Chain or sphere? Perspectives on colony shapes and sizes in microalgae

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Abstract

Some microalgal species can increase their collective size by forming colonies; notable examples are chained colonies in diatoms and *Scenedesmus sp.*, and spherical colonies in *Phaeocystis globosa*. For a given cell specific growth rate, chain formation increases collective length quickly to fend off ciliates, but not against tube- and pallium-feeding heterotrophic dinoflagellates or metazoan grazers with ability to manipulate chains to aid ingestion. Sphere increases in volume relatively slowly but would be difficult to manipulate even for metazoan grazers. Diffusive nutrient supply to a chained colony would be a fixed proportion of that to solitary cells, regardless of chain length, whereas cells within a spherical colony would experience increasing nutrient limitation with increasing colony size. One hemisphere of a spherical colony would inevitably receive less irradiance, creating an auto-light limitation. Experimental data showed that light decreased substantially as it passed through a *P. globosa* colony, and the optical density of the colony increased linearly with colony diameter. However, neither *in situ* nutrient nor light limitation alone can explain an order-of-magnitude difference in colony size between the European and the Asian *P. globosa* populations. Instead, some evidence of different expression of gene(s) involved in colony formation and enlargement suggests genomic variations among the different populations.

Keywords: microalgae; colony formation; nutrient; light; defense

Introduction

Size and shape are fundamental traits that influence an organism's life history (Barnes et al. 2010). Due to the small individual size of microalgae, they are in constant danger of being eaten, and increasing collective size by colony formation can be an effective way to deter grazers. Among the diverse planktonic microalgae, some species can form colonies and some do not, whereas some alternate between solitary form and colonial form (Lampert et al. 1994, Jakobsen & Tang 2002). The two most common forms of microalgal colonies are chain, such as diatoms and *Scenedesmus* (Lürling & Van Donk 1997), and sphere, such as *Phaeocystis globosa* (Rousseau et al. 2007). In this paper, we use these examples to consider the benefits and constraints of the different colonial forms.

Rate of size increase in chain vs. sphere

Chain formation is a simple way to increase the collective size: A cell undergoes simple cell division but without separation, thereby doubling the overall length. Because cell division occurs along one axis, chain size growth is one dimensional. This allows the colony to increase in length as quickly as cell division allows, with minimal requirement of extra structural investment, which is an advantage when responding to grazing threat. For example, when *Scenedesmus acutus* is exposed to a grazer chemical cue, the proportion of 8-celled chains can increase eight-fold within 48 h while the equivalent population growth rate remains unaffected (Lampert et al. 1994).

To form a spherical colony, individual *P. globosa* cells are held within a polysaccharide "colony skin" secreted by the cells (Rousseau et al. 2007). As the cells continue to multiply and produce more colony skin material, the size of the colony increases, but the rate of increase in spherical volume is lower than that in chain length for the same cell division rate. To illustrate this point, we consider a cubic cell of unity dimensions (1 × 1 × 1) with a specific growth rate of μ. To form a chained colony, the relative change in chain length per unit time (R_L) can be expressed numerically as:

$$R_L = e^{\mu} \qquad \text{Eq. 1}$$

To form a spherical colony, relative change in surface area per unit time (R_S) can be expressed numerically as:

$$R_S = e^{\mu} = 4\pi r^2 \qquad \text{Eq. 2}$$

where r is colony radius; the relative change in volume per unit time (R_V) can then be expressed numerically as:

$$R_V = \frac{4}{3}\pi r^3 = \frac{(e^{\mu})^{\frac{3}{2}}}{6\,\pi^{\frac{1}{2}}} \quad \text{Eq. 3}$$

A simulation was run for hypothetical μ values of 0.1 to 3. The results showed that R_L was larger than R_V for the same μ, and the discrepancy increased with increasing μ (Fig. 1); in other words, given the same growth rate and time interval, chain length increases proportionally faster than spherical volume. This begs the question: If the purpose of colony formation is to increase the collective size to fend off grazers, why would *P. globosa* adopt the "slow" strategy of forming spheres instead of the "fast" strategy of forming chains?

Colony formation as defense against grazers

In the marine environment, most of the grazing pressure comes from protozoans such as ciliates and heterotrophic dinoflagellates (Hdino) (Calbet & Landry 2004). Ciliates usually engulf the algal cells whole, whereas some Hdino extract algal cell content via a feeding tube or digest prey extracellularly using a pseudopodial pallium (Hansen and Calado 1999). Because ciliates have limited ability to expand its food intake site or to break a colony into smaller bits (Fig. 2a), chain formation is a quick way to resist engulfment, as has been shown in experiments (Bjærke et al. 2015). This strategy will not be effective against tube feeding and pallium feeding (Fig. 2b) (Sherr & Sherr 2007, Jacobson and Anderson 1986), although these feeding modes are considered a slower process (each feeding event may take hours; Jacobson & Anderson 1986). Spherical colonies in *P. globosa* are effective against ciliates (Jakobsen & Tang 2002), but it is unclear whether the colony skin can defend against tube or pallium feeding by Hdino.

Copepods, as the major marine metazoan grazers, can manipulate and reposition chained colonies with their appendages to aid ingestion (Fig. 3a); therefore, chain elongation is not expected to deter copepod grazing (Bjærke et al. 2015), but it may even allow the copepods to ingest multiple cells more efficiently. Indeed, experiments have shown that diatoms tend to remain as solitary cells when exposed to copepod grazing cues (Bergkvist et al. 2012). Diatoms may also use other defensive strategies such as modifying their cell wall structure and producing chemical deterrents (Pančić and Kiørboe 2018), contributing to their success. In contrast to chains, a spherical colony cannot be repositioned easily to aid ingestion (Fig.

3b), and the tough colony skin may offer the cells additional mechanical protection (Hamm et al. 1999). Experiments have shown that both *P. globosa* colony size and abundance increased when exposed to copepods (Tang 2003). Because a wide range of protozoan and metazoan grazers coexist *in situ*, all with different feeding modes and size preferences, and colony formation and enlargement does not occur instantaneously, it remains an open question how microalgae may respond beyond the single-predator experimental setting.

In freshwater habitats, daphnids are the main metazoan grazers but their feeding appendages are enclosed by the carapace and lacking the same manoeuvrability as copepods' appendages. Therefore, even a simple chain-form colony like *Scenedesmus* is sufficient to defend against daphnids (Lürling & Van Donk 1996).

Nutrient constraints on colony size

In theory, colony size can increase indefinitely. In reality, *Scenedesmus* chains rarely exceed 16 cells and diatom chains rarely exceed tens of cells. *P. globosa* spheres rarely exceed 1 mm in diameter with a few thousands of cells, with the exception of the SE Asian populations, which can reach up to 30 mm in diameter with millions of cells (Qi et al. 2004, Smith et al. 2014). We may ask: What limits the colony size?

We first consider nutrient limitation based on the diffusion models (Berg 1993). Consider a cubic cell of unity dimensions with 6 equal-sized absorbing surfaces. Assuming perfect absorption, nutrient diffusive flux will be a function of ambient nutrient concentration (C_∞) and diffusion coefficient (D). For each absorbing surface, nutrient diffusive flow (F_{total}) is proportional to the linear dimension of the surface (L). For a solitary cell, nutrient supply ($F_{solitary}$) can be expressed as:

$$F_{solitary} \propto 6L \qquad \text{Eq. 4}$$

By forming chain, cells at the chain ends will have only five exposed surfaces for absorption; therefore, nutrient diffusive flow to these cells (F_{end}) equals 5/6 of $F_{solitary}$. Cell between cells has 4 exposed surfaces; therefore, nutrient diffusive flow to these cells ($F_{between}$) equals 67% $F_{solitary}$. The proportionality is independent of chain length and therefore, the nutrient-dependent cell-specific growth rate within a chain should be no worse than 67% that of solitary cells. The nutrient constraint can be further relaxed by ambient turbulence or having intercellular space (Pahlow et al. 1997). Nevertheless, a chained colony may deplete the surrounding nutrients more rapidly than solitary cells, creating localised nutrient limitation— This is supported by laboratory observations where *Skeletonema costatum*

diatom chain length increased with increasing nutrient concentrations (Takabayashi et al. 2006).

For a sphere, nutrient diffusive flow can be calculated as:

$$F_{total} = 4\pi DrC_{\infty} \quad \text{Eq. 5}$$

Therefore, nutrient supply is scaled to the radius *r*. Because cell number increases proportionally to surface area, nutrient demand is scaled to r^2, and the nutrient supply-to-demand ratio decreases as $1/r$. As the sphere size increases, each of the cells will experience an increasing degree of nutrient limitation. This in principle sets a limit to the sphere size.

Light constraints on colony size

Another factor to consider is light. Cells within a chained colony presumably have the same mass density and therefore the natural orientation of a chained colony would be horizontal such that every cell should receive the same average amount of down irradiance (Fig. 4a). For a spherical colony, one hemisphere faces away from the light such that the cells within that hemisphere receive less light than those in the opposite hemisphere (Fig. 4b). If the incident light reaching the near side is L_0, the transmitted light on the far side (L) can be approximated as:

$$L = L_0 e^{-kd} \quad \text{Eq. 6}$$

where d is sphere diameter, and k is attenuation coefficient along the light path through the colony; the average light received the whole colony can be approximated as $(L+L_0)/2$.

The intracolonial fluid of *P. globosa* colonies has an organic carbon concentration much higher than the typical coastal seawater, which would increase its light attenuation (Smith et al. 2014). Additionally, a healthy *P. globosa* colony contains densely packed cells within the colony skin (Fig. 5a), which will create a self-shading effect. The giant *P. globosa* colonies in SE Asia even appear opaque (Fig. 5b). This "auto-light limitation" may limit the overall cell growth rate and hence the overall size of the sphere.

We conducted simple experiments to test our "auto-light limitation" hypothesis. *P. globosa* colonies were collected along the coast of the Guangdong province, China and returned to the laboratory in Jinan University for the experiments. In Experiment 1, we made a plastic black plate with a 5-mm hole at the centre and placed it on an inverted light microscope. We placed a quantum light sensor underneath facing upward. We first measured

the light passing through the plastic plate without colonies (L_o); care was taken to block out any stray light from the surrounding. Next, we placed a colony (> 5 mm diameter) onto the plastic plate and measured the light that passed through (L). The difference between the two readings indicates the extent of light attenuation by the colony itself. The procedures were repeated until a total of 16 colonies were measured (Table 1). Based on the measurements, we calculated the light attenuation coefficient (k) from Eq. 6, assuming negligible light attenuation by air. The results showed that light intensity was decreased considerably by the colonies, and the estimated k was 0.13 ± 0.03 mm^{-1} (Table 1), orders of magnitude higher than even turbid coastal water (Johnson et al. 2014). Extrapolating this k value to a 30-mm colony (the largest *P. globosa* colony observed *in situ*), L/L_o would be 0.021; i.e. only 2% of the incident light would pass through the colony, and the average light received by cells within the whole colony would be 51% L_o.

In Experiment 2, we placed a colony in a cuvette with seawater and measured its optical density at 438 nm (OD_{438}) on a spectrophotometer (pre-zeroed with plain seawater). As the colony slowly sank and crossed the light path, the OD_{438} reading was recorded. A total of 10 colonies were measured. Despite the movement of the colony adding to measurement uncertainty, the data showed a linear increase in OD_{438} with colony size (Fig. 6a). In Experiment 3, we placed the colonies individually in a microplate and measured their OD_{438} using a microplate reader. A total of 12 colonies were used. As in Experiment 2, the OD_{438} reading was linearly correlated with colony diameter (Fig. 6b).

Extrapolating the regression equations from Experiments 2 and 3 to a 30-mm colony, the projected OD_{438} would be 0.92 for both experiments, i.e. only 12% of the incident light could pass through the colony (average 56% L_o for the whole colony). Experiment 1 gave a slightly lower value (51% L_o) likely because the white light used in that experiment should attenuate more strongly than the short wavelength (438 nm) used in other experiments. Taken together, data from all three experiments support the idea that self-shading and light attenuation by the colony constituents would impose strong light limitation on the colonial cells, hence potentially limiting the overall colony size.

Geographical differences in *P. globosa* colony size

Phaeocystis globosa colonies in the N. Atlantic have an upper size limit of ca. 1 mm, whereas the strain in SE Asia can reach up to 30 mm (Qi et al. 2004, Smith et al. 2014).

Based on the available phylogenetics data (18S sequences), there are no discernible differences between the Chinese strain and the European strain of *P. globosa* (Chen et al. 2002). How do we explain their very different colony sizes? One way to relax the size constraint is to increase the ambient nutrient concentration and irradiance. Along the European coasts where *P. globosa* blooms seasonally, the reported dissolved inorganic nitrogen (DIN) concentration is 15-60 μM (Peperzak et al. 1998). Off the coast of Guangdong, China, the ambient DIN concentration averaged ~20 μM when *P. globosa* colonies of > 10 mm occurred (X. Wang; unpubl. data). In Phan Thiet, southern Vietnam where giant *P. globosa* colonies (> 10 mm) are reported (Smith et al. 2014), the seawater DIN concentration averaged ~18 μM (Tâm 2018). Therefore, ambient nutrient concentrations cannot explain the large differences in colony size between the European and the Asian *P. globosa* populations.

Next, we compared the environmental light levels for the typical latitudes and times of year when *P. globosa* blooms occur. The European strain typically blooms in March (Peperzak et al. 1998; Rousseau et al. 2002), the Guangdong population usually blooms in January (Wang et al. 2021) and the Vietnamese population blooms in August (Liu et al. 2015). We used online irradiance calculator to determine the irradiance at the mid-point of the months to represent the average light levels experienced by the *P. globosa* populations (Table 2). While this exercise ignores local conditions such as cloudiness, water turbidity or turbulent mixing, the results showed that differences in irradiance level alone are not sufficient to explain the geographical differences in colony size. For example, the Vietnam location experiences only 34% more sun light than the Europe location, but the maximum colony size differs by 10-fold between the two places.

In a transcriptomic study, Liang et al. (2020) compared two *P. globosa* cultures originated from Chinese waters and found that the culture that formed large colonies (~5 mm) up-regulated the genes for carbon fixation and biosynthesis of exopolysaccharide—both would favor colony formation, whereas the culture that formed only small colonies (<0.5 mm) down-regulated them. Although the factors that triggered different gene expressions were not investigated in their study, their findings suggest that the different colony sizes *in situ* may reflect genetic variations, including possible cryptic species, among the different *P. globosa* populations. Whole genome analysis and comparison will be required to confirm that.

Conclusions

By considering the literature and experimental evidence, we discussed the trade-offs in colony shape, enlargement rate, resource acquisition and grazing deterrence, between chained and spherical colonies in microalgae. Chain formation is a faster way to increase collective size relative to sphere formation for the same cellular specific growth rates, and a sphere is more likely to suffer light and nutrient limitations. However, spherical colonies may have a higher defense efficiency than chained colonies against grazers. Balance between costs and benefits may thereby lead to the different species adopting different colony forms to compete for resources and escape from grazing, and that different trade-offs among functional traits allow different microalgae to co-exist under variable environmental constraints.

Author contributions

X.W. conducted the experiments and edited the manuscript. K.W.T. conceived the study, analyzed the data, and wrote the first draft.

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Table 1. *Phaeocystis globosa* colony diameter (*d*), incident light (L_O), transmitted light (*L*) and the corresponding light attenuation coefficient (*k*) measured in Experiment 1 using an inverted light microscope. Colonies were collected from the Guangdong province, China.

	d (mm)	**Lo (µmol m^{-2} s^{-1})**	**L (µmol m^{-2} s^{-1})**	**k (mm^{-1})**
	11.05	500.0	198.1	0.084
	8.38	507.1	175.2	0.127
	5.45	511.6	171.4	0.201
	7.88	506.1	216.7	0.108
	6.25	515.7	233.1	0.127
	7.13	522.0	178.2	0.151
	8.55	510.6	153.0	0.141
	7.83	526.4	246.7	0.097
	7.05	503.5	182.7	0.144
	8.55	519.7	214.1	0.104
	5.35	515.9	202.1	0.175
	7.63	517.5	218.2	0.113
	8.63	523.7	169.8	0.131
	5.75	513.6	252.6	0.123
	7.68	527.7	225.4	0.111
	8.65	513.7	182.6	0.120
Mean	**7.61**	**514.7**	**201.2**	**0.128**
S.D.	**1.46**	**8.01**	**29.6**	**0.029**

Table 2. Expected daily irradiance experienced by different *Phaeocystis globosa* populations based on their locations and typical bloom periods. Irradiance values are taken from an online irradiance calculator (https://www.pveducation.org/).

P. globosa population	Latitude (°N)	Date	Julian day	Irradiance (kW m^{-2} d^{-1})	Max. colony diameter reported (mm)
Europe	52	15 March	74	7.70	1
China	22	15 January	46	8.04	30
Vietnam	11	15 August	227	10.29	14

Figure captions

Figure 1. Simulation results of rate of increase in chained colony length vs. spherical colony volume, both starting from a cubic cell of unity dimensions with a growth rate (μ) of 0.1 to 3.

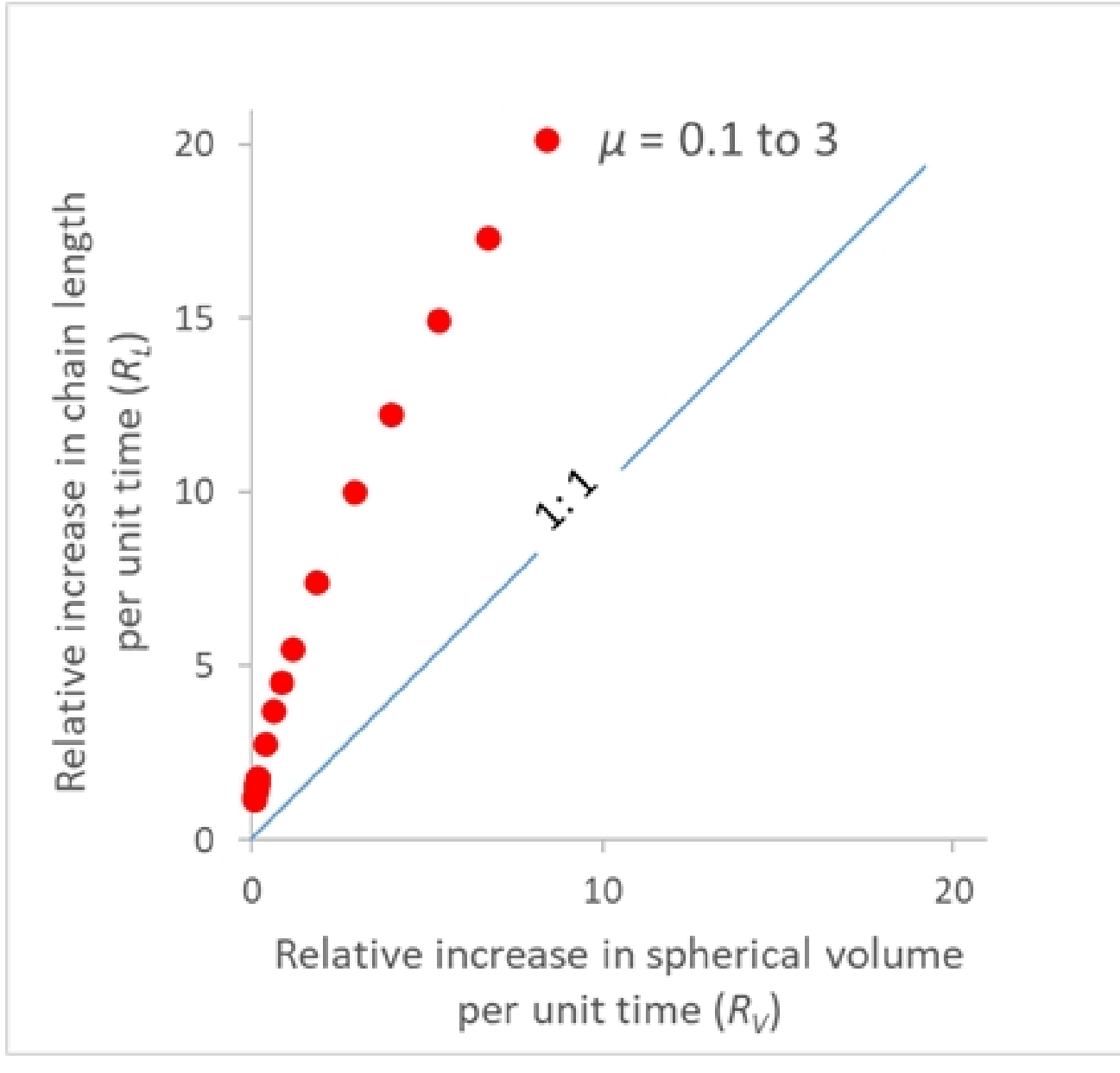
Figure 2. Schematics of interactions between a chained algal colony and protozoan grazers: (a) Chain formation is an effective way to fend off ciliate, which is limited by the prey size it can engulf whole; (b) Chain formation would not be effective against heterotrophic dinoflagellate that uses a feeding extension to extract cell content or digest prey extracellularly.

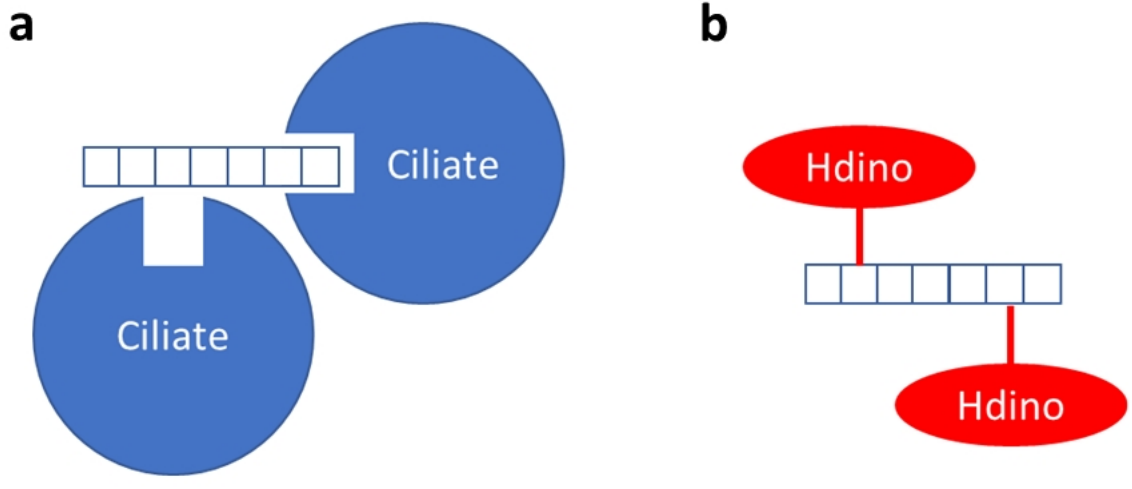
Figure 3. Schematics of interactions between an algal colony and a copepod grazer: (a) Copepod can use its appendages to manipulate and reposition a chained colony to facilitate intake through its month opening (gap between thick arrows); (b) A spherical shape cannot be repositioned to aid ingestion.

Figure 4. Schematics of light reception by colonies: (a) Cells in a chain all receive the same amount of down irradiance through the water column (L_0); (b) Light attenuates when passing through a sphere such that cells in the upper hemisphere will receive a higher irradiance that those in the lower hemisphere ($L_0 > L$).

Figure 5. Photos of *P. globosa* colonies: (a) A healthy *P. globosa* colony with dense distribution of cells within its colony skin; (b) A giant *P. globosa* colony (ca. 12.7 mm diameter) showing its opaque appearance against the background. Photo credits: Y. Wang, Jinan University.

Figure 6. Optical density (OD_{438}) of *Phaeocystis globosa* colonies as a function of colony diameter, measured by (a) spectrophotometer (Experiment 2) and (b) microplate reader (Experiment 3). Colonies were collected from the Guangdong province, China.


μ = 0.1 to 3
1:1
20
15
10
5
0
0
10
20
Relative increase in chain length per unit time (R_L)
Relative increase in spherical volume per unit time (R_V)


a
Ciliate
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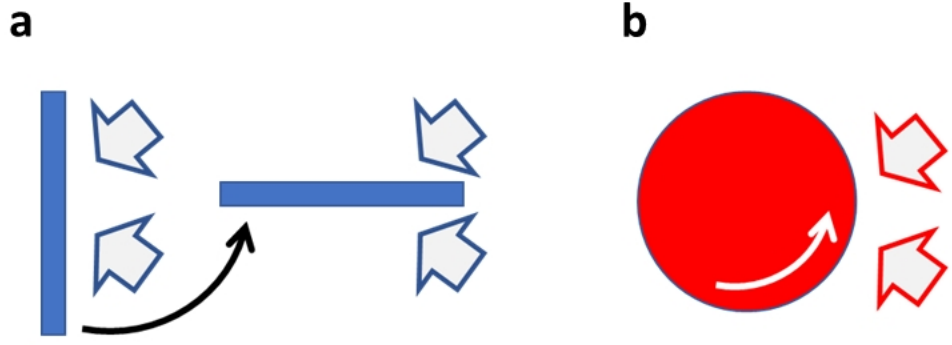
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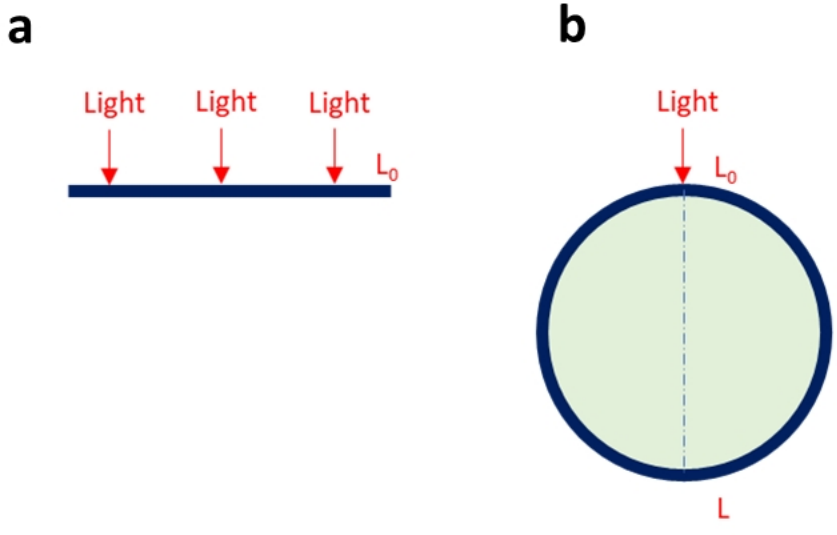
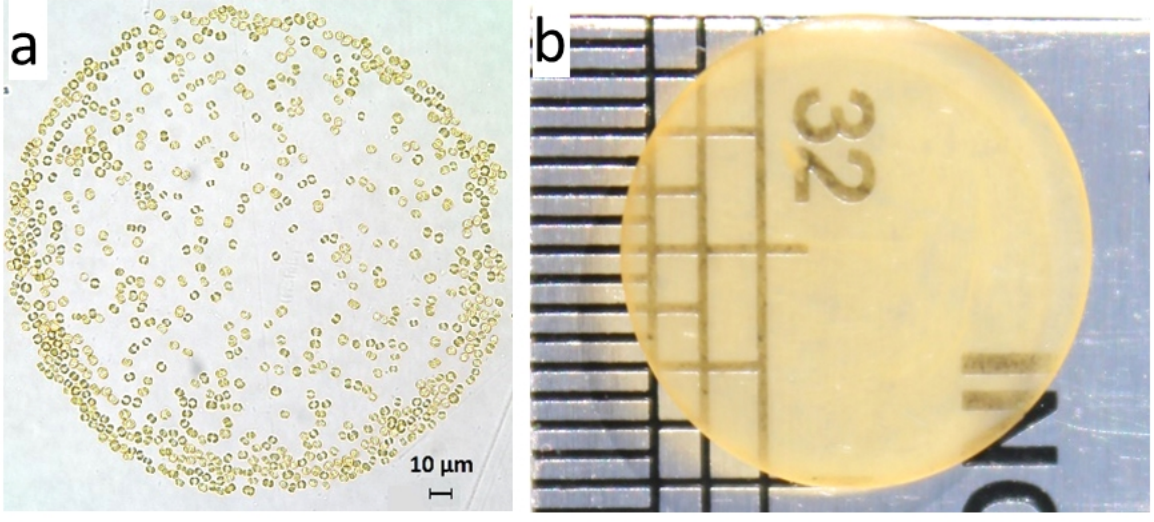
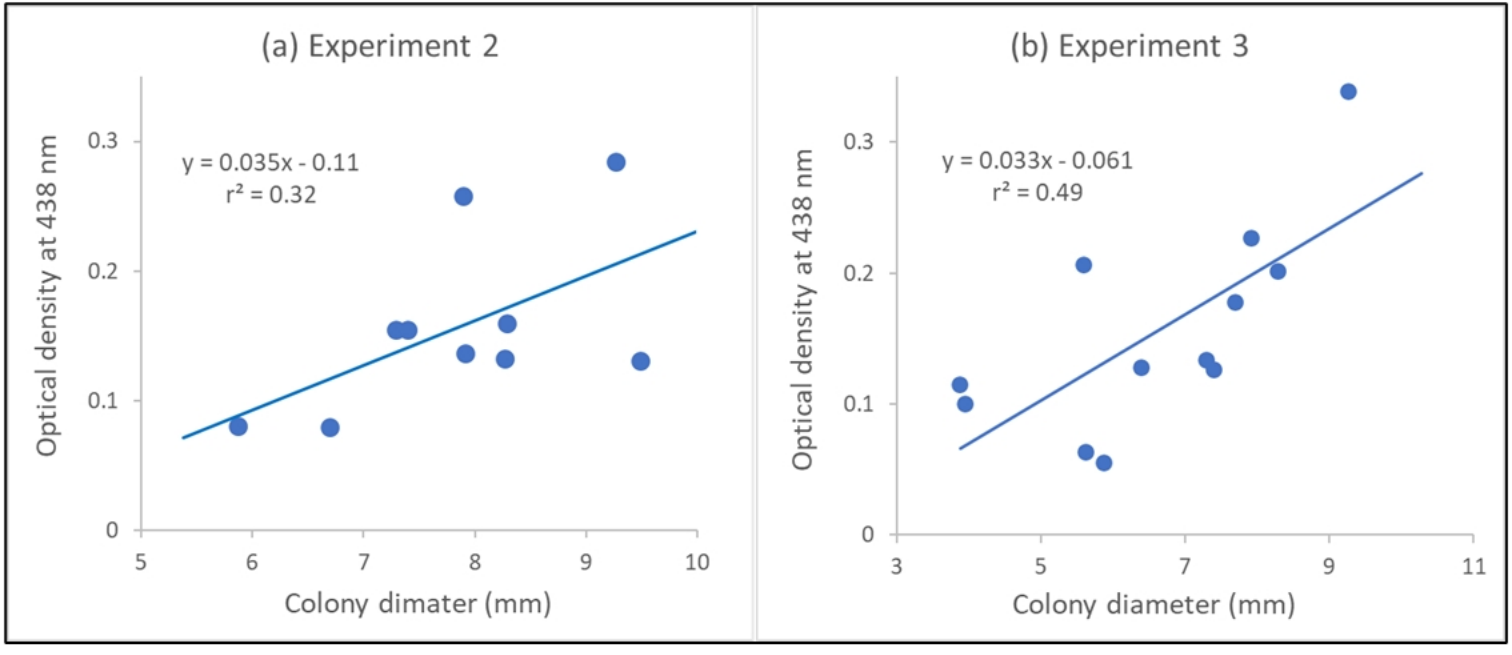

a
Light
Light
Light
L_0
b
Light
L_0
L


a
10 µm
b
32


(a) Experiment 2
y = 0.035x - 0.11
r² = 0.32
Optical density at 438 nm
Colony dimater (mm)
0
0.1
0.2
0.3
5
6
7
8
9
10
(b) Experiment 3
y = 0.033x - 0.061
r² = 0.49
Optical density at 438 nm
Colony diameter (mm)
0
0.1
0.2
0.3
3
5
7
9
11