

Light-induced alteration in pigment signature and growth in a tropical surf zone phytoplankton assemblage (southwestern Bay of Bengal, India)

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Abstract

We present here the first study documenting the variability in growth and pigment signature with fluctuating light in a tropical surf zone (coastal Bay of Bengal) phytoplankton assemblage. In response to an 8.5 times light cut-off for three days, 50% of the particulate organic matter (POC) based growth rate ($\mu_{\text{POC}} \text{ d}^{-1}$) was maintained. Chlorophyll *a* (Chl*a*) and light-harvesting pigment contents exhibited a reverse trend without increasing POC contents suggesting that Chl*a*-based growth rate can be biased under fluctuating light. Certain commonly occurring diatoms like *Skeletonema*, *Leptocylindrus*, and *Chaetoceros* exhibited high light preference, whereas another diatom *Thalassiosira* proliferated at low light. Phytoplankton pigment signature was also monitored on an hourly basis over a day and the lowest light-harvesting pigment contents were associated with the highest photon flux densities; whereas the highest values were noticed during morning and evening hours. The ratio between photo-protective to light-harvesting pigments remained <0.5 indicating that light-harvesting pigment contents were always $>$ photo-protective pigment values and the cells were not severely light stressed. Iron (Fe) supplementation did not alter POC accumulation; photo-protective to light-harvesting pigment ratios also indicated Fe adequacy in this coastal water and the observed variability in pigment signature was entirely attributed to variable light.

Keywords: Surf zone; Bay of Bengal; Phytoplankton; Diatom; Light; Tropical; Pigment

Introduction

Surf zones, a dynamic place for transporting and exchanging materials, are areas of high energy and habitat for diverse biota (Morgan et al. 2018). Despite experiencing substantial variabilities in physicochemical parameters (Lewin and Schaefer, 1983; Kahn and Cahoon, 2012), surf zones within the exposed sandy beaches often show high productivity associated with dense diatom abundance (Campbell et al., 1988; Villac et al., 2008; Odebrecht et al., 2010; 2014; Kahn and Cahoon, 2012; Tedesco et al., 2017). Surf zones also house high faunal diversity (Fiori et al. 2022) and thick diatom abundance that may attract fish larvae at their early stages (McGregor et al. 2021). These zones are usually enriched with nutrients that are continuously replenished through groundwater seepage and coastal sources and the bathymetric rip currents retain the phytoplankton stock (Campbell and Bate, 1988; Kahn and Cahoon, 2012). Continuous-wave action forms foams and creates turbulence with loose sand within the surf zones and photon flux density may vary over a wide range ($440 - 2160 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Cahoon et al., 2017). Besides, light penetration in this water can be impacted through land runoff mediated sediment supply, prolonged overcast (Prasannakumar et al., 2010), etc. However, an earlier study reported that phytoplankton exhibited a significant increase in growth rate within the surf zone most likely due to enhanced exposure to light and higher nutrient uptake potential (Savidge, 1981). Wind-driven currents and wave action may carry the surf zone phytoplankton to the nearshore zone (Fujimura et al., 2018) and also may impose light stress by wind-driven vertical mixing. Surf zone diatoms also showed an interesting feature by producing sticky mucus to remain attached to the sediment during the nighttime and releasing back the mucus with the onset of day and float with the foamy air bubbles (Talbot et al., 1990). Hence, the phytoplankton inhabiting this high-energy area must possess a photoacclimation ability to cope with fluctuating light environments.

Light is crucial for phytoplankton growth, however, irradiance beyond the photosynthetic requirement can be harmful causing photodamage and reduced growth (Lavaud et al., 2002; Ledford and Niyogi, 2005; Lavaud, et al, 2007). In general, coastal phytoplankton possess effective photoacclimation mechanisms to adjust to fluctuating light without compromising photosynthetic rate. Depending on the timescale, the defense mechanisms can either be a short-term or long-term photoacclimation process (Raven and Geider, 2003). The former is usually used over a broad range of time scales from seconds to days; whereas the latter occurs within hours to seasons by changing the structures of photosynthetic apparatus (photosystem I and II) and some physical composition mostly controlled by genes to overcome light stress (Raven and Geider, 2003; Johnsen and Sakshaug, 2007; Brunet et al., 2011). For the short-term photoacclimation process, phytoplankton induce photoprotection via a pigment cycle (Dubinsky and Stambler, 2009) to regulate photosynthetic performance (Lavaud, 2007).

When the light level increases beyond the growth-saturating level (depending on the habitat), phytoplankton produce higher photoprotective pigments than the light-harvesting pigments to maximize photoprotection and it reverses when the light level becomes limiting impeding photosynthesis (Falkowski and Chen, 2003).

The pigment pool as well as the community structure of phytoplankton is sensitive and usually changes in response to daily solar light fluctuations (Brunet et al., 2008; Biswas et al., 2017). Phytoplankton and other photosynthetic organisms use some particular photoprotective pigments (xanthophylls) to dissipate extra energy as heat via a non-photochemical quenching process (Niyogi and Truong, 2013). Some groups of phytoplankton including diatoms mostly use diadinoxanthin which undergoes de-epoxidation in response to light stress forming diatoxanthin via a one-step conversion process within the photosystem II of the light-harvesting pigment complex (Lavaud et al., 2002; Brunet and Lavaud, 2010). Under excess light, light-harvesting pigment synthesis is down-regulated to minimize further light capture and control electron flow within the thylakoid. Simultaneously, the cell up-regulates the synthesis of photoprotective pigments and the lowering of pH within the thylakoid initiates the conversion of diadinoxanthin to diatoxanthin to start non-photochemical quenching (Lavaud and Kroth, 2006). Diatoms possess a well-developed xanthophyll cycle compared to other phytoplankton groups with substantial interspecific variability (Lavaud, 2007). The effective functioning of xanthophyll cycles and non-photochemical quenching protect the cell from photodamage and thus plays a key role in the success of diatoms in a wide range of aquatic environments (Lavaud et al., 2007).

The seasonal reversal of monsoon winds significantly impacts the physicochemical parameters of the coastal Bay of Bengal. The surf zones usually receive a higher quantity of nutrients and sediment flux compared to the nearshore zones. During the summer monsoon, a significant amount of freshwater, nutrients, and sediment load reach this coastal water. Prolonged overcast and massive sediment flux during the summer monsoon months (June - October) also hinder solar light penetration and can slow down photosynthetic performance in coastal phytoplankton. This was evident from the satellite-derived light attenuation coefficient (K_d) and PAR data, that light availability can potentially limit primary production in the northern and coastal parts of this bay during the summer monsoon months (Prasannakumar et al., 2010). Jyothibabu et al., (2018) conducted a 12-day time series observation in the Bay of Bengal during the summer monsoon and noticed a significant decrease in photon flux density under prolonged cloud coverage. However, there is no study available so far showing the responses of surf zone phytoplankton to changing light. In the present study, we show the photoacclimation capacity using pigment signature and growth responses of a diatom-dominated

phytoplankton community to variable irradiance under high and moderate nutrient concentrations on different time scales. Since iron (Fe) plays a key role in the photosynthetic electron transport chain (Raven et al., 1999) and its inadequacy enhances the chances of photodamage (Petrou et al., 2011), we also have tested the impacts of added Fe. Although, this is unlikely that Fe is limited in the coastal waters. The observed changes in biomass, community composition, and pigment signature with changing light and Fe supplement are discussed.

Materials and Methods

Experimental planning

This study was conducted on the natural phytoplankton communities from a coastal station (Visakhapatnam, 17°41' N; 83°13' E) (Fig. 1A) situated on the southwestern Bay of Bengal. The beaches in this area are the sandy exposed type with some rocks. The water samples for conducting the experiments were collected directly from the beach where the water depth is nearly <1.0 m. The first experiment (Fig. 1B) was conducted in August (Exp-IA) and repeated in January (Exp-IB) to document the variability in phytoplankton pigment (LHP, PPP) synthesis and POC-based growth rate under significant interruption in surface light supply. Nutrient concentrations were higher in Exp-IA than that in Exp-IB (Table 1) and the nitrogen to silicate (N: Si) ratio was close to 1:1 for all experiments. In Exp-IA, 200 nM Fe was added and in Exp IB, 50 nM and 200 nM Fe levels were considered. The second experiment (Exp-II) was conducted in April to document the changes in pigment and protein synthesis under changing solar photon flux density over a day [7hrs -17:30hrs]. The detailed information of these two experiments has been described individually in the following sections. Please note that the study location (17°41' N; 83°13' E) is within the tropical low latitude region where the natural day-night cycle varies nearly in a 12:12 hrs pattern with a very minor variability from season to season. We have done all the experiments under natural day-night periods and not using any artificial light sources. The low light levels were achieved using screens and are explained below.

Experiment I (Exp-I)

Coastal water was collected into the acid cleaned 20 L Nalgene bottles (USA) and a 200 µm mesh was used as a prefilter to remove large grazers and particulates. The initial nutrient [nitrate+nitrite (N), silicate (Si), and phosphate (P)] concentrations were measured and additional nutrients were added (Table 1) to avoid nutrient limitations that may induce photodamage. The seawater was uniformly distributed in twelve acid-cleaned Nalgene bottles of 2 L volume. Six bottles were incubated under 100% solar light (Surface light =SL) without (SL-Cont) and with Fe (SL+Fe). Another six bottles were

treated as “low light” (LL) treatment and were also with (LL+Fe) and without Fe (LL-Cont). The bottles were incubated inside a water bath and covered with a black nylon mesh to mimic the LL conditions which were 6 times (Fig. 2a) and 10 times less than the first and the second experiments, respectively (Fig. 2b). Both SL and LL intensities were measured from morning to evening using a digital “Lux meter” (METRAVI 1330, WB, India) fitted with a silicon photodiode filter. The measured light intensities in “Lux” were converted to $\mu\text{mol Einstein m}^{-2} \text{s}^{-1}$ by using a conversion factor of 0.019 for solar light conversion adopted from Thimijan and Heins (1983). The average highest photon flux densities under SL were $\approx 1600 \mu\text{mol Einstein m}^{-2} \text{s}^{-1}$ between 8:30 hrs to 14:30 hrs during the Exp-IA and $\approx 1500 \mu\text{mol Einstein m}^{-2} \text{s}^{-1}$ between 10 hrs to 15:00 hrs during Exp-IB. The corresponding LL highest values were ≈ 200 and $170 \mu\text{mol Einstein m}^{-2} \text{s}^{-1}$ for the same timing during Exp-IA and Exp-IB, respectively. Morning and evening hours were associated with 3 to 4 times lower photon flux densities. In both experiments, the LL intensities were nearly 8 times lower than the SL. The bottles were incubated for 3 days (phytoplankton from this tropical coast usually show exponential growth phase within this time as was noticed in our previous experiments, Biswas et al. 2017; Bandyopadhyay and Biswas, 2021 and references therein) and were gently mixed twice a day to avoid biomass settlement at the bottom. After incubation, subsampling was done for measuring POC and PON, phytoplankton pigments using HPLC, and phytoplankton community composition (only for Exp-IB) using microscopy.

Experiment-II (Exp-II)

Exp-II (Fig. 1B) was conducted only under SL variability without any LL effect to understand the hourly changes in phytoplankton pigments and other parameters over a day (from 7:00 hrs - 17:30 hrs) with natural solar light variability. Unlike Exp-IA and Exp-IB, no nutrients were added (Table 1) and sampling was done after 12 hrs of incubation and continued on an hourly basis. A total of 16 bottles were incubated and subsamples were not collected from the same bottles to avoid any volume loss that may impact the phytoplankton community; individual bottles in duplicate were used for each hour's subsampling which includes phytopygments and total protein analyses.

Analytical methods

The major nutrients (N, Si, P) were analyzed following the colorimetric method using the standard protocol of Grasshoff et al., (2009). Standard solutions of known nutrient concentrations were measured in serial dilution and color was developed and optical density was measured by the spectrophotometer (Shimadzu UV-Vis 1800, Japan). To quantify particulate organic carbon (POC) and nitrogen (PON), an aliquot of water sample (50-200 mL) was filtered onto a pre-combusted GF/F

(25 mm, Adventac, Japan) filter and stored at -20 °C till analysis inside a 47mm Petri-plate. Before analysis, the filters were dried overnight at 60 °C and then exposed to HCl fumes to remove the particulate inorganic carbon if any, and oven-dried again for 5-6 hrs. POC and PON contents were measured by using an elemental analyzer (Thermo, Delta V+) (Sharp, 1974). The POC-based growth rate was calculated using the following formula:

$$\text{Growth rate } (\mu_{\text{POC}} \text{ d}^{-1}) = [\ln(\text{final POC}) - \ln(\text{initial POC})] / \text{incubation time (day)}$$

Total protein content was quantified following the standard Lowry's colorimetric method (Lowry et al., 1951). An aliquot of the sample (100 mL) was filtered onto a 0.2 µm polycarbonate membrane (47 mm; Millipore) and stored inside a cryo-vial at -20 °C until analysis. Thereafter, filters were washed with 6% hot (60°C) trichloroacetic acids to precipitate the protein. A tissue homogenizer (Ultra Turrax, Germany) was used to break the cells followed by centrifugation (Eppendorf, Germany). The supernatant was discarded and the pellets were dissolved in a hot (55°C) alkaline reagent (carbonate solution added with copper sulfate). 1:2 Folin's reagent was used to develop the blue color and optical density was measured at 660 nm wavelength in a UV-Vis spectrophotometer (Shimadzu 1800, Japan). A standard protein solution was prepared using Bovin Serum Albumin (Sigma Aldrich, USA) and an alkaline reagent; a serial dilution method was opted to prepare different concentration solutions. A standard equation was derived against the measured optical density and the unknown concentrations were calculated from the slope of the linear equation.

For analyzing phytoplankton, 1 L of water sample was preserved with Lugol's iodine solution (1%, W/V) and kept in the dark for a minimum of 48 hours for complete settlement. The supernatant was discarded with the help of a siphoning tube which was attached to a 10 µm mesh to avoid any cell loss. The siphoning was done in two steps and the final volume of the phytoplankton cell mixture was made up of 30-40 mL and kept in a closed bottle at 4°C until analysis. Phytoplankton enumeration was done by taking a 1 mL aliquot under 400× magnification using an Olympus microscope fitted with a camera. Phytoplankton enumeration was done following the manuals of Desikachary, (1987) and Hasle and Syversten, (1997), and the total abundance was expressed in terms of relative percentage using the following formula: (Cell abundance of a particular phytoplankton species*100)/Total cell number.

For phytoplankton pigment analysis, an aliquot of (100 -300 mL) seawater sample was filtered onto GF/F filters (25 mm, Adventec, Japan) using a vacuum pump (Rocker 600, Tarsons, India) (< 200 mb) under minimum light to avoid photodegradation. Soon after the filtration, the filters were folded in the quarter and wrapped inside an aluminum foil and kept frozen at -20°C until analysis.

Prior to analysis, pigment extraction was performed by soaking the filters overnight in 3-5 mL ice-cold acetone (90% HPLC grade, Merck chemicals) inside the amber-colored bottle with a screw cap. The filters were homogenized (Ultra Turrax, Germany) followed by cold centrifugation (Eppendorf, Germany) and the supernatant was collected for quantifying the pigment following the method of Heukelem and Thomas, (2001). An HPLC (Agilent Technology; Series 1200) was used to quantify the pigments using a photo-diode-array detector and C8 column (Zorbax). The concentrations of pigments were calculated using the standard pigments procured from Sigma Aldrich (USA) and DHI (Netherlands). Diatoxanthin index (DT-index) was calculated using the formula diatoxanthin/(diatoxanthin+diadinoxanthin) following Lavaud et al., (2002).

Statistical analysis was done using a two-factor Analysis of Variance (ANOVA) with replication at a 95% confidence level ($p=0.05$) in MS office excel. A multiple regression analysis was done using MINITAB 16.

Results

The variability in light supply exhibited considerable impacts on the growth and pigment signature of the experimental phytoplankton communities.

Experiment-I

The initial POC concentration in Exp-IA ($154 \mu\text{mol L}^{-1}$) (Fig. 2A, 2B) was higher ($43 \pm 7.4 \mu\text{mol L}^{-1}$) than in Exp-IB and despite a significant difference in nutrient levels, POC-based growth rates in both experiments were comparable (Fig. 2C and 2D). In Exp-IA (Fig. 2A) SL-Cont treatments, the POC content doubled ($\approx 310 \mu\text{mol L}^{-1}$) from the initial value (corresponding to a growth rate of $0.34 \mu_{\text{POC}} \text{d}^{-1}$), whereas under LL conditions it was 1.28 times lower. And no impact of Fe addition in any treatment was noticed ($p > 0.05$). In Exp-IB, the growth rate was 1.8 times (Fig. 2D) higher ($p < 0.05$) under SL than that of LL treatments. In Exp-IA, PON concentrations (Fig. 2E) showed negligible variability within the treatments ($p > 0.05$), whereas in Exp-IB (Fig. 2F), it was higher under SL ($13.8 \mu\text{mol L}^{-1}$) compared to LL treatment ($p < 0.05$). The initial values of POC: PON ratios in Exp-IA (Fig. 2G) and IB (Fig. 2H) were close to the classical Redfield values (6.63) and increased in all final samples compared to their initial value and showed a noticeable impact of light ($p < 0.05$).

Total pigment concentrations (Fig. 2I, 2J) showed a noticeable difference in response to variation in light ($p < 0.001$) but not to Fe addition ($p > 0.05$). In Exp-IA, the minimum Total pigment content ($5.47 \mu\text{g L}^{-1}$) was in SL-Cont treatment (Fig. 2I) and a 1.71 times increase was noticed in the Fe-supplemented samples ($p < 0.05$). In the LL-treated cells, Total pigment contents were more than three times higher ($20.3 \mu\text{g L}^{-1}$ in LL-Cont and $34.76 \mu\text{g L}^{-1}$ in LL+Fe treatment) than that of SL

treatments. In Exp-IB, the average Total pigment contents in the SL treated sample ($1.82 \mu\text{g L}^{-1}$) (Fig. 2J) doubled under the LL condition ($p < 0.001$) without any significant change due to Fe addition. Chla concentrations (Fig. 3A, 3B) changed substantially with changing light ($p < 0.05$). The minimum Chla values were noticed in the SL-Cont ($2.8 \mu\text{g L}^{-1}$ in Exp-IA and $4.91 \mu\text{g L}^{-1}$ in Exp-IB) and the highest value was found under LL treatments ($p < 0.02$). Consequently, Chla: POC ratios (Fig. 3C, 3D) were significantly higher (5 and 4 times higher in Exp-IA and Exp-IB, respectively) under LL compared to that of SL treatments without any effect of Fe ($p > 0.05$). The average light-harvesting pigment contents under SL ($6 \mu\text{g L}^{-1}$, $10.23 \mu\text{g L}^{-1}$) were 4 and 2 times lower relative to the LL treatments in Exp-IA (Fig. 3E) and Exp-IB (Fig. 3F), respectively. The average photoprotective pigment concentrations showed light significantly higher values under LL relative to SL (Fig. 3G) in Exp-IA, however, such difference was absent in Exp-IB (Fig. 3H). In both experiments, the ratio of light-harvesting to photoprotective pigment (Fig. 3I, 3J) showed $> 80\%$ higher values under SL light conditions ($p < 0.05$) than that of LL, and no impact of Fe addition was noticed ($p > 0.05$).

The minimum concentrations of Fuco (Fig. 4A, 4B) and Chlc₂ (Fig. 4C, 4D) were seen in SL treatments and increased considerably ($p < 0.05$) under LL conditions. Fuco (Fig. 4A, 4B) contents also increased ($p < 0.05$) to Fe addition under both light treatments in Exp-IA and Exp-IB. The ratio of Fuco: Chla (Fig. 4E, 4F) and Chlc₂:Chla (Fig. 4G, 4H) showed no statistically significant difference in response to light level or Fe addition. The concentrations of diadinoxanthin showed higher values under LL compared to SL in Exp-IA (Fig. 5A) ($p < 0.05$) but no such difference was seen in Exp-IB. The ratio of diadinoxanthin: Chla (Fig. 5C; 5D) decreased under LL relative to the SL ($p < 0.05$). No diadinoxanthin was detected in Exp-IA indicating the absence of non-photochemical quenching. β -carotene contents in Exp-IA (Fig. 5E) increased ($p < 0.05$) in response to decreased light and Fe supplementation and in Exp-IB (Fig. 5F) this trend was noticed only under LL. The ratios of β -carotene: Chla showed the highest values in SL-Cont treatment in both experiments (Fig. 5G, 5H) and in Exp-IB the light-induced impact was significant ($p < 0.05$) but not for Exp-IA ($p > 0.05$).

The microscopic analysis of the phytoplankton community revealed the dominance of diatoms in both experiments. During Exp-IA, 50% of the community was dominated by large chain-forming centric diatoms *Skeletonema* sp., a common genus repeatedly reported from this coastal water. The next contributing genera were *Leptocylindrus danicus* (12.5%), *Thalassiosira* sp. (8.3%), *Chaetoceros* spp. (6.3%), and *Pseudo-nitzschia* sp. (6%). The final samples were not analyzed for this experiment. During Exp-IB (Fig. 6), the initial community was also dominated by *Skeletonema* sp. (29.5%), *Chaetoceros* (22.72%), and *Helicotheca* (24.32%) with a relatively lower number of *Leptocylindrus danicus* (10%) and *Thalassiosira* (5%). Under SL-cont treatment, the relative abundance of

Leptocylindrus danicus (15.42%) and *Skeletonema* sp. (33.79%) increased, whereas *Chaetoceros* (18.82%) and *Thalassiosira* (0.45%) decreased. 50 nM Fe addition under SL doubled the relative abundance of *Chaetoceros* along with the slight increase in the relative abundance of *Skeletonema* sp.. Under SL, in response to 200 nM Fe treated samples the former showed a 37% decrease, and the latter increased by 31%. *Helicotheca* relative abundance was reduced to half in both Fe treated samples. *Leptocylindrus danicus* did not show any definite trend in SL; however, decreased significantly ($p < 0.05$) under LL condition from 6.3% (LL-Cont) to $< 1.5\%$ (LL-200nM Fe). The average relative abundance of *Skeletonema* sp. was about 20% under LL-Cont and increased 37% and 58% in response to 50 and 200 nM Fe addition. *Chaetoceros* did not show any major change between LL-Cont and 50 nM Fe treatment; though, increased slightly with 200 nM Fe addition. Interestingly under all LL treated samples the relative abundance of *Thalassiosira* was higher than that of SL ($p < 0.05$) and decreased to almost half in both Fe+LL treated samples. *Helicotheca* abundance was higher under LL and showed enhancement under Fe treated samples compared to LL-Cont.

Experiment- II

During this experiment, the phytoplankton community was diatom-dominated. However, the community was different than the first experiment. Here *Thalassiosira* was the major contributing species (79.5%) and *Skeletonema* sp. became the second dominant species (6.7%) followed by *Pseudonitzschia* sp. (5%), *Chaetoceros* spp. (4.8%), and *Nitzschia* spp. (1.5%). The photon flux density varied from nearly 450 $\mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$ from 7 am into 1300 $\mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$ between 11 -13:00 hrs. The minimum photon flux density (15 $\mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$) was recorded during 17:30 hrs. In this experiment, most of the nutrients were consumed with a parallel increase in Chl a and total pigment values (Table 1). There was a significant impact of N depletion on pigment synthesis patterns. The maximum drawdown (equivalent to $3.76 \pm 0.4 \mu\text{M}$) of N was noticed during the morning hours. During 13:00 - 14:30 hrs (highest photon flux density) the concentrations of N became $< 1 \mu\text{M}$. Si uptake was maximum from 7:00 -8:30 hrs and again in the evening after 14:30 - 16:30 hrs. P did not get exhausted but depleted significantly with the progression of the day and increasing biomass.

Marker pigments like fucoxanthin, Chl c_2 , diadinoxanthin, and β -carotene were noticed with a very less amount of lutein, zeaxanthin, and Chl b ; but due to their insignificant contribution was not discussed further. The concentrations of total pigment (Fig. 7A), Chl a (Fig. 7B), and light-harvesting pigment (Fig. 7C) increased significantly over the day with a clear decrease around midday under the highest light intensities. The initial total pigment concentration ($17.22 \pm 1.32 \mu\text{g L}^{-1}$) doubled within 12 hours of incubation and kept increasing in the next 1.5 hrs followed by a drop around midday and enhanced further ($43.2 \pm 1.12 \mu\text{g L}^{-1}$) by 17:30 hrs. Chl a concentration got doubled at 7:00 hrs from its initial

value ($11 \pm 0.9 \mu\text{g L}^{-1}$) followed by a slight enhancement (6%) in the next hours and decreased considerably ($\approx 55\%$) during the highest photon flux density ($1300 \mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$ at 11:30 hrs) (Fig. 7B). Light-harvesting pigment concentration got more than doubled at 7:00 hrs from its initial value ($16.8 \mu\text{g L}^{-1}$) and decreased by 44% by midday followed by a further enhancement with decreasing photon flux density. The concentrations of photoprotective pigment (Fig. 7D) enhanced with increasing photon flux density and the highest value ($1.7 \pm 0.58 \mu\text{g L}^{-1}$) was observed at 16:00 hrs and was corresponding to a 1.86 times enhancement from its initial value. The ratios of photoprotective to light-harvesting pigments (Fig. 7E) were always < 1 indicating higher light-harvesting pigment synthesis relative to photoprotective. The ratios of photoprotective to light-harvesting pigments varied positively with photon flux density from 8:30 - 14:30 hrs ($R^2=0.878$; $p<0.05$) and showed a significant negative correlation ($R^2=0.80$; $p<0.05$) with N concentrations.

Fucoxanthin concentrations showed a similar pattern to Chla and decreased significantly with increasing light (Fig. 7F) and increased again by 35% at 17:30 hrs. The ratio of fucoxanthin:Chla increased gradually and stabilized in the evening (Fig. 7G). The final concentrations of Chlc₂ (Fig. 7H) showed 87% higher values relative to its concentration at 7:00 hrs. The ratios of Chlc₂:Chla (Fig. 7I) increased gradually with time indicating the relatively higher level of down-regulation in Chla synthesis in response to increasing photon flux density. The ratios of Chlc₂:Chla showed a steady enhancement and the final value doubled at 17:30 hrs.

Diadinoxanthin contents increased steadily with time and the final value ($0.88 \pm 0.09 \mu\text{g L}^{-1}$) was 77% higher than the concentration noticed at 7:00 hrs (data not shown here) without any decrease around midday. Diatoxanthin was detected in this experiment and was below the detection limit at 7:00 hrs, but increased with increasing photon flux density reaching its highest value ($0.58 \mu\text{g L}^{-1}$) at 14:30 hrs corresponding to the highest photon flux density ($1300 \mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$). Diatoxanthin-index (Lavaud, 2002) showed a direct linear correlation with increasing photon flux density up to 13:00 hrs (Fig. 7J) ($R^2=0.91$, $p < 0.05$) and started to decrease after 16:00 hrs when photon flux density decreased to $\approx 200 \mu\text{mol Einstein m}^{-2} \text{ s}^{-1}$. Diatoxanthin-indices were negatively correlated ($R^2=0.986$; $p<0.05$) with N concentrations and photon flux densities. A multiple regression analysis was done including both N concentrations and photon flux density and it showed that reduced nitrogen supply and an increase in photon flux density both were associated with diatoxanthin production.

The initial protein concentration ($0.62 \pm 0.09 \text{ mg L}^{-1}$) showed an almost 43% increase at 10:00 hrs and decreased slightly (4.5%) during the midday period (Fig. 7K). With decreasing solar light, protein content started to increase further finally reaching a value of $1.29 \pm 0.07 \text{ mg L}^{-1}$ at 17:30 hrs. Within 11:30 hrs -13:00 hrs, the concentrations of protein remained almost constant (0.88 mg L^{-1}). The

ratio of light-harvesting pigment: protein contents showed (Fig. 7L) the highest value at 7:00 hrs and decreased considerably from 10:00 hrs onward. The ratio again increased at 14:30 hrs with a fall in the following hrs.

Discussions

The presence of fucoxanthin, chlorophyll c_2 (Chl c_2), diadinoxanthin, and β -carotene as major pigments indicated the dominance of diatoms in the experimental phytoplankton community. Moreover, the ratios between Fuco: Chl a (0.49 ± 0.16) also strengthened this fact. The microscopic analysis of Exp-IB also showed the dominance of diatoms and is described separately at the end of the section. Very low concentrations of zeaxanthin and chlorophyll b were also present only in Exp-IA indicating the presence of green algae and probably some cyanobacteria too. However, those were not found in Exp-IB. Since the contributions of green algae and cyanobacteria were $<1\%$, they are not further discussed. The concentration of light-harvesting pigment was calculated by summing up the values of Chl a , Chl c_2 , Chl b , and zeaxanthin; The concentration of photoprotective pigment was calculated by combining the values of diadinoxanthin, diatoxanthin, β -carotene, and zeaxanthin.

Impacts of light on growth and pigment signature

Marine phytoplankton can be exposed to highly variable light intensities from a light-limiting to a light-inhibited environment over a day due to dynamic solar radiation, vertical mixing, cloud coverage, and turbidity. Therefore, to maintain steady-state photosynthesis under variable light intensities they possess a wide range of photo-acclimation processes (reviewed by Geider, 1993) which is likely to be linked to their ecological niches (Lavaud et al., 2007). Surf zones are usually occupied by high diatom density as has been reported from the studies on other tropical beaches (Tedesco et al., 2017). There are some particular community distribution patterns seen in many Brazilian tropical beaches where the dominance of certain species was related to the type of beaches (Villac et al., 2008). The exposed beaches (with turbulent surf zone where high energy is expended to transport water and sediment) exhibited the dominance of certain diatoms, whereas the arrested beaches (where wave action is relatively less creating low turbulence) showed some other genera. Such type pattern can also be related to their photophysiology. A similar study is rare on Indian sandy beaches although India has more than 7000 km of coastline. The scorching sun in the tropical exposed sandy beaches can be deleterious for the cells and thus the diatoms inhabiting this dynamic zone possess a high level of light adaptability. The community we encounter is quite different from the one reported from the other tropical beaches. However, how these diatoms can cope with such high light variability is poorly understood. "Photo-acclimation" is a cumulative term used to address a combination of cellular

processes related to the changes in physiological, biochemical, and biophysical activities of the cell exposed to variable light (Anning et al., 2000; Raven and Geider, 2003). Our results demonstrate that the surf zone diatoms from this tropical sandy beach possess very high photo-acclimation capability by altering their pigment pool. Among different phytoplankton groups, diatoms are exceptionally adapted to fluctuating light and this could be a reason for their ecological success (Brunet and Lavaud, 2010). In the case of Exp-IA, a significant increase in photoprotective pigment concentrations under SL (Fig. 3G, 3H) was a clear sign of the up-regulated photo-protection process enabling avoidance photodamage. Several cultural experiments reported a similar trend (Schlüter et al., 2000; 2006). Under such conditions, the flow of electrons within the chloroplast reaches its maximum value and hence creates excess electron flow and oxidative stress (Ledford and Niyogi, 2005; Asada, 2006). In the following steps, the cell tries to down-regulate the flow of electrons within the thylakoid to reduce light capture which is a passive process without any biochemical or genetic control. Therefore, the light-stressed cells down-regulate the total pool of light-harvesting pigments to reduce the total electron flow (Rodriguez et al., 2006).

Phytoplankton usually adjust their intracellular pigment pool to cope with fluctuating irradiance levels (Brunet et al., 2011). In diatom cells, all light-harvesting pigments (Chl*a*, Chl*c*, and fucoxanthin) form complexes in the light-harvesting photosystem (Friedman and Alberte, 1984) and therefore respond similarly to fluctuating light. Our experiments also documented the same trend. In the first experiment under LL, high light-harvesting pigments, Chl*a* as well as very low photoprotective to light-harvesting pigment ratio (Fig. 3E- 3J) indicated severe light limitation. Reduced POC content (Fig. 2A, 2B) indicated light-limited photosynthesis. This is likely that under such light-limited conditions, the experimental phytoplankton community maximized light-harvesting pigments and minimized photoprotective contents; despite photoacclimation, the POC-based growth rates have reduced under LL conditions (Fig. 2C, 2D). A similar trend was noticed in the marine diatom *Phaeodactylum tricorutum* (Nymark et al., 2009). The authors showed that under LL, the genetic expression responsible for Chl*a* biosynthesis was rapidly reduced. Anning et al., (2000) also reported a significant increase in Chl*a* content in a marine diatom when the high light acclimated cells were shifted to a low light environment. The increased ratios of fucoxanthin:Chl*a* (Fig. 4C, 4D) and Chl*c*₂:Chl*a* (Fig. 4G, 4H) usually indicate light limitation and were noticed in both experiments. A similar trend with increasing depth indicating light-limited conditions was seen in some field studies (Descy et al., 2009) and is consistent with our observation.

Chl*a*:POC ratio is usually inversely correlated with light intensities and this is a well-known photo-acclimation strategy (Wang et al., 2009). Under LL, the high Chl*a*:POC ratio (Fig. 3C, 3D)

indicated a strong up-regulation in *Chla* synthesis, and POC accumulation was probably hindered due to restricted photosynthesis induced by light limitation. A model study showed that under nutrient-replete conditions phytoplankton can adjust their light-harvesting apparatus (mainly chlorophyll) to energy storage reservoirs to cope with different irradiance levels (Geider et al., 1996). At growth saturating light level (depending on the habitat and the type of phytoplankton), cells perform steady-state photosynthesis if nutrients are sufficient; contrarily, when light exceeds that level, photoprotection is maximized by cutting down the light capture potential (Fig. 7B). The storage matter usually remains unaffected under such conditions (Fig. 8). When the incident light becomes limiting, the rate of carbon accumulation becomes slow and the energy pool is mostly diverted to maximize light-harvesting pigment synthesis to acquire maximum light energy (Fig. 7C). When the acquired light energy is not enough to support steady photosynthetic rate, total storage matter or biomass can be reduced. The surf zone of the coastal Bay of Bengal often receives land runoff with sediment flux and experiences prolonged cloud coverage during the monsoon months which have been thought to limit phytoplankton primary production (Prasannakumar et al., 2010). Sarma et al., (2009) and Acharyya et al., (2012) showed that enhanced transportation of suspended particles during the SW monsoon significantly reduced phytoplankton growth and suppression in river discharge enhanced phytoplankton bloom in the Godavari estuary (situated close to our study area). Recently, Jyothibabu et al., (2018) showed that per cell *Chla* quota is directly dependent on cloud coverage and light limitation during the monsoon months in the Bay of Bengal. It was also noticed under a prolonged cloudy period that the SL ($600 \mu \text{ Einstein m}^{-2}\text{s}^{-1}$) that usually reaches 2 m depth became as low as $50 \mu \text{ Einstein m}^{-2}\text{s}^{-1}$. Our results suggested that this kind of light-limited situation impacts carbon assimilation and the growth of the phytoplankton community in the coastal Bay of Bengal. Besides, the wind-driven rapid mixing process may also change the light availability much faster than the normal solar variability over the day and may alter non-photochemical quenching and xanthophyll cycle activities at different magnitudes in marine phytoplankton (Giovagnetti et al., 2014).

Diadinoxanthin is one of the major xanthophylls in diatoms. The reduced ratio of diadinoxanthin: *Chla* (Fig. 5C, 5D) in Exp-IB under LL conditions suggests the enhanced *Chla* synthesis relative to diadinoxanthin production. This could be attributed to the higher cellular demand for synthesizing the major light-harvesting pigments, that is *Chla* to run photosynthesis on a steady state. Contrarily, the need of increasing diadinoxanthin synthesis may not be required under LL and hence there was an insignificant change in diadinoxanthin contents. The key role of the photoacclimation process is to dissipate extra energy or electron flow as heat via non-photochemical quenching (diadinoxanthin>diatoxanthin) (Lavaud, 2007; Niyogi and Truong, 2013) and any detection

of diatoxanthin indicated the occurrence of non-photochemical quenching in phytoplankton cells (Lohr, 2011). In the second experiment, diadinoxanthin and diatoxanthin were the major xanthophylls and the diatoxanthin-index (Fig. 7J) showed a significant negative correlation with increasing light availability. Fujiki et al., (2003) reported a significant positive correlation between (diadinoxanthin+diatoxanthin): Chl a ratio with increasing photon flux density in a phytoplankton bloom in Sagami Bay, Japan suggesting a simultaneous enhancement in diadinoxanthin+diatoxanthin production and decrease in Chl a content to minimize light capture. In our experiment also, the highest detected diadinoxanthin+diatoxanthin contents and minimum Chl a values corresponded with the highest light intensities. The negative correlation with N is usually due to the lack of nitrogen resources for photo-protection, synthesizing new chlorophylls, and protein. It has been shown in a species of the marine diatom *Thalassiosira* that N limitation reduced the repair capacity of PS-II and increases photodamage (Li et al., 2021). The increase in diadinoxanthin content with a simultaneous reduction in the diatoxanthin level even after decreasing photon flux density (16:00 hrs onwards) was likely due to the reverse reaction from diatoxanthin \rightarrow diadinoxanthin (epoxidation) (Goss et al., 2006a; 2006b). A similar result was noticed in the coastal diatom *Pseudo-nitzschia multistriata* (Giovagnetti et al., 2014) and a model diatom *Phaeodactylum tricornutum* (Nymark et al., 2009).

A significant amount of D1 protein of PS II can be degraded due to light stress and a simultaneous repair process is necessary to run and maintain the steady photosynthetic rate (Baene-González and Aro, 2002). In our second experiment, reduced protein content (Fig. 7K) and light-harvesting pigment: protein contents ratio (Fig. 7L) associated with the highest light intensities also supports this fact. The rate of D1 protein repair relative to the rate of degradation can be higher under SL and may potentially reduce the photosynthetic rate in marine diatoms (Domingues et al., 2012). It has been shown that under high light the electron transport rate from PS-II and carbon assimilation can be decoupled (Schuback et al., 2015). And for this reason, a midday depression in the primary productivity profile in the tropical ocean is a common phenomenon (Mackey et al., 2008). Hence, under maximum oxidative stress, the cell first attempts to repair the pigment system by spending substantial resources and energy (Alderkamp et al., 2010). It was observed in a model marine diatom (Dong et al., 2016) that the expression levels of different proteins involved in light-harvesting/photo-protection including the production of diadinoxanthin+diatoxanthin were directly linked to high light exposure for 10 hrs. Brunet et al., (2008) reported a clear variability in the photobiological parameters in a natural phytoplankton population over a natural day-light cycle from the Mediterranean Sea.

Impacts of Fe

Fe addition did not show any significant impact except on *Chla* and total pigment production under SL (Fig. 2I, 2J). The coastal water of the Bay of Bengal is unlikely to be Fe limited. An average dissolved Fe concentration of > 50 nM was reported from the coastal stations near our study area during the winter and summer monsoons (Rejomon et al., 2008; Rejomon et al., 2010). A lower photoprotective to light-harvesting pigment ratio >0.25 also indicated a Fe-sufficient condition in this water. In our earlier study on a coastal diatom, it was noticed that Fe supplement from 200 nM - to 1 μ M led to a decrease in photoprotective to light-harvesting pigment ratio to <0.2 (Biswas and Bandyopadhyay, 2013). Further, the first experiment was conducted during a summer monsoon month (August) when the coastal waters usually receive a considerable quantity of land runoff containing diverse trace metals. The absence of diatoxanthin in the first experiment also suggested the possibility of a Fe replete condition, because, under such conditions, the photosynthetic electron transport can be done efficiently. This will reduce the chance of accumulation of excess electrons and the generation of reactive oxygen species in the chloroplast as well as the need for de-epoxidation from diadinoxanthin to diatoxanthin. The second experiment was conducted in April which is a pre-monsoon month without any rainfall and the availability of Fe in the coastal waters of the Bay of Bengal can be relatively lower than that in monsoon months. It was noticed in our previous study (Biswas et al., 2013) that diatoxanthin production decreased significantly with Fe addition in a coastal diatom from the Bay of Bengal. The supplied Fe was probably utilized for chlorophyll biosynthesis (Spiller et al., 1982). But the Fe quota to maintain the electron transfer chain reactions was probably enough and hence carbon biomass build-up was not impacted. Hence, POC production under SL or LL was not impacted by Fe addition, although *Chla* and total pigment increased. The change in *Chla* increased *Chla*:POC ratio in the first experiment. The addition of extra nitrate in the first experiment may also increase some Fe demand for metabolizing nitrate (Schoffman et al., 2016). The enhanced total pigment (Fig. 2I), photoprotective as well as light-harvesting pigments (Fig. 3E, 3F) in response to Fe addition in Exp-IA could be due to higher nitrogen levels than Exp-IB as nitrogen metabolism needs a substantial amount of Fe (Schoffman et al., 2016).

Phytoplankton community shift

The dominance of *Skeletonema* sp. was noticed during Exp-IA and IB. During Exp-II, the community shifted significantly which must be related to the availability of major nutrients and other physicochemical parameters. The relative % of *Skeletonema* sp. was higher in Exp-IA and could be due to higher Si availability as this species is well known to grow on high Si levels, particularly during freshwater discharge (Shaik et al., 2015). The shift in the phytoplankton community was noticed both

in response to changing light and Fe addition. Although Fe did not show any significant impact on the growth of the experimental phytoplankton community, the species shift was significant. The dominance of *Skeletonema* sp. under Fe enriched samples indicated that this diatom has a higher Fe requirement. Bizsel and Uslu, (2000) reported the dominance of *Skeletonema* sp. in association with high Fe concentrations in Aegean Sea bay water. Occurrence of *Skeletonema* sp. bloom during a freshwater discharge period was also noticed in our earlier study (Shaik et al., 2015) and this could be also due to freshwater-induced Fe input in the coastal waters. *Chaetoceros* spp. showed a similar trend and was likely to be influenced by added Fe. Relatively higher *Thalassiosira* sp. abundance under LL suggested low light adaptability of this genus. Mallin and Paerl, (1992) noticed a higher abundance of *Thalassiosira* sp. during the time of cloud coverage in a shallow estuary. Bharathi et al., (2018) noticed *Thalassiosira* sp. is one of the dominating centric diatoms associated with monsoonal river discharge in the western coastal Bay of Bengal. Jyothibabu et al., (2018) also noticed the enhanced abundance of *Thalassiosira* sp. under LL conditions. The higher relative abundance of *Leptocylindrus danicus* under SL also indicated that the light requirement of this species could be high. *Helicotheca* sp. abundance was higher under LL suggesting their low light preference. Under SL, *Chaetoceros* spp. and *Skeletonema* sp. seemed to have outcompeted *Helicotheca* sp. in the presence of excess Fe. This is likely that fluctuating light conditions, which frequently occur in the coastal Bay of Bengal may significantly impact phytoplankton succession in the surf zones.

Conclusions

Usually, coastal diatoms show a high physiological plasticity relative to other phytoplankton to cope with a rapidly changing light environment. Surf zones are extremely dynamic and light changes very frequently. We show here that the experimental phytoplankton from a tropical surf zone (sandy exposed beach) exhibit very quick photo-acclimation turnover when photon flux density changes even over a very short period. This study also indicated that in the case of prolonged overcast or very high turbidity (likely to happen during monsoon period due to heavy rainfall), photosynthetic rate and organic carbon accumulation can be significantly affected. The present study shows that the POC-based growth rate can be maintained above 50% in response to 8.5 times decrease in light supply for three consecutive days. However, it does not show the threshold light level below which the photosynthetic function gets severely impeded and needs to investigate in future studies. This investigation also shows that *Chla* can be highly sensitive to light and considering *Chla* as a potential biomass indicator may introduce some major bias in the natural data set. Particularly, during the monsoon months under prolonged cloud coverage/turbidity, the phytoplankton may significantly enhance their *Chla* content per cell resulting in a total increase in *Chla* concentrations. High *Chla*

values may not be necessarily associated with a high photosynthetic rate and organic carbon accumulation. We also show that Fe was sufficient to support good amount of POC production and the observed changes in the pigment signature were mostly due to light. Light and additional Fe supplements could potentially alter the phytoplankton community in this surf zone. This is important to note that experimental studies on surf zone phytoplankton are rare on a global basis. The level of light stresses in high and low latitude surf zones are not similar. In the case of high latitude seas, the stress related to light limitation as well as photoinhibition could be high due to prolonged dark winter times as well as long summer days. Contrarily, tropical seas experience, the 12:12 hours light-dark natural cycle and insignificant variability in light level during summer and winter. This may further differ due to regional climate, like the influence of monsoon in the case of Indian coastal seas. Due to such differences, the responses of tropical surf-zone phytoplankton to light fluctuation may not be comparable with high latitude or other tropical or subtropical surf zones. Hence, this is recommended that more experimental studies are needed to improve our understanding of the responses of surf-zone phytoplankton to different stress factors most importantly, considering the climate change variable shall be prioritized.

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Table 1: Initial and final concentrations of dissolved inorganic nutrients during the experiments conducted in August, January, and April, respectively (value±SD; n =2-4). The final nutrient concentrations of Exp-IB are not provided due to some technical error during analysis.

		Treatments	Dissolved inorganic nutrients(μmolL^{-1})		
			Phosphate (P)	Silicate (Si)	Nitrate+nitrite (N)
August'12	Initial		16.48±0.0	94.01±0.84	111.55±1.47
	Final	SL- Cont	7.21±1.09	69.61±3.58	75.25±3.02
		SL+200nMFe	9.34±0.31	55.26±11.16	59.77±3.46
		LL-Cont	5.56±1.29	77.89±5.78	91.79±2.31
		LL+200nMFe	6.94±0.12	61.49±11.99	79.09±1.95
January'13	Initial		1.55±0.44	12.5±1.6	14.81±1.1
	Final		-	-	-
April'15	Initial		1.11±0.09	9.91±1.26	14.15±1.17
	Final	7:00hrs	0.57±0.095	8.50±0.404	12.07±0.172
		8:30hrs	0.49±0.014	5.42±0.323	9.97±0.109
		10:00hrs	0.45±0.014	5.62±0.040	8.49±0.313
		11:30hrs	0.43±0.041	4.99±0.040	6.14±0.109
		13:00hrs	0.55±0.014	5.11±0.121	4.38±0.407
		14:30hrs	0.39±0.014	5.08±0.081	0.62±0.000
		16:00hrs	0.39±0.014	1.48±0.081	0.48±0.047
		17:30hrs	0.34±0.014	0.40±0.081	0.23±0.016

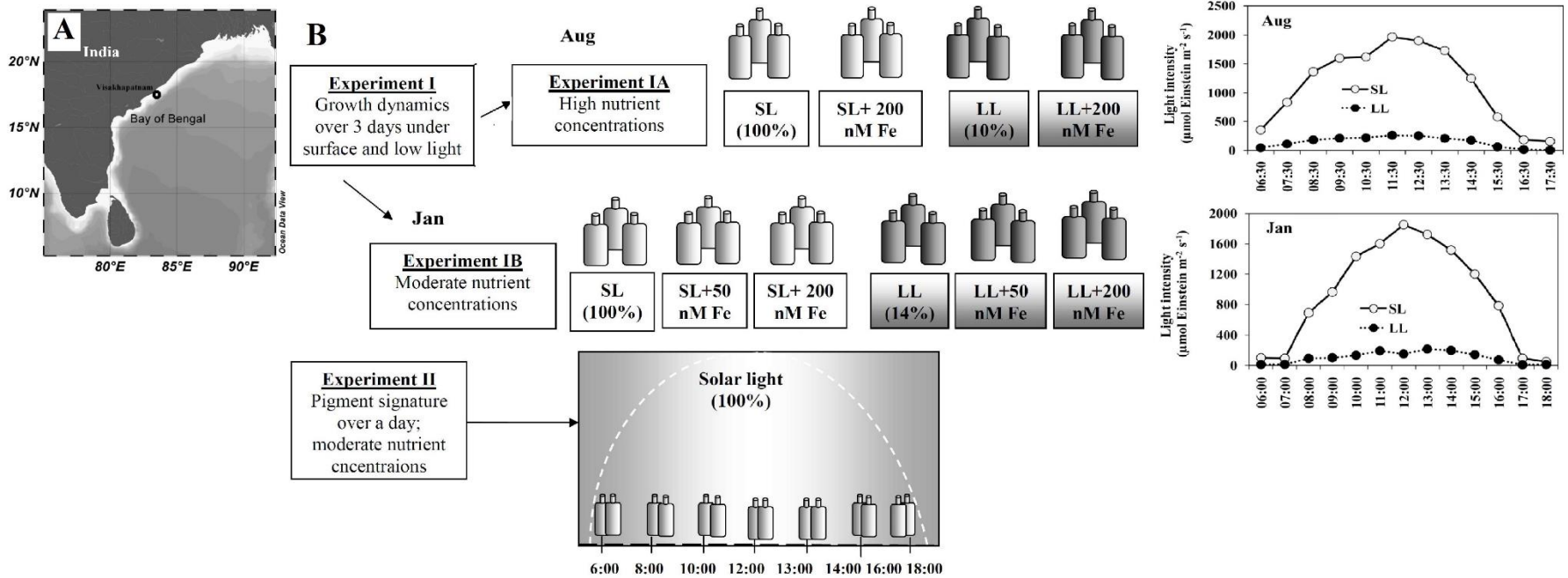


Figure 1: A) Map showing the water sampling location situated at the Visakhapatnam beach (southwestern coastal Bay of Bengal); B) schematic representation of the experimental plan showing three individual experimental settings; experiment-I and II were conducted during August and January over three days. The corresponding variabilities in solar photon flux density are shown on the right panel; the grey circle represents the “surface light” (SL, 100%) and the black circle shows the “low light” (LL) intensities (nearly 8.5 times lower than the SL). Experiment-II was conducted over a day with varying solar photon flux density in April (the solar light level is shown in Fig. along with other parameters and hence not displayed here).

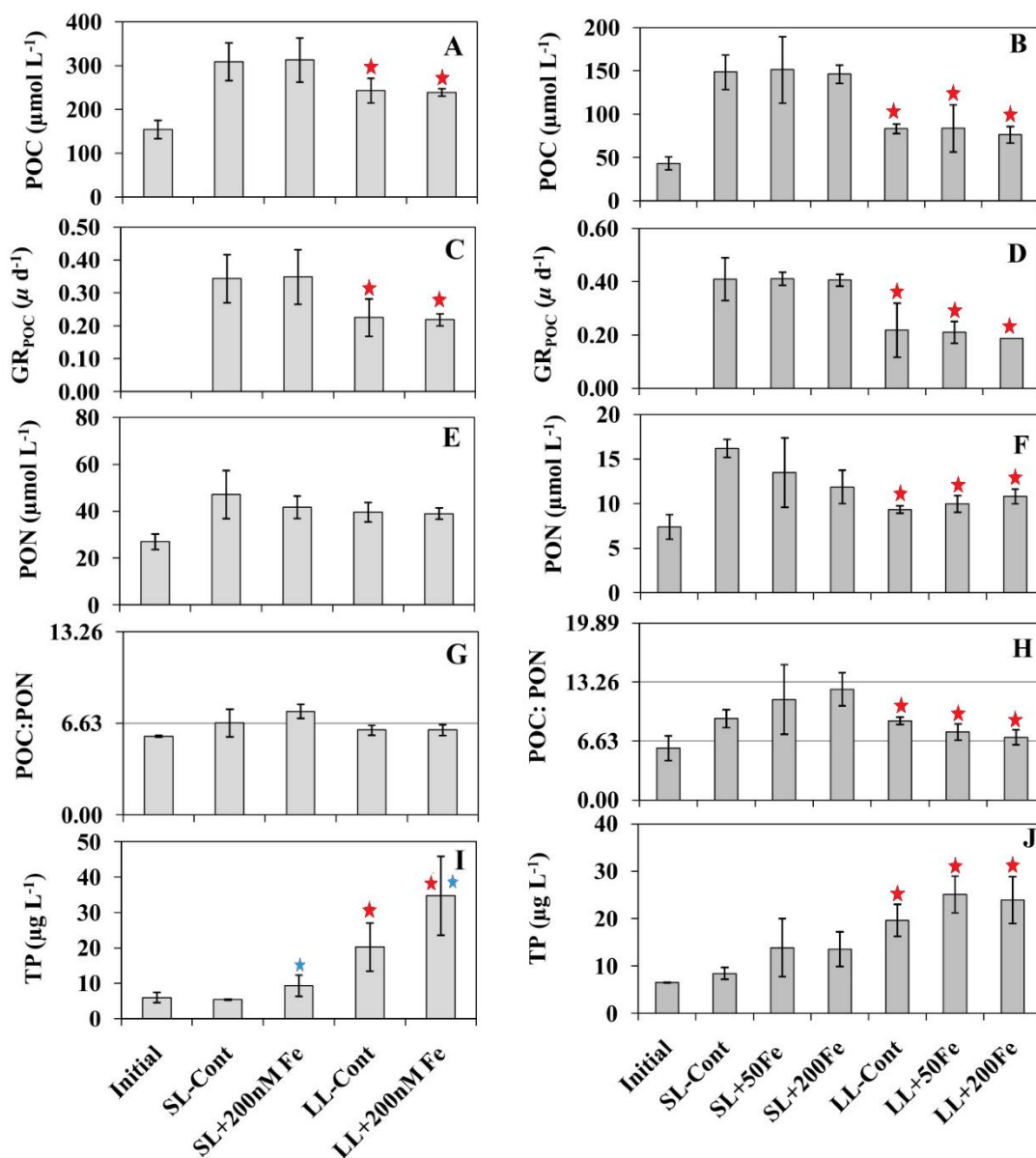


Figure 2: The concentrations of particulate organic carbon (POC) during Exp-IA (A) and Exp-IB (B); POC-based growth rate in Exp-IA (C) and Exp-IB (D); the concentrations of particulate organic nitrogen (PON) in Exp-IA (E) and Exp-IB (F); the ratios of POC:PON in Exp-IA (G) and Exp-IB (H); the concentrations of total pigment (TP) in Exp-IA (I) and Exp-IB (J) under “surface light” (SL), “low light” (LL), and Fe addition over the experimental period (values \pm SD; $n = 3$). The red stars indicate statistical significance ($p < 0.05$) between the mean values due to light and the blue stars show the significance against Fe addition.

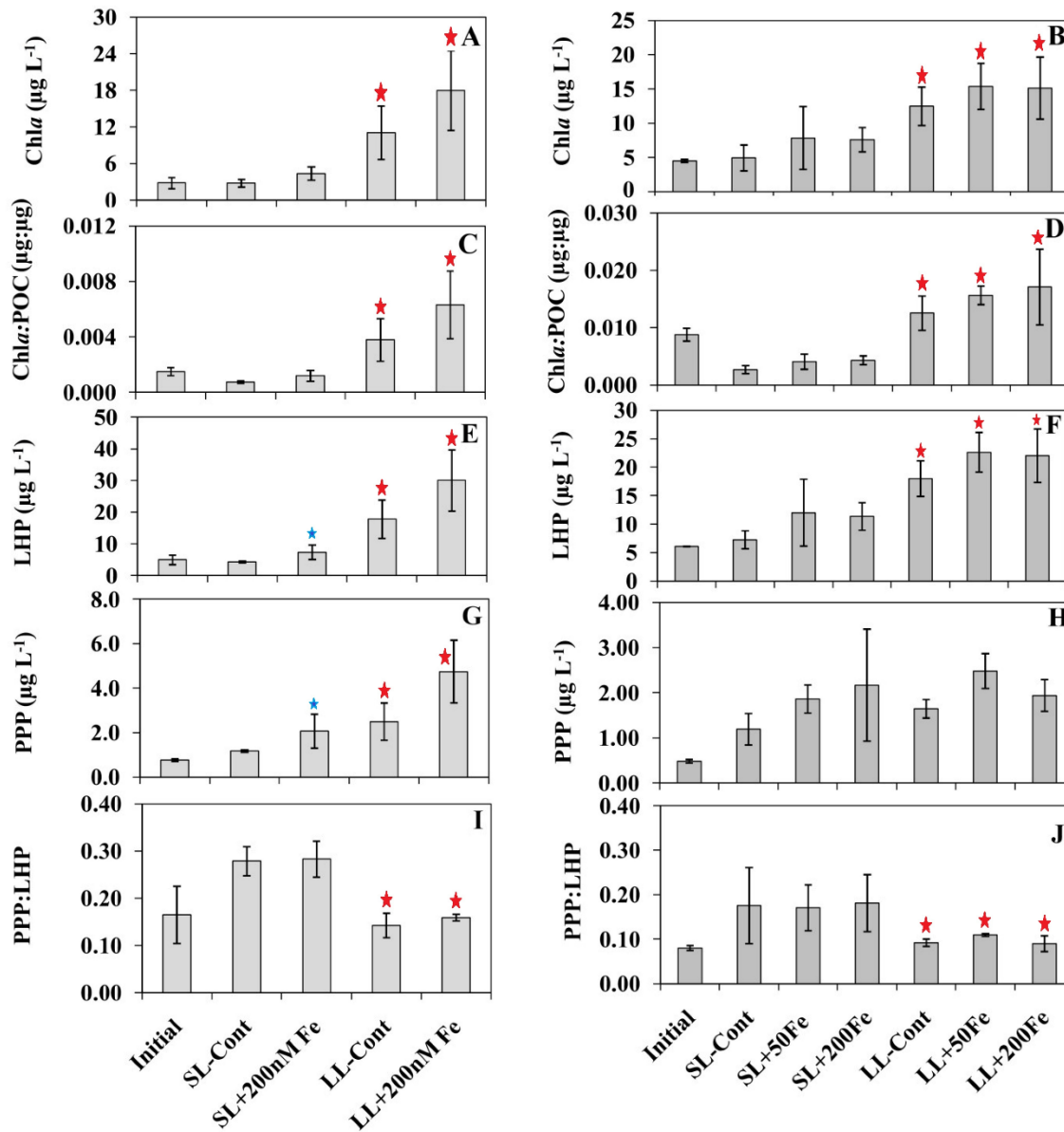


Figure 3: The concentrations of Chla in Exp-IA (A); Exp-IB (B); the ratio of Chla: POC in Exp-IA (C) and Exp-IB (D); the concentrations of light-harvesting pigments (LHP) in Exp-IA (E) and Exp-IB (F); the concentrations of photo-protective pigments (PPP) in Exp-IA (G) Exp-IB (H); the ratios of PPP:LHP in Exp-IA (I) and Exp-IB (J) under “surface light” (SL), “low light” (LL), and Fe addition over the experimental period (values \pm SD; n =3). The red stars indicate statistical significance ($p < 0.05$) between the mean values due to light and the blue stars show the significance against Fe addition.

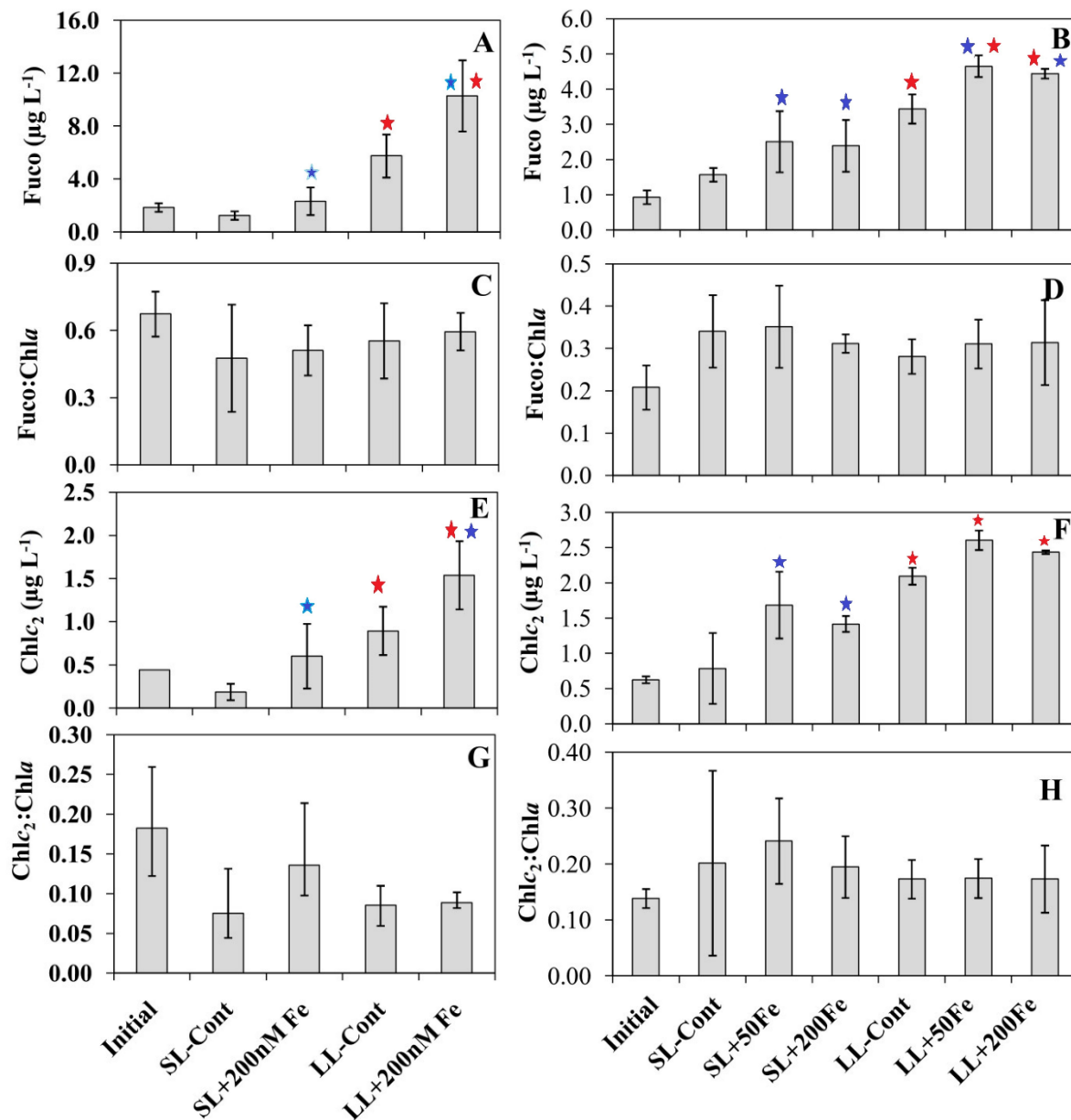


Figure 4: The concentrations of fucoxanthin (Fuco) in Exp-IA (A) and Exp-IB (B); the ratios of Fuco:Chla in Exp-IA (C) and Exp-IB (D); the concentrations of Chlc₂ in Exp-IA (E) and Exp-IB (F); the ratios of Chlc₂: Chla in Exp-IA (G) and Exp-IB (H) under “surface light” (SL), “low light” (LL), and Fe supplemented treatments over the experimental period (values \pm SD; $n = 3$). The red stars indicate statistical significance ($p < 0.05$) between the mean values due to light and the blue stars show the significance against Fe addition.

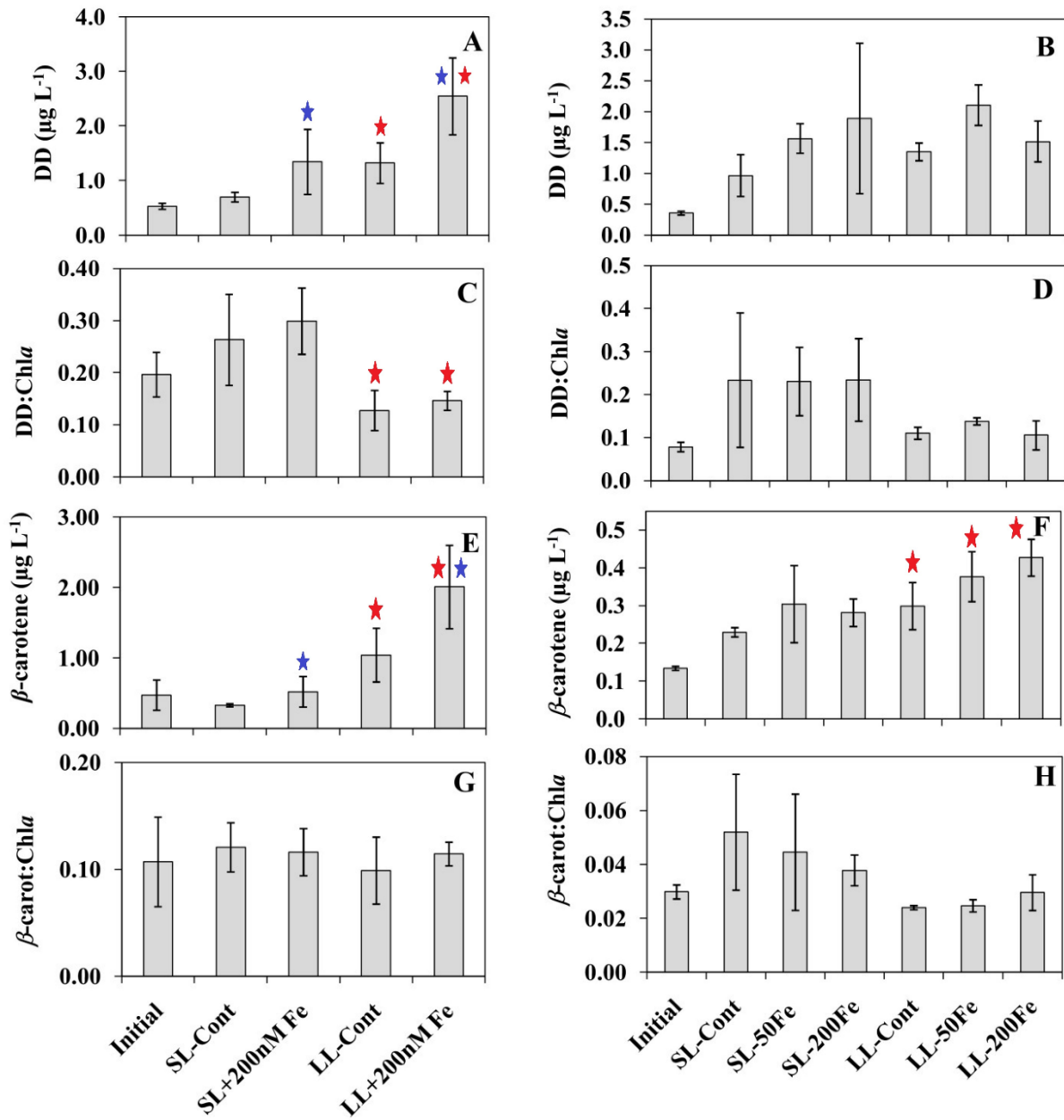


Figure 5: The concentrations of diadinoxanthin (DD) in Exp-IA (A) and Exp-IB B); the ratio of DD:Chla Exp-IA (C) and Exp-IB (D); the concentrations of β -carotene in Exp-IA (E) and Exp-IB (F); the ratio of β -carotene:Chla in Exp-IA (G) and Exp-IB (H) under “surface light” (SL), “low light” (LL) and Fe supplemented treatments over the experimental period (values \pm SD; n =3). The red stars indicate statistical significance ($p < 0.05$) between the mean values due to light and the blue stars show the significance against Fe addition.

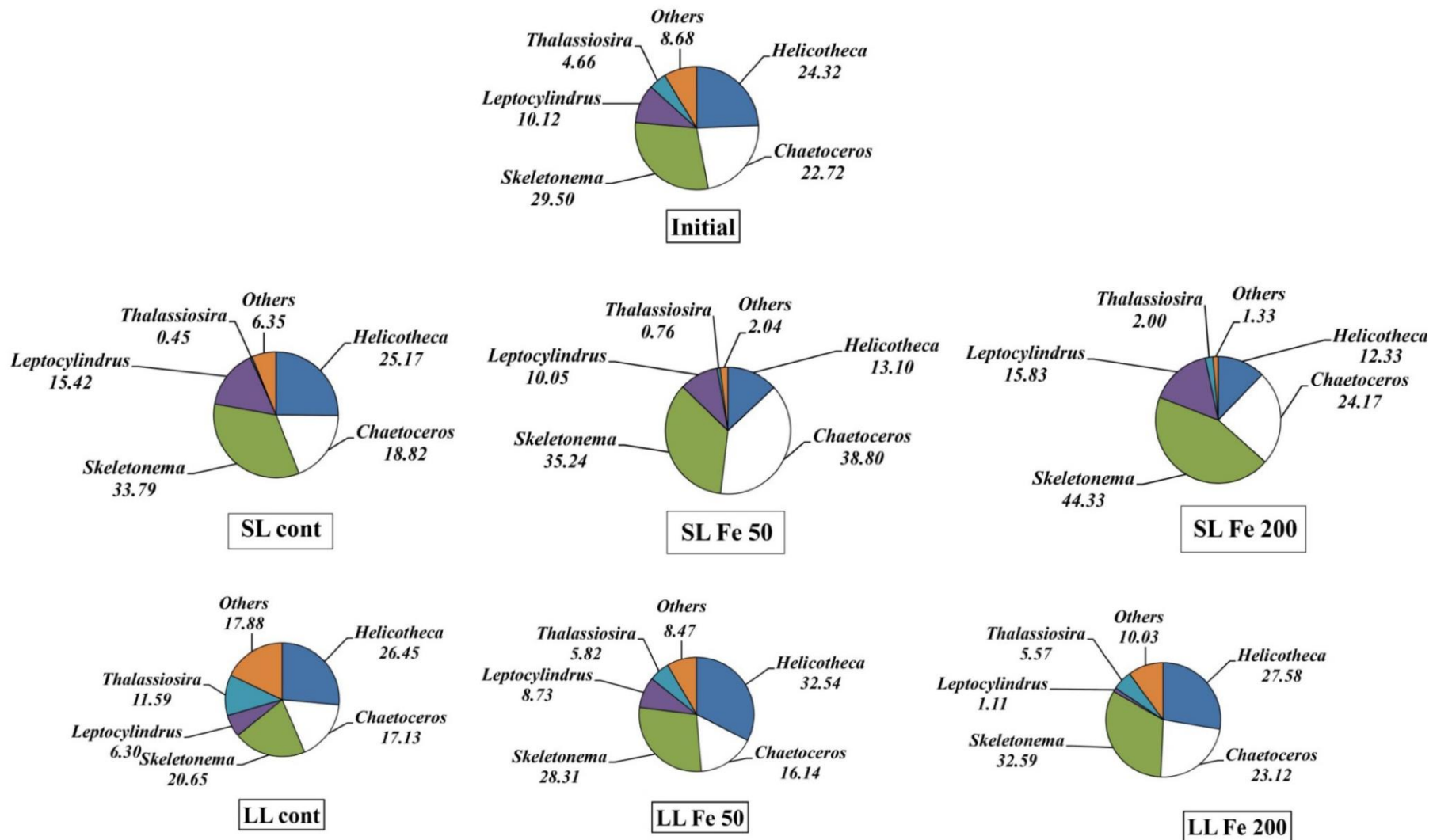


Figure 6: Phytoplankton community composition representing the relative % of various diatom species under “surface light” (SL), “low light” (LL), and Fe-treated samples in Exp-IB.

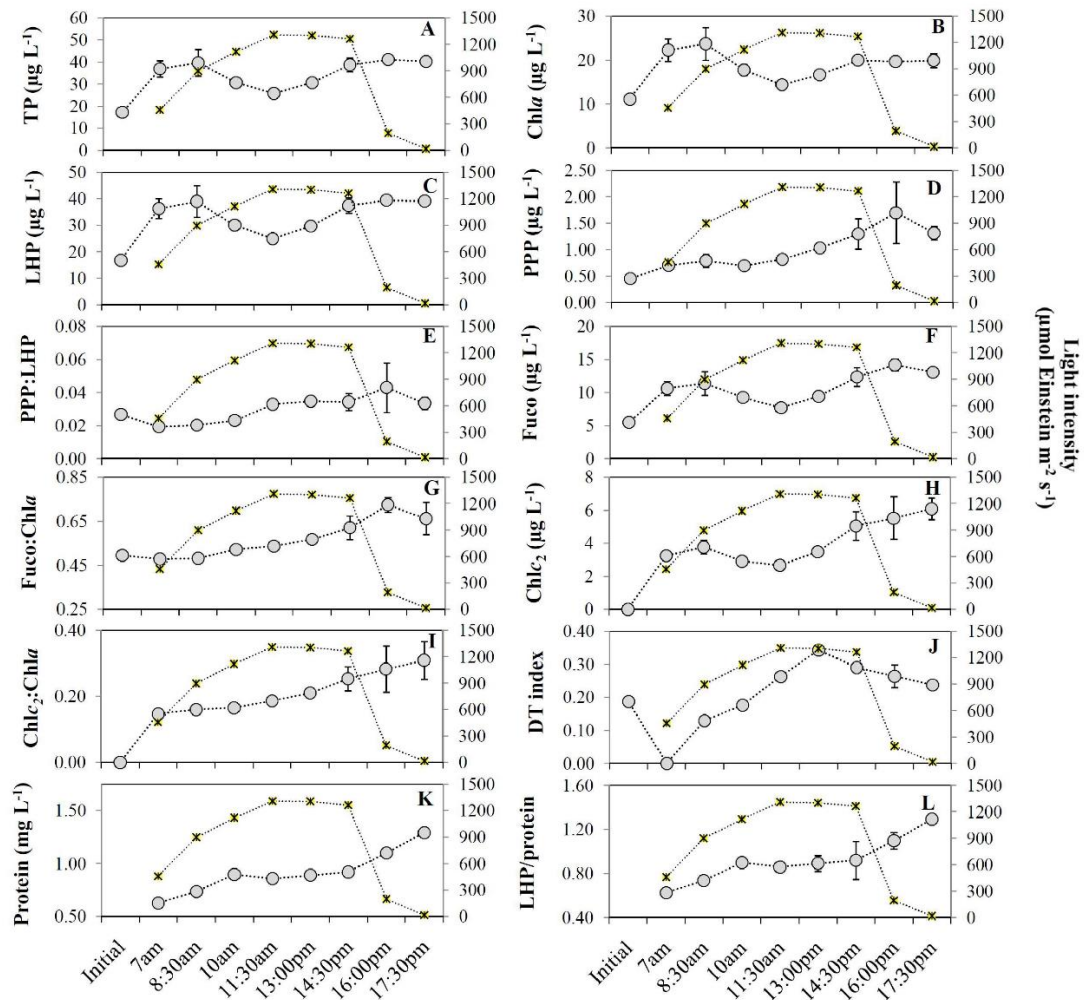


Figure 7: The overall variability in phytoplankton pigment and total protein contents with dynamic solar light (photon flux density) during Exp-II (April). The cross marks with dotted lines represent the solar photon flux density displayed in each panel against Y-axis during the sampling day. The closed circles are used to denote the variability of individual parameters. The concentrations of (A) total pigments (TP); (B) *Chla*; (C) light-harvesting pigments (LHP); (D) photoprotective pigments (PPP); the ratios of (E) light-harvesting to photoprotective pigments (LHP:PPP); the concentrations of (F) fucoxanthin (Fuco); (G) the ratios of Fuco:*Chla*; (H) the concentrations of *Chlc2*; (I) the ratios of *Chlc2*:*Chla*; (J) Diatoxanthin (DT)-index; (K) the concentrations of protein and the (L) ratios of light-harvesting (LHP) :protein in relation to changing solar photon flux density over the sampling period.

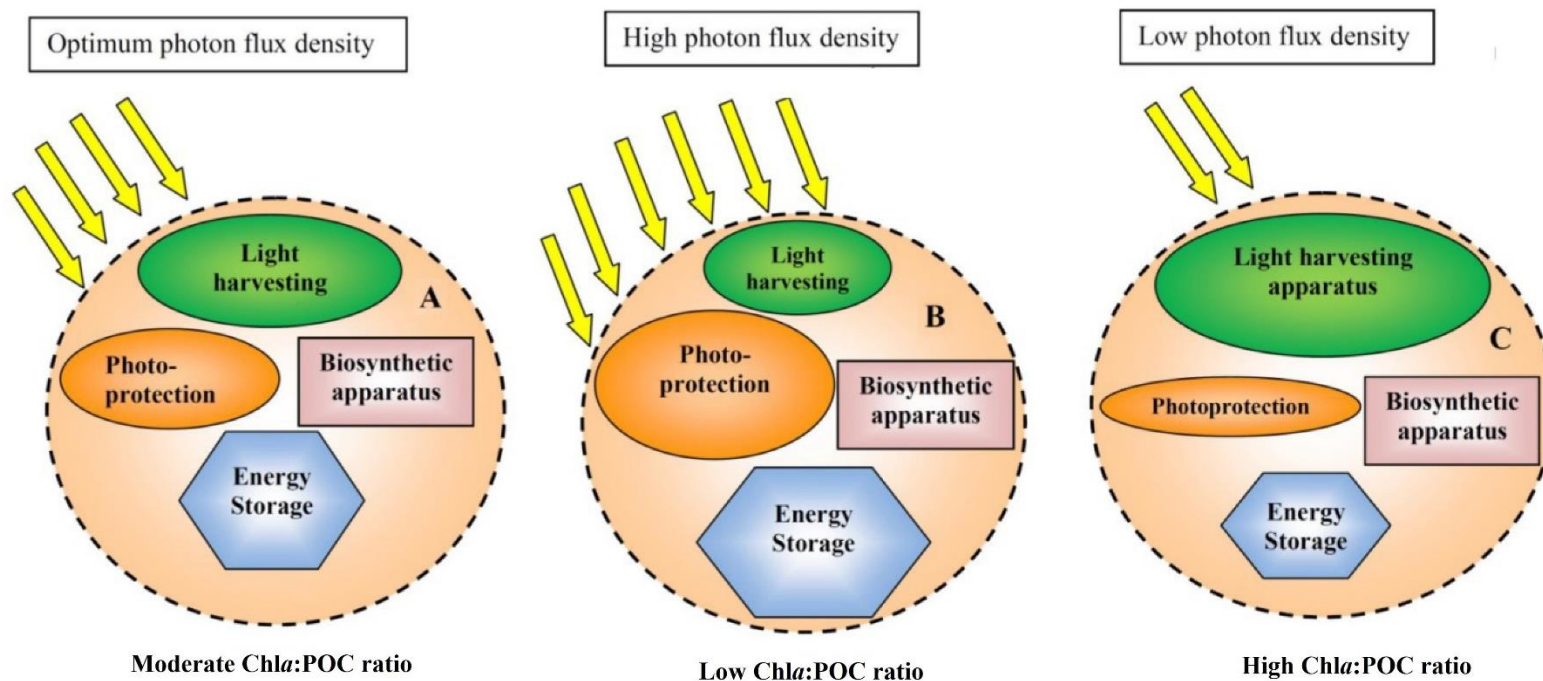


Figure 8: The schematic represents short-term photo-acclimation processes in marine phytoplankton in response to changing irradiance (redrawn from Geider et al., 1991). (A) phytoplankton cells under growth-saturating photon flux density perform a steady photosynthetic rate (moderate Chl a:POC ratio); (B) under excess light phytoplankton cells maximize photosynthetic capacity, cellular storage, and photo-protective machinery, and minimize light-harvesting pigment pool to avoid photodamage (low Chl a:POC ratio); (C) the cell under light-limited condition performs limited photosynthesis and reduce storage matter. To enhance their photosynthetic performance, the cell maximizes the light-harvesting apparatus and minimizes photo-protective machinery (high Chl a:POC ratio).