Nutrient and temperature constraints on primary production and net phytoplankton growth in a tropical ecosystem

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Abstract

The Red Sea depicts a north-south gradient of positively correlated temperature and nutrient concentration. Despite its overall oligotrophic characteristics, primary production rates in the Red Sea vary considerably. In this study, based on five cruises and a 2-year time series (2016–2018) sampling in the Central Red Sea, we determined phytoplankton photosynthetic rates (PP) by using $^{13}$C as a tracer and derived phytoplankton net growth rates ($\mu$) and chlorophyll a-normalised photosynthesis ($P_B$). Our results indicate a 9-fold variation (14–125 mgC m$^{-2}$ h$^{-1}$) in depth-integrated primary production and reveal a marked seasonality in PP, $P_B$ and $\mu$. Depth-integrated PP remained <30 mg C m$^{-2}$ h$^{-1}$ during spring and summer, and peaked in autumn-winter, particularly in the southernmost stations (~17°N). In surface waters, phytoplankton grew at a slow rate (0.2 ± 0.02 d$^{-1}$), with the population doubling every 3.5 days, on average. However, during the autumn-winter period, when chlorophyll-a concentrations peaked in the central and southern regions, $\mu$ increased to values between 0.60 and 0.84 d$^{-1}$, while $P_B$ reached its maximum rate (7.8 mgC [mg Chl-a]$^{-1}$ h$^{-1}$). We used path analysis to resolve direct vs indirect components between correlations. Our results show that nutrient availability modulates the photosynthetic performance and growth of phytoplankton communities and that $P_B$ and $\mu$ fluctuations are not directly associated with temperature changes. Our study suggests that similarly to other oligotrophic warm seas, phosphorus concentration exerts a key role in defining photosynthetic rates and the biomass levels of phytoplankton communities in the region.
Introduction

Phytoplankton photosynthesis is the primary process responsible for microalgal growth and energy flow to the pelagic food web (Falkowski 1994; Cullen 2001). Photosynthetic rates are typically constrained by the availability of resources such as light and nutrients (Falkowski and Owens 1980; Cullen et al. 1992; Marañón et al. 2014), temperature (Eppley 1972; Regaudie-De-Gioux and Duarte 2012), and the primary producers' capacity to acclimate to changes in these conditions (Falkowski and Laroche 1991; Collos et al. 2005).

Reduced nutrient availability is associated with reducing cell nutrient quotas, light-harvesting pigments and carbon fixation (Geider et al. 1998). Fluctuations in irradiance drive changes in the chlorophyll per cell, for example, acclimation to low irradiance is accompanied by a five- to tenfold increase in the cellular quota of pigments (Falkowski 1981; MacIntyre et al. 2002). Temperature affects specific enzymatic activities (e.g., carboxylase activity of RuBisCO), the transport of substances across membranes (Raven and Geider 1988), and to a large degree determines respiratory demands (Barton et al. 2020). Both high temperature and reduced nutrient availability lead the community toward dominance of picoautotrophs and low phytoplankton primary production (Agawin et al. 2000). However, disentangling the effects of nutrients and temperature has proven cumbersome due to the inverse correlation that commonly exists between both variables across the ocean (Agawin et al. 2000).

The Red Sea is a natural experiment to assess nutrient and temperature constraints on net primary production, as opposite to the general trend for the global ocean-, it presents a gradient of positively-correlated nutrient availability and temperature, with values > 30ºC in the southern Red Sea (Chaidez et al. 2017) and extreme oligotrophy, due to the lack of nutrient inputs, in the northern Red Sea (Triantafyllou et al. 2014; López-Sandoval et al. 2019). In contrast, nutrients
are advected into the southern Red Sea from upwelling regions in the Arabian Sea and the Gulf of Aden, resulting in higher nutrient availability in the warmer, southern region (Churchill et al. 2014; Wafar et al. 2016). Accordingly, Red Sea phytoplankton abundance, biomass and primary production declines from south to north (Ismael 2015; Raitos et al. 2015; Kheireddine et al. 2017).

The chlorophyll-a concentration generally peaks between autumn and winter, with the higher values mostly restricted to the basin's southern part (Kheireddine et al. 2017). However, occasional peaks have also been reported for the central Red Sea (Qurban et al. 2017). Overall, phytoplanktonic productivity can take values from 3 to 58 mgC m$^{-2}$ h$^{-1}$ (Qurban et al. 2014; Qurban et al. 2017), and for most of the year, autotrophic metabolism prevails, except during the spring and summer in the northern Red Sea (López-Sandoval et al. 2019). As temperature enhances the metabolic response of phytoplanktonic cells in the presence of abundant nutrient supply compared to nutrient-limiting conditions (Marañón et al. 2014), we, therefore, expect the temperatures in the nutrient-sufficient waters of the southern Red Sea to affect phytoplankton primary production and growth rates to a greater extent than in the northern Red Sea.

At light saturating conditions, the rate of chlorophyll a-normalised photosynthesis is a measure of phytoplankton communities' photosynthetic efficiency (Falkowski 1981; Cullen et al. 1992). This rate, also known as the assimilation number ($P^B$), describes the asymptotic maximum rate of change in carbon fixation per unit of Chl-a. $P^B$ is equivalent to $P^B_{\text{max}}$ in a P-E relationship (Platt and Jassby 1976; Cullen et al. 1992); a photosynthetic parameter commonly used in algorithms retrieving satellite-based primary production estimates (Platt and Sathyendranath 1993). $P^B$ tends to be maximal when the cell to chlorophyll ratio or the carbon to chlorophyll ratio is high and decreases with increasing pigment content as light is less efficiently collected.
due to self-shading (Falkowski 1981). P\textsuperscript{B} values are also affected by nutrient (Osborne and Geider 1986; Kolber et al. 1988) and light availability (Marañón and Holligan 1999) and are sensitive to temperature changes (Eppley 1972). Here, we report primary production, assimilation numbers, and phytoplankton growth rates along the Eastern Red Sea, and assess how nutrient concentration and temperature affect microalgae photosynthetic rates and growth along the south to north Red Sea temperature and nutrient gradients, and through different seasons in the central Red Sea resolved between July 2016 and March 2018.

**Methods**

**Sample collection**

We collected samples fortnightly between July 2016 and March 2018 at a fixed time-series station located ~10 km from Thuwal, Saudi Arabia (22.30 °N, 38.99 °E, n = 42), and as part of five oceanographic surveys (October and November 2016, January 2017, August 2017 and March 2018) across the Eastern Red Sea (Figure 1A, B) onboard the R/V Thuwal and the R/V Al Azizi (n = 40). During the cruises, we collected four to five water samples between the first optical depth and the bottom of the photic layer (from 100–1 % of the photosynthetically active radiation) to determine primary production rates throughout the water column (depth-integrated primary production) and to derive surface assimilation numbers and growth rates. The water was collected between 07:00 and 09:00 local time with a General Oceanic rosette sampling system. The rosette was fitted with 12 L Teflon Niskin bottles provided with silicone O-rings and seals. Water from the coastal station was manually collected at the surface with a Niskin bottle (10 L) deployed around noon.
At the time-series station, sea surface temperature and salinity were measured for 5–15 min by using an Ocean Seven 305 Plus CTD (Idronaut, Brugherio, Italy), and water samples were collected and processed as described in Prabowo and Agusti (2019). During the cruises, temperature and salinity were recorded with a Sea-Bird SBE 911 plus CTD profiler (Sea-Bird Electronics, Bellevue, WA, United States). The CTD was equipped with additional sensors to measure in vivo fluorescence (WetLabs ECO-FL fluorometer), the attenuation of photosynthetically active radiation PAR (400–700 nm) (Biospherical Instruments, San Diego, CA, United States). Additionally, PAR was also recorded around noon by using a BIC radiometer (Biospherical Instruments, San Diego, CA, United States) and a C-OPS (Compact-Optical Profiling System) radiometer (Biospherical Instruments, San Diego, CA, United States) (n = 67) as detailed in Overmans and Agusti (2019).

**Sample processing**

Water samples were transferred directly from the Niskin bottles into acid-washed carboys. The collected water was enriched with 50 mL of $^{13}$C-labelled sodium bicarbonate solution (2.18 g of NaH$^{13}$CO$_3$, 99.8% $^{13}$C, in 1 L of de-ionised water) and carefully shaken to homogenise the sample. We distributed the enriched water into sets of three light and one dark acid-washed (2 L or 500 mL) polycarbonate bottles (PC). The final concentration of $^{13}$C in each bottle was ~153 µmol $^{13}$C L$^{-1}$. Before each incubation, all PC bottles were covered with neutral density mesh to attenuate light intensity according to the corresponding optical depth. At the end of the incubation period (<5 h), the entire bottle content was filtered onto a pre-combusted Whatman GF/F filter (25 mm diameter for 2 L samples, and 15 mm diameter for 500 mL samples). We removed any residual carbonate by adding 70–100 µL of HCl 50% directly to the filters. After 12–14 h, the filters were frozen at -80 °C.
Phytoplankton carbon fixation rates and growth rates

Phytoplankton primary production rates were determined by following the $^{13}$C-CRDS-PP method described by López-Sandoval et al. (2019). Briefly, the carbon content and isotopic analysis ($\delta^{13}$C ) of samples were analysed by using a cavity ring-down spectrometer system by Picarro (CM-CRDS G2201-i, Picarro Inc, Santa Clara CA, USA) attached to a combustion module ( elemental analyser) (Costech Analytical Technologies Inc., California, USA). The ratios between $^{13}$C:$^{12}$C are expressed in delta $\delta^{13}$C notation as follows:

$$\delta^{13}C (\text{‰}) = ((R_{\text{sample}}/R_{\text{VPDB}}) - 1) \times 1000 \quad (1)$$

where $R = ^{13}C/^{12}C$ for $\delta^{13}$C values in the sample and the international standard, Vienna Pee Dee Belemnite.

We calculated phytoplankton growth rates based on the changes in PO$^{13}$C isotopic labelling as in Dijkman et al. (2009):

$$\mu (h^{-1}) = \frac{1}{t} \ln \left(1 - \frac{\Delta\delta^{13}C_{\text{POC}}}{\Delta\delta^{13}C_{\text{DIC}}} \right) \quad (2)$$

where $^{13}$C$_{\text{POC}}$ and $^{13}$C$_{\text{DIC}}$ are the $^{13}$C content of carbon measured in the particulate fraction (PO$^{13}$C obtained from the filters) and estimated from the dissolved inorganic pool (DI$^{13}$C) as in López-Sandoval et al. (2019), and $t$ is the incubation time in hours. $\Delta\delta^{13}$C represents the enrichment of $^{13}$C in the samples (i.e., the difference in $\delta^{13}$C$_{\text{POC}}$ at the end of the incubation and the $\delta^{13}$C$_{\text{POC-dark}}$ obtained at the end of the incubation). $\delta^{13}$C$_{\text{DIC}}$ represents the enrichment of the DIC pool. Daily phytoplankton growth rates ($\mu \text{ d}^{-1}$) were extrapolated from hourly rates by taking into account the total length of daylight.
Nutrient and chlorophyll-a concentration

We collected 50- and 200-mL water samples to quantify nutrient and Chl-a concentrations, respectively, at the same depths as the $^{13}$C-PP samples. Samples for nutrient analyses were measured with a SEAL AA3 Segmented Flow Analyzer (SEAL Analytical Inc., WI, USA) using standard methods (Hansen and Koroleff 1999). Chl-a concentration was estimated with the non-acidification technique using a Trilogy Fluorometer equipped with CHL-NA module (Turner Designs, San Jose, USA), previously calibrated with pure Chl-a as described in López-Sandoval et al. (2019) and Prabowo and Agusti (2019).

Statistical analysis

Statistical analyses and figures were done using the statistical and machine learning toolbox in Matlab version R2018b (Mathworks Inc, Natick, MA, USA) and with the R statistical computing package R version 4.0.2 (2020-06-22). To characterise all potential relationships between photosynthetic rates and phytoplankton growth rates with environmental variables (temperature, salinity, nutrient availability [nitrate, phosphate, silicate]) or Chl-a concentration, we performed a Spearman rank correlation test by using cor function (stats 4.0.2 package in R) and visualised by using ggpairs function (GGally, 2.0.0 package). The variability of PP, $\mu$, $P^B$ and nutrient concentration between seasons was statistically tested by using the non-parametric Kruskal-Wallis test (kruskal.test, stats 4.0.2 package). We performed a Dunn's test of multiple comparisons following a significant Kruskal-Wallis (dunnTest, FSA 0.8.21 package). Mean values and their standard error (SE) are reported throughout the text.

The Spearman correlation results combined with previous knowledge about the basin dynamics helped us test a series of hypothetic pathways that acted as a framework to build a multivariate model where all parameters could work as endogenous (dependent) or exogenous
(predictor) variables. We used confirmatory path analysis, a form of structural equation modelling (SEM), to evaluate the potential relationships between environmental variables (e.g., temperature or nutrient concentration) and $P_B$ or $\mu$ by using the Piecewise Structural Equation Modelling (piecewiseSEM 2.1.0) (Lefcheck 2016), and nlme 3.1-149 (Pinheiro et al. 2020) packages for R. We tested if $P_B$ or $\mu$ data were normally distributed by using plotNormalHistogram (rcompanion). Because growth data were right-skewed, we applied a Tukey's Ladder of Powers to transform our data set (transformTukey, rcompanion).

In piecewise SEM the path diagram is framed as a set of linear equations that are solved individually. Therefore, it is possible to evaluate assumptions for each structure separately and with only enough data to fit and estimate individual regressions (Lefcheck 2016). This flexibility allowed us to address spatial and temporal autocorrelation in our data set. We did so by incorporating the sampling site (ºN) position as a random structure and including an autocorrelation structure of order 1 with a continuous-time covariate (corCAR1 function, nlme package) into a series of linear mixed models describing each relationship.

Piecewise SEM is stepwise and tests the assumption that there are no missing relationships among unconnected variables that could change the overall model's interpretation. This assumption is tested by using Shipley's test of direct separation (psem function in the piecewiseSEM package) (Shipley 2000; Lefcheck 2016). Due to the inherent bidirectional relationship between salinity and temperature, and between nutrients availability (e.g., phosphate and silicate, Figure 4), we assumed correlated errors between those variables and included them in the original hypothesised models (Figures 6A and 6D, double-headed arrows). Correlated errors describe a relationship among variables that are not presumed to be causal and unidirectional; instead, it might be explained by some underlying driver. Piecewise SEM allows
running a significance test on those bivariate correlations and allows removing their influence before computing the correlation between variables (Lefcheck 2016).

We included all statistically significant and ecologically relevant paths missing to optimise the initial models. We tested each new model by evaluating the Akaike Information Criterion (AIC), which indicates the robustness of the optimised model against the original one. Additionally, we performed a Chi-square difference test between models. Whenever the comparison between the two models was not significantly different, we selected the model with fewer parameters. The global goodness-of-fit of the optimised model (with the lowest AIC) was evaluated by using $\chi^2$ statistics. If the $\chi^2$ was non-significant ($p>0.05$), then we can conclude that the optimised structure is supported by our data (Lefcheck 2016). For each model, we visualised the residuals' linearity (residuals vs predicted plot) and normality (quantile-quantile plots "qq plot"). Residuals normality was also confirmed performing a Shapiro-Wilkinson normality Test (shapiro.test, stats package). The total number of samples for each model was 79. The ratios between the number of samples to the number of parameters from the initial hypothesised models (Figures 6A and 6D) were 13.1 and 11.3, which are within the advisable range (Grace et al. 2015). The path structures were visualised by using the plot function in R. Figures were optimised for publication by using Affinity Designer 1.8.4 (Serif (Europe) Ltd, 2020).

Results

Environmental variability and primary production rates

A marked decrease of temperature with latitude (Figure 2A), opposite to the increase in salinity (Figure 2B) prevailed over the different seasons (cruises). The warmer surface waters measured in autumn and summer ranged from 29.5 to 32.5°C, while in spring and winter sea
surface temperature (SST) remained below 28.5°C. SST seasonality at the time-series station (Figure 3) showed minimum temperatures in February (24.4°C), followed by rapid warming that exceed 30°C between the end of May and mid-October, to subsequently cool again during the winter period (between November and March).

Overall, phosphate and silicate concentration decrease with latitude (Figure 4) and significantly differed between seasons (Kruskal-Wallis $\chi^2 = 24.4$, df = 3, $p < 0.001$), peaking during the winter-spring period (Table 1). In contrast, nitrate concentration did not reveal any significant seasonal patterns (Kruskal-Wallis $\chi^2 = 2.87$, df = 3, $p = 0.41$) nor showed a significant relationship with changes in latitude ($p>0.05$) (Figure 4). At the coastal station, in the central region of the basin, phosphate, silicate and nitrate concentrations increased between winter and early spring, when sea surface temperature was the lowest (Figure 3). Generally, phosphate, silicate and nitrate concentration remained below 0.2, 1.5 and 2 $\mu$M, respectively (Figure 3A and 3D). However, we detected extremely high phosphate and nitrate values in June and November 2017 (Figure 3B). These anomalous values agreed with sampling dates that took place 2–5 days after a dust storm event.

Along the Eastern Red Sea, primary production rates mirrored the latitudinal and seasonal patterns observed with phosphate and silicate concentration. During the cruises, we observed a nine-fold increment of depth-integrated primary production rates (14-125 mg C m$^{-2}$ h$^{-1}$) and a 20-fold change in surface carbon fixation rates (< 1 to 4.2 mg C m$^{-3}$ h$^{-1}$) from the northern to the southern part of the basin (Figure 5). Primary productivity along the basin was highest in the nutrient-enriched southern region (Figure 5A and B), particularly between autumn and winter when phytoplankton productivity throughout the water column exceeded 100 mg C m$^{-2}$ h$^{-1}$ (Figure 5B). The time-series data revealed that at the coastal station, chlorophyll-a
concentration and primary production rates peaked between November and February, matching the cooling of surface waters and the increase of chlorophyll-a, phosphate and silicate concentration (Figure 3). During the summer months, when SST was highest (up to 32.5ºC), primary production rates remained below 2 mg C m\(^{-3}\) h\(^{-1}\). The anomalously high phosphate and nitrate concentrations detected in June 2017, were not followed by an increase in PP or Chl-a concentration. When taking both time series and cruises data in concert, our results indicate that PP rates and PO\(^{13}\)C concentration increased with increasing Chl-a concentration (\(R^2 = 0.57, F = 110.5,\) df = 82, \(p<0.001\) and \(R^2 = 0.53, F = 95.8,\) df = 82, \(p<0.001\)), with a slope (± SE) of 0.12 ± 0.01 and 251.5 ± 25.7, respectively.

*Chlorophyll a-normalised photosynthesis (P\(^B\))*

Analysis of the entire dataset revealed that assimilation numbers also depicted significant seasonal differences (Kruskal-Wallis, \(\chi^2 = 21.70,\) df = 3, \(p<0.001\)). On average, P\(^B\) rates in spring (5.6 ± 0.4 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\)) and winter (5.1 ± 0.4 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\)) were significantly higher than in autumn (3.4 ± 0.3 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\)) and summer (3.6 ± 0.3 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\)) (post hoc Dunn-test, \(p<0.05\)), when mean SST was >30ºC and phosphate concentration was generally close to detection limits (Figure 3). Similarly to PP, the higher P\(^B\) values (i.e., >6 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\)) were found in the central (22.5 ºN) and southern-most stations (17.5 ºN) (Figure 6), decreasing towards the north of the basin (Spearman correlation, \(r = -0.35,\) \(p<0.01\)) and towards the most saline waters (Spearman correlation, \(r = -0.42,\) \(p<0.001\)) (Figure 4). At the time-series station, P\(^B\) rates averaged 4.3 ± 0.3 mgC [mg Chl-a]\(^{-1}\) h\(^{-1}\). The seasonal pattern at the central station was also significant (Kruskal-Wallis, \(\chi^2 = 8.23,\) df = 3, \(p = 0.04\)), albeit less pronounced (Figure 3G).
By using path analysis, we tested an initial theoretical model (Figure 6A) that related assimilation numbers to temperature and nutrient concentration, variables that were highly correlated to $P^B$ (Figure 4). The global goodness-of-fit of the initial analysis indicated that the model was significant (Fisher’s $C = 2.57$, $p = 0.28$, df = 2, $AIC = 62.57$) and pointed to a direct (significant) and positive effect of phosphate concentration over $P^B$ rates (Standardised coefficient $= 0.61$, $p<0.001$) (Table 2). The initial confirmatory analysis also revealed that both temperature and salinity had a direct negative effect over phosphate (Standardised coefficient ($\text{SST}$) $= -0.40$, $p<0.001$, standardised coefficient ($\text{sal}$) $= -0.60$, $p<0.001$) and silicate concentration (Standardised coefficient ($\text{SST}$) $= -0.38$, standardised coefficient ($\text{sal}$) $= -0.39$, $p<0.001$) (Table 2). These results likely explained the significant (positive) correlation found between silicate and phosphate concentration (Figure 4).

We used the d-separation test to reach to an optimised version of the initial model (Figure 6B and C). The final model (Figure 6C) confirmed the significant direct effect of temperature and salinity over the phosphate concentration, which in turn relates significantly to $P^B$ (Standardised coefficient $= 0.59$, $p<0.001$) (Table 2). Moreover, the comparison of models suggest that temperature acts indirectly through reduced nutrient concentrations with warming. The chi-square difference test showed no difference between the initial model (Fisher's $C = 2.57$, $p = 0.28$) (Figure 6A) and the final optimised model (Figure 6C). However, the final optimised model (Figure 6C) was the most parsimonious, with a lower AIC value (32.1), and a robust fit to our data (Fisher's $C = 6.12$, $p = 0.19$).
Growth rates (µ) of Red Sea phytoplankton communities

Throughout the study, µ averaged 0.2 ± 0.02 d⁻¹ (0.15 d⁻¹ median), corresponding to a doubling time (ln 2/µ) of phytoplankton populations in the eastern side of the basin of 3.5 days. At the southernmost stations, phytoplankton growth rates were fastest during the winter (0.7 to 0.84 d⁻¹), with doubling times < 1 day (0.82–0.99 d⁻¹). At the central station, the fastest doubling times (1.7-1.9 days) of phytoplankton populations were between February and March when growth rates ranged between 0.35-0.4 d⁻¹ (Figure 3H).

Our initial path analysis (Figure 6D) indicated a strong and significant positive relationship between growth rates and phosphate (standardised coefficient = 0.39, p = 0.001) and chlorophyll-a concentration (standardised coefficient = 0.45, p <0.001) (Table 3). Similarly to P⁺ models, the initial hypothesised model for µ (Figure 6D) corroborated that, as expected, physical properties of the water column (i.e., sea surface temperature) significantly affect the concentration of phosphate and silicate in surface waters (Figure 6D). With no significant paths found between temperature and growth rates (Figure 6D). Overall, the initial model provided a robust fit to the data (Fisher’s C = 3.79, df = 4, p = 0.44, AIC =83.79). By using the d-separation test, we further optimised the initial model (Figure 6E-F) (Fisher’s C = 6.64, df = 8, p = 0.58, AIC = 46.64).

Discussion

The results presented here confirm that a reduction of primary production, phytoplankton growth rates, and chlorophyll-a normalised photosynthesis concurred with a decrease in nutrient concentrations. This finding agrees with previous reports that indicate that chlorophyll-a concentration and primary production rates generally peaked whenever nutrient concentration in
the water column increases, particularly at the southern Red Sea and its central region (Qurban et al. 2014; Qurban et al. 2017). In contrast, phytoplankton biomass and productivity are remarkably low in the northern Red Sea (Raitos et al. 2015; Kheireddine et al. 2017); a region also characterised by the periodical dominance of heterotrophic metabolism (López-Sandoval et al. 2019). Our data add to those prior assessments and also provide a seasonally-resolved primary production time series, which has been lacking to date for the Red Sea.

Assimilation numbers varied widely across the basin, while growth rates remained generally low, particularly in the basin's northern part. The average $P^B$ and $\mu$ obtained in the Eastern Red Sea ($4 \pm 0.2 \text{ mgC [mg Chl-a]$^{-1}$ h$^{-1}$ and $0.2 \pm 0.02 \text{ d}^{-1}$, respectively) were comparable to those reported for oligotrophic surface waters of the South Atlantic Subtropical Gyre and the North Atlantic Subtropical Gyre (Marañón and Holligan 1999; Marañón 2005). However, we should note that grazers were not excluded in our incubation, which can remove a significant fraction of the production per day in oligotrophic ocean ecosystems (Jackson 1980; Calbet and Landry 2004; Teira et al. 2019). Therefore, $\mu$ values presented here correspond to the net population growth rate (reproductive rate minus mortality).

Our results revealed that the highest growth rates concurred with the highest $P^B$ values found during the winter at 17.2°N (highest $\mu$ ranged from 0.8–0.84 d$^{-1}$ whereas $P^B$ went from 6.42 – 7.75 mgC [mg Chl-a]$^{-1}$ h$^{-1}$), and at the central Red Sea station (22.3°N), where $P^B$ and $\mu$ reached values between 6.2–6.3 mgC [mg Chl-a]$^{-1}$ h$^{-1}$ and 0.3–0.4 d$^{-1}$, respectively. Both regions are characterised by the seasonal inflow of nutrient-enriched water coming from the Indian Ocean (Churchill et al. 2014; Wafar et al. 2016), and by mesoscale features (Zarokanellos et al. 2017) that at times enhance primary production rates to up to 950 mg C m$^{-2}$ d$^{-1}$ (Qurban et al. 2017). The nutrient supply cascades over the entire trophic structure of the southern and even central
regions of the Red Sea, fuelling the metabolism of planktonic communities (López-Sandoval et al. 2019), favouring the presence of nano- and microphytoplankton (Ismael 2015; Kheireddine et al. 2017), and supplying food to the massive filter-feeding communities in the extensive coral reefs of the Red Sea.

Due to the almost negligible terrestrial inputs, aeolian dust and aerosol deposition represent an additional source of nutrients into the basin (Chase et al. 2011; Engelbrecht et al. 2017). On the 4th of July and the 6th of November 2017, surface phosphate concentrations in the central Red Sea was above 0.5 µM (Figure 3). These anomalously high phosphate concentrations coincided with dust storm events that preceded our sampling. However, in agreement with Torfstein and Kienast (2018) that found no correlation between dust storm events in the basin and the increase of chlorophyll-a concentrations during the winter period, we did not find a response in phytoplankton productivity or phytoplankton growth during those sampling days. Therefore, it is likely that the increase in photosynthetic activity observed during spring-winter is primarily due to the upward flow of nutrients from deeper layers.

The analysis we present here outlines that overall, phytoplankton communities in the region are generally growing below 0.5 d\(^{-1}\) so that population biomass doubles approximately every three days. This finding is consistent with what we would expect from an oligotrophic region (Gasol et al. 2016). However, our results emphasise that relatively faster growth rates also occur in the Red Sea, particularly in the southern and central region, as observed during the time series sampling in winter. In particular, relatively faster growth rates and higher primary production, comparable to those commonly found in highly productive waters (>1gC m\(^{-2}\) d\(^{-1}\)) (Carr 2001), are occasionally found across the basin, and they are not restricted to the more productive
southern Red Sea. Therefore, although the basin can generally be considered a low productive marine system, episodes of relatively high primary production occur.

When inorganic nutrient concentrations and overall $P^B$ rates were relatively high in spring, phytoplankton communities were growing at a slow pace (on average $0.14 \pm 0.02 \text{ d}^{-1}$). Hence, in addition to nutrients, there are other factors controlling phytoplankton growth. Laboratory work with cultured species and studies carried out with natural phytoplankton communities have shown that a general relationship between temperature and metabolic rates such as photosynthesis or phytoplankton growth exists (Agawin et al. 1998; Regaudie-De-Gioux and Duarte 2012). However, the strength of temperature effects on metabolic rates may rely on the extent of nutrient supply to microalgae communities (Stæhr et al. 2002; Tadonléké 2010; Marañón et al. 2014; Marañón et al. 2018).

By combining data of a time-series and surveys, we could disentangle the main correlations between environmental variables and the growth rates of phytoplankton communities in the region. Our results, consistent with previous findings for planktonic metabolic rates in the Red Sea (López-Sandoval et al. 2019), indicated that temperature did not exert a direct statistical effect over $\mu$ nor $P^B$ rates. Instead, the correlation between temperature and $\mu$ (or $P^B$) rates seems to be mostly indirect, operating mainly through a relationship between temperature (and salinity) and nutrients, that will define nutrients availability in the water column, which will then directly correlate with the metabolic rates of phytoplanktonic communities in the Red Sea.

It is worth noting that when we run a similar path structure as in Figure 6C, with samples collected in the warmer southern region ($<20^\circ\text{N, n = 20}$), where nutrient concentration was relatively higher, the temperature was also not directly correlated to changes in $\mu$, while the significant correlation between phytoplankton growth rates and phosphate concentration
prevailed. These findings led us to reject our initial hypothesis that posited that temperature might significantly affect microalgal metabolic rates in the southern Red Sea. Our confirmatory path analysis also highlighted that nutrient concentration (particularly phosphorous and silicate) decreases whenever the Red Sea’s surface waters are warmer and saltier (Figure 6). These results summarise two main processes that defined the structure of the water column during our study: (1) the seasonal temperature changes (i.e., nutrient concentration was significantly higher during the cooler period, Table 1) and (2) the latitudinal changes in salinity (i.e., nutrient concentration was higher in the southern region, where salinity was the lowest, Figure 4). However, we note that the results from the path analysis help disentangle the direct and indirect components of the correlations with phytoplankton growth and productivity along the Red Sea, where parallel gradients in temperature, nutrient and salinity preclude deriving inferences from simple correlations. Nevertheless, the resulting components (direct and indirect) of the correlations remain statistical relationships, and cannot be used to reject causal relationships between temperature and phytoplankton growth and productivity, even though these are not the main drivers of the latitudinal patterns arising in the Red Sea.

Quantifying phytoplankton carbon-specific growth rates are challenging to address due to bacterial or detrital carbon present in field samples. The interference of non-photosynthetic sources of carbon may alter the $^{13}\text{C}_{\text{POC}}:^{13}\text{C}_{\text{DIC}}$ ratio; affecting the estimated growth rates. Although we must acknowledge this methodological caveat, previous studies done with natural communities and with cultured species indicate that growth rates derived from POC isotopic labelling compare well with those obtained from isotopic labelling of cellular compounds like photosynthetic pigments (Welschmeyer and Lorenzen 1984; Riemann et al. 1993) or fatty acids
(Dijkman et al. 2009). Thus, it seems unlikely that methodological constraints can resolve the growth rates found here.

To explain the generally low growth rates estimated in the present work, we must account for loss factors in addition to the availability of resources. The net growth of phytoplankton populations is the balance between cell division, which can be controlled by environmental variables, such as nutrients or light availability, and losses resulting from grazing or viral lysis. Across diverse marine regions, micrograzers consumption is the primary source of phytoplankton mortality in the oceans, consuming up 75% of the primary production in the tropical-subtropical regions (Calbet and Landry 2004). In the Red Sea, information of grazers activity is scarce. However, measurements from the northern region suggest that bottom-up control of medium-sized algae is more important than top-down control, whereas protozoan grazing rates on algae <6 µm is usually high, except for Synechococcus spp. where the grazer pressure is relatively low (Sommer et al. 2002). The growth rates derived here during short incubations (<5 h) may somewhat reduce the effect of grazing (Dijkman et al. 2009). However, loss processes (such as grazing and viral lysis) during the incubations may explain the slow growth rates observed in the presence of relatively high nutrients and temperatures below the annual maximum.

The decreases in chlorophyll-a, primary production, growth rates and nutrient concentrations toward the northern Red Sea suggest nutrient limitation of phytoplankton production and growth in the northern region, as proposed in the past (Sommer 2000; Rahav et al. 2015). Nutrient limitation has been demonstrated through large blooms developing in mesocosms holding central Red Sea communities, after receiving nutrient additions (Pearman et al. 2016), although these experiments did not add nutrients factorially. The high nitrate to phosphate ratios found,
generally above 50, are a common feature of many oligotrophic regions such as the Eastern Mediterranean Sea (Thingstad et al. 2005), and the subtropical and tropical Atlantic Ocean (Vidal et al. 2003), and further, suggest a phosphorus deficiency in the northern Red Sea. This finding is consistent with the path analysis results, which showed strong and direct effects of phosphorus concentration on the growth and photosynthetic performance of phytoplankton communities and their biomass (seen as Chl-a concentration). Overall, the current study highlights the crucial role that the availability of nutrients (phosphorus in particular) exerts on conforming the latitudinal gradients in productivity and growth rates of planktonic autotrophic communities along the Red Sea, while also provides a comprehensive set of measurements of these physiological traits that ultimately define the biomass levels in the basin.
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Conflict of Interest

None conflict of interest
Figure 1. Map indicating the position of the sampled stations along the Red Sea during the cruises (black dots) and the coastal time-series station in the Central Red Sea (red dot) sampled between 2016 and 2018.
Figure 2. Relationship between [A] sea surface temperature and [B] salinity with latitude during spring (green triangles), summer (red squares), autumn (yellow circles) and winter (blue crosses) cruises. The lines represent the ordinary least square linear regression (OLS). OLS $R^2$ and p-values for temperature: spring ($R^2 = 0.83, p<0.001$), summer ($R^2 = 0.94, p<0.001$), autumn ($R^2 = 0.93, p = 0.001$). OLS $R^2$ and p-values for salinity: spring ($R^2 = 0.97, p<0.001$), summer ($R^2 = 0.91, p<0.001$), autumn ($R^2 = 0.46, p<0.001$).
Figure 4. Scatter plot matrix, histograms (diagonal) and Spearman correlation coefficient (above the diagonal) between, primary production rates (PP), chlorophyll a-normalised photosynthesis ($P_B$) and phytoplankton growth rates ($\mu$) with chlorophyll-a concentration (Chl-a), sea surface temperature (SST), salinity (Sal), silicate (SiO$_2$), phosphate (PO$_4$) and nitrate (NO$_3$) concentrations and their latitudinal distribution. The anomalous high phosphate and nitrate concentration found in July and November 2017 at the central station (Figure 3) were not included in the analysis. Significant correlations are indicated with (*). * $p<0.05$; ** $p<0.01$, $p<0.001$.
Figure 5. [A] Surface (PP$_{surf}$) and [B] depth-integrated primary production (PP$_{Zint}$) measured between spring and summer and between autumn and winter during the oceanographic surveys.
Figure 6. Structural equation models that describes potential direct effects between environmental variables [sea surface temperature (SST), salinity (SAL), or dissolved inorganic nutrient concentrations [nitrate (NO$_3$), phosphate (PO$_4$) or silicate (SiO$_2$)] with Chlorophyll-a normalised photosynthesis (P$_B$) and phytoplankton growth rates (μ). Direction and magnitude of the path coefficients are colour coded. The green and red colour indicate positive and negative relationships, respectively. Double-headed dashed arrows link variables with correlated errors and Black arrows indicate non-significant effects. Standardised coefficients (values from arrows) are shown in Tables 2 and 3. A and E, depict the full initial theoretical models. B and F are improved models based on d-separation test. C and D are the final optimised models. The anomalous high phosphate and nitrate concentration found in July and November 2017 at the central station were not included. The coefficient of determination, the Akaike Information Criterion (AIC) and the global goodness-of-fit for each model are provide
Table 1: Mean and standard error of the mean (SE) of Chlorophyll-a concentration (Chl-a, mg m$^{-3}$), sea surface temperature (SST, ºC), salinity, and silicate (SiO$_2$, µM), phosphate (PO$_4$, µM) and nitrate (NO$_3$, µM) concentrations by season.

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<th>Chl-a</th>
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<th>Salinity</th>
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<th>PO$_4$</th>
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Table 2. Summary of results from the path analyses built for Chlorophyll a-normalised photosynthesis ($P^B$). Estimates shown here are standardised path coefficients. Model description refers to Figure 7.

<table>
<thead>
<tr>
<th>Model</th>
<th>Response</th>
<th>Predictor</th>
<th>P-Value</th>
<th>Estimate</th>
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Table 3. Summary of results from the path analyses built for phytoplankton growth rates ($\mu$).

Estimates shown here are standardised path coefficients. Model description refers to Figure 7.

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