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Protein and carbohydrate contents related to varying light levels and chlorophyll-a in selected freshwater and marine phytoplankton

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ABSTRACT

This study investigated correlations between chlorophyll-a (CHLa) and certain biomass parameters (protein and two forms of carbohydrates) under the influence of light intensity. These findings are applicable to the estimation of metabolizable biomass in water bodies, which is important for understanding the nutritional value of phytoplankton and their impact on aquatic food webs. Furthermore, these determined biomass relationships can also assist in the prediction of the generation of anoxia during and following algal blooms. That is, one could relate the standing crop of metabolizable organic matter (proteins and carbohydrates) to existing conditions of water depth, currents, dissolved oxygen trends and other parameters. Results from this study indicate that protein, colloidal carbohydrates, and storage carbohydrate concentrations in phytoplankton can be broadly estimated by multiplying chlorophyll-a amounts (pg/cell or mg/L) by 202.6, 17.7, and 144.9, respectively. The methodology presented can therefore serve as a means of approximating the standing crop of metabolizable phytoplankton organic matter (viz. protein and two forms of carbohydrates).

Keywords: Pigment analysis, Algal protein, Algal carbohydrates, Water column, Algal metabolizable organic matter, Irradiance levels, Algal bloom

Introduction

As primary producers, phytoplankton form the base of aquatic/marine food webs. Photosynthetically produced organic matter (OM) is grazed and consumed by zooplankton and other microconsumers (Schaffner et al., 2019) which are then consumed by macroconsumers, such as fish and even, via krill, to whales (Miller et al., 2019). The quality of the organic matter (~' food') produced by phytoplankton therefore has significant impacts on zooplankton and other consumers (Gulati and Demott, 1997).

Chlorophyll-a (CHLa) is the most widely used estimator of algal biomass. It is accepted as a fairly accurate and convenient measure of algal 'biomass' (weight and volume) and can serve to indicate interactions between nutrient concentration and several biological phenomena (Huot et al., 2007; Mineeva, 2011). The review by Mineeva (2011) concluded "*Taking into account high pigment diversity in algae, the high-performance liquid chromatography method seems to be the most perspective method for assessment of total biomass of algal cenoses and of the relative abundance of representatives of large taxonomic groups*." High performance liquid chromatography (HPLC) also allows the full separation and quantitation of chlorophylls and carotenoids and allows for taxon-specific carotenoids to be used in the pigment-based chemotaxonomic assessment of microalgal taxa present in water samples (Louda, 2008; Millie et al., 1993; Wright et al., 1996). Changes in algal physiology are not confined to ratios of CHLa to pigments but is also reflected in other indices of biomass such as proteins, carbohydrates, and organic carbon. The determination of the protein and carbohydrate content of microalgae can provide important information for phytoplankton biomass assessment, which can in turn be used to investigate protein and carbohydrate dependent physiological processes in cells as well as with studies of nutritional value of phytoplankton and their impacts on food webs (Bhavya et al., 2018; Liao, 2024).

Carbohydrates are major products of photosynthesis and include polysaccharides and storage structure compounds. Carbohydrates play important roles in biogeochemical cycles in the water column and water-sediment interface, in cellular metabolism and structure, and are major storage compounds in autotrophic organisms. Carbohydrates, particularly polysaccharides, contribute significantly to the organic matter of diatoms, green algae, and cyanobacteria (Fernandez et al., 1992). Carbohydrates have also been previously suggested as a measure of phytoplankton abundance (Marshall and Orr, 2009).

The two major groups of carbohydrates in microalgae are extracellular, loosely bound colloidal carbohydrates and intracellular storage polysaccharides (glucans and starch). Several groups of microalgae have been shown to secrete copious amounts of carbohydrates and are involved in the transfer of nutrients in lower food webs (Decho, 1990). Colloidal carbohydrate fractions have been shown to contain mucopolysaccharides, extracellular polymeric substances (EPS), transparent exopolymers (TEP), and others, each with their own function. However, these secretions have largely been ignored in studies regarding microalgal production and trophic energy transfer. Epipelic diatoms secrete mucopolysaccharides to facilitate movement. These secretions then represent sources of food for bacteria and invertebrates (Decho 1990). EPS also contributes to the aquatic flocculent organic matter (aka floc), such as in the coastal Everglades (Neto et al., 2006); and excreted polysaccharide EPS forms the basis of hydrogels that stabilize the fine-grained carbonate sediments in Florida Bay and elsewhere (Louda et al., 2004). In addition to function in the mucous matrix of diatoms, mucopolysaccharides have also been reported to serve as storage polysaccharides (Lancelot & Mathot, 1985). Few studies have been done to investigate the influence of light on mucopolysaccharide production in phytoplankton. Studies carried out on *Cyanospira capsulate* and *Synechococcus* strains grown under various light/dark cycles showed that both produced smaller amounts of mucopolysaccharides in comparison to control cultures grown under continuous light (Philips et al., 1989). Decreased production of mucopolysaccharides equated to shorter light periods. Therefore, it could be concluded that the synthesis and release of these polysaccharides is light dependent (Philips et al., 1989). The effect of light on the production of carbohydrates and proteins therefore forms the basis of this study.

Proteins are essential biomolecular components of cells that have the following roles: regulating metabolic activities, providing structural support and existing as pre-cursors as well as end-products of macromolecular synthesis and catabolism (Yun et al., 2015). Therefore, knowledge of the quantity of total protein present is important for understanding a broad range of biological processes in phytoplankton cells. With respect to biomass, the proteins in phytoplankton cells are also important to the secondary consumers (e.g. zooplankton, benthic microbes etc.) that feed on them (Gulati and Demott, 1997). The quantitative information of the protein content in phytoplankton cells, as well as their relationships to chlorophyll-a, is important to a variety of studies that are directly and indirectly related to various aspects of cellular ni-

trogen metabolism as well as predictors of phytoplankton dynamics and physiological state. Few studies have reported generalized relationships between algal protein and chlorophyll-a. For example, a weight- to- weight ratio for protein/CHLa of 8.57:1 has been reported and is often used in the literature (Meyers and Kratz, 1955). However, that reported work focused only on one species of a cyanobacterium, *Anacystis nidulans*. The study by Moal et al. (1987) examined many species across 5 major taxa and found protein/CHLa weight ratios between 20-385:1 and carbohydrate to protein weight ratios between 0.16-2.36:1. However, the two studies mentioned above did not examine the effects of light intensity on protein/CHLa relationships. We report herein, the effects of varying light intensities on the concentrations of protein and CHLa in freshwater and marine phytoplankton.

Traditionally, algal cell volume measurement via microscopy has been the method of choice and is a relatively good indicator of algal biomass. However, the method is laborious and highly dependent on the skills of the researchers (Dunker et al., 2018; Karlson et al., 2010). Moreover, samples become altered, and measurements are biased by the very preservative that the samples are fixed in prior to microscopic analysis (Zarauz & Irigoien, 2008). In the present study, correlations between CHLa and biomass parameters (protein and two forms of carbohydrates) under the influence of light intensity were investigated to assess if the determined relationships could aid biomass estimation since phytoplankton have significant nutritional contribution to food webs.

Therefore, the working hypothesis for this study is that chlorophyll-a *per se* is not the ultimate descriptor of phytoplankton biomass, especially 'food' biomass for transfer amongst food webs. Large variations in 'true' biomass, defined here as metabolisable organic matter (proteins, carbohydrates, lipids), exist between phytoplankton groups (taxa) and within each taxon by variations in light and/or nutrient availability. Thus, taxonomic information would also be required to convert chlorophyll-a to biomass. Alternately, the null hypothesis would be that chlorophyll-a alone works perfectly to estimate phytoplankton biomass.

Materials and Methods

The overall experimental design: algal growth, harvesting, pigment analysis, protein and carbohydrate analysis is shown as schematic 1.

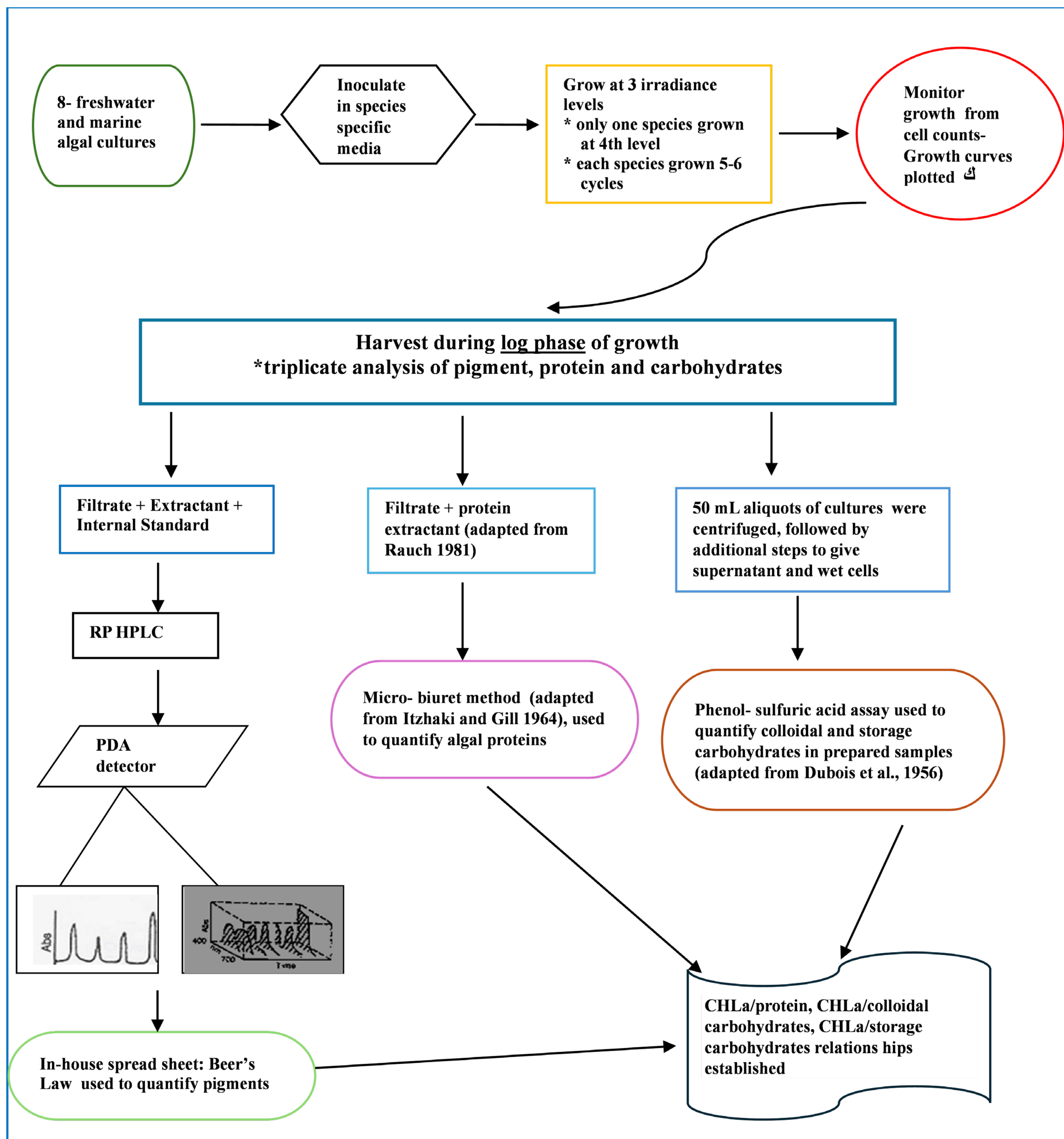
Experimental organisms: The following eight freshwater and marine microalgal species were purchased from the Carolina Biological Supply Company (Burlington, N.C.): Cyanobacteria; *Synechococcus elongatus* (marine), *Microcystis aeruginosa* (freshwater), Chrysophyta; *Thalassiosira weissflogii* (marine), *Cyclotella meneghiniana* (marine), Chlorophyta; *Scenedesmus sp.* (freshwater), Pyrrophyta, Dinophyceae; *Amphidinium carterae* (marine). The following species were purchased from the University of Texas (UTEX) algal culture collection (Austin, TX): Cryptophyta; *Rhodomonas salina* (marine), Chlorophyta; *Dunaliella tertiolecta* (marine).

Selected details of the experimental species: This section is included to stress certain characteristics of each species and include: (a) harmful algal bloom (HAB) formation leading to high inputs of metabolizable organic matter such as proteins and carbohydrates; and (b) importance as primary producers supporting heterotrophic growth in food chains/webs.

Synechococcus elongatus is a common cyanobacterium. *Synechococcus* is the main source of primary production in oligotrophic, pelagic marine, open, warm waters. They have been known to cause non-toxic harmful algal blooms in Florida Bay (Phlips et al., 1999). Harmful here includes blooms leading to anoxia, disruption of socio-economic function and environmental change. The dominance of this species in the center of the Florida Bay may be attributable to its physio-chemical characteristics: small size, buoyancy, and tolerance to high light intensity (Phlips et al., 1999).

Microcystis aeruginosa is a common unicellular colonial cyanobacteria found in freshwater environments. The existence of intracellular structures such as gas vesicles provides cells with buoyancy. *M. aeruginosa*, used in this study, occurs in large amounts on the surface waters of lakes and reservoirs in the spring and summer months. This species synthesizes a variety of toxins of which the three most abundant are microcystin-LR, cyanopeptolin-A, and aerucyclamide-A (Ricca et al., 2024). It is one of the most damaging species, due to its toxicity to aquatic and terrestrial organisms and is known to occur worldwide (Ross et al., 2006).

Thalassiosira weissflogii grows primarily in marine waters with some species within the genus being found in estuaries, high-conductance waters and rivers, polluted ponds, and other aquatic systems that have been impacted by human activities (Spaulding and Edlund, 2009).



Schematic 1. Experimental design

Cyclotella meneghininana, synonymous with *Stephanocyclus meneghinianus* (Kützing) Kulikovskiy (Guiry, 2024), used in this study, is perhaps the best-known species of this genus and is widely used in growth experiments (Mitrovic et al, 2010). *C. meneghininana* is a very common freshwater diatom in many places across the world (Kiss & Nausch 1988, Murakami et al. 1994) and may predominate over other diatoms in silica-rich environments (Hori et al. 1969).

Scenedesmus quadricauda is very common in eutrophic freshwater ponds and has planktonic forms in rivers and lakes; it is reported worldwide in all climates and is rarely found in brackish water (Wehr and Sheath, 2003).

Amphidinium carterae is a brown tide organism forming harmful algal blooms, releasing toxins, and physical irritants and creating noxious events. Most species produce toxins that affect humans as well as fish (ichthyotoxic). This species, as well as others in the genus are CFP (ciguatera fish poisoning) producers (Hallegraeff, 1993).

Rhodomonas salina is a marine photoautotrophic flagellate. They occurs in marine and brackish water. This species contains fragile cell membranes and has the name hidden-plant (crypto-phyte). Cryptophytes are very fragile and are often lost in fixed samples (Jeffrey and Vesk, 1990).

Dunaliella tertiolecta is a unicellular, ovoid, biflagellate, naked green marine alga (Morais Jr. et al., 2020). Twenty-eight species of *Dunaliella* are presently recognized (Jayappriyan et al., 2010). The unique morphological feature of *Dunaliella* is that it lacks a cell wall. The cell is enclosed by a thin plasma membrane or periplast, which permits rapid changes in cell shape and volume in response to osmotic changes. To survive, these organisms have high concentrations of β-carotene to protect against the intense light and high concentrations of glycerol to provide protection against osmotic pressure.

Algal culturing: All species were grown in 2 L batches in 4L cylindrical polycarbonate containers (CAMBRO. Huntington Beach, CA). Zephyrhills ® Natural Spring Water was used for the freshwater cultures (*M. aeruginosa, Scenedesmus sp., C. meneghiniana)* and the media was prepared according to Guillard's (1975) f/2 medium recipe. The seawater for the marine cultures was collected from coastal water (FAU Gumbo Limbo Environmental Complex and Nature Center, Boca Raton, Florida). Media were autoclaved (122º C and 2 atm.) after filtering. The addition of nutrients, including vitamins and trace metals were also based on Guillard's (1975) f/2 medium for the marine species *S. elongatus, T. weissflogii,* and *A. carterae.* Erdschreiber's (Schreiber, 1927) marine medium was used to culture *Dunaliella tertiolecta* and *Rhodomonas salina*.

Culture conditions: Light intensity (photosynthetically active radiation (PAR) radiation: 400 – 700 nm) was measured with a 4π spherical radiometer and Li-Cor LI-250 Light Meter. PAR transmission through the polycarbonate flasks was measured at 90% and subsequent measurements were adjusted by 0.9. Light levels are defined here as: high (HL: 180-200 µmol photons·$m^{-2}·s^{-1}$), moderate (ML: 70-75 µmol photons·$m^{-2}·s^{-1}$), low (LL: 35-37 µmol photons·$m^{-2}·s^{-1}$), and dim (DL:10 µmol photons·$m^{-2}·s^{-1}$). It is noted here that "high light" in this context refers only to the conditions utilized in this study and is not meant to mimic natural environment light conditions in surface waters (~ 1,500+/- µmol photons·$m^{-2}·s^{-1}$). For this study, light conditions were achieved within three separate temperature-controlled (25°C) growth chambers: a Revco-Harris growth chamber was used for the high light experiments, while two Precision Illuminator 818 growth chambers were used for the remaining light levels. All growth was achieved at 25°C with a 12-hour light: 12-hour dark diurnal cycle. Temperature control was observed within + 1.5°C. The samples in the two Precision growth chambers were illuminated from the front only (fluorescent tubes vertically attached to the inside door) with two 34W Econo (Philips) 120 cm long fluorescent tubes, covered by a diffuser screen for the medium light experiments and without a diffuser screen for the low light experiments. Samples for the high light experiments (Revco-Harris growth chamber) were illuminated from the top as well as both sides with sunlight quality (Verilux Instant Sun™), full Spectrum™ (ValuTek) and "aquarium" quality (Sylvania Gro-Lux™) fluorescent tubes. Three 8W (Westwek 20121) cool white, fluorescent tubes were attached horizontally on the inside door of the Precision growth chamber and used for illumination in the dim light experiments. Only one of the species in the study: *Synechococcus elongatus*, grew at the dim light level, therefore, dim light experiments were discontinued for the remaining species. All species exhibited increasing specific growth rate constants with increasing light intensity. This indicated that the light intensities used did not limit or inhibit the growth of the algal cells.

Cell counting: Cell counts were taken with a Coulter Counter model ZM electronic cell counter. Cell counting was done every two to three days during and on the same day that the algal samples were to be harvested. ISOTON® II diluent (electrolyte solution) was pipetted (20 mL) into the counting vial (Fisher 'Accuvette') and 100 µL of suspended algal cells were added. The three most consistent counts out of six were averaged and used as the corrected count. The dilution factor (DF) was determined, and the number of cells per milliliter was calculated by multiplying the corrected count by the di-

lution factor (DF x corrected counts = cell mL^{-1}). The following equation was used for calculating the dilution factor: [DF = (S + E) ÷ (MS x S)], where S = mL sample, E = mL electrolyte, MS = manometer setting. The number of cells per milliliter was determined from cell counting and the concentration of each analyte (pigments, proteins, carbohydrates, organic carbon) in pg/cell or µg/L could then be calculated. Samples for the analyses of chlorophyll-a, proteins, colloidal and storage carbohydrates were taken from cultures in the log phase of growth.

Analyses overview: Relationships between protein-to-CHLa, colloidal CHO-to-CHLa, and storage CHO-to-CHLa, in relation to light treatments were analyzed for each species. Cellular concentrations of chlorophyll-a, protein, and the two functional classes of carbohydrates, as well as their relationship to biovolume for each species, at each light level were determined. Throughout this report and in figures, the acronyms DL, LL, ML and HL will be used for dim, low, medium, and high light levels, respectively.

Pigment Analyses: All pigment, notably chlorophyll-a, analyses were carried out under dim, yellow light conditions to prevent photo-oxidative alterations such as pigment isomerization. For harvesting and for pigment monitoring during growth, cultures were filtered onto glass microfiber filters (Whatman GF/F, 0.7-micron pore size borosilicate glass fiber). The filters were removed from the filter funnel, folded in half, and blotted between paper towels. The filters were then folded into quarters, re-blotted and wrapped in aluminum foil, then immersed in liquid nitrogen for quick freezing. The individual samples were removed from the liquid nitrogen and stored in a refrigerator at -80ºC until extraction. The filters were extracted in pre-chilled glass tissue grinders (Kontes “Duall” 15 mL) as reported previously (Grant and Louda, 2010). The UV/Vis absorption spectra (350-800 nm) of the extracts were recorded on a Perkin Elmer Lambda - 2 UV/Vis Spectrophotometer, calibrated for wavelength and absorbance vs. holmium oxide. Pigments were separated via reversed phase high performance liquid chromatography (RP-HPLC) as previously reported (Grant and Louda, 2010; Louda 2008). This allowed full quantitation of total chlorophyll-a (CHLa) as the sum of chlorophyll-a, chlorophyll-a-epimer, chlorophyll-a-allomer, chlorophyll-a-allomer-epimer, chlorophyllide-a, and pyro-chlorophyllide-a, all of which would be commeasured by normal spectrophotometric (UV/Vis, fluorescent) measurements.

Algal protein extraction: The procedure was adapted from Rausch (1981) with some modifications, as reported herein: 100 mL aliquots of algal culture were filtered onto pre-combusted glass fiber filters. The filters were then folded in halves, then quarters and refrigerated at -80˚C until analysis. Analyses were performed within one week of filtering. Samples were extracted in 0.5 M sodium hydroxide (NaOH), by grinding the filters in 12 mL tissue grinders (glass mortar with Teflon® pestle e.g. Kontes Duall). The tubes were next heated at 80℃ for 10 minutes to further extract the proteins. After this step, the tubes were quickly cooled to room temperature, then centrifuged (Fisher Scientific, Centrific Model 228) for 5 minutes. The resulting supernatant was transferred to 10 mL graduated tubes for subsequent protein analysis. A second extraction was carried out on the remaining filter debris (extraction in 0.5 M NaOH at 80℃ for 10 minutes, followed by cooling and centrifugation), and the supernatants were combined in the 10 mL graduated tubes. A third extraction was carried out (0.5 M NaOH at 100℃ for 10 minutes) for green algae and cyanobacteria, as prescribed by Rausch (1981). The combined supernatants were then made up to a definite volume (6-10 mL) with 0.5 M NaOH and used for protein measurement. This methodology was applied to all eight species of this study.

Algal protein measurement: The micro-biuret method for estimating proteins as adapted from Itzhaki and Gill (1964) was slightly modified. The procedure used is as follows: 2 mL of algal protein extract was assayed with 1 mL of 0.21% $CuSO_4.5H_2O$ in 30% NaOH at 310 nm in a 1 cm quartz cuvette and another 2 mL of algal protein extract was assayed with 1 mL of 30% NaOH at 310 nm in a 1 cm quartz cuvette. The absorbance of the protein was obtained from the difference between the absorbance of the sample in 30% NaOH and that from the reaction in 0.21% $CuSO_4.5H_2O$ in 30% NaOH. All samples were measured against a distilled water reference. Bovine serum albumin was used as the calibration standard.

Algal colloidal and storage carbohydrate extraction: The following method was used: aliquots of approximately 50 mL of the algal cultures in the logarithmic stage of growth were collected in 50 mL centrifuge tubes and centrifuged (Dynac Centrifuge, Becton Dickinson and Co, Parsippany N.J.) at 3300 rpm for 30 minutes. The supernatant was decanted to leave ~ 0.5-1 mL of wet cells, plus 2 mL of the supernatant, for colloidal carbohydrate analysis. The wet cells, minus supernatant, were re-suspended in 30 mL ultra-pure water (Milli-Q® Ultra-pure water systems, Millipore Corporation) and heated in a water bath for an hour, with stirring at 10-minute intervals. The solutions were then sonicated for 5 minutes (Burdloff et al., 2001), followed by pelleting the cells via centrifugation for 30 minutes. The resulting supernatant containing mostly the water-soluble storage carbohydrates was then filtered through 0.22 µm membrane filters (Fisher Scientific).

The filtrates were then lyophilized, and the dried material used for carbohydrate analysis.

Algal colloidal and storage carbohydrate measurement: The two extracted carbohydrate fractions were analyzed using the phenol-sulfuric acid assay (Dubois et al, 1956). The lyophilized samples were dissolved in exactly 2 mL of ultra-pure water and pipetted into 10 mL disposable test tubes. Using an extracted colloidal carbohydrate fraction as an example: exactly 2 mL of the initial supernatant was pipetted into 10 mL disposable test tubes. Next, 0.05 mL of 80% phenol was added to each tube followed by the rapid addition of 5 mL concentrated H_2S0_4. The tubes were allowed to stand for 10 minutes, after which they were placed for approximately 20 minutes in a water bath at 25-30°C with occasional shaking. The resulting champagne-dark orange solutions were then measured at 485 nm against distilled water in a Perkin Elmer UV/Vis Lambda 2 spectrometer. Alpha-D (+)-Glucose was used as the calibration standard.

Results and Discussion

The appendix contains Figures A1 - A8 which detail the concentrations of chlorophyll-a (CHLa), protein (PROT), colloidal carbohydrates (C-CHO), and storage carbohydrates (S-CHO) versus light intensities and includes cross plots of PROT, C-CHO and S-CHO versus CHLa concentrations. It is the cross plots versus CHLa that are suggested for use in generating a method for estimating these organic matter types from total CHLa data gathered in the field and/or laboratory. Plots of CHLa, PROT, C-CHO, and S-CHO versus light intensity contain data points that are the mean plus standard deviations of triplicate trials.

Linear regressions of PROT, C-CHO and S-CHO relationships to CHLa gave R-squared (r^2) values greater than 0.8 – 0.9 for all species except *A. carterae* ($r^2 \sim 0.2$) and *D. tertiolecta* ($r^2 \sim 0.6$-0.7), likely from natural variability in the sample. Table 1 contains the linear regression equations for the estimation of protein, colloidal carbohydrates, and storage carbohydrates from chlorophyll-a data in these eight species. Additionally, the averaged values of each estimation regression are given for each compound classification estimation. Protein to carbohydrate ratios from Table 1 averaged 1.9. As a comparison to our reported data, the ratios of protein to carbohydrates in the twelve species reported by Sassi et al. (2019) grown at 150 mmol photons $m^{-2}\cdot sec^{-1}$ averaged 1.4.

The potential use of this method as a tool to aid in the estimation of metabolizable biomass can now be assessed. For example, if a water body was sampled and found to have a total chlorophyll-a concentration of 8.8 mg/L, then PROT, C-CHO and S-CHO could be estimated to be: PROT = (202.5 x 8.8 mg/L) – 9.2 = 1,863.9 mg/L; C-CHO = (17.7 x 8.8 mg/L) + 0.4 = 156.2 mg/L; and S-CHO = (144.9 x 8.8 mg/L) – 6.2 = 1,268.9 mg/L. Total carbohydrates would then equal the sum of C-CHO and S-CHO or 156.2 mg/L + 1,268.9 mg/L = 1,425.1 mg/L.

It must be noted that growth conditions may alter total chlorophyll-a, protein and carbohydrate contents and ratios. The constitution of microbiomes, also called the interactome, is known to affect many aspects of the growth and constitution of *M. aeruginosa* (Cook et al., 2020) and likely applies to other species as well.

Table 1. Data compendium: Estimation of protein, colloidal carbohydrates, and storage carbohydrates from chlorophyll-a concentrations.

		Averaged relations to chlorophyll-a.			
	High Light		y = Colloidal	y = Storage	
	CHL-a	y =Protein	Carbohydrates	Carbohydrtaes	Protein
Organism	~ pg / cell	pg / cell	pg / cell	pg / cell	to Carbs
Synechococcus elongatus	7.2	y = 188.0xC - 54.9	y = 12.3xC - 3.5	y = 57.1xC - 5.6	**2.7**
Microcystis aeruginosa	0.6	y = 87.1xC- 3.2	y = 7.4xC - 0.1	y = 107.3xC-2.6	**0.8**
Thalassiosira weissflogii	10.5	y = 194.6xC - 2.4	y = 16.1xC - 1.8	y = 229.5xC - 33.1	**0.8**
Cyclotella meneghiniana	1.5	y = 132.9xC - 10.2	y = 20.9xC - 0.1	y = 242.8xC - 26.6	**0.5**
Scendesmus quadricauda	5.8	y = 116.8xC + 46.6	y = 6.6xC + 3.9	y = 14.1xC + 28.4	**5.6**
Amphidinium carterae	0.34	y = 319.6xC + 26.1	y = 51.6xC + 5.6	y = 242.7xC + 8.9	**1.1**
Rhodomonas salina	0.48	y = 357.6xC - 5.2	y = 18.8xC + 0.6	y = 151.2xC - 2.2	**2.1**
Dunaliella tertio;ecta	0.9	y = 223.5xC - 70.4	y = 7.7xC - 1.1	y = 114.7xC - 16.9	**1.8**
Averaged values	N/A	**y = (202.6 xC) -9.2**	**y = (17.7 xC) +0.4**	**y = (144.9 xC)-6.2**	**1.9**
		C = Chlorophyll-a			

Conclusion

Correlations between CHLa and biomass parameters (protein and two forms of carbohydrates) under the influence of light intensity were investigated to ascertain if this could aid biomass estimation, especially as it potentially pertains to food chain considerations. This may also assist the prediction of the generation of anoxia during and following algal blooms. That is, one could relate the standing crop of metabolizable organic matter (proteins and carbohydrates) to existing conditions of water depth, currents, dissolved oxygen trends and other parameters.

The data given in the Appendix (Figures A1-A8) reveals that CHLa per cell increased linearly up to 200 mmoles photons m^{-2} sec^{-1} except for *Amphidinium carterae* (Fig. A8). In this species CHLa stayed at 0.30 – 0.35 pg/cell across all light levels. However, protein and the carbohydrate concentrations did increase with light. Previously, we found that CHLa per cell levels off or decreases at light levels above 200-300 mmoles photons m^{-2} sec^{-1} in several species of cyanobacteria, chlorophytes, chrysophytes and prymnesiophytes (Grant and Louda, 2010). The estimation of colloidal carbohydrates is most likely affected by sampling. That is, extracellular polymeric substances (EPS), such as polysaccharides, are likely sloughed away from cells during sampling and filtration prior to analysis.

We realize that, in nature, the presence of multiple species or a predominance of a single species, such as during harmful algal blooms, will affect the resultant estimation of the quantities of protein and carbohydrates present and available for food chain considerations. However, we offer these results as a broad-brush method by which to estimate the standing crop of metabolizable phytoplankton organic matter.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethics committee approval is not required for this study.

Data availability: Data will be made available on request.

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Disclosure: No ethically important comments regarding the scientific research and/or article preparation exist.

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APPENDIX (Figures A1 – A8)

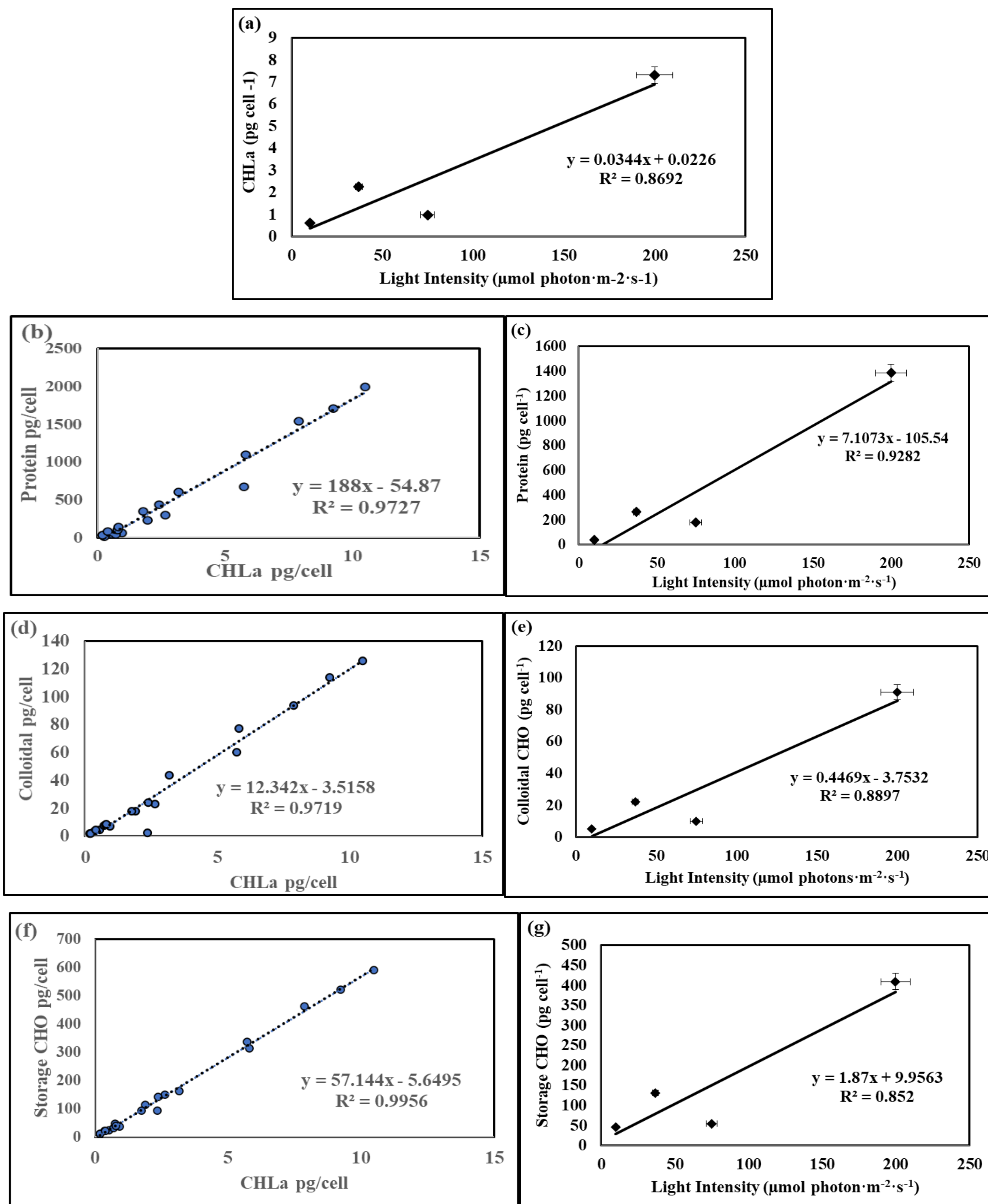

Figure A1: ***Synechococcus elongatus*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Storage carbohydrates related to CHL-a; (g) Storage carbohydrates as a function of light intensity.

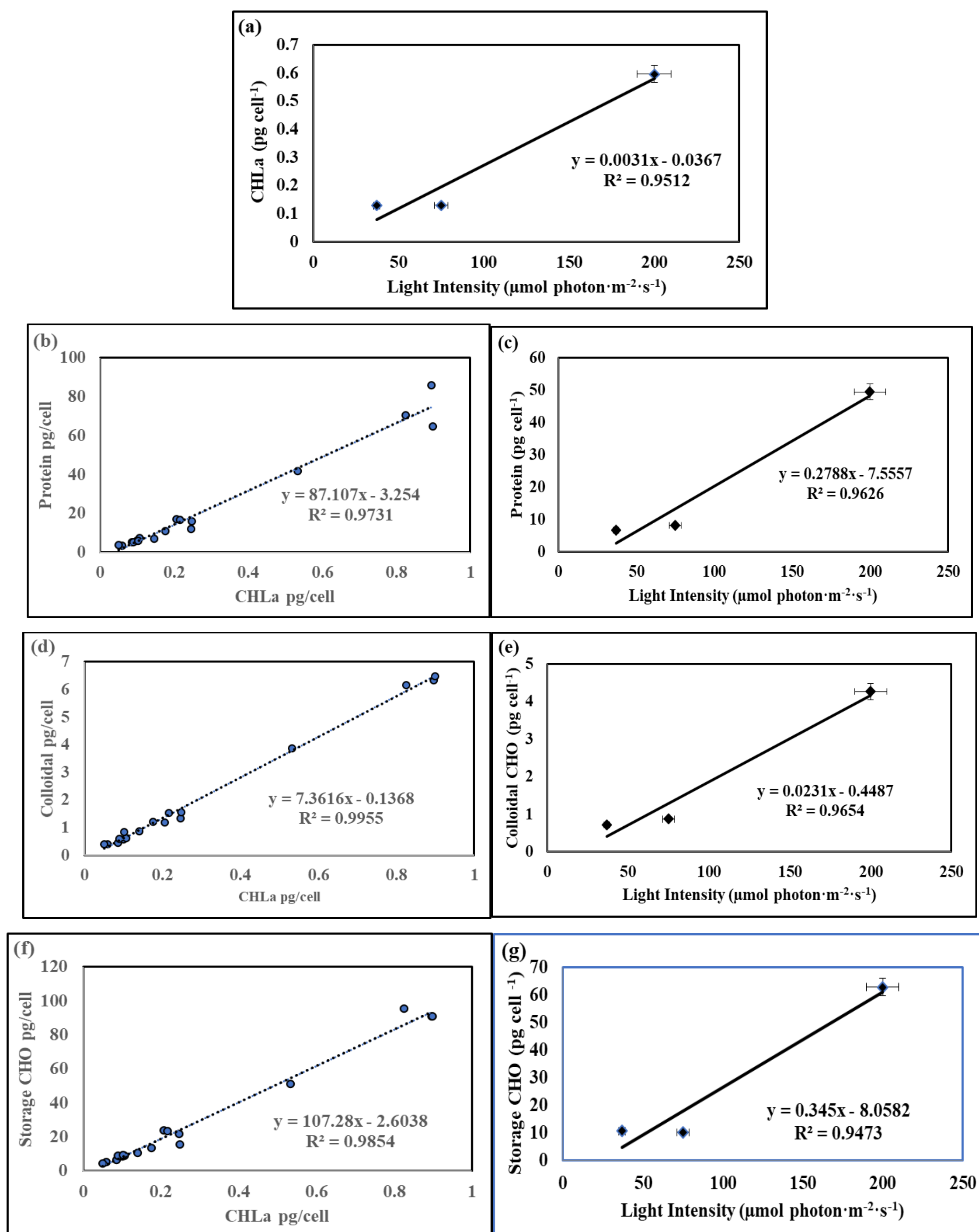

Figure A2. ***Microcystis aeruginosa*** (a) Chlorophyll-a concentration per cell as a function of light intensity. (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.

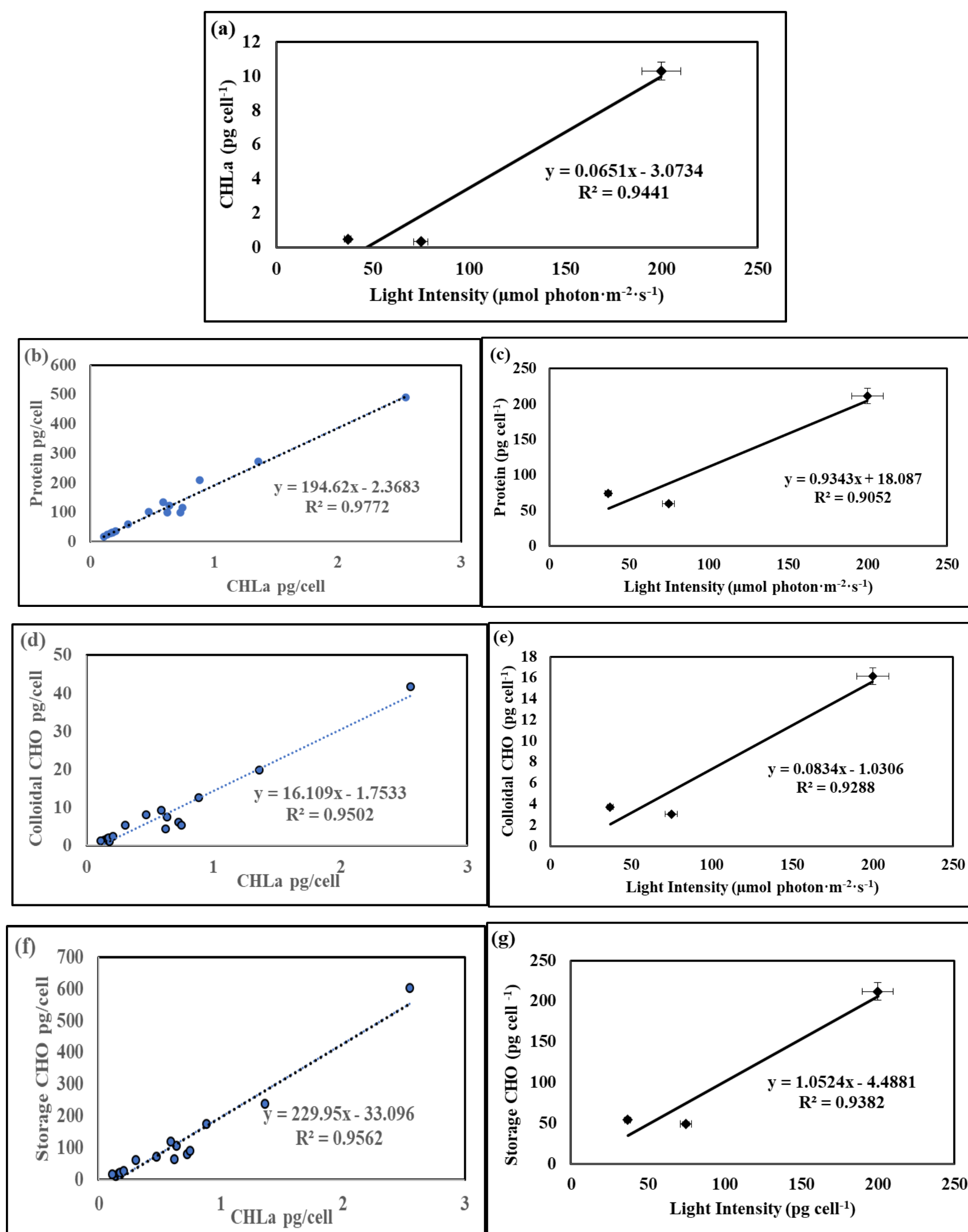

Figure A3. ***Thalassiosira weissflogii*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.

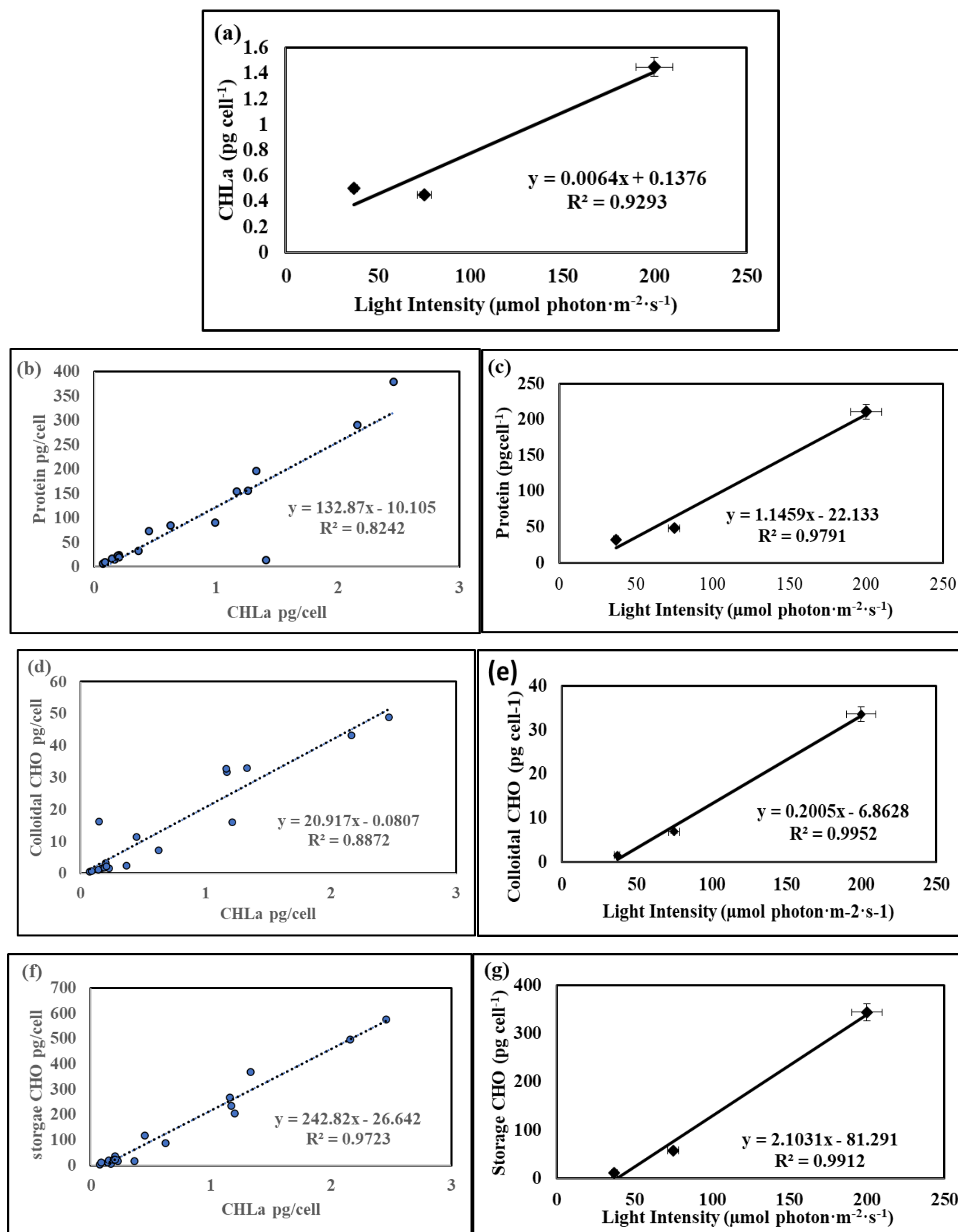

Figure A4. ***Cyclotella meneghininana*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.

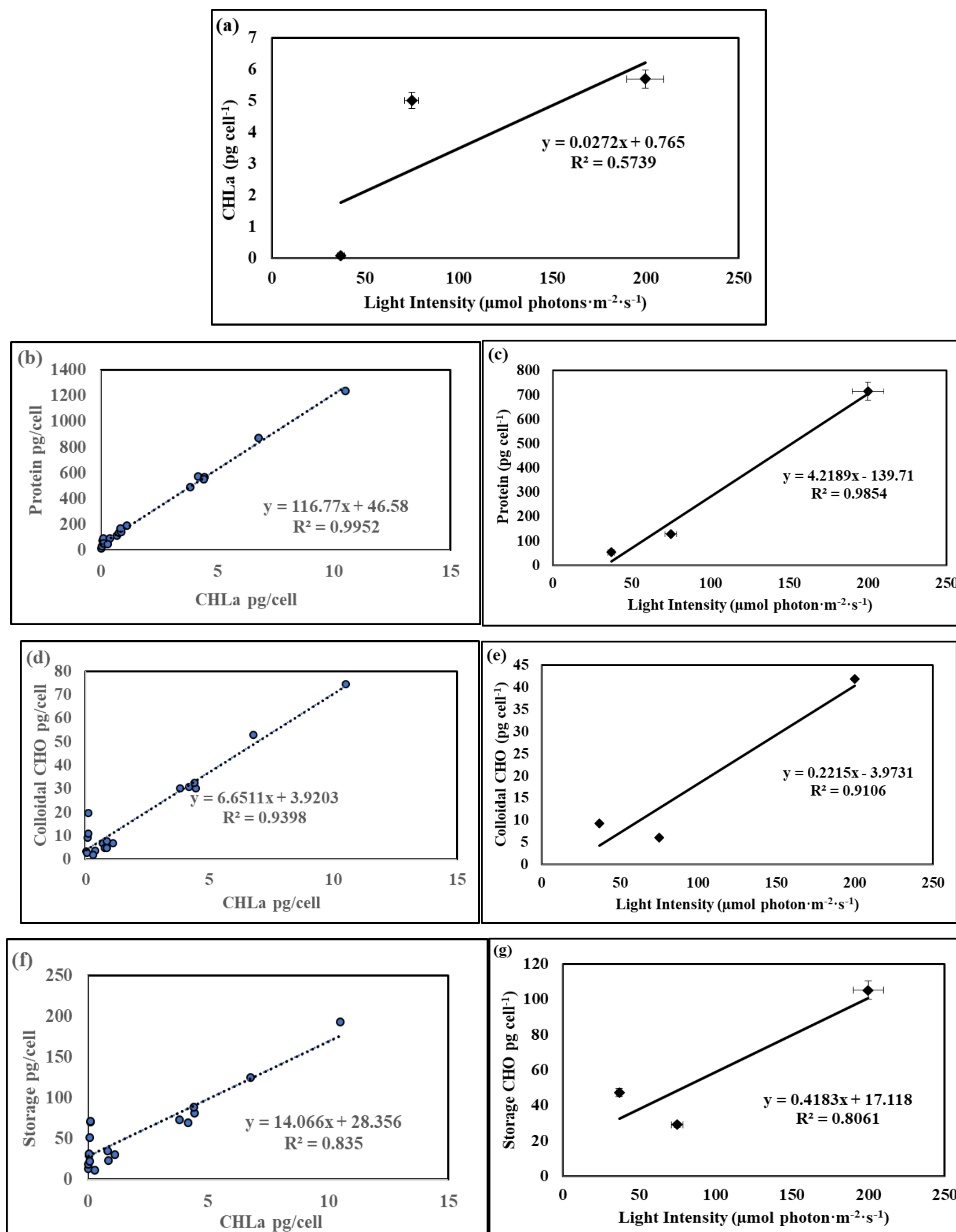

Figure A5. ***Scenedesmus quadricauda*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.

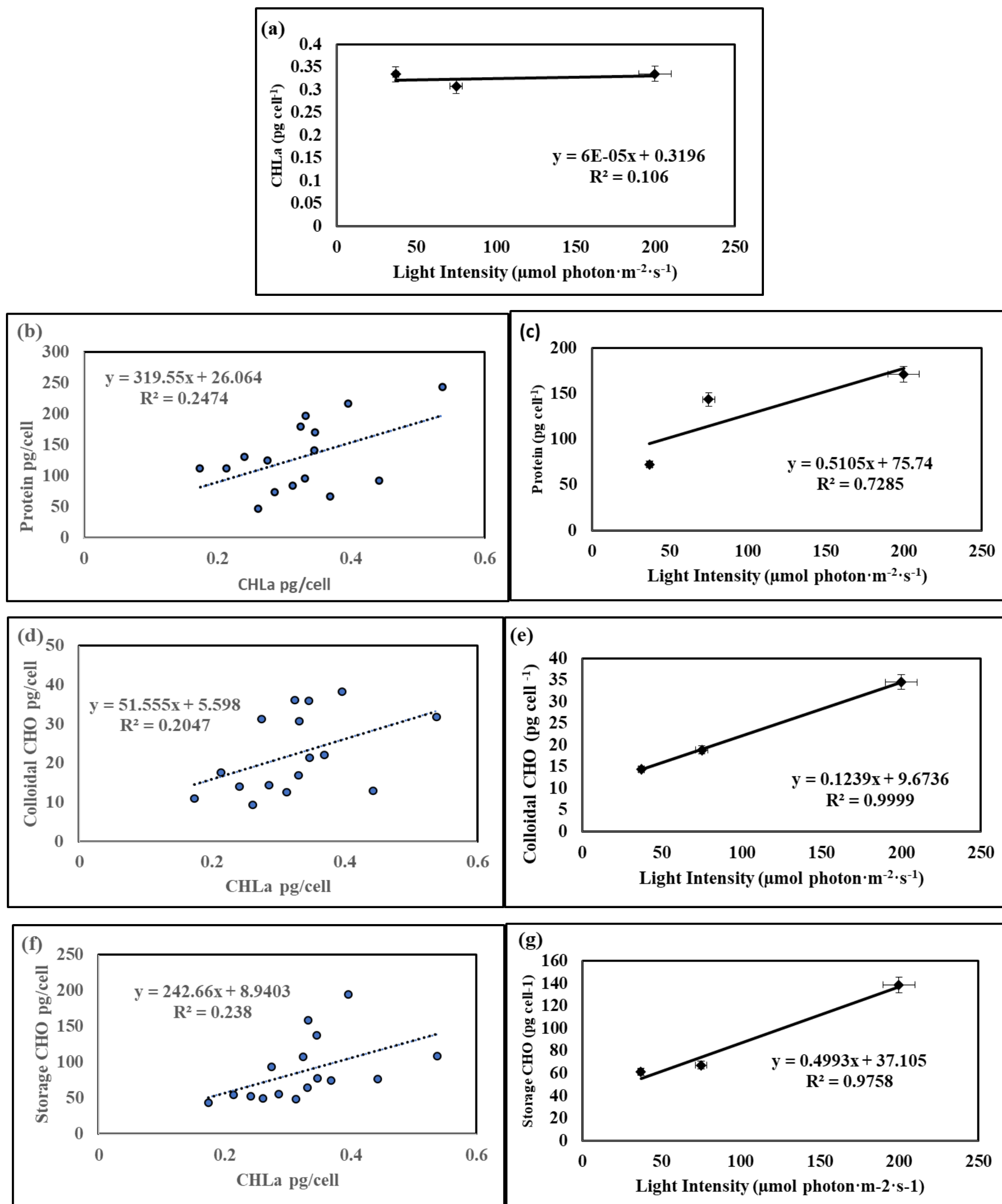

Figure A6. ***Amphidinium carterae*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.

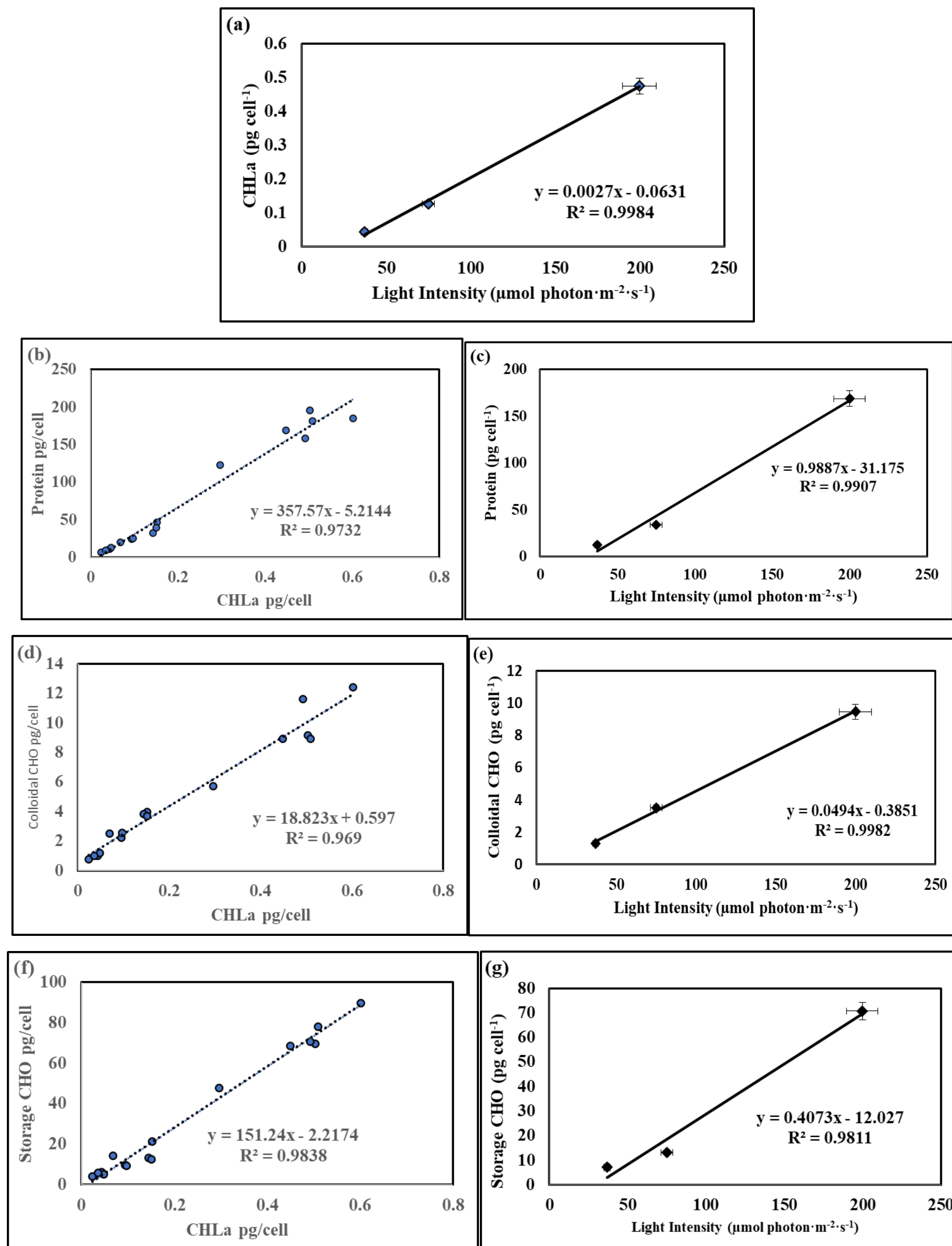

Figure A7. ***Rhodomonas salina*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity; (d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Storage carbohydrates related to CHL-a; (g) Storage carbohydrates as a function of light intensity.

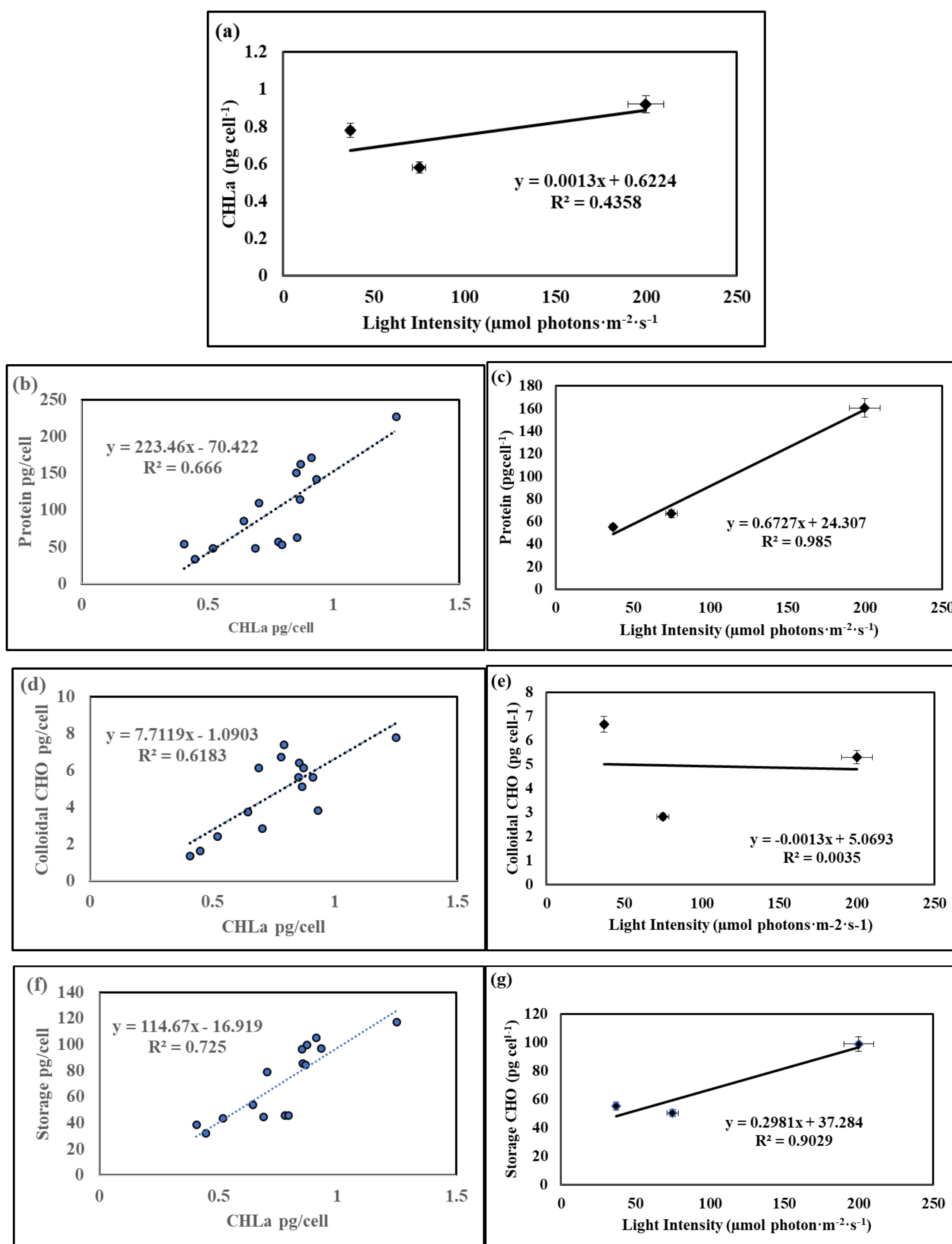

Figure A8. ***Dunaliella tertiolecta*** (a) Chlorophyll-a concentration per cell as a function of light intensity; (b) protein concentration related to CHLa; (c) Protein concentration as a function of light intensity;
(d) Colloidal carbohydrates related to CHL-a; (e) Colloidal carbohydrate as a function of light intensity; (f) Structural carbohydrates related to CHL-a; (g) Structural carbohydrates as a function of light intensity.