Giantism and its role in the harmful algal bloom species *Phaeocystis globosa*

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**ARTICLE INFO**

**ABSTRACT**

The cosmopolitan alga *Phaeocystis globosa* forms large blooms in shallow coastal waters of the Viet Nam coast, which impacts the local aquaculture and fishing industries substantially. The unusual feature of this alga is that it forms giant colonies that can reach up to 3 cm in diameter. We conducted experiments designed to elucidate the ecophysiological characteristics that presumably favor the development of giant colonies. Satellite images of chlorophyll fluorescence showed that the coastal bloom was initiated in summer and temporally coincident with the onset of monsoonly driven upwelling. While determining the spatial distribution of *Phaeocystis* was not feasible, we sampled it in the near-shore region. A positive relationship was found between colony size and colonial cell densities, in contrast to results from the North Sea. Mean chlorophyll a concentration per cell was 0.45 pg cell⁻¹, lower than in laboratory or temperate systems. The contribution of mucous carbon ranged from 63 – 95% of the total carbon; furthermore, mucous carbon per unit of colony surface area appeared to decrease with colony size, suggesting that the mucoid sheath became thinner as colonies grew larger. Sinking rates averaged 189 m d⁻¹, strongly suggesting that giant colonies could only persist in shallow, turbulent environments. No relationship between colony size and sinking rates was observed. DOC concentrations of intracolonial fluid averaged 5940 µM, 25 times greater than ambient concentrations. Estimated diffusion coefficients of ions across the mucous envelope were ca. 1.0 ± 0.3 × 10⁻⁷ cm² s⁻¹ for colonies with diameters of ca. 1.0 cm. In total, the characteristics of the giant colonies suggest that the Vietnamese strain is substantially different from that found in temperate environments, and that it has a number of unusual features that influence its growth and development in coastal Vietnamese waters.

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1. Introduction

The species *Phaeocystis globosa* has a cosmopolitan distribution and forms large blooms in a number of locations in the ocean, such as the waters of the North Sea and coastal China (Schoemann et al., 2005). Although one strain has been reported to have hemolytic properties (He et al., 1999), the species is considered a harmful algal bloom species due to the significant indirect effects it has on local systems via its high biomass and oxygen depletion upon its degradation. All of the three major species in this genus have a complex life cycle that includes stages of solitary cells and large colonies (Schoemann et al., 2005). The balloon-like colonies are formed by small cells embedded in an organic mucilage that encloses a fluid-filled intracolonial space. These colonies are spherical during rapid growth, and generally are from 0.03 – 1.0 mm in diameter. However, there have been reports of *P. globosa* colonies forming blooms in Southeast Asia and reaching diameters of up to 3 cm (Qi et al., 2004), an order of magnitude larger than colonies that are usually found. Such “giant” colonies represent a major deviation from “normal”-sized phytoplankton. A potential major advantage of such large colony size is that the grazing by herbivores is largely reduced; however, possible disadvantages include increased sinking rates, decreased nutrient uptake capabilities, self-shading, physical disruption due to turbulence, and large photosynthetic carbon requirements to produce the mucoid sheath. Given that the dominant size of phytoplankton in the ocean is on the order of a few microns, the adaptations of giant colonies of *P. globosa* are of broad interest.

Today it is generally acknowledged that blooms of harmful algae are correlated with and can be triggered by inputs of nutrients (Anderson et al., 2002; Glibert et al., 2005), especially in stratified waters. Enhanced concentrations of harmful dinoflagellates in Vietnamese coastal waters were first noted 15 years ago (Nguyen et al., 2004), but the haptophyte *Phaeocystis globosa* was not observed until 2002 (Tang et al., 2004); however, it has bloomed along the south central coast every summer since then (Doan et al., 2010) as well as in Ha Long Bay (Nguyen et al., 2012). Its general appearance is similar to that noted in the North Sea, in that it reaches a high biomass, and eventually generates large amounts of organic foam that appears on beaches (Blauw et al., 2010). These blooms are characterized by the appearance of giant colonies. The outbreaks appear to be associated with the southwest monsoon, which is characterized by upwelling, substantial riverine discharges due to precipitation on land, and stratified water columns that result from solar heating. During 2002 nearly 90% of all animal and plant species in the tidal reefs of Phan Ri Bay (Binh Thuan Province, Viet Nam) were eliminated by the *P. globosa* bloom, causing a substantial loss to the local economy (Doan et al., 2003). Blooms of giant colonies have been reported throughout much of Southeast Asia, including China (Qi et al., 2004), Viet Nam (Tang et al., 2004; Doan et al., 2010), Thailand, Pakistan, Myanmar (P. Harrison, pers. comm.) and Arabian Sea (Madhupratap et al., 2000). Interestingly, giant colonies have not been reported from temperate waters, contrary to the suggestion of Verity et al. (2007) that cold water enhances colony formation.

Large size can be both an advantage and disadvantage. The obvious advantage over solitary cells (diameter ca. 7 µm) is the uncoupling from grazing by small zooplankton (size mismatch: Jakobsen and Tang, 2002). A corollary to this concept is that as colonies form, the cells are encased in a polysaccharide envelope that also may deter grazers due to its chemical and physical properties. The sheath has been reported to be extremely permeable to oxygen (Ploug et
al., 1999), so presumably it does not act as a barrier to nutrient and solute exchange. Additionally, the envelope has been suggested to be a reserve for carbon and other elements required for cellular metabolism that can be utilized during periods unfavorable for net photosynthesis (Lancelot and Mathot, 1985; Lancelot et al., 1987; Davidson and Marchant, 1987). It has a tough polysaccharide composition (Hamm, 2000) that may be resistant to microbial degradation and alter the overall carbon:nitrogen ratio of the pelagic ecosystem (Lancelot et al., 1998; Verity et al., 2007). Possible disadvantages of giantism, however, are many. If colonies sink according to Stroke’s Law, their vertical fluxes should be large, although most phytoplankton reduce their sinking rates to very low speeds by a variety of chemical and physical means (Bienfang et al., 1982; Kiørboe et al., 1996). Furthermore, nutrient uptake rates may be reduced in colonies if the mucous is a barrier to nutrient diffusion. Increased ambient nutrients, therefore, may be an important stimulus of P. globosa blooms. Finally, as a colony grows in size, the mucous envelope surface area also increases. This requirement might result in thinner envelopes that are structurally unstable, or alternatively an increased carbon allocation demand that limits cellular growth.

We conducted measurements on a variety of aspects of the ecology of Phaeocystis globosa in coastal south central Viet Nam to begin to understand the adaptations that giant colonies (> 0.5 cm) have developed to grow and accumulate in Asian coastal waters. We hypothesized that sinking rates were similar to those of other large phytoplankton (less than 1 m d−1) and biologically controlled (i.e., deviate from Stoke’s Law), and that as colonies grew larger, colonial cell density and particulate organic carbon per unit area of colony surface would decrease asymptotically, thereby increase the amount of carbon per cell required for envelope generation and ultimately determine the maximum colony size possible. We also used satellite information to place the blooms in the context of the larger coastal ecosystem. Our experiments were far from all-inclusive, and focused on sinking, envelope permeability, internal fluid composition, and carbon allocation. We found that giant colonies of Phaeocystis globosa are highly unusual in their adaptations, and suggest that giantism in the colonies is an adaptation to the turbulent coastal environment. The adaptations allow the species to reach high concentrations and have harmful impacts on the coastal ecosystem.

Fig. 1. Picture of a giant Phaeocystis globosa colony.

2. Materials and Methods

Experimental Procedures

Giant Phaeocystis globosa colonies (Fig. 1) were sampled in July 2009, August 2010 and July 2011 off the coast of Phan Thiet, Binh Thuan province, south central Viet Nam (Fig. 2). Once observed, colonies were collected very gently with small containers in the coastal surf zone, placed in ambient seawater at ambient temperature, and maintained in a shaded area. Colonies were replaced by newly collected ones ca. every 3 h. Only spherical, non-collapsed colonies were used in experiments. Abundance of colonies in the water column was not quantified due to the difficulty of quantitative sampling, but visual observations suggested an approximate abundance of ca. 1 giant colony L−1.
Colonies of a wide size range were selected and transferred to a Petri dish filled with filtered seawater and allowed to settle to the bottom (but retain their spherical shape). A ruler was placed under the Petri dish to measure the diameters of individual colonies (resolution 0.2 mm). Immediately after colony size measurement, individual colonies were placed under a Nikon inverted microscope for quantifications of colonial cell size and colonial cell abundance (Jakobsen and Tang, 2002). Total cell abundance in the entire colony was determined by multiplying cell abundance per unit area (cell density) and total surface area of a colony. Colonial cell size was estimated with a calibrated reticule.

After sizing, colonies were filtered under low vacuum through Whatman GF/F filters for measurements of either chlorophyll or particulate organic carbon (POC). Chlorophyll samples were placed in 90% acetone and extracted for at least 24 h in cold and dark conditions; the chlorophyll was then measured on a Turner Designs TD-700 fluorometer (JGOFS, 1996). For measurement of POC in colonies, individual colonies were filtered onto combusted GF/F filters, rinsed with ca. 5 mL 0.01N HCl in filtered seawater, and dried at 60°C. POC samples were analyzed via pyrolysis on a Costech ECS 4010 elemental analyzer. Blanks were filters through which filtered seawater had been run (ca. 5 mL) and treated in the same manner (Gardner et al., 2000). POC of the mucous envelope for an individual colony was estimated by subtracting the total cellular carbon determined from cell abundances estimated from microscopic observations and converted to carbon using a value of 13.5 pg C cell⁻¹ (Rousseau et al., 1990) from the total colony POC measured by pyrolysis.

Sinking rates were determined using the visual method of Smayda and Boylen (1965). A 2-L transparent graduated cylinder (50 cm high, diameter 10 cm) was filled with filtered seawater, its surface covered, and maintained undisturbed at ambient temperature for at least 1 h to reduce convection. A giant colony was selected and its diameter and cell density quickly measured, and individual colonies were then gently released into the top of the graduated cylinder. The sinking velocity was calculated as the vertical distance traveled per unit time (m h⁻¹). Some turbulence was introduced by the addition of the colonies, but sinking rate determinations were initiated only after the turbulence dissipated and sinking rates visually appeared to be constant.

Estimates of diffusion of salts across the envelope into the intracolonial fluid were conducted by measuring the change of salinity of the intracolonial fluid through time. Colonies of similar diameter (ca. 1.0 cm) were selected, and a salinity gradient was generated by placing individual colonies into a 24-well culture plate filled with 3 mL diluted seawater (extracolonial fluid made by taking 750 mL filtered, ambient seawater and diluted with distilled water to 1 L). Colonies were removed from the salinity gradient at different time intervals (t = 2.5, 5, 10, 15, 20, 30, 40, 60, 90 minutes), and the extracolonial fluid sampled with a 5-mL pipette. Immediately thereafter, ca. 0.3 mL
intracolonial fluid was extracted using a 1mL syringe fitted with a fine gauge needle. All time points were conducted in duplicate or triplicate. Filtered ambient seawater, extracolonial fluid, and intracolonial fluid were stored in small containers for later assessment of salinity using an Amprobe WT-30 digital salinity meter. The mass balance of salt ions was examined by comparing the mass changes in intra- and extracolonial spaces (by multiplying the volume of intra- or extracolonial fluid with the corresponding salinity change for each colony). Diffusion coefficients of ions \( D \) within the colony mucus envelope under the experimental conditions were estimated by Fick’s Law:

\[
J = -D \frac{dC}{dx} = \frac{Q}{4\pi r^2} \tag{Eq. 1}
\]

where \( J \) is the net flux of ions (that is, the net number of ions passing per unit surface area of colony per unit time; mol m\(^{-2}\) s\(^{-1}\)), \( Q \) is the flow of the salt ions (i.e., the net number of ions transported across the mucus envelope per unit time; mol s\(^{-1}\)), \( r \) is the radius of a colony, \( \frac{dC}{dx} \) is the concentration gradient of ions within the mucus envelope, and \( dx \) is the thickness of the envelope, assuming that the diffusive boundary layer between envelope surface and bulk ambient environment is negligible. The minus sign indicates that the net transport is from the internal to the external environment. To integrate Eq. 1:

\[
\int_0^x \frac{Q}{4\pi r^2} \cdot dx = \int_0^x -dC \tag{Eq. 2}
\]

\[
\frac{Qx}{4\pi r^2} = -dC = C_{in} - C_{out} \tag{Eq. 3}
\]

where \( C_{in} \) and \( C_{out} \) are the concentrations of ions at the internal and external surfaces of the mucus envelope, respectively. To use the Eq. 3 for a specific time interval \( t \), the concentration gradient was derived by averaging the values at the beginning \( (t_0) \) and at time \( t \), and \( Q \) was estimated by multiplying the bulk volume of the colony and the difference between concentrations of intracolonial ions at time \( t \) and \( t_0 \) then being divided by the total time \( t \):

\[
\frac{1}{2}[(C_{in0} - C_{out0}) + (C_{int} - C_{out,t})] = \frac{Q}{4\pi Dr^2} x \tag{Eq. 4}
\]

\[
Q = \left(\frac{C_{in0} - C_{out0}}{t}\right) \frac{4}{3} \pi r^3 \tag{Eq. 5}
\]

so that

\[
D = \frac{2}{3} \left(\frac{C_{in0} - C_{out0}}{C_{in,t} - C_{out,t}}\right) \frac{rx}{t} \tag{Eq. 6}
\]

\( D \) was then calculated as mean of all the derived \( D \) values.

The intracolonial fluid was sampled for dissolved organic carbon (DOC) directly. Intracolonial fluid was carefully extracted with 2-mL syringes fitted with small gauge needles, transferred to a 2-mL vials, and mixed with the intracolonial fluid from colonies of a same size to increase the sample volume to 2.0 mL. Samples were then transferred to 15-mL HDPE bottles to which 13.0 mL distilled water was added, and preserved by adding a few drops of 10% HCl to reduce the pH to 3. Ambient seawater and distilled water samples were collected and treated in the same manner for assessment of their DOC concentrations. DOC samples were analyzed at the University of California, Santa Barbara on a custom-built high-temperature pyrolysis combustion analyzer calibrated with known seawater standards (Carlson et al., 2000). The samples were not filtered and may include some particulate matter that originated from the cells or envelope.

Pearson’s correlation coefficients were calculated to assess relationships among observed sinking rates, colonial cell abundance, intracolonial DOC, colonial cell
and colony size, chlorophyll, and POC. Statistical tests were all performed using R (Version 2.8.1).

Remote Sensing Analyses

To place the *P. globosa* bloom in the context of a larger area, data from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and the Moderate Resolution Imaging Spectroradiometer (MODIS) were analyzed (http://oceancolor.gsfc.nasa.gov/). Level-3 global Standard Mapped Images at 4 km (MODIS) and 9 km (SeaWiFS) resolutions from January 2003 to December 2010 were selected, and monthly averaged pigment concentrations, SST, and standard errors extracted and binned from 10.4-11.4°N, 107.6-109.2°E to generate monthly climatologies of SST and pigment concentrations along the coast. Climatologies of wind speed and direction were derived from a blend of Quick Scatterometer (QuikSCAT) and NCEP reanalysis ocean winds from Colorado Research Associates (http://dss.ucar.edu/datasets/ds744.4/) and binned from 10.4-11.4°N, 107.6-109.2°E. Image analyses and data extraction were performed using Matlab and WIM/WAM (version 6.58).

Fig. 3. Climatologies of a) monthly averaged MODIS estimates of sea surface temperature (°C), b) weekly QUIKScat estimates of wind speed, c) weekly QUIKScat estimates of wind direction, and d) monthly MODIS estimates of surface chlorophyll (µg L⁻¹), binned from 10.4 – 11.4°N, 107.6 – 109.2°E for 2003 – 10. Bars represent standard errors of the means.

Fig. 4. Monthly averaged sea surface temperature of the south central coast of Viet Nam derived from MODIS satellite estimates for 2003 – 10.

3. Results

Spatial Variations in Temperature and Chlorophyll

Sea surface temperatures varied in both time and space (Fig. 3a, 4). In general, the water was warm over the continental shelf of Viet Nam, with monthly averaged SST exceeding 24°C throughout the year. Monthly SST was highest in May and lowest in January (29.1 and 24.3°C, respectively; means represent data from 10.4-11.4°N, 107.6-109.2°E). Compared to the neighboring regions, noticeably cooler waters were observed off south-central Viet Nam in July and August (Fig. 4), temporally coincident with the occurrence of southwest monsoon winds (Fig. 3b,c). Seasonal variations of the chlorophyll were assessed by analyzing monthly averaged ocean color images of chlorophyll concentrations from the period January 2003 to December 2010. Chlorophyll concentrations were greatest in August, with average values of 2.41 µg L⁻¹ (Fig. 3d). Pigment accumulation was detected over the shelf, as indicated by elevated chlorophyll concentrations that exceeded 6 µg L⁻¹ (Fig. 5). The blooms were centered at 11°15’N, 108°35’E with a ca. 100
km filament extending northeast to the shelf break and into the ocean interior. Elevated levels of chlorophyll were observed near-shore over much of the region analyzed during the summer months.

**Fig. 5.** Monthly averaged chlorophyll concentrations off the south central coast of Viet Nam derived from MODIS satellite estimates for 2003 – 10.

**Phaeocystis globosa colony morphology**

The giant *P. globosa* colonies collected from the coastal zone of south central Viet Nam displayed visible variations in both size and color. Colony diameter ranged from 0.07 to 1.3 cm among 55 colonies that were measured for size. The colors varied from bisque to brown, but were not correlated with colony size. Colonies maintained a spherical shape in seawater, but became flattened when removed from their environment (Fig. 1). The skin of a colony was slimy, elastic and tough and was ca. 50 µm in thickness. Cells were distributed rather evenly within the mucilage.

**Relationships among colony size, colonial cell size, and cell abundance**

Colonial cell diameters ranged in size between 7.4 to 12.4 µm, with a mean of 10.3 (± 1.6 µm; n = 32). This was larger than previously reported values for *P. globosa* solitary cells (3-9 µm; Rousseau et al., 1994) and colonial cells (5.6 - 8.3 µm; Peperzak et al., 2000). No significant correlation was found between colony size and colonial cell size (n = 19, $R^2 = 0.04$), suggesting that the elevated colonial cell size may be associated with a strain-specific characteristics rather than colony size. In contrast to observations on the North Sea strain of *P. globosa* (Jakobsen and Tang, 2002), a positive correlation (rather than a negative one) was found between colonial cell density and colony size for the Vietnamese strain (Fig. 6a; Fig. 6. a) Log-log relationship between colonial cell density and colony size for the giant *P. globosa* colonies. Solid circles represent samples collected in 2010, and open circles are samples from 2011. The line represents the significant linear correlation for the pooled data ($Y = 0.936X – 0.500$, n = 64, $R^2 = 0.38$, p < 0.0001). b) Log-log relationship between total colonial cell abundance and colony size for the giant *P. globosa* colonies. Solid circles represent samples collected in 2010, and open circles are samples from 2011. The line represents the significant linear correlation for the pooled data ($Y = 2.94X – 0.0026$, n = 64, $R^2 = 0.86$, p < 0.0001).
n = 64, $R^2 = 0.38$, p<0.0001). A linear relationship for the pooled data was subsequently derived between log total colonial cell abundance and log colony diameter with a slope of 2.94 (Fig. 6b; n = 64, $R^2 = 0.86$, p<0.0001), which was greater than previous observations (i.e., slope = 1.61, Jakobsen and Tang, 2002; slopes ranging from 1.51 to 1.57, Wang and Tang, 2010). The slope for 2010 only (the year with the widest range of sizes) was 2.44 (n = 32, $R^2 = 0.91$, p < 0.001).

Fig. 7. a) Relationship between chlorophyll per unit area of colony surface and colony size for the giant P. globosa colonies in 2010. The dashed line represents the significant linear correlation ($Y = 0.34X - 0.17$, n = 13, $R^2 = 0.44$, p < 0.01). b) Relationship between cellular chlorophyll and colony size for the giant P. globosa colonies. The dashed line represents the significant linear correlation ($Y = 0.72X - 0.34$, n = 31, $R^2 = 0.42$, p < 0.01).

**Colony chlorophyll and POC**

Cellular chlorophyll scaled with colony size (Fig. 7a; n = 13, $R^2 = 0.44$, p<0.01). However, the positive regression was primarily driven by the four colonies with relatively small sizes that had extremely low cellular chlorophyll (< 0.05 pg chl cell$^{-1}$). It is possible that a large fraction of colonial cells were liberated from the mucous envelope during handling of these few colonies, which resulted in an underestimation of total and cellular chlorophyll. Mean cellular chlorophyll in giant P. globosa colonies was 0.45 ± 0.09 pg chl cell$^{-1}$ when the four potential “outliers” were excluded, still substantially lower than the cellular chlorophyll values reported for the North Sea strain of P. globosa (2.97 pg chl cell$^{-1}$; Hansen and van Boekel, 1991). The lower chlorophyll values per cell might reflect the high irradiance growth habitat of the colonies, or physiological stress induced by environmental conditions. A positive regression was found between chlorophyll per unit area of colony surface and colony size in 2010 (Fig. 7b; n = 13, $R^2 = 0.42$, p < 0.01), but this correlation again may be biased due to the loss of colony integrity in several samples.

Particulate organic carbon (POC) per unit area of colony surface decreased with colony size (Fig. 8a; n = 17, $R^2 = 0.36$, p < 0.01), although data collected from the two smaller colonies dominated the negative relationship. It is at present impossible to liberate colonial cells from the mucous envelope without disrupting colonial integrity; hence, direct measurements of cellular carbon and mucous carbon in a P. globosa colony are not possible. Assuming that the carbon per cell does not vary within and among colonies, and assuming a carbon concentration of 13.5 pg C cell$^{-1}$ (Rousseau et al., 1990), the total mucous carbon was estimated from the difference in total measured POC and cellular carbon determined microscopically. A weak negative relationship was found between mucous POC per unit area of colony surface and colony size (Fig. 8b; n = 17, $R^2 = 0.38$, p < 0.01), suggesting that the mucous envelope may become thinner as a colony grows larger. The contribution of mucous carbon to total colony carbon ranged between 62.7 to 94.7%
(Table 1), and hence potentially represents the major pool of particulate carbon in a colony. It decreased with an increase in colony size, in that the cell density and thus total cellular carbon were both positively correlated with colony size.

Fig. 8. a) Relationship between POC concentration per unit area of colony surface and colony size for the giant *P. globosa* colonies. The line represents the significant linear correlation for the pooled data (Y = -40.2X + 57.5, n = 17, R² = 0.36, p < 0.01). b) Relationship between mucous POC concentration per unit area of colony and colony size for the giant *P. globosa* colonies. The line represents the significant linear correlation (Y = -43.3X + 55.0, n = 17, R² = 0.38, p < 0.01).

**Relationship between colony size and sinking velocity**

Measured sinking rates of giant *P. globosa* colonies were extremely fast, with an average speed (\(v_s\)) of 189 ± 102 m d\(^{-1}\) (range from 29.4 – 516 m d\(^{-1}\); n = 46). A positive correlation was observed between sinking rates and colony size (Fig. 9; n = 46, R² = 0.33, p < 0.001). Three sinking rates from 2011 were omitted in the analysis given their extremely rapid sinking rates (>1,000 m d\(^{-1}\)). We believe these colonies may have been damaged in handling but retained their spherical shape. If the sinking rate of a colony were approximated by Stokes’ Law, internal density (\(p_i\)) can be then determined. The average \(p_i\) for the giant *P. globosa* colonies is 1022.1 ± 0.03 kg m\(^{-3}\) (n = 46), which is only slightly higher than the ambient seawater (1022.0 kg m\(^{-3}\)) but much lower than previously published empirical values for smaller colonies (1035 – 1047 kg m\(^{-3}\); Peperzak et al., 2003).

![Fig. 9. Relationship between sinking rate and diameter of the *P. globosa* colonies. Solid circles represent samples collected in 2010, and open circles are samples from 2011. The line represents the linear regression of all data (Y = 250X – 1.59, n = 46, R² = 0.33, p < 0.01).](img)

**Intracolonial DOC**

The average concentrations of dissolved organic carbon (DOC) in ambient seawater in 2010 and 2011 were 217 ± 52.8 (n = 3) and 281 ± 4.43 (n = 3) µM, values which are substantially enhanced relative to the open ocean values (ca. 45 – 65 µM; Carlson 2002). The elevated DOC concentrations in Vietnamese coastal waters may be a product of allochthonous inputs from rivers, run-off and coastal sediments. DOC concentrations within the colonies were extremely high, with a mean value from both years of 5,940 ± 1,800 µM (n = 21), more than 25-fold higher...
Table 1. Estimated mucous carbon in giant Phaeocystis globosa colonies. Mucous carbon was determined by the difference between POC (from elemental analysis) and the amount of carbon contributed by living cells (13.5 pg C cell$^{-1}$; Rousseau et al., 1990).

<table>
<thead>
<tr>
<th>Colony diameter (cm)</th>
<th>POC per unit surface (µg cm$^{-2}$)</th>
<th>Total cell abundance ($10^6$)</th>
<th>Total cellular POC (µg)</th>
<th>POC per unit mucous (µg cm$^{-2}$)</th>
<th>Contribution of mucous POC (%)</th>
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Fig. 10. a) Relationship between intracolonial DOC concentration and colony size for the giant P. globosa colonies. Open circles represent data collected in 2011, and filled circles those from 2010. The line represents the significant linear correlation of the 2010 data ($Y = -2850X + 9650$, $n = 13$, $R^2 = 0.46$, $p < 0.01$). b) Relationship between total intracolonial DOC and colony size. The line represents the significant linear correlation for the pooled data ($Y = 8.99X - 5.36$, $n = 21$, $R^2 = 0.93$, $p < 0.0001$).

than ambient concentrations (Fig. 10a). The high levels of DOC likely resulted from the photosynthesis of colonial cells and internal accumulation. There was a negative correlation between intracolonial DOC concentration and colony size in 2010 (the year with a large range of colony sizes; Fig. 10a; $n = 13$; $R^2 = 0.46$, $p < 0.01$). As expected, total DOC accumulated in the interior of a P. globosa colony (estimated by multiplying the volume of a colony and the intracolonial DOC concentration) was positively correlated with colony size (both years; Fig. 10b; $n = 21$; $R^2 = 0.93$, $p < 0.0001$).

**Diffusion experiment**

The ions of seawater consist of a wide range of sizes and ionic states, and these have variable properties, such as diffusive fluxes. Despite these known variations, we assumed
Fig. 11. Relationship between a) changes in salt mass in the intracolonial fluid and the external seawater, b) salinity change in the external seawater and time, c) salinity change in the external seawater and time. Open circles represent values which were excluded from calculations and analyses.

that all ions diffuse across the colony mucous at an identical rate, and thus the diffusive coefficient reported hereinafter is an integrated value. *P. globosa* colonies did not exhibit an evident expansion in size after being placed in 75% seawater, indicating that the mucous envelope was permeable to ions. Changes in salt mass in external seawater and intracolonial fluid were positively correlated (Fig. 11a; n = 9, $R^2 = 0.74$, $p < 0.01$). The salinity changes vs. time in both extracolonial and intracolonial environments exhibited a markedly reduced rate of change at ca. 60 min (Fig. 11b), which suggests that this was the point when a salinity equilibrium was reached. Estimated diffusion coefficients of ions across the mucous envelope from Eq. 4 were ca. $1.0 \pm 0.3 \times 10^{-7}$ cm$^2$ s$^{-1}$ (n = 9) for colonies with diameters from 0.9 – 1.0 cm and an assumed constant mucous envelope thickness (50 µm).

4. Discussion

As a harmful algal bloom species, *Phaeocystis globosa* in Vietnamese waters has direct, harmful effects on marine organisms, killing filter-feeding organisms (Nguyen et al., 2012) and causing extensive economic damage to aquaculture facilities (Doan et al., 2003). It also has indirect, ecological impacts on coastal systems (Lu & Huang, 1999; Peperzak, 2002; Doan et al., 2003). These (such as the generation of hypoxia) can be substantial, and have serious impacts on coastal regimes and economies, especially in developing countries. Blooms of *P. globosa* have been associated with eutrophic conditions in the Baltic and North Seas, and blooms dominated by colonial forms of other *Phaeocystis* species occur in the North Atlantic and along the coasts of Norway (Lancelot et al., 1998). As such, it was suggested that colony formation was enhanced by cold temperatures (Verity et al., 2007), a trend that was confirmed by experiments with *P. antarctica* (Wang et al., 2010). However, blooms of colonial *P. globosa* have been reported in the past two decades in tropical and semi-tropical regions, including warm, coastal waters of China (*Qi*...
et al., 2004), Viet Nam (Tang et al., 2004), and the Arabian Sea (Madhupratap et al., 2000). All of these blooms had substantial concentrations of colonies, and suggests that temperature has only a minor influence on the overall distribution and appearance of colonial forms of *Phaeocystis*.

In addition to the relatively recent reports in Asia of *P. globosa*, colonies have been reported to reach extreme sizes – up to 3 cm in diameter. Such giant phytoplankton are extremely unusual in marine systems, and their size would encompass a transition from a viscous environment to one dominated by turbulent processes. Indeed, we hypothesized that such colonies must have some unusual adaptations to survive and proliferate in a turbulent environment, and conducted experiments designed to understand and quantify some of its unusual characteristics.

Substantial accumulations of chlorophyll in a narrow band along the coast were noted from satellite images. Chlorophyll began to increase in these waters at the onset of monsoonally driven upwelling, indicated by changed wind direction and speeds and decreased temperatures in June through August (Figs. 3 – 5). Narrow bands of enhanced chlorophyll (up to 6 μg chl a L⁻¹) were observed by satellite in near-shore waters. Our direct sampling of *P. globosa* colonies at Phan Thiet suggested that this coastal enhancement could have been at least in part contributed by colonial *P. globosa*. For example, if colony density were 1 L⁻¹, then the contribution to total chlorophyll of a single 1 cm colony would be ca. 0.4 μg L⁻¹ (Fig. 7). The colonies we observed were of variable sizes and colors, but were common enough to easily sample in the near-shore environment. We are not suggesting that the blooms observed by satellites were completely dominated by *P. globosa*, but believe that the blooms (especially those near the shore) could have substantial contributions by colonial *Phaeocystis*.

**Influence of Colony Size on the Ecology of *P. globosa***

Increased colonial size potentially has substantial impacts on the growth and survival of *P. globosa*. Examples of parameters that likely would be influenced include sinking rates and nutrient uptake. We reasoned that as colonies increased in size, the amount of carbon required to generate the mucous envelope would increase relative to the number of cells, unless the density of cells remained the same or increased. Regardless, the carbon “demand” of envelope production (given the proportion of the entire POC of a colony) could be substantial and negatively impact colonial growth and survival. If upon size increases the envelope became thinner, colonies might rupture due to the turbulent forces on the colony. Additionally, a thinner envelope might alter its diffusive characteristics, and therefore impact nutrient availability. Our experiments were designed to try to understand some of the factors that changed as colony size increased.

In general, phytoplankton sinking rates are 1 m d⁻¹ or less (K írboe et al., 1996). Peperzak (2000) reported positive buoyancy in small *P. globosa* colonies (0.1 m d⁻¹), but we never observed any positive buoyancy in our samples. To the contrary, the measured colony sinking rates were extremely high, ranging from 29.3 to 516 m d⁻¹ and averaging 189 m d⁻¹, which were as fast as, or faster than, zooplankton fecal pellets and large aggregates (Turner, 1979). Indeed, even if colonies were at the surface, such sinking rates would remove them completely from the euphotic zone within one day, unless resuspension was strong. Given the rapid sinking rates, it is likely that large *P. globosa* colonies effectively utilize a benthic strategy, whereby colonies are continually resuspended in shallow water. Such a strategy would confine the largest colonies to near-shore (a few meters deep), high energy environments, which is the exact environment from which...
we sampled colonies. It is unknown how far the colonies are distributed off-shore, but during the same period we did not observe the colonies in waters ~100 m from shore (although our sampling may have missed colonies growing offshore). Clearly, the high sinking rates pose a significant challenge for the colonies to maintain their population within the water column, and necessitate a modified distribution and strategy for survival.

Another potential limitation of colony size could be nutrients, in that nutrient fluxes could be limited both by diffusive boundary layers and the rates of flux across the envelope. Ploug et al. (1999) suggested that the diffusive boundary layer would increase in thickness with colony diameter, but also found that the mucous envelope was extremely permeable to oxygen (diffusion coefficients across the envelope were essentially the same as those in seawater). As such, we did not expect that the envelope would restrict the movement of ions or nutrients into or out of the intracolonial fluid. If the envelope did restrict ionic fluxes, then nutrients could be depleted within the intracolonial fluid by cellular growth, and thus restrict the uptake of nutrients to extracolonial seawater. We conducted an experiment designed to assess the permeability of the envelope by altering the external salinity and measuring the change in salt internally through time. This experiment showed that the envelope was indeed permeable, and that an equilibrium was reached (i.e., internal and external salt concentrations became equal) in ca. 60 minutes. The estimated diffusion coefficients were $1.0 \pm 0.3 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$, similar to ions (i.e. Ca$^{2+}: 0.84 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$, Mg$^{2+}: 0.54 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$; Chen et al., 2002) and low molecular-weight proteins ($2 \times 3 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$; Verkman, 2002) in cytoplasm, and orders of magnitude lower than diffusion coefficients in seawater. Based on both the length of time required to establish equilibrium, as well as the magnitude of estimated diffusion coefficients, we conclude that the envelope does indeed restrict the flux of salts, and hence potentially can restrict the growth of colonial cells. Experiments measuring the flux of nutrients are needed to examine the diffusion coefficients of individual ions.

**Carbon Partitioning in Colonies**

If the envelope restricts salt flux into and out of the colony, there is a potential that the intracolonial fluid could act as a reservoir for materials that could be utilized during unfavorable growth conditions. This has been suggested previously (Lancelot and Mathot, 1985; Davidson and Marchant, 1987), but the intracolonial fluid has never been chemically analyzed for reduced carbon. Our measurements of the internal fluid showed that it was exceptionally enriched with dissolved organic carbon – more than 30 times greater than the ambient DOC. Such concentrations strongly suggest that DOC does not rapidly diffuse across the envelope and diffusive boundary layer. Because the DOC samples were not filtered, the values should actually be considered to be total organic carbon. Samples undoubtedly included POC that arose from envelope, from cells released from the mucilage, and products of cellular degradation, but most likely a vast majority of the material was truly dissolved. Given the levels found, it is instructive to consider its origin and rates of production, especially relative to the carbon allocation patterns of the entire colony.

Colony diameter was positively correlated with total intracolonial DOC, but negatively with intracolonial DOC concentration. Using the relationship established in this study (Fig. 6b), we estimated colonial cell abundance from colony diameter. The estimated colonial cell abundance ($X$) was significantly correlated with the total intracolonial DOC ($Y$) according to the equation:

$$Y = 3 \times 10^6 X + 0.095 \quad (R^2 = 0.96; \ p < 0.001).$$
The linear relationship suggests that the amount of DOC released per cell remained nearly constant and was independent of colony size.

In addition, as the colony increases in size, intracolonal DOC would also be increasingly diluted due to the increasing volume. This relationship can be deduced as follows: DOC production was a function of colonial cell abundance, which in turn scaled to $L^{2.44}$ (Fig. 6b), whereas colonial volume scaled to $L^3$, where $L$ represents colony diameter. At steady state, intracolonal DOC concentration would scale to $L^{-0.56}$.

Over the range of colony diameters used in our DOC measurements (0.64 – 1.4 cm), this scaling relationship predicts a reduction of intracolonal DOC concentration by 35% due to dilution effect alone. This prediction is largely consistent with observations: measured intracolonal concentrations in 2010 were 4.86 (largest colonies) – 7.78 (smallest colonies) mM, equivalent to a reduction of 37.5%. Thus, even with an increasing number of cells releasing DOC as colonies grew, intracolonal DOC concentrations would still decrease with colony size due to this dilution effect. The slightly larger reduction than predicted could be a result of increased permeability of the mucous envelope. The intracolonal DOC concentration was on average 30-fold higher than ambient DOC concentration, and therefore the strong diffusion gradient would drive a DOC flux out of the colony through the mucous envelope. One notable observation is that mucous POC per unit surface area decreased as colony size increased (Fig. 8b), suggesting that the mucous envelope in larger colonies was thinner, and therefore presumably more permeable to DOC leakage.

The colonies sampled were substantially different between the two years, in that in 2010 the colonies were quite robust in appearance and were larger than those observed in 2011. Additionally, in 2011 there were a large number of broken colonies in the water column; furthermore, colonies were nearly absent in the water during the early morning, and were found towards local noon after the winds had increased. This suggests the colonies were resuspended from the sediments, and may imply they were in some manner “senescent”. Part of that phase might include having less intact (thinner, broken) mucilage, which in turn allowed the release of DOC into the extracolonial water. Such an explanation would also be consistent with the 2010 trend of greater DOC values in smaller colonies. Ambient DOC concentrations in 2011 were significantly greater (by 64 µM) than those in 2010, which might partially reflect DOC losses from broken and damaged colonies that appeared to be more common in 2011, although coastal run-off also likely was different.

If we were to consider a colony 1 cm in diameter, it can be predicted (Fig. 6) to have $0.90 \times 10^6$ cells, representing 12.1 µg C (1.01 μmol C). Mucilage carbon would equal 0.40 µg C (0.033 μmol), and the DOC internal sap would represent 44.4 µg C (3.70 μmol). Thus the internal DOC carbon pool represents 78% of the total organic carbon of a 1 cm colony. Given the relative contributions of mucilage and cellular material, even the inclusion of part of this organic material could not represent a major contribution to the colonial reduced carbon pool. Thus, intracolonal DOC represents the major pool of organic carbon to giant colonies.

**Estimation of Maximum Colony Size**

As colonies grow larger from sizes of 200 µm to greater than 1 cm, they undergo a transformation from a viscous environment dominated by diffusive processes to one that is dominated by turbulence. With that transition, they also are influenced by substantially different physical factors, which in turn likely influence their growth and survival. For example, smaller colonies must acquire nutrients that potentially can be
limited by diffusive exchanges and boundary layer dynamics. In contrast, larger colonies must survive forces that can damage colonial integrity. Larger colonies have the advantage of being released from grazing pressure as well. The question remains as to why the Vietnamese strain reaches such large sizes, and what controls the maximum size.

Based on our derived relationship between colonial cell density and colony diameter (Fig. 6), and using the ratio between the total colony surface area taken by colonial cells and totally colony surface (which cannot exceed 1), we estimated that the maximum colony size possible is 9.77 cm (Liu, 2011). Clearly this exceeds any colony size that has ever been observed. Similarly if we extrapolated the relationship of total POC per unit surface area (Fig. 8) to the x-intercept, this suggests that the colony diameter at zero POC would be ca. 1.43 cm, much smaller than that estimated from the colonial cell density – diameter relationship discussed above, but also likely an underestimate, as colonies larger than this have been observed (Qi et al., 2004). It is interesting that most of the colonies we observed in Viet Nam were smaller than 1.5 cm, but these represent those that could easily be collected and were in apparently good condition for us to experimentally manipulate.

The decreasing mucous POC with increasing colony size suggests a reduction in envelope integrity as the colony grew, which might lead to increased susceptibility to microbial invasion and infection (Peperzak et al., 2000, Brussaard et al., 2005), thus limiting the maximum colony size. Finally, given the demands of carbon allocation upon colonial cells, it is probable that as colonies get larger, the requirements of DOC production and for mucilage generation becomes so large that the number of cells cannot increase proportionately, and thus the viability of intact colonies becomes limited by reduced carbon availability. Accurate carbon budgets for these colonies are needed to fully assess the role of carbon allocation in giant *P. globosa* colonies.

5. Conclusions

Giant *Phaeocystis* colonies represent an unusual growth and survival strategy for marine phytoplankton and harmful algal blooms species. While *Phaeocystis* has only recently been recognized as a HAB, its global distribution suggests it has significant impacts in a variety of coastal systems. Furthermore, its growth in tropical systems like that of Viet Nam has only recently been documented and represents a phenomenon that is restricted to the past decade. Combined with its impacts on aquaculture and coastal ecology, its sudden appearance represents a serious problem to developing countries, and knowledge of the causes of giantism and its effects on plankton ecology may help mitigate its deleterious impacts. Our experiments represent an initial attempt to understand the ecology of these giant colonies, but clearly a great deal more work is needed to place this unusual HAB species in the context of tropical coastal systems.

Acknowledgements. This research was supported by NSF grant OCE 0850910 (WOS, KWT) and NAFOSTED (National Foundation of Science and Technology, Ministry of Science and Technology, Viet Nam) under project 106.13.35.09. We thank J. Dreyer and C.T. Nguyen for their help in the field, and Dr. C. Carlson for processing the DOC samples. This is VIMS contribution XXXX.

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