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Body size, light intensity, and nutrient supply determine plankton stoichiometry in mixotrophic plankton food webs

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ABSTRACT:

Trophic strategy determines stoichiometry of plankton. In general, heterotrophic zooplankton have lower and more stable C:N and C:P ratios than photoautotrophic phytoplankton whereas mixotrophic protists, which consume prey and photosynthesize, have stoichiometry between zooplankton and phytoplankton. As trophic strategies change with cell size, body size may be a key trait influencing eukaryotic plankton stoichiometry. However, the relationship between body size and stoichiometry remains unclear. Here, we measured plankton size-fractionated C:N ratios under different intensities of light and nutrient supply in subtropical freshwater and marine systems. We found a unimodal body size-C:N ratio pattern with a maximum C:N ratio at $\sim 50 \mu\text{m}$ diameter in marine and freshwater systems. Moreover, the variation in C:N ratios is mainly explained by body size, followed by light intensity and nutrient concentration. To investigate the mechanisms behind this unimodal pattern, we constructed a size-based plankton food web model in which the trophic strategy and C:N ratio is an emerging result. Our model simulations reproduce the unimodal pattern with C:N ratio of photoautotrophs $\leq 50 \mu\text{m}$ increasing with body size due to increase of photosynthetic carbon, whereas C:N ratios of organisms $> 50 \mu\text{m}$ decreases with size due to decreasing photoautotrophic but increasing heterotrophic uptake. Based on our field observations and simulation, we extend the classic “light-nutrient” theory that determines plankton C:N ratio to include body size and trophic strategy dependency. We conclude that body size and size-dependent uptake of resources (light, nutrients and prey) determine plankton stoichiometry at various light and nutrient supplies.

Introduction

Difference in elemental composition is often observed between consumers and their resources. In pelagic ecosystems, between herbivorous zooplankton consumers and their autotrophic phytoplankton prey, phytoplankton have higher than average carbon to nitrogen (C:N) and carbon to phosphorus (C:P) ratio (Dobberfuhl and Elser 2000; Elser et al. 2000; Sterner and Elser 2002). In contrast, zooplankton and heterotrophic protists have lower C:N or C:P ratios and maintain their stoichiometric composition homeostatically (Elser et al. 2000; Golz et al. 2015). These studies point out that the stoichiometry of plankton, here defined as unicellular eukaryotic phytoplankton, protists, and multicellular zooplankton, is closely related to its trophic strategy (photoautotrophy or heterotrophy). This “stoichiometric imbalance”, or elemental imbalance, between predators and prey determines assimilation efficiency and influences growth rate of zooplankton in aquatic food webs (Sterner and Elser 2002; Hessen et al. 2013).

A stoichiometric perspective on trophic interactions and trophic transfer in plankton food webs also requires consideration of mixotrophy. Mixotrophs are common: they gain carbon not only by photosynthesis (photoautotrophy) but also by consuming living or dead particulate matters (here we consider only mixotrophy by phagotrophy and not by taking up dissolved organic matter) (Jones 2000). Mixotrophs critically control pico- and nanophytoplankton density and are prey to crustacean zooplankton (Ptacnik et al. 2004; Katechakis et al. 2005), thereby mediating biomass flow from small phytoplankton to zooplankton. More importantly,

mixotrophs have more nutrient-rich body composition (lower C:N and C:P ratios) than photoautotrophs (Katechakis et al. 2005; Moorthi et al. 2017), i.e., mixotrophs are more stoichiometrically balanced. Thus, the intake of mixotrophs is likely to alleviate nutrient-limitation of crustacean grazers (Ptacnik et al. 2004). Delving into the variation of mixotroph stoichiometry and mechanisms determining their stoichiometry can improve our understanding of elemental imbalances across trophic positions in plankton food webs.

The trophic strategy of planktonic organisms is closely related to body size. Small- to intermediate-sized organisms tend to be photoautotrophic and mixotrophic (simultaneously photoautotrophic and phagotrophic, i.e. eating other organisms) (2-200 μm), while large organisms (>200 μm) tend to be heterotrophic (Jones 2000; Flynn et al. 2013; Andersen et al. 2016; Chakraborty et al. 2017), except for some large (>200 μm) planktonic photoautotrophs such as diatoms *Coscinodiscus* and *Ethmodiscus* (Sieburth et al. 1978; Beardall et al. 2009). This size-trophic strategy pattern has been explained by differences in plankton size dependency in harvest of light, uptake of inorganic nutrients and feeding on food particles (Andersen et al. 2016): Photoautotrophic carbon production decreases with size due to self-shading of chloroplasts within the cells (Morel and Bricaud 1981; Falkowski et al. 1985) and inorganic nutrient uptake also decreases with size due to diffusion limitation (Marañón 2015). Therefore, phototrophy becomes increasingly inefficient as size increases. Large organisms therefore tend to obtain carbon from prey, so becoming more heterotrophic than photoautotrophic. As photoautotrophs are generally more carbon-rich than heterotrophs, C:N and C:P ratios should

therefore decrease with body size as heterotrophy increases with body size; however, this pattern has not been revealed empirically, nor explained theoretically.

Body size and trophic strategy also influence how plankton stoichiometry varies along gradients of light and inorganic nutrient supply. The “light-nutrient hypothesis” predicts that increased light intensity enhances photoautotrophic carbon accumulation and increases the C:N and C:P ratios in small photoautotrophs, while increased inorganic N and P nutrient supply decreases C:N and C:P (Geider et al. 1996; Leonardos and Geider 2004; Dickman et al. 2006). Although the light-nutrient hypothesis describes the stoichiometry within single species or aggregate assemblages of phytoplankton (Arrigo 2005; Dickman et al. 2006), it does not describe whether there are systematically stoichiometric variations between cells of different size or different trophic strategies.

To investigate the relation between body size and stoichiometry, we analyze size-fractionated field samples of plankton and develop an explanatory size-based food web model. We focus on the C:N ratio but discuss phosphorous briefly. We address two hypotheses: (H1) plankton C:N ratio decreases with body size, because carbon surplus from photoautotrophy decreases with body size; (H2) plankton C:N ratio increases with light and decreases with inorganic N supply, as predicted by the light-nutrient hypothesis, but the magnitude of change decreases with body size. We first present methods and results from the empirical investigation and then introduce the model equations and simulation results.

Field observations

Size-fractionated C:N ratios of plankton in natural systems

We characterized changes in C:N ratio with body size in both a freshwater and marine system. We collected size-fractionated plankton at a freshwater station in the Feitsui Reservoir (100 sampling dates) and at 145 cruise-stations in the East and South China Sea areas (details in Appendix A). In the Feitsui Reservoir we sampled biweekly at 9 a.m.. Freshwater plankton samples were size-fractionated in 6 groups: (1) 0.7–10 μm particles in 10- μm sieve-filtered water (500 ml) is collected at chlorophyll-*a* maximum depth by 20 L Go-Flo bottles; larger-size plankton were collected by 10- and 50- μm mesh Norpac nets and divided into five size fractions (2) 10–44, (3) 44–74, (4) 74–177, (5) 177–500, (6) >500 μm . Marine plankton samples were size fractionated into 8 groups: (1) 0.7–50 μm particles from 50- μm sieve-filtered water (5 L water was filtered to obtain enough biomass for elemental analysis) were collected at 3-4 depths from water surface to chlorophyll-*a* maximum depth by 20 L Go-Flo bottles; marine plankton larger than 50 μm were collected by 50- and 200- μm mesh Norpac nets and divided into seven size fractions (2) 50–104, (3) 104–200, (4) 200–363, (5) 363–500, (6) 500–1000, (7) 1000–2000, (8) >2000 μm . The sampling of marine sites was conducted whenever the research vessel arrived at the site. Regarding that marine zooplankton are larger than freshwater species, we chose sieves of larger mesh sizes when sampling marine plankton community. Before proceeding to analysis, all samples were acidified by 1M HCl and dried for at least 24 hours to remove the inorganic carbon. We used elemental analyzer (EA1108, Fisons, Italy, and FLASH 2000, Thermo

SCIENTIFIC, USA) to measure the C and N mass and calculated the atomic C:N ratio of each size class.

Light intensity and inorganic N supply in the freshwater system (Feitsui Reservoir)

To investigate the effect of light and inorganic N supply on plankton C:N ratios, we measured surface (5 m depth) light intensity and inorganic N concentration along with the size-fractionated plankton C:N ratios in Feitsui Reservoir. Light intensity (photosynthetically active radiation PAR; $\mu\text{E s}^{-1} \text{m}^{-2}$) was recorded by the PAR sensor equipped on a CTD (conductivity-temperature-depth sensor; IDRONAUT) rosette. Nitrite and nitrate concentrations (μM) were measured by spectrophotometry (Parsons et al. 1984).

Statistical analysis

To illustrate the pattern of C:N ratio with plankton body size, we used box plots to present marine and freshwater size-fractionated C:N ratios. Extreme C:N ratios > 20 or < 4 were excluded for statistical analyses. We applied 1-way ANOVA and Tukey's test (the functions *aov* and *TukeyHSD* in R) to marine and freshwater size-fractionated plankton C:N ratios.

To investigate the effect of light (PAR = 0-707 $\mu\text{E s}^{-1} \text{m}^{-2}$) and inorganic N nutrient ($[\text{NO}_2^- + \text{NO}_3^-] = 10.18\text{-}70.2 \mu\text{M}$) on the change of C:N ratio along size classes, we showed the C:N ratio distribution of each size class by boxplots under four combinations of light intensity (high and low light; PAR $\geq 75\%$ and $\leq 25\%$ quantile) and inorganic N (high and low N

concentration; $[\text{NO}_2^- + \text{NO}_3^-] \geq 75\%$ and $\leq 25\%$ quantile) in Feitsui Reservoir: (1) high light but low N (9 samplings); (2) low light and low N (5 samplings); (3) high light and high N (7 samplings); (4) low light but high N (10 samplings).

Furthermore, we used a general additive model (GAM) (the function *gam* in R package *mgcv*) to include the non-linear effect of mean body length of each size class (thin plate regression spline of $\log(\text{size})$; mean body size = (upper limit of size class + lower limit of size class)/2) on plankton C:N ratio. For the effect of light intensity and inorganic N nutrient, we combined linear effects of these two environmental factors with the non-linear effect of body size on plankton C:N ratio in GAM.

Results: Changes of C:N ratio along body size in freshwater and marine plankton food webs

In both the freshwater and marine systems, the C:N ratio exhibits an almost unimodal relationship with body size (Fig. 1). In the freshwater system, the median C:N ratio increases with size for the size classes smaller than 44–74 μm , and then decreases with size in larger organisms (middle bars in Fig. 1a). For marine plankton, the median C:N ratio of each plankton size class reaches maximum around 50–104 μm and then decreases (middle bars in Fig. 1b). There are significant differences among size-fractionated C:N ratios based on 1-way ANOVA in freshwater and marine systems (Freshwater: $F = 77.24$, $p < 0.001$; Marine: $F = 32.5$, $p < 0.001$).

Results: Light intensity and inorganic N supply affect plankton C:N ratio in freshwater system

Freshwater plankton C:N ratio increases with light intensity (Fig. 2 magenta versus blue curves), but light intensity does not explain the variation of C:N ratio within size-classes much (similar R^2 in GAM with size classes and light effect compared to GAM with only size effect; Table 1, Freshwater). The C:N ratios decrease with inorganic N concentration (lower C:N ratios shown by solid curves than dashed curves in Fig. 2), and inorganic N concentration further explains the variation of C:N ratio (increased R^2 in GAM with size and inorganic N effect comparing with GAM with only size class effect; Table 1, Freshwater). Based on AIC, the best model includes size class, inorganic N, and light intensity (Table 1, Freshwater). This indicates that light and N supply influences the element and energy transferring in Feitsui Reservoir, though N-limitation is less common than P-limitation in freshwater systems (Guildford and Hecky 2000; Maberly et al. 2002).

Mixotrophic food web model

Mixotrophic food web model structure and simulation

Our field observations revealed an approximately unimodal pattern of molar C:N ratio with plankton body size, with the overall C:N ratio modified by light and nutrient supply. To investigate the biological mechanisms behind the pattern, we constructed a size-based mixotrophic food web model that is driven by light and inorganic nutrient supply, loosely based on Ward and Follows (2016). The model is rooted in fundamental processes that are expected to

be similar in freshwater and marine systems. The food web is described by $i = 1-20$ size groups with nitrogen body masses M_i from 10^{-5} to 10^3 $\mu\text{g N}$, each size class group with carbon and nitrogen biomasses $B_{C,i}$ and $B_{N,i}$ ($\mu\text{g C}$ and $\mu\text{g N}$ per liter). We assume that the nitrogen mass is a measure of the biologically active biomass and consider excess carbon as an inactive reserve. All plankton organisms are considered generalists that can harvest sunlight, take up inorganic nutrients, and feed on smaller organisms (Fig. 3a). Light harvesting and inorganic nutrient uptakes lead to influxes of carbon and inorganic nitrogen for photosynthesis, J_L and J_N ($\mu\text{g C ind}^{-1} \text{ d}^{-1}$ and $\mu\text{g N ind}^{-1} \text{ d}^{-1}$; blue dotted and green solid lines in Fig. 3b). Feeding on smaller organisms leads to influxes of heterotrophic carbon and nitrogen, J_{FC} and J_{FN} , (magenta dashed lines) whose ratio depends on the carbon and nitrogen mass ratio of the prey. The following describes the governing equations; all parameters are given in Table 2.

The influxes of resources into an individual organism are determined by the availability of resources X , body size-dependent affinities α_X , and maximal uptake rates $g_{\max,X}$ via a classical type II functional response (with a slight complication for uptake of N from food; see appendix B):

$$J_X = g_{\max,X} \frac{\alpha_X X}{\alpha_X X + g_{\max,X}} . \quad (1)$$

The resources X are light (L), inorganic nutrients (N) or food (F). Prey are only selected according to their size. A predator of size M_i 's preference for prey of size M_j is described by a bell-shaped prey size preference function:

$$\phi(M_j, M_i) = \exp\left[-\left(\ln\left(\frac{M_i/(\beta M_j)}{2\sigma^2}\right)\right)^2\right] , \quad (2)$$

with optimal predator:prey mass ratio β and width σ . The available food C and N mass to size class i is therefore $F_{C,i} = \sum_j B_{C,j} \phi(M_j, M_i)$ and $F_{N,i} = \sum_j B_{N,j} \phi(M_j, M_i)$. The encountered resources ($\text{liter}^{-1} \text{ day}^{-1}$) are $\alpha_X X$, where α_X is the affinity for resource X . The affinities scale allometrically with nitrogen body mass M : $\alpha_X = c_X M^{\theta_X}$, with exponents θ_X depending on the type of resource (Fig. 3c). The scaling exponents of the affinities are determined by the process that limits the uptake of each resource (Andersen et al. 2016): the uptake of light is limited by self-shading of chlorophyll inside the cell, so light encounter is limited by the cell surface, leading to a scaling exponent $\theta_L = 2/3$ (Morel and Bricaud 1981). The encounter with nutrients is limited by diffusion towards the cell with exponent $\theta_N = 1/3$ (Munk 1952). The encounter with prey is observed to be proportional to cell mass $\theta_F = 1$ (Kiørboe 2011). These differences in exponents of affinities turn out to be crucial for understanding the stoichiometry of cells of different sizes. The maximal uptake rate is proportional to mass: $g_{\max,X} = G_X M$, where G_X is the nitrogen mass-specific maximum uptake rate.

The synthesis of new nitrogen and carbon biomass is regulated by the inverse C:N mass ratio, the “quota” of the cell, calculated as the ratio of the nitrogen and carbon biomasses, $Q = B_N/B_C$ ($\mu\text{g N } \mu\text{g C}^{-1}$). We follow the standard Droop model formalism (Droop 1973, 1983) which constrains the quota via regulation factors (Fig 3b):

$$q_N = \left(\frac{Q_{\max} - Q}{Q_{\max} - Q_{\min}} \right)^{h_N} \quad (3)$$

$$\gamma_X = \left(\frac{Q - Q_{\min}}{Q_{\max} - Q_{\min}} \right)^{h_X}, \quad (4)$$

where q_N regulates the uptake and assimilation of nitrogen (N) and γ_X regulates the assimilation of carbon from photoharvesting (L) and food (F). The factors gradually reduce the assimilation of N and C as the quota Q approaches $Q_{\max}$ and $Q_{\min}$ respectively, with the constants h_X controlling how gradual the reduction tapers off. For nitrogen and light uptake and assimilation $h_N = h_L = 0.1$ indicates that uptake is only reduced slightly, until the quota is close to the limits ($Q_{\max}$ and $Q_{\min}$ respectively) (Geider and Roche 2002), while $h_F = 1$ indicates a linear reduction of carbon assimilation from prey (Ward and Follows 2016). Overall, this formalism ensures that the molar C:N ratio ($\mu\text{mol C } \mu\text{mol N}^{-1}$) of the cell is constrained between $Q_{\max}^{-1} \times 14/12$ and $Q_{\min}^{-1} \times 14/12$.

The dynamics of N and C biomass of size class i ($B_{N,i}$ and $B_{C,i}$) are described by standard biomass differential equations where the change in population biomass (the left-hand sides) are equal to the synthesis rate minus losses to predation, respiration (only for carbon), and a background mortality:

$$\begin{aligned} \frac{dB_{N,i}}{dt} &= \overbrace{n_i q_{N,i} (J_{N,i} + J_{FN,i})}^{\text{synthesis}} - \overbrace{\sum_j n_j Q_i P_{i,j}}^{\text{predation}} - \overbrace{\mu B_{C,i} B_{N,i}}^{\text{background loss}}, \quad (5) \\ \frac{dB_{C,i}}{dt} &= \overbrace{n_i (\gamma_{L,i} J_{L,i} + \gamma_{F,i} J_{FC,i})}^{\text{synthesis}} - \overbrace{\sum_j n_j P_{i,j}}^{\text{predation}} - \overbrace{\mu B_{C,i}^2}^{\text{background loss}} - \overbrace{RB_{C,i}}^{\text{respiration}}. \quad (6) \end{aligned}$$

The total amount of synthesized nitrogen and carbon biomass is the assimilated mass per individual are $q_{N,i} (J_{N,i} + J_{FN,i})$ and $\gamma_{L,i} J_{L,i} + \gamma_{F,i} J_{FC,i}$, with $q_{N,i}$ (equation 3) and $\gamma_{X,i}$ (equation 4) being the regulation factors, multiplied by the numeric density $n_i = B_{N,i}/M_i$ (individual $\times$ liter $^{-1}$). The total loss of nitrogen and carbon by predation from larger organisms in

the food web are $\sum_j n_j Q_i P_{i,j}$ and $\sum_j n_j P_{i,j}$, with predation loss of carbon of organism i by individual predator j being (see Appendix C):

$$P_{i,j} = \frac{g_{\max F,j} \alpha_{F,j} B_{C,i} \phi(M_i, M_j)}{\alpha_{F,j} F_{C,j} + g_{\max F,j}} . \quad (7)$$

The organisms are further exposed to a background mortality $\mu B_{C,i}$ that increases with abundance, which leads to background N and C losses $\mu B_{C,i} B_{N,i}$ and $\mu B_{C,i}^2$ (Edwards and Yool 2000). Such density dependent mortality is commonly used in plankton models to represent larger predators and viral lysis outside the model that specifically target high prey concentrations, and to dampen oscillations. The value of the constant μ is adjusted such that the dominant mortality is caused by predation of larger organisms in the model (i.e. “predation” >> “background loss” in equations 5 and 6), except for large cells with no predators in the model. Finally, carbon losses due to respiration for structure maintenance (“respiration” in equation 6) are assumed to be proportional to the carbon mass (Tang and Peters 1995; Glazier 2006), where R (d^{-1}) is the carbon mass-specific respiration rate.

The dynamic of inorganic N concentration in the water (N in Fig. 3a; $\mu\text{g N L}^{-1}$) is described as a chemostat:

$$\frac{dN}{dt} = \overbrace{\kappa(N_0 - N)}^{\text{mixing}} - \overbrace{\sum_i n_i J_{N,i}}^{\text{nutrient uptake}} + \overbrace{\varepsilon \sum_i \mu B_{C,i} B_{N,i}}^{\text{remineralization}} . \quad (8)$$

The chemostat represents the upper mixed layer of a water column where water is exchanged through mixing κ with a deeper layer of high nutrient concentrations N_0 (Evans and Parslow 1985). The plankton cells are assumed to avoid being mixed down in the deeper layer due to buoyancy or active motion. A fraction $\varepsilon = 0.5$ of the nitrogen background loss is assumed to be

released and quickly remineralized from dead plankton biomass (Garber 1984); the remainder is assumed to sink to the deep layer where it is not available to the plankton food web.

We define the trophic strategy of size class i by the relative autotrophic and heterotrophic C influx ratios:

$$\text{autotrophic influx ratio} = \frac{\gamma_L J_L}{\gamma_L J_L + \gamma_{FC} J_{FC}}, \quad (9)$$

$$\text{heterotrophic influx ratio} = \frac{\gamma_{FC} J_{FC}}{\gamma_L J_L + \gamma_{FC} J_{FC}}. \quad (10)$$

When an organism obtains C predominantly from photosynthesis or from phagotrophy (autotrophic influx or heterotrophic influx ratio > 95% of total C influx, respectively), it is defined as an obligate photoautotroph or a heterotroph, respectively; otherwise it is classified as a mixotroph.

To make the simulation results comparable with field data, we calculate body size in terms of the equivalent spherical diameter (ESD; μm), which is $323(M_i/Q_{\text{Redfield}})^{1/3}$ with M_i measured in units of $\mu\text{g N}$ and nitrogen quota Q_{Redfield} in accordance to Redfield ratio ($1/5.68 \mu\text{g N } \mu\text{g C}^{-1}$) (Appendix D). The molar C:N ratio ($\mu\text{mol C } \mu\text{mol N}^{-1}$) is calculated from the carbon and nitrogen biomass in size class i : $(B_{C,i}/12)/(B_{N,i}/14)$, assuming that all the individuals in size class i have the same carbon and nitrogen mass.

We numerically solved the model with the ODE solver *ode45* in MATLAB (m files for model simulation are deposited in the Dryad Digital Repository,

<https://doi.org/10.5061/dryad.c2fqz6145>). We used a range of light and nutrient conditions that

ensured a viable plankton community and within the range of conditions in the Feitsui Reservoir

($L = 91.4\text{--}457 \mu\text{E s}^{-1} \text{ m}^{-2}$ and $N_0 = 12.86\text{--}35.71 \mu\text{M}$). We ran each simulation until biomass of each plankton size class stopped oscillating, and used the results of the last simulation day for analyses.

Results: Simulated trophic strategy, C:N ratio, and cellular N and C influxes along body size

As an example, we show the trophic strategy and molar C:N ratio under average conditions in our freshwater system, i.e., high light and low N supply (Fig. 4). Simulations under different conditions exhibit similar patterns (not shown). The molar C:N ratio increases and reaches a maximum C:N ratio about 12 at a size around $50 \mu\text{m}$ and decreases for larger sizes towards the 6.625 Redfield ratio, forming a unimodal C:N ratio-size pattern (Fig. 4c).

A cell's encounter rates with light, inorganic N, and food change systematically with size (Fig. 4a). Small cells have a higher encounter rate with carbon from photo harvesting than with dissolved nutrients (the green line is higher than the blue line in Fig. 4a). Small cells are therefore limited more by nutrients than carbon. Cells and organisms larger than about $50 \mu\text{m}$ have high encounter rates with food, which supplies both nitrogenous nutrient and carbon (magenta lines in Fig. 4a). As some of the carbon is used for metabolism, larger organisms increasingly become limited by carbon. These differences in encounter rate with size are driven by the differences in allometric size scaling of the affinities to light, nutrient, and food. The change in availability of food determines the trophic strategy of the cells (Fig. 4b): smaller cells

are phototrophic, while cells and organisms larger than about 50 μm are mixotrophic, with the heterotrophic input of carbon and nutrients increasing with size.

The changes in C- and N-limitation and trophic strategy with size shapes how the C:N ratio changes with size (Fig. 4c). The nutrient-limited small photoautotrophic plankton ($\leq 50 \mu\text{m}$) encounter a surplus of carbon that is not used for protein synthesis (Fig. 4c). Instead the surplus is used to increase their C:N ratio. The intensity of N-limitation and surplus of carbon increases with size and consequently the C:N ratio also increases with size (Fig. 4a). Larger mixotrophic plankton ($> 50 \mu\text{m}$) obtain nutrients from phagotrophy. Their photoautotrophic carbon surplus decreases and they become increasingly more C-limited, which leads to a decrease in C:N ratio (Fig. 4a and c). The C:N ratio of mixotrophs is further shaped by the C:N ratio of the diet. Mixotrophs smaller than about 500 μm consume autotrophs in the size range 10-50 μm (the optimal predator-prey mass ratio $\beta = 500$ corresponds to an optimal predator-prey mass diameter ratio of $\approx 500^{1/3} \approx 8$) with a high C:N ratio and thus have a carbon-rich diet. They use some of the carbon for metabolism; however, they also have a sizeable influx of carbon from photoautotrophy to compensate for that loss and helps increase the C:N ratio. The mixotrophs larger than about 500 μm obtain a relatively small amount of photoautotrophic C ($< 30\%$ autotrophic influx ratio) and consume larger prey with lower C:N ratio, and consequently their C:N ratio is lowered. The carbon input from photoautotrophy in mixotrophs combined with a carbon-rich diet act to reduce the decline in C:N ratio with size – without mixotrophy the peak in C:N ratio around 50 μm would be more pronounced. Overall, the carbon-rich diet and the influx

of carbon from photoautotrophy makes the C:N ratio decline slowly for organisms larger than 50 μm .

The general allometric relationships are modulated by changes in prey biomass with size, which changes the availability of food for different sizes. The biomass distribution shows a strong undulation with local minima around 50 and 500 μm (Fig. 4d). The troughs and peaks in the biomass distribution are spaced roughly by the predator:prey mass ratio, which shows that the undulations are due to a trophic cascade. The cascade is likely initiated at the first local minimum around 50 μm . This size corresponds to the largest autotrophs, whose growth is strongly limited by N (low inorganic N uptake and no heterotrophic N supply; Fig. 4a). The second local minimum at size class 494 μm is caused by insufficient prey supply: the biomass of its preferred prey size classes is low (size classes 51 and 71 μm ; Fig. 4d).

Results: Light intensity and inorganic N supply affect simulated plankton C:N ratio

The models' response to changing light and nutrient conditions are similar to the observations in the freshwater system: the simulated C:N ratios of all size classes increase with light intensity and decrease with inorganic N supply (Fig. 5). GAM regression reveals that size class is the main factor determining C:N ratio of plankton ($R^2 = 0.740$; Table 1, Model simulations). Light intensity and inorganic N concentration further explain the variation of C:N ratio ($R^2 = 0.928$; Table 1, Model simulations). The C:N ratios of plankton $\leq 357 \mu\text{m}$ increase with light intensity and decrease with inorganic N supply, whereas the C:N ratios of large organisms increase with

light intensity but change little with nutrient supply. The change of C:N ratio with light and nutrient is the largest in 51 μm size class.

Discussion

Molar C:N ratio as a function of body size

Our observations in marine and freshwater systems show a unimodal body-size pattern of molar C:N ratio with a maximum at approximately 50 μm (Fig. 1). The similarity of the results in both systems suggests that the stoichiometry of plankton along body size is determined by fundamental processes unrelated to the type of system (freshwater or marine). According to our mechanistic food web model, the unimodal pattern is a result of autotrophic plankton having increasing C:N ratio with size and mixotrophic and heterotrophic plankton having a decreasing C:N ratio with size. We assert that these two effects are a result of how encounter rates for light, nutrients and food change with size. Our model shows that while small autotrophs encounter more carbon than nitrogen, the amount of surplus carbon is less than it is for larger autotrophic plankton (plankton around 10 μm have less excess light encounter than those around 50 μm in Fig. 4a). The smallest autotrophs therefore tend to have a lower C:N ratio than larger ones. The size of the carbon surplus increases with size and gives way to strict nutrient limitation for larger sized autotrophs. This effect explains the C:N ratio increase with size, until around 50 μm . The decreasing C:N ratio of plankton larger than 50 μm occurs as the larger mixotrophic plankton rely on predation on smaller plankton, while losing carbon to respiration. Also, mixotrophs and

heterotrophs larger than about 500 μm consume prey with lower C:N ratio, and consequently their C:N ratio is lower. The decreasing C:N ratio with size corroborate our first hypothesis. However, the increasing C:N ratio of small plankton was unexpected.

Effects of light intensity and inorganic N supply on plankton C:N ratio

Consistent with our second hypothesis, freshwater and simulated C:N ratios of photoautotrophs increase with high light intensity and decrease with high inorganic N supply, in accordance with the classic light-nutrients hypothesis and with observations (Sterner and Elser 2002; Dickman et al. 2006; Fig. 2). The multiple linear regression model with light and inorganic N is also significant for both freshwater and model system ($p < 0.001$; regression parameters are shown in Table 1). However, in the freshwater system the positive effect of light on the C:N ratio is weak (-1.003×10^{-4} for the multiple linear regression; Table 1). One possible explanation of the relatively small effect of light intensity on plankton C:N ratio in the freshwater system could be that the light is generally low ($< 100 \mu\text{E s}^{-1} \text{ m}^{-2}$) and thus it is difficult to observe the positive effect of high light intensity seen in the model, which is run for the full range of light intensities (Appendix A, Fig. A1a). An alternative explanation may be light acclimation (Geider et al. 1996, 1997), which would lower the light affinity of the observed plankton as light levels increase, and which is not included in the model. Some empirical support is lent by Frenette et al. (1998) who observed that large phytoplankton have higher C:N ratio and surplus carbon uptake when light intensity increases. Though effects of light intensity and

inorganic N do follow the light-nutrient hypothesis, adding non-linear size effect to the light and inorganic N nutrient model minimizes the AIC and increases R^2 from 0.052 to 0.440 and 0.186 to 0.740, respectively, for freshwater and simulated C:N ratio (Table 1). While light and inorganic nutrient generally explain plankton C:N ratio, the importance of size cannot be dismissed.

Our second hypothesis proposed that the C:N ratios of large mixotrophic and heterotrophic size classes are less affected by light and nutrients than photoautotrophs. In our freshwater system, C:N ratio changes with light and nutrient supply are the largest among the 10-74 μm size classes (Fig. 2). Our model simulation showed that the change of C:N ratio with light and nutrient supply is the largest at autotrophic size classes 27-51 μm and C:N ratio change is the smallest at size classes larger than 1000 μm and smaller than approximately 20 μm , partly supporting our second hypothesis (Fig. 5). The small C:N ratio change in small size classes of photoautotrophic phytoplankton may be caused by their relatively high affinity to inorganic nutrient. These small phytoplankton efficiently uptake ambient inorganic nutrient in oligotrophic environments and may maintain a lower carbon to nutrient ratio (Friebele et al. 1978; Sournia 1982; Hein et al. 1995).

Overall, our identification of a size-stoichiometry pattern adds to the existing light-nutrient hypothesis about plankton C:N ratios (Sterner and Elser 2002; Leonardos and Geider 2004; Dickman et al. 2006). The light-nutrient hypothesis states that the C:N ratio is determined by the supplies of carbon and dissolved nutrients, with higher light or lower N leading to increased C:N ratios (Dickman et al. 2006). Our understanding is also rooted in nutrient quota-limited growth as

the model is based on Droop dynamics; however, we add two additional hypotheses. First, we hypothesize that the degree of autotrophic C and N uptakes are modulated by size, as size determines whether cells are more likely to be nutrient limited – leading to a high C:N ratio – or carbon limited with a lower C:N ratio. The strongest nutrient limitation occurs among large autotrophs, leading to the peak in C:N ratio around 50 μm . Smaller autotrophs are still nutrient limited, though to a lesser degree, thus having smaller C:N ratios. Second, we extend the light-nutrient hypothesis to higher trophic levels by showing how the C:N ratio declines with size and trophic level through predation on autotrophs by mixo- and heterotrophy. These insights emphasize how body size and trophic interactions critically influence the stoichiometry in plankton community.

Deviation of simulated C:N ratio from natural plankton community

We found higher simulated C:N ratios than observed in our marine and freshwater systems (Fig. 5). Four important reasons could be: the lack of bacteria, underestimation of respiration, $\overline{\tau}$ underestimation of the parameter $Q_{\min}$, and overemphasis of the role of mixotrophy in large size classes. Lower C:N ratio in small size classes in natural systems may be caused by the existence of small size ($< 20 \mu\text{m}$), bacterivorous mixotrophs such as *Micromonas* (Unrein et al. 2014). These mixotrophs feed on bacteria with lower C:N ratio (Jansson et al. 1996), which are not considered here. In addition, the C:N ratios decrease with respiration (Fig. E1 in Appendix E). This suggests that respiratory C demand (Castellani et al. 2005) could be underestimated in the

model. Furthermore, increasing $Q_{\min}$ lowers the maximum level of the C:N ratios (Fig. E2 in Appendix E). The high simulated C:N ratios indicate that our assumed $Q_{\min}$ is too small. We need empirical knowledge of $Q_{\min}$ of photoautotrophs (Shuter 1977; Edwards et al. 2012) and less studied mixotrophs. Finally, mixotrophy is overemphasized for the organisms larger than $10^3 \mu\text{m}$ (Fig. 4b and Fig D1), which are multicellular heterotrophs in nature (Hansen et al. 1997). Resolving these issues would bring the model better in line with the observations.

Extension of plankton stoichiometric research to P-limited systems

We have only considered the C:N ratio and not included the C:P ratio. This choice is mainly for practical reasons: in our observations we had only access to nitrogen and we considered two (C and N) instead of three (C, N, and P) elements in our model to simplify the system in this study. The “N” compartment here can be considered generally as a limiting nutrient, and the results would be similar for P since inorganic P utilization should follow a similar physiological mechanism as inorganic N (Edwards et al. 2012).

Conclusions

We have shown that plankton stoichiometry is structured by body size in freshwater and marine food webs. More importantly, our model reveals how fundamental allometric size-scaling of resource affinity and mixotrophy shape these patterns. We have used the insights to formulate an extension to the classical light-nutrient hypothesis to include the effects of size and trophic

level. We call for more field observational researches of plankton size-based stoichiometry and trophic strategy in other regions and for the C:P ratio to test the extended hypothesis. Further, the model could be strengthened by better empirical knowledge of the storage strategies and limits of nutrient quota ($Q_{\min}$ and $Q_{\max}$), which also influence the stoichiometry and stoichiometric homeostasis (Meunier et al. 2017). The extended hypothesis provides a framework to understand global and seasonal patterns in plankton stoichiometry along the entire trophic spectrum of eukaryotic plankton, from phytoplankton to zooplankton.

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Statement of authorship: P-C. H., K. H. A., and C-H. H. conceived the research question. C-W. C., P-C. H., C-H. H., and F-K. S. sampled and processed marine and freshwater size-fractionated plankton. P-C. H. and P-L. W. measured the C and N contents of plankton. F-K. S. measured environmental data in Feitsui Reservoir. P-C. H. analyzed the data. P-C. H. and K. H. A. constructed the theoretical model. P-C. H. wrote the manuscript and all authors contributed to the revision.

Appendixes

Appendix A: Size-fractionated C:N ratios of field plankton and environmental conditions in the freshwater and marine systems

We collected samples at the Feitsui reservoir and in the East China Sea. The fixed station is near the dam of Feitsui Reservoir, a subtropical freshwater reservoir in Taipei, Taiwan (24.9090° N, 121.5798° E). In Table S1 (TableS1_FreshwaterCN_Size.csv; deposited in the Dryad Digital Repository, <https://doi.org/10.5061/dryad.c2fqz6145>), we present the sampling date, molar C:N ratio of 100 samplings in 6 plankton size classes (0.7-10, 10-44, 44-74, 74-149, 149-500, >500 μm), light intensity (photosynthetically active radiation PAR; $\mu\text{E s}^{-1} \text{ m}^{-2}$), concentration of nitrite and nitrate (NO_2 and NO_3 ; μM). The histogram of PAR distribution indicates that most of the PARs are below 100 $\mu\text{E s}^{-1} \text{ m}^{-2}$ (Fig. A1a), and thus the positive effect of high light intensity on plankton C:N ratio may not be clearly seen in this dataset (Table 1). The histogram of $[\text{NO}_2^- + \text{NO}_3^-]$ shows that inorganic nitrogen nutrient concentrations are mostly within 10 – 50 μM and the distribution is more even than PAR (Fig. A1b); this may be the reason why the negative effect of inorganic nitrogen supply on plankton C:N ratio is better shown (Table 1).

The marine pelagic habitats include the East China Sea and the South China Sea (145 cruise-stations). In Table S2 (TableS2_OceanCN_Size.csv; deposited in the Dryad Digital Repository, <https://doi.org/10.5061/dryad.c2fqz6145>), we present the sampling date, latitude and longitude ($^\circ$), and molar C:N ratio of 8 size classes (0.7-50, 50-104, 104-200, 200-363, 363-500, 500-1000, 1000-2000, >2000 μm) in the East China Sea and near Dongsha Atoll.

Appendix B: Prey carbon and nitrogen consumption rates in the model food web

Accounting for the differences in the C:N ratio of food requires a modification of the simple functional response in equation 1. As the largest part of the food is carbon, we assume that it is the available prey carbon biomass that limits the uptake rate. We first write the uptake of carbon as:

$$J_{FC} = g_{\max} f \text{ with } f = \frac{\alpha_F F_C}{\alpha_F F_C + g_{\max}} , \text{ (B1)}$$

where $g_{\max}$, α_F , and F_C are the maximal uptake rate of food, affinity to food, and the available food carbon mass.

From that relation, the ratio between food carbon mass uptake and encountered ($\alpha_F F_C$) is:

$$c = \frac{g_{\max} f}{\alpha_F F_C} . \text{ (B2)}$$

This factor defines the reduction of uptake due to satiation. The uptake of nitrogen is then the food nitrogen mass encountered ($\alpha_F F_N$, where F_N is the available food N mass) reduced with the same factor to become:

$$J_{FN} = c \alpha_F F_N = J_{FC} \frac{F_N}{F_C} . \text{ (B3)}$$

J_{FN} and J_{FC} appear in the “synthesis” part in equations 5 and 6, respectively, as the uptake of prey nitrogen and carbon biomass.

Appendix C: Predation pressure from large plankton

In our food web model, each plankton size class suffers the predation pressure from plankton larger than itself (except for the largest size class). Let size class i be consumed by plankton of size class j that is smaller than itself. The amount of prey i available to size class j is:

$$F_{C,(i,j)} = B_{C,i} \phi(M_i, M_j) \quad , \quad (C1)$$

where $B_{C,i}$ is the carbon biomass of size class i and $\phi(M_i, M_j)$ is the preference of predator of size M_i for prey of size M_j .

The total food available to plankton of size class j is:

$$F_{C,j} = \sum_k B_{C,k} \phi(M_k, M_j) \quad , \quad (C2)$$

where k is any size class smaller than j . According to the classical type II functional response, the total prey biomass consumed by predator of size class j is:

$$J_{FC,j} = \frac{g_{\max F,j} \alpha_{F,j} F_{C,j}}{\alpha_{F,j} F_{C,j} + g_{\max F,j}} \quad , \quad (C3)$$

where $g_{\max F,j}$ and $\alpha_{F,j}$ are size class j 's maximal uptake rate of food and affinity to food.

The biomass of prey size class i consumed by plankton j is $J_{FC,j} r_i$, where $r_i = F_{C,(i,j)} / F_{C,j}$ is the biomass ratio between prey i and total prey biomass available to plankton of size class j .

Thus, the biomass of size class i ingested by plankton of size class j is:

$$P_{i,j} = \frac{g_{\max F,j} \alpha_{F,j} F_{C,j}}{\alpha_{F,j} F_{C,j} + g_{\max F,j}} \frac{F_{C,(i,j)}}{F_{C,j}} = \frac{g_{\max F,j} \alpha_{F,j} B_{C,i} \phi(M_i, M_j)}{\alpha_{F,j} F_{C,j} + g_{\max F,j}} \quad . \quad (C4)$$

The predation pressure $P_{i,j}$ is seen in the “predation” part in equation 5 and 6.

Appendix D: Autotrophy to heterotrophy transition with size under different light to inorganic nutrient supply

To compare the results with field observations, the body size of modeled plankton is represented as equivalent spherical diameter (ESD), which is transformed from the body volume $= (4/3)\pi(\text{ESD}/2)^3$. To estimate the body volume, the C mass is first transformed to wet mass by carbon mass = $0.42 \times \text{dry weight}$ (Beers 1966) and dry weight (DW) = $0.135 \times \text{wet mass}$ (Postel et al. 2000), and the mass density of the organism is assumed $1\text{g/cm}^3 = 10^{-6} \mu\text{g}/\mu\text{m}^3$. In general, organisms smaller than $71 \mu\text{m}$ are obligate autotrophs under all light: nutrient supply ratios (Fig. D1, dark blue region at the bottom). Most of the organisms larger than $71 \mu\text{m}$ engage mainly in heterotrophy. However, under high light intensity (Fig. D1b) and/or low nutrient condition (Fig. D1, $N_0 \sim 14 \mu\text{M}$) some large size classes (494 and $4740 \mu\text{m}$) can be mixotrophs with only 60-70% heterotrophic C influx ratio. This is mainly caused by the low density of preferred prey and thus low C influx from prey consumption. In natural aquatic systems, these size classes should be obligate heterotrophs (dominated by crustaceans; Hansen et al. 1997) and cannot survive due to insufficient prey supply. But in our model these size classes are mixotrophic and can keep a low population biomass due to the C gain from photosynthesis.

Appendix E: Sensitivity tests of the model

Sensitivity test 1: Effect of maintenance respiration on the C:N ratio of plankton

Respiration for structural maintenance is the pivotal factor that controls plankton C:N ratio in our model, especially in large organisms that mainly depend on heterotrophic C uptake. In the main simulations, carbon mass-specific R is set 0.03 d^{-1} (Fig. 5). Here, we tested the effect of respiration by lowering R to 0.01 (Fig. E1a) and increasing R to 0.06 d^{-1} (Fig. E1b). We chose maximal $R = 0.06 \text{ d}^{-1}$, considering that large mixotrophs will go extinct if their respiration rate is too high. We found that C:N ratio in all size classes decreases as R increases (Fig E1a to b). This infers that increase of respiration and respiratory loss of carbon is one important force that lowers C:N ratio in plankton.

Sensitivity test 2: Effect of $Q_{\min}$ on the C:N ratio of plankton

The value of $Q_{\min}$ determines the minimal N content per unit C mass (minimum N/C mass in a cell) needed to sustain the growth of plankton (Flynn 2008), and is the potential factor that influences the maximum C:N ratio. Here, we examined the effect of $Q_{\min}$ on the distribution of C:N ratio along body size under different light: inorganic N supply. We changed $Q_{\min}$ to 0.0778 and $0.0467 \mu\text{g N } \mu\text{g C}^{-1}$ (equals to molar C:N ratio 15 and $25 \mu\text{mol N } \mu\text{mol C}^{-1}$) in this sensitivity test. We found that lower $Q_{\min}$ increases the upper limit of plankton C:N ratio (higher C:N ratio especially at size class $51 \mu\text{m}$ in Fig. E2b compared with Fig. E2a).

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Table 1. GAM analyses of freshwater and simulated size-fractionated C:N ratio.

Freshwater							
Model	Intercept	Light (PAR ($\mu\text{E s}^{-1} \text{ m}^{-2}$))	Inorganic N ([N (μM)])	K	R^2	AIC	ΔAIC
C:N = spline(log(size))	6.69			3	0.385	2226.90	136.58
C:N = spline(log(size)) + L	6.66	0.0001		4	0.378	2183.54	93.22
C:N = spline(log(size)) + [N]	7.89		-0.039	4	0.449	2129.87	39.55
C:N = spline(log(size)) + L + [N] [§]	7.88	-7.46×10^{-5}	-0.038	5	0.44	2090.32	0.00
C:N = L + [N]	7.87	-1.003×10^{-4}	-0.038	3	0.052	2374.63	284.31
Model simulations							
Model	Intercept	Light (PAR ($\mu\text{E s}^{-1} \text{ m}^{-2}$))	Inorganic N ([N (μM)])	K	R^2	AIC	ΔAIC
C:N = spline(log(ESD))	8.60			3	0.740	3539.33	2066.08
C:N = spline(log(ESD)) + L	7.28	0.0048		4	0.902	1954.91	481.66
C:N = spline(log(ESD)) + [N]	9.25		-4.26	4	0.754	3452.53	1979.28

$C:N = \text{spline}(\log(\text{ESD})) + L + [N]^{\S}$	8.11	0.0050	-5.77	5	0.928	1473.25	0.00
$C:N = L + [N]$	8.10	0.0050	-5.74	3	0.186	5380.36	3907.11

Note: The regression model is presented in column 1: $\text{spline}(\log(\text{size}))$ is the spline regression of logarithm body size (equivalent spherical diameter in μm), L is light intensity and $[N]$ is inorganic nutrient concentration. K is the number of parameters including intercept and smoothing parameter (fitting to body size) in GAM. The model with the lowest AIC is labeled with $\S$.

Table 2. Symbols and model parameters. Subscripts N, L, F indicate inorganic nitrogen, light, and prey (food), respectively.

Symbol	Name	Value	Unit
$B_{N,i}$	Nitrogen biomass concentration of size class i		$\mu\text{g N}^{-1}\text{liter}^{-1}$
$B_{C,i}$	Carbon biomass concentration of size class i		$\mu\text{g C}^{-1}\text{liter}^{-1}$
N	Inorganic nitrogen concentration		$\mu\text{g N}^{-1}\text{liter}^{-1}$
c_L	Coefficient of light affinity	0.00959 ^a	$\mu\text{g C d}^{-1} (\text{W m}^{-2})^{-1} (\mu\text{g N}^{2/3})^{-1}$
c_N	Coefficient of inorganic N affinity	0.000289 ^a	$\text{liter d}^{-1} (\mu\text{g N}^{1/3})^{-1}$
c_F	Coefficient of prey affinity	0.0704 ^a	$\text{liter d}^{-1} (\mu\text{g N})^{-1}$
$\theta_L, \theta_N, \theta_F$	Allometric scaling exponent of light, inorganic N, prey affinity	2/3, 1/3, 1 ^b	—
G_L	N specific maximal photosynthetic rate	4.54 ^c	$\mu\text{g C d}^{-1}\mu\text{g N}^{-1}$
G_N	N specific maximal inorganic N uptake rate	1.14 ^c	d^{-1}
G_F	N specific maximal prey uptake rate	25.55 ^c	$\mu\text{g C d}^{-1}\mu\text{g N}^{-1}$
μ	Quadratic mortality coefficient	0.01 ^d	$\mu\text{g C}^{-1}\text{liter d}^{-1}$
R	C mass specific respiration rate	0.03 ^e	d^{-1}

h_N, h_L, h_F	Exponents for uptake reduction	0.1, 0.1, 1 ^f	-
κ	Mixing rate	0.01	d ⁻¹
$Q_{\min}, Q_{\max}$	Minimal and maximal N:C mass ratio (quota)	0.0583 and 0.198 ^f	$\mu\text{g N } \mu\text{g C}^{-1}$
β	Preferred predator-prey mass ratio	500 ^g	
σ	Width of prey size preference	1 ^g	

^aModified from Andersen et al. (2016) to nitrogen-based constant by the Redfield ratio; ^b Andersen et al. (2015); ^cTransformed from C- to N-specific by the Redfield ratio (Chakraborty et al. 2017); ^dAdjusted such that quadratic mortality is less than predation mortality.

^eBased on the percentage of carbon loss to respiration in protists from Stoecker and Michaels (1991). ^f Ward and Follows (2016).

^gApproximately the optimal predator-prey mass ratio of unicellular ciliates (Hansen et al. 1994).

Figure legends

Figure 1. Molar C:N ratios with respect to size classes from the (a) freshwater reservoir and (b) subtropical marine plankton community. Extreme C:N ratios (> 20 or < 4 molar C:N ratio) are excluded. Upper case letters (*A, B, C, D, E*) represent the significant difference of C:N ratios between size classes based on Tukey's post hoc test. The lines in the middle, upper and lower ends of the box represent the median, 25% and 75% quantiles of C:N ratios. The upper and lower whiskers represent the maximal and minimal values and circles represent outliers of C:N ratios.

Figure 2. Freshwater plankton molar C:N ratio under different light intensity and inorganic N conditions. The C:N ratios under high and low light are PAR of 25 and 185 $\mu\text{E s}^{-1} \text{m}^{-2}$ (blue and magenta lines); high and low nutrients represent concentrations of $\text{NO}_2^- + \text{NO}_3^-$ of 21.2 and 38.1 μM (dashed and solid lines). The vertical bars are ± 1 standard deviation of the C:N ratios of each size class under the 4 conditions.

Figure 3. Schematic representation of the mixotrophic plankton food web model (a), carbon and nitrogen fluxes in an individual cell (b), and resource affinity scaled with body size (c). Plankton in each size class (represented by its carbon biomass $B_{C,i}$) takes up sunlight (L ; green) and inorganic N from ambient water (N ; blue) and eats smaller cells (food F ; magenta). In panel (b), J_N (equation 1) and J_{FN} (equation B3 in Appendix B) represent the flux of inorganic N nutrient and prey N biomass, which is allocated to nitrogen (N) biomass. J_L (equation 1) and J_{FC}

(equation B1 in Appendix B) represent the flux of photosynthetic C and prey C biomass, which is allocated to carbon (C) biomass. Nitrogen quota-based factor q_N regulates the uptake and assimilation of nitrogen (equation 3) and γ_X regulates the assimilation of carbon from photoharvesting and food (equation 4). Panel (c) shows how the affinities for uptake of light, nutrients and food (α_L , α_N , and α_F) scales with body mass with allometric exponents θ_L , θ_N , θ_F (Table 2).

Figure 4. Results from a model simulation with light $L = 228.5 \mu\text{E s}^{-1} \text{m}^{-2}$ and supply source of inorganic nitrogen nutrient $N_0 = 12.86 \mu\text{M}$. The white area indicates obligate photoautotrophs and shading indicates mixotrophs and heterotrophs, as determined by the heterotrophic influx being below or above 5% of total C influx respectively (see equation 10). Panel (a) shows how the encounter rate of inorganic nitrogen, light, and food changes with size. Encounter rate is the affinity from Fig. 3c multiplied by resource abundance: $\alpha_X X$, where X = inorganic nitrogen (N), light (L), and food (F) (blue, green and magenta lines). The solid and dotted magenta lines are calculated by multiplying size-based food affinity with the prey mass averaged over all size groups and prey mass for each size class, respectively. The nitrogen nutrient is converted to units of carbon by multiplying with the Redfield ratio. Panel (b) shows the fraction of auto- (green line) and heterotrophic (magenta line) C influx ratios (equations 9 and 10). Panel (c) shows the molar C:N ratio, which equals to the inverse of nitrogen quota Q divided by the atomic mass

between C and N (12/14). Panel (d) shows carbon biomass B_C , with the magenta line indicating the mean.

Figure 5. Simulated plankton molar C:N ratio under different light intensity and inorganic N supply schemes. The simulated C:N ratios under the high light and low supply of inorganic N nutrient ($L = 457 \mu\text{E s}^{-1} \text{ m}^{-2}$; $N_0 = 12.9 \mu\text{M}$), and high light and high supply of inorganic nutrient ($L = 457 \mu\text{E s}^{-1} \text{ m}^{-2}$; $N_0 = 35.7 \mu\text{M}$ low) are represented by magenta dashed and solid lines, respectively. The simulated C:N ratios under the low and high light are $L=91.4$ and $457 \mu\text{E s}^{-1} \text{ m}^{-2}$ (blue and magenta); low and high nutrients are $N_0 = 12.9$ and $35.7 \mu\text{M}$ (dashed and solid). The white and shaded regions represent photoautotrophs versus mixo- and heterotrophs as in Fig. 4.

Figure A1. The light intensity (PAR, photosynthetically active radiation) and surface inorganic nitrogen nutrient concentration ($[\text{NO}_2^- + \text{NO}_3^-]$, nitrite and nitrate concentration) in Feitsui Reservoir. The 25% quantile, median, and 75% quantile PARs are 23.76, 59.00, and $182.50 \mu\text{E s}^{-1} \text{ m}^{-2}$, respectively in panel (a). The 25% quantile, median, and 75% quantile $[\text{NO}_2^- + \text{NO}_3^-]$ are 21.24, 27.63, and $38.10 \mu\text{M}$, respectively in panel (b).

Figure D1. Fraction of heterotrophic to total carbon influx under low (a) and high (b) light intensity. The heterotrophic influx ratio is calculated according to equation

10: heterotrophic influx ratio = $\frac{\gamma_{FC}J_{FC}}{\gamma_LJ_L + \gamma_{FC}J_{FC}}$, where $\gamma_{FC}J_{FC}$ is the carbon assimilated from prey consumption and γ_LJ_L is the carbon produced by photosynthesis. L is the light intensity at water surface and N_0 is the supply source concentration of inorganic nitrogen. ESD is equivalent spherical diameter (μm) of plankton. The region between two dash lines (ESD = 494 and 4740 μm) represents the size classes that should be obligate heterotrophs in natural plankton communities.

Figure E1. Molar C:N ratio with respect to size of plankton with C mass-specific respiration rate $R = 0.01$ (a) and 0.06 d^{-1} (b). Equivalent spherical diameter is transformed from the nitrogen body mass by the method described in Appendix D and used to represent the body size of plankton. The way to present light and nutrient supply conditions follows Fig. 5.

Figure E2. Molar C:N ratio with respect to size of plankton with $Q_{\min}$ equals to (a) 0.0778 , and (b) 0.0467 ($\mu\text{g N } \mu\text{g C}^{-1}$). ESD (equivalent spherical diameter) is transformed from the nitrogen biomass body mass by the method described in Appendix C and used to represent the body size of plankton. The way to present light and nutrient supply conditions follows Fig. 5.

a Freshwater

b Marine

Figure 2

Figure 3

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Figure A1

a

b

Figure D1

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a

b

a

b