |  |
| \(m\) | Internal predation (larger protists) | \(d^{-1}\) |  | Equation 18 |
| \(m_2\) | Quadratic viral lysis | 0.01 | \((\mu C L^{-1})^{-1} d^{-1}\) |  |
| \(\theta\) | Prey Size preference | 0-1 |  | Equation 17 |
| \(\kappa\) | Predator-prey size ratio | 500 |  |  |
| \(\sigma\) | width of size preference | 1.22 |  |  |
| \(S_0\) | Background Si concentration | \(\mu M Si\) |  |  |
| \(N_0\) | Background N concentration | \(\mu M N\) |  |  |
| \(P_0\) | Background P concentration | \(\mu gC L^{-1}\) |  |  |
| \(\mu_R\) | Resource (N/Si) uptake | \(\mu gN/Si L^{-1} d^{-1}\) |  | Equation 21 |
| \(\eta_R\) | Resource (N/Si) leakage | \(\mu gN/Si d^{-1}\) |  | Appendix D |
| \(\epsilon_{remin,R}\) | Resource (N/Si) remineralization rate | Si:0.8 | N:0.1 |  |

Table 1
Figure 1
Figure 2

Figure 3
Figure 4

Figure 5
Appendix A: Physical properties of the cells: equivalent spherical radius, volumes, shell thickness and C:Si ratio of vacuolated cells

The modelled cells are considered spherical. The total volume $V \ (\mu m^3)$ is:

$$V = \frac{4}{3} \pi r^3,$$  \hspace{1cm} (A.1)

where $r$ is the equivalent spherical radius (excluding the shell).

The volume of the vacuole $V_v$, the plasma membrane $V_{MO}$ and the inner membrane $V_{MI}$ are:

$$V_v = \nu^3 V = \frac{4}{3} \pi (r\nu)^3,$$  \hspace{1cm} (A.2)

$$V_{MO} = \frac{4}{3} \pi \left[ r^3 - (r - t_M)^3 \right]$$  \hspace{1cm} (A.3)

$$V_{MI} = \frac{4}{3} \pi \left[ (r\nu + t_M)^3 - (r\nu)^3 \right]$$  \hspace{1cm} (A.4)

where $\nu$ is the radius fraction of the vacuole and $t_M$ is the thickness of the membranes.

Finally, the volume of the cytoplasm $V_c$ (excluding vacuole, membranes and shell) is:

$$V_c = \frac{4}{3} \pi r_0^3 = \frac{4}{3} \pi \left[ (r - t_M)^3 - (r\nu + t_M)^3 \right]$$  \hspace{1cm} (A.5)

where $r_0 \ (\mu m)$ is the radius of a sphere without vacuole:

$$r_0(x) = \left( \frac{4}{3} \pi \right)^{\frac{1}{3}} \left( \frac{x}{\rho_c} \right)^{\frac{1}{3}}$$  \hspace{1cm} (A.6)

where $\rho_c$ is the carbon density in the cytoplasm.

The composition of cytoplasm and membranes are different with the density of membranes ($\rho_M = 0.6 \ 10^{-6} \ \mu g \ \mu m^{-3}$) being higher than density of the cytoplasm ($\rho_c = 0.11 \ \mu gC \ \mu m^{-3}$) (Strathmann, 1967; Raven, 1987).
Therefore, the total carbon content of the cell (µgC) is:

\[
x_{\text{tot}} = V_c \rho_c + (V_{MI} + V_{MO}) \rho_M = \frac{4}{3} \pi [r^3 (1 - v^3) \rho_M + (r - t_M)^3 - (vr + t_M)^3] (\rho_c - \rho_M)
\] (A.7)

By solving Eq. A.7, we can derive \(r\) from \(x_{\text{tot}}\) and \(v\) (Fig. A.1). The exact solution including the thickness of the membranes can only be found numerically but if \(t_M = 0\), \(r\) can be approximated by as:

\[
r \approx \left(3 x_{\text{tot}} \frac{1-v^3}{4 \pi \rho_c}\right)^{1/3}
\] (A.8)

The vacuolated cells are surrounded by a silicate shell with a thickness \(t_s\) and a volume \(V_s\). The relation between the thickness of the shell and the total volume of the cell \(V\) is given by equation A.9 derived from data of size dependent silicon content in diatoms (Brzezinski, 1985; Eppley et al., 1967; Harrison et al., 1977 and Parsons et al., 1961):

\[
t_s = 1.62 V^{0.24}
\] (A.9)
Therefore, the volume of the shell, the corresponding silica content in the shell and the Si:C ratio depend on both the carbon mass \( x \) and the size of the vacuole \( v \) (Fig. A.2).

The carbon density of the cell \( (\rho_{\text{tot}} \, \mu g C \, \mu m^{-3}) \) is:

\[
\rho_{\text{tot}} = \frac{V_c \rho_c + (V_{MI} + V_{MO}) \rho_M}{V_c + V_{MI} + V_{MO}} \quad (A.11)
\]

The silicate density of the shell \( \rho_S \) is fixed at \( 10.3 \times 10^{-6} \, \mu g Si \, \mu m^{-3} \) (Hansen and Visser, 2019), and the Si:C ratio \( (\mu g Si \, \mu g C^{-1}) \) is thus:

\[
c_{S:C} = \frac{\rho_S}{\rho_{\text{tot}}} \frac{V_s}{V_c + V_{MI} + V_{MO}} \quad (A.12)
\]

Figure A.2: Shell thickness (nm) (a) and S:C ratio (b) according to the carbon mass \( x \) (x-axis) and the vacuole size \( v \) (% of radius) (y-axis).
Appendix B: Investment in membranes $\phi_M$

The creation of membranes requires a fraction $\phi_M$ of the carbon produced by the cell. The fraction of carbon used for membranes depend of membranes related to the total carbon content of the cell:

$$\phi_M = \frac{\rho_M V_{MI} + V_{MO}}{\rho_c V_c + (V_{MI} + V_{MO})\rho_M}$$  \hspace{1cm} (B.1)$$

where $V$ (\SI{}{\mu m^3}) is the volume and $\rho$ (\SI{}{\mu g \cdot \mu m^{-3}}) the density. Subscripts ‘c’ represents the carbon mass in the cytoplasm, ‘MO’ the outer (plasma) membrane and ‘MI’ the vacuole inner membrane.

The remaining carbon for uptake processes: $\phi_L + \phi_F = 1 - \phi_M - \phi_{struct}$ depends on the radius of the cell and the size of the vacuole (Fig. B.1). As vacuolation takes place, the ‘cost’ of membranes may be important for small vacuolated cells as a large fraction of cell’s carbon is located in vacuole’s membrane.

![Figure B.1: Maximum investment in resources ($\phi_L + \phi_F = 1 - \phi_M - \phi_{struct}$) according to the carbon mass $x$ (x-axis) and the vacuole size $v$ (y-axis).](image-url)
Appendix C: Light affinity

The absorption of photons $Q \, (\mu E \, s^{-1})$ by a non-vacuolated spherical cell of radius $r$ is (Duyens, 1956):

$$Q(r, \lambda) = 2\pi r^2 L \int_0^1 \xi \left(1 - e^{-2\lambda r \sqrt{1 - \xi^2}}\right) d\xi = \frac{\pi L}{2\lambda^2} \left[(1 + 2\lambda r)e^{-2\lambda r} + (2\lambda^2 r^2 - 1)\right]$$  \hspace{1cm} (C.1)

where $L \, (\mu E \, \mu m^{-2} \, s^{-1})$ is the light intensity (PAR), and $\lambda \, (\mu m^{-1})$ is the light attenuation coefficient, assumed to be constant throughout the volume of the cell. The light attenuation coefficient can be decomposed as $\lambda = a \cdot c$, where $a \, (\mu m^2)$ is the optical cross section of a single plastid, and $c \, (\mu m^{-1})$ is the concentration of plastids within the cell (Raven, 1984). In a diatom, and potentially in other cells, the distribution of plastids is not uniform, but concentrated within a layer close to the cell surface. For our spherical shell model of a vacuolated cell, where no plastids, and hence no absorption occurs in the vacuole, the absorption is:

$$Q(r, \lambda) = 2\pi r^2 L \int_0^1 \xi \left(1 - e^{-2\lambda r v}\right) d\xi $$ \hspace{1cm} (C.2)

where (Papageorgiou, 1971):

$$I(\xi, v) = \text{Re} \left[\sqrt{1 - \xi^2} - \sqrt{v^2 - \xi^2}\right]$$ \hspace{1cm} (C.3)

Unfortunately, while this can be solved numerically, it does not yield to a simple analytical expression. We can recast the cellular model into a simpler form that captures the effect of vacuolation on light absorption. In particular, we consider a flat cylinder with the same physical radius $r$ as the vacuolated cell, and an equivalent bio-volume. Thus the absorption of light hitting the surface of the cylinder is:

$$Q(r, \lambda) = \pi r^2 L \left(1 - e^{-2h}\right) $$ \hspace{1cm} (C.4)

where $h \, (\mu m)$ is the cylinder height. The equivalency of bio volume means that:

$$h = \frac{4}{3} (1 - v) r$$ \hspace{1cm} (C.5)

This gives a much simpler analytic expression for light affinity $A_L \, [\mu mol \, C \, (\mu E \, \mu m^{-2})^{-1}]$, namely
where $q$ is the photosynthetic yield ($\mu$mol C $\mu$E$^{-1}$).

Investment in photosynthesis $\phi_L$, is linked to the light attenuation coefficient as:

$$\lambda = ac = 9\phi_L$$  \hspace{1cm} (C.7)

where an increase in investment, increases the concentration $c$ of plastids; the return on investment is governed by the parameter $\theta$ ($\mu$m$^{-1}$). Typical values (Raven, 1984) suggest that $\lambda = 0.1$ $\mu$m$^{-1}$ when about half of the cell’s carbon is associated with photosynthesis. This suggests a value of $\theta = 0.2$ $\mu$m$^{-1}$.

The resulting light affinity as a function of the two-dimensional trait space is shown on Fig. C.1 for both vacuolated and non-vacuolated cells.

---

**Figure C.1:** Light affinity ($\mu$Ein m$^{-2}$ s$^{-1}$ d$^{-1}$) as a function of carbon mass $x$ (x-axis) and (a) the vacuole size $v$ (y-axis) for vacuolated cells or (b) investment in light $\phi_L$ for non-vacuolated cells.
Appendix D: Liebig’s law of minimum

The harvesting of light (photosynthesis) $J_L$ (µgC d$^{-1}$) and uptake of nitrogen $J_N$ (µgN d$^{-1}$), silica $J_S$ (µgS d$^{-1}$) and food $J_F$ (µgC or µgN d$^{-1}$) have corresponding metabolic costs, $\beta_R$ (µgC/µgN/S$^{-1}$), that are all paid in carbon (Table 1). In order to maintain the stoichiometric balance between carbon and nitrogen $c_{CN}$ and between carbon and silica ratio $c_{SC}$ given by the shell/carbon biomass ratio in the case of vacuolated cells, the uptake of nutrient has to be down-regulated when carbon is limiting, i.e., when potential nutrient fluxes exceed net carbon flux into the cell. It is assumed that the cells are unable to down-regulate the amount of carbon taken up and that it must do photosynthesis according to the amount of available sunlight but they can reduce the control the amount of dissolved nutrient taken up. Therefore, the down-regulation of nutrient uptake prevents the cell to take-up (and pay the cost for) nutrient that cannot be used for growth.

In the case of non-vacuolated cells, the nutrient uptake $J_N$ is reduced by a factor $\epsilon$ that restricts the nitrogen uptake to not exceed the net carbon flux into the cell (after removal of respiration $J_{resp} = \beta_0 x$ (µgC d$^{-1}$) and carbon uptake costs). $\epsilon$ is obtained by equating the carbon and nitrogen net fluxes (from different sources) (in carbon unit):

$$J_L - \beta_L J_L - J_{resp} + J_F - \beta_F J_F - \beta_N \epsilon J_N = c_{CN} \epsilon J_N + J_F$$  

(D.1)

And solve for $\epsilon$:

$$\epsilon = \max \left[ 0, \min \left( 1, \frac{J_L - \beta_L J_L - \beta_F J_F - J_{resp}}{(\beta_N + c_{CN}) J_N} \right) \right]$$  

(D.2)

After downregulation of nutrient uptakes, the light harvesting, nutrient uptake and food fluxes are combined following Liebig’s law to compute the effective uptake (Eq. 10). This may lead to further leakage of nutrients (from food uptake) that are in excess compared to carbon when costs are implemented. The excess nitrogen $\eta_N$ that is leaked by the cell and released to the nitrogen pool is:
\[ \eta_N = \max \left( 0, \varepsilon_N J_N - \frac{\max(0, J_L(1 - \beta_L) - J_{\text{resp}}) - \beta_F J_F - \beta_N \varepsilon_N J_N, c_{CN} \varepsilon_N J_N}{c_{CN}} \right) \] (D.3)

When nitrogen is in excess and growth is carbon-limited (light is limiting), this term is equal to the difference between nitrogen uptake (after down-regulation) and carbon uptake (second term of Eq. D.3).

In the case of vacuolated cells, we introduce three reduction factors \( \varepsilon_L \), \( \varepsilon_N \), and \( \varepsilon_S \), such that the cell do not take up more nutrients that it can use for maintaining the stoichiometric balance. We solve for the three reduction factors by setting all fluxes equal according to appropriate \( c_{CN} \) and \( c_{C:S} \), thereby assuming co-limitation of the three elements.

\[ \varepsilon_L J_L - \varepsilon_L \beta_L J_L - J_{\text{resp}} - \varepsilon_N \beta_N J_N - \varepsilon_S \beta_S J_S = c_{CN} \varepsilon_N J_N \] (D.4a)

\[ \varepsilon_L J_L - \varepsilon_L \beta_L J_L - J_{\text{resp}} - \varepsilon_N \beta_N J_N - \varepsilon_S \beta_S J_S = c_{C:S} \varepsilon_S J_S \] (D.4b)

\[ c_{CN} \varepsilon_N J_N = c_{C:S} \varepsilon_S J_S \] (D.4c)

As three equations with three unknowns cannot be solved immediately, we solve this system over three times: when carbon is limiting \( \varepsilon_L = 1 \), when nitrogen is limiting \( \varepsilon_N = 1 \), when silica is limiting \( \varepsilon_S = 1 \). Thereby we have three different solutions for the three reduction factors. The condition when \( \varepsilon_1 = 1 \) and \( \varepsilon_2, \varepsilon_3 < 1 \) occurs when \( \varepsilon_L \) is limiting. As only one substrate will be limiting, only one of the three solutions fulfil the previous condition at each set of traits under each set of environmental conditions. In case one of the nutrients are limiting and \( \varepsilon_L < 1 \) it is manually set it to 1, as the photosynthetic uptake cannot be reduced. The value \( 1 - \varepsilon_L \) would determine the leaked carbon that cannot be used for synthesis because of the limited nutrient availability.

Liebig’s law of minimum in the case of vacuolated cells is given by Eq. 9. Leakages of nitrogen and silica (\( \eta_N \) and \( \eta_S \)) when other elements are limited are found as the difference between the uptake of the limited element and nitrogen/silica uptake:

- When carbon is limiting:
\[ \eta_N = \epsilon_N J_N - \frac{\max(0, J_L (1 - \beta_L) - J_{\text{resp}} - \epsilon_N \beta_N \eta_N - \epsilon_S \beta_S \eta_S)}{c_{C:N}} \] (D.5a)

\[ \eta_S = \epsilon_S J_S - \frac{\max(0, J_L (1 - \beta_L) - J_{\text{resp}} - \epsilon_N \beta_N \eta_N - \epsilon_S \beta_S \eta_S)}{c_{C:S}} \] (D.5b)

- When nitrogen is limiting:

\[ \eta_N = 0 \] (D.6a)

\[ \eta_S = \epsilon_S J_S - \frac{\epsilon_N J_N c_{C:N}}{c_{C:S}} \] (D.6b)

- When silica is limiting:

\[ \eta_N = \epsilon_N J_N - \frac{\epsilon_S J_S c_{C:S}}{c_{C:N}} \] (D.7a)

\[ \eta_S = 0 \] (D.7b)