



Impact of nutrients on photoacclimation of phytoplankton in an oligotrophic lake measured with long-term and high-frequency data: implications for chlorophyll as an estimate of phytoplankton biomass

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Abstract Chlorophyll measurements are commonly used to estimate phytoplankton biomass. However, phytoplankton readily acclimate to variations in light through a range of phenotypic responses, including major adjustments in chlorophyll pigmentation at the cellular level. The ratio of pigment chlorophyll to carbon concentration (Chl:C) is a commonly used metric in the oceanographic community to explore photoacclimation responses to varied light levels, yet is relatively rare in freshwater studies. Here we explore how nutrient variability impacted summertime Chl:C ratios of a natural phytoplankton community throughout the water column of a stratified oligotrophic lake. We utilized both long-term (18–24 years) and high-frequency (daily) data from Crater Lake, Oregon, a deep mountain lake with little anthropogenic disturbance. As expected, fluctuation in nutrients had a strong impact on phytoplankton

particle density, primary productivity, light penetration, and water clarity. However, chlorophyll concentration did not register predictable changes even though the vertical location of the deep chlorophyll maximum was responsive to the overlying algal density. The impact of elevated nutrients on the Chl:C ratio was further complicated by upward shifts in chlorophyll distribution. The muted response of chlorophyll concentration to nutrients may be partially explained by variations in phytoplankton community composition or iron stress.

Keywords Chlorophyll:carbon ratio · Deep chlorophyll maximum · Primary production · Crater Lake

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Introduction

Phytoplankton in freshwater and marine environments experience a highly variable light environment due to changes in day–night cycles, cloud cover, season, position within a stratified water column, and/or vertical movements within a surface mixed layer. Phytoplankton readily acclimate to these variations in light level through a combination of non-photochemical quenching (NPQ), regulation of photosystem II (PSII) reaction centers, and changes in cellular pigmentation, including chlorophyll (Falkowski & LaRoche, 1991, Halsey & Jones, 2015). Collectively,

these complex physiological processes are referred to as photoacclimation.

Regulation of cellular pigment density in response to changes in growth irradiance is the most documented form of photoacclimation (Graff et al., 2016). Analogous to large leaf areas populating the understory of a mature forest, phytoplankton cells increase chlorophyll density to more effectively capture the available light (Halsey & Jones, 2015). As such, increased pigment content at the cellular level over-inflates estimates of algal biomass based on laboratory calibration of chlorophyll concentration at higher light levels. Consequently, conventional assessments of chlorophyll concentration as a measure of algal biomass are known to be inaccurate in low-light, lentic environments (Cullen, 1982). Careful interpretation of depth-induced irradiance effects on cellular pigment density must compliment measurements of chlorophyll if algal biomass is to be estimated accurately, yet these comparisons are rarely employed in freshwater studies.

Nutrient availability is also an important variable driving phytoplankton production. Not only do nutrients impact cell division rates and thus algal density, nutrient availability can also impact cellular pigment density. In general, cellular chlorophyll density increases with added nutrients to facilitate rapid cell division and increased photosynthetic demand. Marine field studies show cellular pigment density increases with increased nutrients, particularly nitrogen (Bellacicco et al., 2016; Graff et al., 2016; Burt et al., 2018). Similarly, researchers showed a long-term decrease in cellular pigment density following reduced nitrate loading (Jakobson & Markager, 2016). Cellular light-harvesting apparatuses are rich in nitrogen and energetically expensive to produce. Thus, cellular chlorophyll tends to decrease when nitrogen is scarce (Halsey & Jones, 2015). The results of these marine studies could be extended to oligotrophic lakes as nitrogen can be limiting. However, none of these marine-based studies assessed the impact of nutrients on cellular chlorophyll density throughout a stratified water column, but were focused on the well-mixed surface water.

In the oceanographic field, the ratio of chlorophyll-a to algal carbon concentration (Chl:C) is a commonly used metric with which to explore cellular chlorophyll density responses to light and nutrients. This ratio is used to approximate the amount of chlorophyll per

algal cell and allows for comparison of phytoplankton occupying different light environments. Both chlorophyll concentration and carbon content can be estimated using laboratory techniques or in situ measurements with newer generation optical sensors. Laboratory methods used to measure chlorophyll concentration include extraction and High Performance Liquid Chromatography (HPLC). Methods for estimates of carbon include particulate organic carbon (POC) and biovolume via microscope or flow cytometry. Optical sensors (e.g., beam transmissometers, backscatter sensors, and satellite-based radiometers) allow in situ proxies of chlorophyll concentration and POC with the advantage of high vertical or temporal resolution that is typically not feasible with traditional boat based sampling. In situ optical proxies of POC have correlated well with conventional POC measurements in ocean (Behrenfeld & Boss, 2006; Mignot et al., 2014) and select freshwater (Boss et al., 2007) environments where inorganic particles are lacking.

Elevation of cellular pigment density under low irradiance conditions is likely to be an important consideration any time a deep chlorophyll maxima (DCM) forms below a thermocline in meso to oligotrophic systems (Cullen, 2015), or when vertical mixing transports phytoplankton to greatly reduced irradiance, such as during fall or spring isothermal conditions in the absence of ice cover (Letelier et al., 1993; Mignot et al., 2014). Elevated pigment density under low irradiance conditions in freshwater systems has been described in studies of lakes Superior (Barbiero & Tuchman, 2004, White & Matsumoto, 2012), Michigan (Fahnenstiel & Scavia, 1987), Tahoe (Andrews, 2010), Crater (Fennel & Boss, 2003), Redo (Felip & Catalan, 2000), and several of the Finger Lakes of central New York State (Kalenak et al., 2013). However, no lake study has specifically explored the impact of variable nutrient levels on the photoacclimation response even though nutrients clearly have a fundamental impact on algal biomass and Chl:C ratios. Characterizing the effects of nutrient fluctuations in a wide and replicated range of lake systems is fundamental for determining the accuracy of chlorophyll concentration as a surrogate for algal biomass and important for understanding the formation of the DCM.

Our objective here was to evaluate how annual fluctuation in nutrient availability affected both chlorophyll and carbon concentrations throughout

the photic zone of an oligotrophic lake during summer stratification. Because DCMs commonly form below thermoclines in less productive lakes, we sought to describe impacts of nutrients throughout the photic zone and not just in the surface mixed layer. We predicted that elevated nutrients would increase both phytoplankton density and chlorophyll concentration throughout the photic zone but the increase in chlorophyll would be proportionally greater given that cellular chlorophyll density is predicted to increase with added nutrients. This analysis was unique as it exploited both long-term (18–24 years) and high-frequency (daily) measurements of Chl:C ratios. Nutrient fluctuations were driven by natural variability in deep-water mixing in winter and the corresponding upwelling of nitrate from deep-water storage, quantified via deep-water temperature and nitrate measurements. Readings of chlorophyll for Chl:C ratios included both extracted chlorophyll-a as well as in situ profiling fluorescence sensors with 1 m vertical resolution. To validate the impact of nutrients on the algal community, we compared patterns in Chl:C ratios to additional long-term measures of primary productivity, light penetration, water clarity, and taxonomic composition of the phytoplankton community.

Methods

Study site

Data were collected from Crater Lake, a deep (590 m) ultra-oligotrophic lake near the crest of the Cascade Mountains in Oregon. Protected within a National Park since 1902, Crater Lake is one of the clearest lakes in the world, with optical characteristics similar to vast regions of the open ocean (Boss et al., 2007). The lake is facultatively dimictic, with periods of vertical mixing in the upper half of the water column in spring and late fall, reverse stratification in winter, and strong thermal stratification in summer. Onset of stratification typically occurs in May or June, depth of mean summer surface mixing is 10–15 m, and summer temperature difference between surface and hypolimnion is typically 9–12°C (summer Schmidt Stability \approx 15–25 kJ/m²). The lake's high transparency (mean summer Secchi depth = 31 m) and strong summer stratification combine with frequently

clear summer skies to create a broad and stable depth gradient of light exposure for phytoplankton (Hargreaves et al., 2007). Nitrogen is the primary nutrient limiting algal growth along with limitation by trace metals, likely iron (Groeger, 2007). Phosphorus and silica are always in excess (Larson et al., 2007a, b). Rarely freezing over completely, upward flux of nitrate stored in the deep-lake occasionally occurs during episodic deep-water mixing events in winter. These deep-water mixing events are well described, quantified, and correlated with direct measurements of deep-water nitrate storage (Larson et al., 2007a, b).

Long-term data

Crater Lake has a long-term (34 years) limnological monitoring program (LTLMP) providing consistent physical, chemical, biological, and climatological data collected during summer months (Jul–Sep). Here, LTLMP data related to the phytoplankton community, under water light environment, and nutrient flux were used to quantify photoacclimation responses of algae. Data set length varied by measurement (Table 1).

Phytoplankton data included measurements of algal particle density as the in situ particulate beam attenuation coefficient (C_p) and in situ chlorophyll fluorescence (Fchl). Laboratory measurements included extracted chlorophyll-a (Chl-a), primary productivity (PPR), and phytoplankton species composition. In situ measurements were made with sensors mounted to and controlled by a Seabird CTD (model 19) deployed via a wire winch from an 11 m research vessel. Measurements of C_p were collected with a WETLabs CSTAR or SeaTech beam transmissometer (25 cm), with data adjusted by subtracting attenuation due to water (C_w ; Pegau et al., 1995) and attenuation of particle free water for each cast (interval 300–310 m) based on methods developed for Crater Lake (Fennel & Boss, 2003). Similar to C_p , Fchl was measured with a WETLabs WetStar fluorometer and adjusted for particle free water by subtracting the average Fchl over the depth interval 300–310 m. Fluorescence values were then converted to Chl-a concentration through a manufacturer provided scale factor, produced through calibration with *Thalassiosira weissflogii* (Grunow) cultures. Boss et al. (2007) demonstrated that Fchl and Chl-a were highly correlated in Crater Lake, with the exception of reductions in near-surface values during daytime due

Table 1 Study years and the corresponding availability of high-frequency profiler and summer long-term monitoring variables

Year	580 m temp change	550 m NO ₃ change	Rank	Profiler Fchl and Bbp	Cp 93-16 In situ	Fchl 99-16 In situ	Chl-a 93-16 lab	Kd PAR 95-16 In situ	Secchi 93-16 In situ	PPR 96-16 lab	Phytotaxa 93-13 lab
1995	− 0.108	0.009	1		••		••		••		••
2004	− 0.073	− 0.003	2		••	••	••	••	••	••	••
2006	− 0.065	− 0.008	3		••	••	••	••	••	••	••
1999	− 0.054	− 0.002	4		••	••	••	••	••	••	••
1998	− 0.040	− 0.003	5		••		••	••	••	••	••
2011	− 0.039	− 0.004	6		•	••	•	••	•	••	•
2009	− 0.033	0.002	7		•	••	•	•	•	•	•
2012	− 0.016	0.000	8		•	•	•	•	•	•	•
2010	− 0.004	− 0.002	9	••	•	•	•	•	•	•	•
1993	0.010	0.000	10		•		•		•		•
1994	0.012	0.002	11		•		•		•		•
2008	0.018	− 0.001	− 13		•	•	•	•	•	•	•
2016	0.020	0.006	− 12		•	•	•	•	•	•	•
2003	0.023	0.007	− 11		•	•	•	•	•	•	•
2007	0.025	0.004	− 10		•	•	•	•	•	•	•
1996	0.027	0.008	− 9		•		•	•	•	•	•
2000	0.034	0.004	− 8		•	•	•	•	•	•	•
2001	0.040	0.004	− 7		•	•	•	•	•	•	•••
2013	0.040	0.003	− 6	•	•	•••	•	•	•	•	•••
2002	0.042	− 0.009	− 5		•••	•••	•••	•••	•••	•••	•••
2005	0.045	0.005	− 4		•••	•••	•••	•••	•••	•••	•••
1997	0.045	− 0.001	− 3		•••		•••	•••	•••	•••	•••
2014	0.054	0.004	− 2	•	•••	•••	•••	•••	•••	•••	
2015	0.063	0.001	− 1	•••	•••	•••	•••	•••	•••	•••	

Study years are in hierarchal order of largest winter mixing (positive rank) to least winter mixing (negative rank) based on 580 m annual temperature change. Two dots show the five biggest mixing years used in analyses and three dots shows the least winter mixing years. Headings for summer variables show period of record (years) and type of data (in situ or laboratory)

to non-photochemical quenching. Laboratory Chl-a samples were determined from 17 depths (0–300 m) via extraction using 0.45 µm filters with acetone and a Turner designs Model 10 fluorometer (McIntire et al., 2007). Net primary productivity was estimated at 13 depths (0–180 m), using the carbon-14 light–dark bottle method with a 4 h midday incubation time, 0.45 µm filters, and counted in a Beckman Liquid Scintillation Counter (McIntire et al., 2007). Phytoplankton species composition was determined from 17 depths via settling of 500 ml preserved samples and counting/identification with an inverted microscope and 300 cell count minimum. Two taxonomists were

involved over the study period (Robert E. Truitt and Deborah A. Hunter).

Light penetration and water clarity included measurements of underwater PAR [(attenuation coefficient (Kd) and 1% depth] and Secchi disk depth. Light penetration was measured within the upper 180 m using submersible radiometers (Licor LI1800UW scanning radiometer and Biospherical PRR2600 profiling reflectance radiometer) following methods outlined in Hargreaves et al. (2007). Measurements were made between 10:00 and 15:00 local time, under clear sky conditions. Secchi disk depth was determined as the mean of three descending and ascending readings

taken from the shaded side of a research vessel with calm surface conditions (Larson et al., 2007b).

High-frequency data

High-frequency data were available near year-round on a daily basis throughout the full water column for select years (Table 1). Measurements were collected with a WETLabs bio-optical sensor (BBFL2) and Seabird CTD (SBE41cp) attached to an automated profiling instrument (McLane Research Labs, Ice Tethered Profiler). The profiler was battery powered and traveled nightly along a wire mooring from 560 m to near-surface and back to 560 m (see Krishfield et al., 2008 for profiler details). Minimum sampling depth was 8 m in 2010 and 4 m in 2015 due to slight changes in mooring design. The CTD measured conductivity, temperature, pressure, and dissolved oxygen and sampled once per second resulting in approximately four readings per meter. The bio-optical sensor measured Fchl, optical backscatter (Bbp), and dissolved organic matter fluorescence (FDM) once every 6 s resulting in approximately one reading every 1.5 m. Similar to long-term data, measurements of Fchl were converted to Chl-a concentration through the scale factor provided by the manufacturer, based on calibration with *Thalassiosira weissflogii* cultures. For Bbp, backscatter raw voltage minus dark voltage was converted to the volume scattering function, beta (124°, 700 nm), using the factory provided scale factor. The particulate backscattering coefficient (Bbp) was calculated by subtracting the contribution of water as defined in Zhang et al. (2009) and multiplying resulting values by $2\pi X$, using a modified X factor of 1.076 (Sullivan et al., 2013).

Calculations

We calculated Chl:C ratios (Geider et al., 1998) as an estimate of chlorophyll density per algal particle. Chlorophyll was based on either extracted Chl-a (long-term data) or sensor-based Fchl (long-term and high-frequency data). Two methods from optical sensors were used as proxies of phytoplankton carbon: (1) the particulate beam attenuation coefficient (Cp) (long-term data) similar to Fennel & Boss (2003), and (2) particulate backscattering coefficient (Bbp) at 700 nm similar to Graff et al. (2016). Cp values in

Crater Lake were previously shown to be highly correlated with measurements of particulate organic carbon ($r^2 = 0.91$, Boss et al., 2007).

We determined the depth of the DCM for all available in situ Fchl profiles (1–1.5 m vertical resolution) in both long-term and high-frequency data (1999–2017). The depth of DCM corresponded to the maximum Fchl observation. The Fchl of algae residing in the epilimnion never approached those growing below the thermocline. The deep biomass maximum (DBM) was similarly determined but based on particle density from Cp (long-term) or Bbp (high-frequency) profiles. The depth of the DBM corresponded to the maximum Cp or Bbp value below a depth of 30 m so as to exclude algae growing in the epilimnion.

Annual nutrient flux

Crawford & Collier (1997, 2007) summarize how deep ventilation events due to thermobaric instabilities in winter transport significant amounts of nitrogen out of the hypolimnion and into the upper water column of Crater Lake. This upward mixing of deep-water nitrate can account for more than 85% of new nitrogen input to the euphotic zone (Dymond et al., 1996). Deep-water mixing events are characterized by sudden cooling at the lake bottom as some of the cold, reversely stratified water, floating in the upper water column, sinks to the lake floor (Crawford & Collier, 2007). Significant deep-water mixing events have occurred in 12 of 24 study years (Supplementary Figure S1). In years with no deep-water mixing events, water temperature at the bottom slowly increases due to a geothermal input of 1.4 W/m^2 (Wood et al., 2016). The magnitude of winter mixing as measured by annual temperature change at the lake bottom is positively correlated with annual change in deep-water nitrate storage ($R^2 = 0.52$, $N = 24$, Supplementary Figure S2) and there is a fivefold variation in annual nitrogen flux at the extremes of mixing and non-mixing years. Wood et al. (2016) further studied the frequency of deep ventilation events using a 1-dimensional deep ventilation model (1DDV) to simulate the ventilation of deep-water initiated by thermobaric instability and showed that such deep-water mixing events could significantly decline in Crater Lake in the future due to failure of wintertime reverse stratification to form in a warming climate.

We quantified annual upward nutrient flux due to deep-water mixing events in winter based on average annual water temperature change in the deep-lake (500–550 m) per methods by Crawford & Collier (2007). Annual temperature change was calculated with temperature profiles measured in August with a Seabird SBE19 CTD for the period 1993–2017. Temperature resolution for the CTD measurements is $\pm 0.0001^\circ\text{C}$ with an absolute accuracy estimated at 0.005°C (Crawford & Collier, 2007). Sampling years were ranked according to the degree of annual temperature change (Table 1) and the average of the five biggest and five least mixing years were compared for Cp, Fchl, Chl-a, Fchl:Cp ratio, Chl-a:Cp ratio and several additional LTLMP parameters (PPR, light extinction (Kd PAR), 1% PAR depth, and Secchi disk depth). Not all LTLMP parameters were available for all years (Table 1), so the five highest and five lowest nutrient years were not necessarily the same between data sets. Likewise, the two high-frequency years with daily water column data span a range in upward nutrient flux (Table 1).

Results

DCM and DBM depths

Elevated chlorophyll with decreasing irradiance at depth was consistently evident during summer stratification in Crater Lake based on vertical separation of DCM and DBM depths. Over a 19-year period, the DCM, where chlorophyll concentration was greatest, was consistently deeper than the DBM, where particle density was greatest (Fig. 1). On average, summer DCM depth was 52 m deeper than DBM depth ($N = 111$). Overlap of DCM and DBM depths was rare, occurring only when DBM depth was uncharacteristically deep. Daily measurements from high-frequency profiling supported the decoupling of DCM below DBM while also capturing distinct seasonal shallowing of DCM depth from summer to fall (Fig. 2). Overall, DCM was deepest in early summer (120–170 m) and subsequently shallowed, ending 65–85 m closer to the surface by November. Conversely, DBM depth was comparatively shallow (average 75 m) and more consistent seasonally.

The substantial shallowing of DCM depth from summer to fall corresponded with an increase in algal

biomass in the overlying water and a subsequent reduction in light penetration, combined with seasonal reductions of incident irradiance that naturally occurs from reduced sun angle. The percent PAR light level at the DCM was nearly constant ($0.25 \pm 0.21\%$, $N = 65$), even though vertical location of DCM fluctuated over a 106 m range during the 19 years of study (62–168 m). Seasonal shallowing of DCM in both long-term and high-frequency data and the corresponding reduction in light penetration appeared to be driven by seasonal increases in algal biomass in the overlying water. Both long-term and high-frequency DCM depth were strongly correlated with particle density measured at the overlying DBM depth (Fig. 3).

Nutrient impacts on carbon

A comparison of high versus low nutrient years in various long-term and high-frequency data sets is provided in Fig. 4. Here, the left hand column of figures shows carbon content (Cp or Bbp) as a surrogate for algal carbon content, the middle column of figures shows chlorophyll readings, and the right hand column shows the ratio of those two (Chl:C). Upwelling of nutrients associated with deep-water mixing events were linked to distinct elevation of algal carbon throughout the upper water column in both long-term (Cp) and high-frequency data (Bbp) (Fig. 4a, c). Summer Cp was 50% higher above 80 m in the five high nutrient years compared to the five low nutrient years. Likewise, carbon values estimated on a daily basis were consistently elevated throughout the summer and fall in the year with higher nutrients (Fig. 4d).

Further evidence for elevated algal biomass in higher nutrient years is apparent in several additional long-term data sets. Primary productivity above 80 m was $\approx 100\%$ higher in the 5 high nutrient years (Fig. 5a). Elevated algal biomass in higher nutrient years decreased water clarity as indicated by higher light extinction (Fig. 5b), shallower Secchi depth (Fig. 5c) and shallower euphotic zone (Fig. 5d).

Nutrient impacts on chlorophyll

Contrary to carbon and clarity parameters, long-term chlorophyll concentration was not higher in higher nutrient years as one might expect, although the

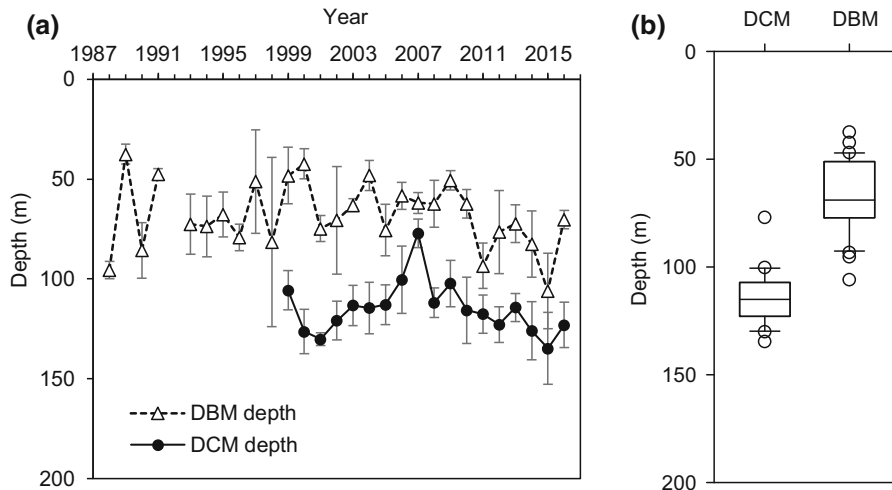


Fig. 1 **a** Mean summer (Jul–Sep) DCM and DBM depth in Crater Lake over 25-year period. **b** Box-and-whisker plots showing summer DBM and DCM depth

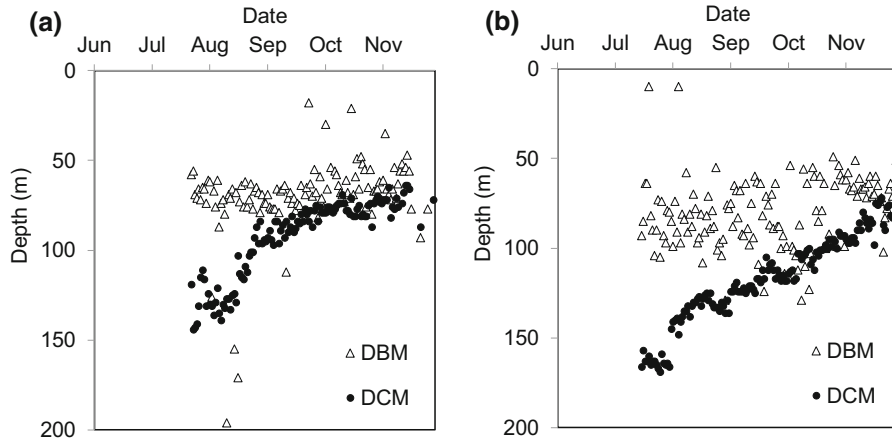


Fig. 2 Daily DCM and DBM depth from high-frequency profiler data during summer and fall. **a** 2010 and **b** 2015

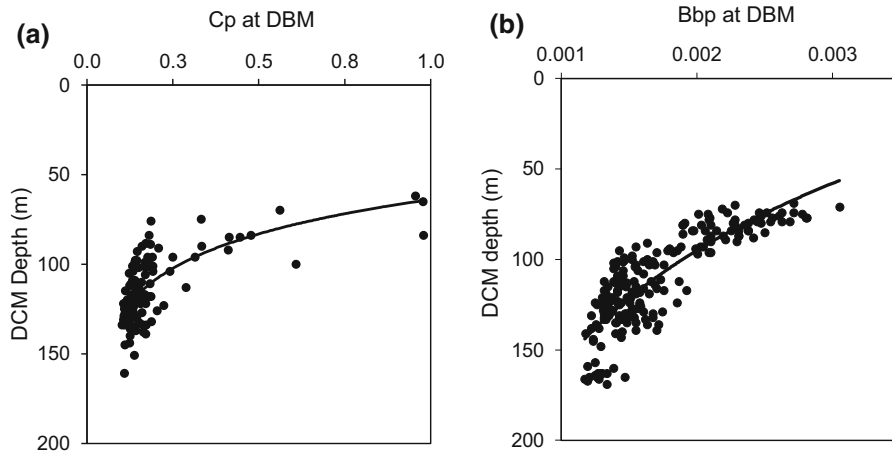
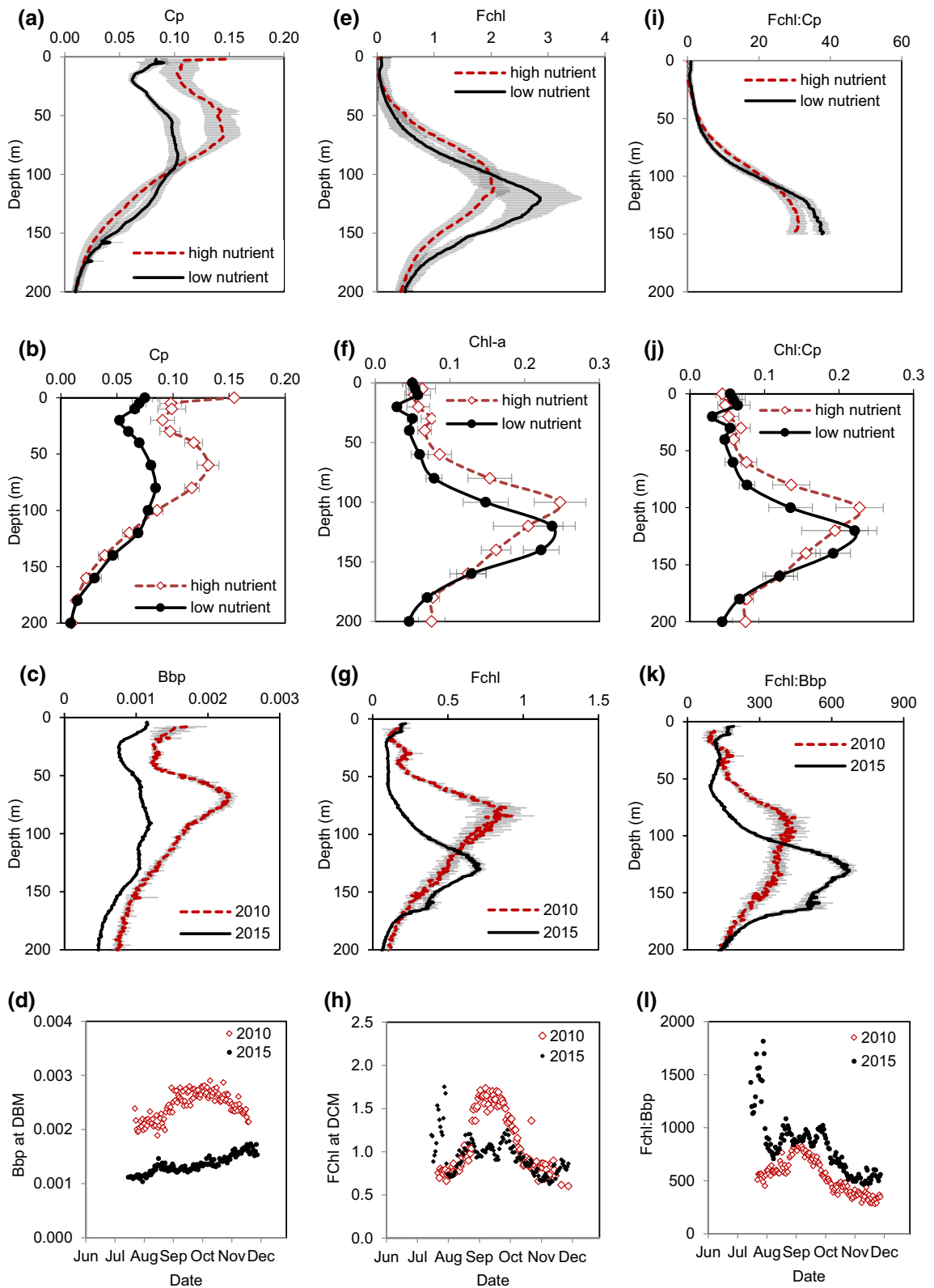


Fig. 3 **a** Long-term DCM depth versus particle density (C_p) at the DBM depth during summer (Jul–Sep), 1999–2016. **b** Daily DCM depth versus particle density (B_{bp}) at the DBM depth for summer and fall (Jun–Nov), 2010 and 2015



◀ **Fig. 4** Measurements of carbon (left column), chlorophyll (middle column), and Chl:C ratio (right column) from both long-term (rows 1 and 2) and high-frequency data (rows 3 and 4). Long-term water column data are average summer (Jul–Sep) shown for the five highest and five lowest nutrient years based on Table 1. High-frequency water column data (row 3) are mean summer (Jul–Sep) shown for a high nutrient year (2010) and a low nutrient year (2015). Seasonal high frequency (row 4) shows the daily pattern over summer and fall for **d** particle density at the DBM, **h** Fchl at the DCM, and **i** Fchl:C ratio at the DCM. Error bars show 95% confidence intervals. Note: Cp data in **b** is the same as **a** but only shown for depths that have corresponding filtered Chl-a samples

vertical distribution of both Fchl and Chl-a were shifted upward consistent with reduced light penetration (Fig. 4, middle column of figures). In situ Fchl concentration at the DCM depth actually tended to be lower in the higher nutrient years (Fig. 4e) and the depth of maximum (DCM) was ≈ 20 m shallower. Extracted Chl-a values at the DCM were no different between high and low nutrient years (Fig. 4f). There was a similar upward shift in Chl-a vertical distribution when nutrients were elevated; Chl-a above the DCM depth tended to be higher yet Chl-a values below the DCM were lower.

High-frequency Fchl based on daily measurements showed more variability over summer and fall in the high and low nutrient years. Measurements of Fchl at the DCM depth were higher in the high nutrient year in late August and September but not early in the summer or later in the fall (Fig. 4h). Consistent with long-term data, the vertical distribution of Fchl from the high-frequency sensor was clearly shifted upward in the high nutrient year compared to the low nutrient (Fig. 4g).

Nutrient impacts on Chl:C ratio

Since quantifying the degree of photoacclimation requires measurements of both chlorophyll and algal carbon, the effects of nutrients on both variables need to be considered when assessing the net effect on Chl:C ratios. The impact of nutrients on Chl:C were variable within the water column and variable between data sets due to the upward shift in chlorophyll vertical distribution and the overall muted response of chlorophyll concentration to nutrient fluctuations. The Chl:C ratio was nearly zero in the upper 50–60 m and no consistent differences were apparent between high and

low nutrient years in either long-term (Fig. 4i, j) or high-frequency data (Fig. 4k). As depth increased, cellular pigment concentrations increased such that Chl:C ratios were 3–9 times higher at peak DCM depths compared to the upper 50–60 m. Peak Chl:C values in the water column occurred at the DCM and years with elevated nutrients showed lower Chl:C ratio at the DCM depth (Fig. 4i, k) or no difference (Fig. 4j). Because peak chlorophyll was shifted upward due to a reduction in light penetration resulting from greater algal density at higher nutrients, the Chl:C ratio also shifted upward with higher nutrients. The result of this upward shift is that Chl:C ratio with elevated nutrients tended to be higher when approaching its DCM depth compared to the same depth in low nutrient years, yet Chl:C ratio in high nutrient years was reduced compared to low nutrient years when below the DCM.

Lower or equal Chl:C ratio at the DCM depth (where percent of surface light level is consistent) in high versus low nutrient years, suggested that elevated particle density with elevated nutrients was proportionally greater than any increase in chlorophyll concentration with elevated nutrients. The only data set where actual chlorophyll readings were higher when nutrients were higher was the deep-water mixing year (2010), where Fchl was only elevated in late Aug and Sep (Fig. 4h). Even in this example where Fchl at the DCM was higher at higher nutrients, the actual Chl:C ratio was still consistently lower throughout the summer and fall at higher nutrients (Fig. 4l), thereby demonstrating that added nutrients had a larger impact on increasing carbon content via cell division than increasing cellular chlorophyll pigment levels.

Phytoplankton taxonomy

Phytoplankton community composition during summer varied vertically within the water column in both high and low nutrient years similar to previous studies (McIntire et al., 2007). Overall, the epilimnion (above ≈ 20 m) was dominated by diatoms and dinoflagellates, whereas deeper depths had higher diversity (Fig. 6). When comparing years with high versus low nutrients, diatoms dominated at higher nutrients, both shallow in the epilimnion and deeper in the water column. Between 60 and 160 m, diatoms were 12% higher on average in the 5 highest nutrient years and were 29% higher in the epilimnion.

Fig. 5 Mean summer water column measurements of **a** primary productivity, and **b** light extinction (K_d PAR) in the five highest and five lowest nutrient years. Seasonal measurements of **c** Secchi depth and **d** 1% PAR depth in the five highest and five lowest nutrient years. Error bars show 95% confidence intervals

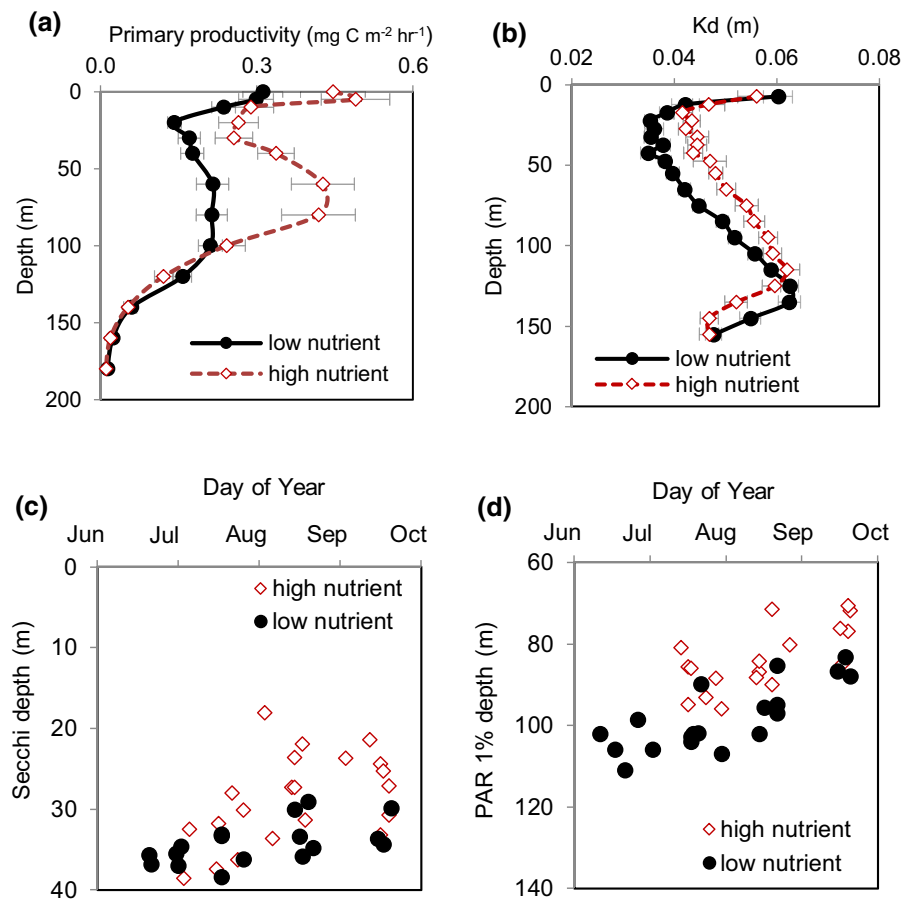
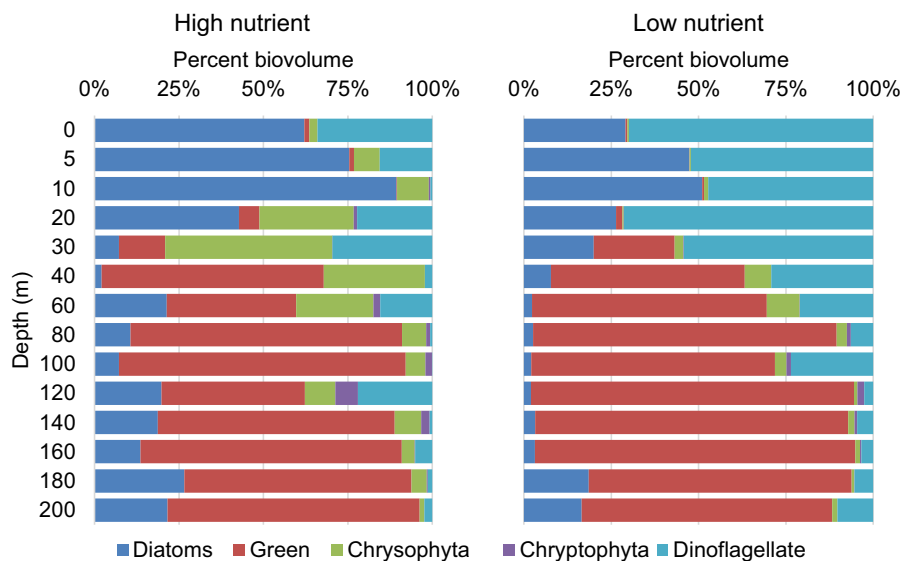


Fig. 6 Community composition (class) of phytoplankton by depth during August in the five biggest and five least mixing years



Chrysophytes also tended to be more numerous throughout the water column in high nutrient years. In low nutrient years, dinoflagellates dominated more in the epilimnion and upper hypolimnion.

Discussion

From a practical standpoint, chlorophyll concentration was not a functionally accurate measure of algal biomass within Crater Lake given that neither extracted Chl-a nor in situ Fchl responded to increased nutrients in a predictable or quantitative fashion. Likewise, chlorophyll values were not a readily useful metric even after estimating cellular pigment density using the Chl:C ratio. On the contrary, added nutrients from winter mixing events had strong positive effects on phytoplankton particle density in both long-term and high-frequency data (Fig. 4a–c), further validated by clear positive effects on long-term net primary productivity, water column light extinction, depth of the euphotic zone, and Secchi disk depth (Fig. 5). Reduced or no change in Chl:C ratios at higher nutrients in Crater Lake does not corroborate studies in marine systems where higher Chl:C ratios occurred at higher nitrate levels, either implied seasonally (Bellacchio et al., 2016), spatially (Graff et al., 2016, Burt et al., 2018), or from changes in nitrate runoff (Jakobsen & Markager, 2016). We found that the effects of nitrate availability on photoacclimation in Crater Lake were not easily predicted due to the following: (1) a muted response of chlorophyll concentration to higher nutrients and (2) the confounding effect of an upward shift in vertical chlorophyll distribution caused by a reduction in light penetration at higher algal biomass.

Exactly why chlorophyll did not respond to nutrient fluctuations in Crater Lake is unclear. We present two possible hypotheses. First, the muted response of chlorophyll to nutrient upwelling may be partially explained by variations in phytoplankton community composition between high and low nutrient years. Diatoms, which tend to be lower in Chl-a per unit biovolume (Deblois et al., 2013), were more dominant in high nutrient years throughout the water column (Fig. 6). A higher proportion of diatoms in years following deep-water mixing could make it appear that chlorophyll concentration was not responsive to nutrient upwelling by lowering the average Chl-a per

unit biovolume. Likewise, dinoflagellates were more abundant throughout the water column when nitrate was lower (Fig. 6). Halsey et al. (2014) suggested that motile species like dinoflagellates may have a competitive advantage by maintaining excess light-harvesting capacity, particularly when nitrogen is limiting. Motile species may maintain excess chlorophyll that is detached from the reaction centers when growth rates are low. This is suggested to be an important growth strategy for flagellates by rapidly responding to microscale nitrogen patches associated with viral lysis, fecal pellets, or excretion by grazers (Halsey & Jones, 2015). The detached chlorophyll is still present but currently unusable causing a mismatch between apparent chlorophyll levels and the actual light-harvesting capacity of the cell. If flagellates in Crater Lake have excess chlorophyll when less nitrogen is available, the higher density of dinoflagellates in the low nutrient years would give an artificially high chlorophyll content relative to years with higher nutrients.

Secondly, a lack of chlorophyll response to higher nutrients might be associated with iron stress and the peculiar way in which algal cells can form unphotosynthetically available chlorophyll under iron-limited conditions, which occurs in high-nitrate low-chlorophyll (HNLC) regions of the world's oceans (Behrenfeld & Milligan, 2013) and similar to that suggested for flagellates as discussed above. Iron is one of the most important micronutrients for photosynthesis and can be limiting in various lake and ocean environments (Norman et al., 2014). When iron and nitrogen are colimiting, chlorophyll is downregulated and the excess chlorophyll is energetically detached from photosystem reaction centers causing a reduction in growth (Halsey & Jones, 2015). The chlorophyll is still present, but currently unusable causing a mismatch between apparent chlorophyll levels and the actual light-harvesting capacity of the cells. Many questions still remain about evolutionary advantages of maintaining a pool of non-functional pigment that requires significant resource investment. Phytoplankton may have adapted to periodic deposition of iron containing dust that allows for rapid incorporation of excess pigments for additional photosynthetic capability (Behrenfeld & Milligan, 2013), thus priming the cell for future conditions. Although, Crater Lake is colimited by iron and nitrogen (Groeger, 2007), whereas over expression of photosynthetic pigments

in the ocean is usually limited to conditions where nitrogen is in excess (Behrenfeld et al., 2006, Schrader et al., 2011). Further studies using Fast Repetition Rate Fluorometry (Behrenfeld & Milligan, 2013) may be useful in Crater Lake to explore this discrepancy between iron stress and nitrate in more detail.

The use of profiling optical fluorescence sensors to investigate physical, chemical, and biological processes within lakes has been increasing rapidly (Brentrup et al., 2016). Although chlorophyll fluorescence within Crater Lake was not functionally correlated with nutrient availability, the depth of the DCM during summer was tightly coupled to the depth of percent PAR light penetration and responsive to the overlying algal density. The DCM depth at or near 1% of surface PAR is consistent with other studies looking at a diverse set of lakes (Leach et al., 2018, Modenutti et al., 2013, Hamilton et al., 2010). In general, mechanisms controlling DCMs in less productive systems are attributed to phytoplankton balancing the need for light from above and nutrients supplied from below (Abbott et al., 1984, Cullen, 2015). Utilizing optical fluorescence sensors to track the physical location of DCMs in meso to oligotrophic systems may be an especially useful indicator of the overlying water column conditions even if absolute chlorophyll concentrations are complicated by photoacclimation responses to reduced irradiance, nutrients, or changes in phytoplankton community composition.

Crater Lake is relatively well studied for a lake system and is fortunate to have a variety of algal, water clarity, and light penetration metrics at both long-term and high-frequency timescales. Failure of chlorophyll readings to increase with elevated nutrients underscores the challenges that limnologists and lake managers need to recognize when using chlorophyll or chlorophyll fluorescence sensors as an estimate of algal biomass. Although it has long functioned as a measure of algal biomass, chlorophyll variability can be dominated by physiological shifts in intracellular pigmentation in response to growth conditions, especially light and nutrients (Geider et al., 1998, MacIntyre et al., 2002, Behrenfeld & Boss, 2006). This study suggested that estimates of photoacclimation in freshwater lakes using the Chl:C ratio may not respond to added nutrients as expected based on similar oceanographic studies. Furthermore, the impact of nutrients on light penetration and corresponding shifts

in vertical chlorophyll distribution under stratified conditions need to be considered. It is difficult to compare these results to other lake systems because few studies have published basic results documenting photoacclimation impacts of low irradiance in freshwater environments (Fahnenstiel & Scavia, 1987; Felip & Catalan, 2000; Fennel & Boss, 2003; Barbiero & Tuchman, 2004; Andrews, 2010; White & Matsumoto, 2012; Kalenak et al., 2013) and even fewer have examined the impact of growth conditions on cellular pigment levels. Knowledge of the world's oceans have benefited greatly from extensive Chl:C measurements as observing and understanding photoacclimation responses in natural ocean conditions has allowed more accurate estimates of global net primary productivity, phytoplankton growth rates, and carbon cycling from readily available chlorophyll sources, including satellite-based radiometers (Behrenfeld et al., 2005, Westberry et al., 2016, Lyngsgaard et al., 2017). The freshwater community could similarly benefit by pairing chlorophyll readings with an independent measure of biomass (POC, biovolume via microscope, flow cytometry, Cp, Bbp) and characterizing specific physical and chemical conditions that influence Chl:C ratios in a wide and replicated range of lake systems. Assessing drivers of Chl:C ratios is fundamental for understanding the basic formation of DCMs, spatial and temporal variability in carbon cycling, and the reliability of commonly collected chlorophyll measurements as a surrogate for algal biomass.

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References

- Abbott, M. R., K. L. Denman, T. M. Powell, P. J. Richerson, R. C. Richards & C. R. Goldman, 1984. Mixing and the dynamics of the deep chlorophyll maximum in Lake Tahoe. *Limnology and Oceanography* 29: 862–878.
- Andrews, S. W., 2010. Measurement and Interpretation of Light Scattering by Suspended Particulates in an Oligotrophic Lake. Dissertation thesis, University of California, Davis.
- Barbiero, R. P. & M. L. Tuchman, 2004. The deep chlorophyll maximum in Lake Superior. *Journal of Great Lakes Research* 30: 256–268.
- Behrenfeld, M. J. & E. Boss, 2006. Beam attenuation and chlorophyll concentration as alternative optical indices of phytoplankton biomass. *Journal of Marine Research* 64: 431–451.
- Behrenfeld, M. J. & A. J. Milligan, 2013. Photophysiological expressions of iron stress in phytoplankton. *Annual Review of Marine Science* 5: 217–246.
- Behrenfeld, M. J., E. Boss, D. A. Siegel & D. M. Shea, 2005. Carbon-based ocean productivity and phytoplankton physiology from space. *Global Biogeochemical Cycles*. <https://doi.org/10.1029/2004GB002299>.
- Behrenfeld, M. J., K. Worthington, R. M. Sherrell, F. P. Chavez, P. Strutton, M. McPhaden & D. M. Shea, 2006. Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics. *Nature* 442: 1025.
- Bellacicco, M., G. Volpe, S. Colella, J. Pitarch & R. Santoleri, 2016. Influence of photoacclimation on the phytoplankton seasonal cycle in the Mediterranean Sea as seen by satellite. *Remote Sensing of Environment* 184: 595–604.
- Boss, E. S., R. Collier, W. S. Pegau, G. Larson & K. Fennel, 2007. Measurements of spectral optical properties and their relation to biogeochemical variables and processes in Crater Lake, Crater Lake National Park, OR. *Hydrobiologia* 574: 149–159.
- Brentrup, J. A., C. E. Williamson, W. Colom-Montero, W. Eckert, E. de Eyto, H. P. Grossart, Y. Huot, P. D. Isles, L. B. Knoll, T. H. Leach & C. G. McBride, 2016. The potential of high-frequency profiling to assess vertical and seasonal patterns of phytoplankton dynamics in lakes: An extension of the Plankton Ecology Group (PEG) model. *Inland Waters* 6: 565–580.
- Burt, W. J., T. K. Westberry, M. J. Behrenfeld, C. Zeng, R. W. Izett & P. D. Tortell, 2018. Carbon: Chlorophyll ratios and net primary productivity of subarctic Pacific surface waters derived from autonomous shipboard sensors. *Global Biogeochemical Cycles* 32: 267–288.
- Crawford, G. B. & R. W. Collier, 1997. Observations of a deep-mixing event in Crater Lake, Oregon. *Limnology and Oceanography* 42: 299–306.
- Crawford, G. B. & R. W. Collier, 2007. Long-term observations of deepwater renewal in Crater Lake, Oregon. *Hydrobiologia* 574: 47–68.
- Cullen, J. J., 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 791–803.
- Cullen, J. J., 2015. Subsurface chlorophyll maximum layers: Enduring enigma or mystery solved? *Annual Review of Marine Science* 7: 207–239.
- Deblois, C. P., A. Marchand & P. Juneau, 2013. Comparison of photoacclimation in twelve freshwater photoautotrophs (chlorophyte, bacillaryophyte, cryptophyte and cyanophyte) isolated from a natural community. *PLoS ONE* 8: 57139.
- Dymond, J., R. Collier, J. McManus & G. L. Larson, 1996. Unbalanced particle flux budgets in Crater Lake, Oregon: Implications for edge effects and sediment focusing in lakes. *Limnology and Oceanography* 41: 732–743.
- Fahnenstiel, G. L. & D. Scavia, 1987. Dynamics of Lake Michigan phytoplankton: The deep chlorophyll layer. *Journal of Great Lakes Research* 13: 285–295.
- Falkowski, P. G. & J. LaRoche, 1991. Acclimation to spectral irradiance in algae. *Journal of Phycology* 27: 8–14.
- Felip, M. & J. Catalan, 2000. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: Decoupling in their spatial and temporal maxima. *Journal of Plankton Research* 22: 91–106.
- Fennel, K. & E. Boss, 2003. Subsurface maxima of phytoplankton and chlorophyll: Steady-state solutions from a simple model. *Limnology and Oceanography* 48: 1521–1534.
- Geider, R. J., H. L. MacIntyre & T. M. Kana, 1998. A dynamic regulatory model of phytoplankton acclimation to light, nutrients, and temperature. *Limnology and oceanography* 43: 679–694.
- Graff, J. R., T. K. Westberry, A. J. Milligan, M. B. Brown, G. D. Olmo, K. M. Reifel & M. J. Behrenfeld, 2016. Photoacclimation of natural phytoplankton communities. *Marine Ecology Progress Series* 542: 51–62.
- Groeger, A. W., 2007. Nutrient limitation in Crater Lake, Oregon. *Hydrobiologia* 574: 205–216.
- Halsey, K. H. & B. M. Jones, 2015. Phytoplankton strategies for photosynthetic energy allocation. *Annual Review of Marine Science* 7: 265–297.
- Halsey, K., A. Milligan & M. Behrenfeld, 2014. Contrasting strategies of photosynthetic energy utilization drive life-style strategies in ecologically important picoeukaryotes. *Metabolites* 4: 260–280.
- Hamilton, D. P., K. R. O'Brien, M. A. Burford, J. D. Brookes & C. G. McBride, 2010. Vertical distributions of chlorophyll in deep, warm monomictic lakes. *Aquatic Sciences* 72: 295–307.
- Hargreaves, B. R., S. F. Girdner, M. W. Buktenica, R. W. Collier, E. Urbach & G. L. Larson, 2007. Ultraviolet radiation and bio-optics in Crater Lake, Oregon. *Hydrobiologia* 574: 107–140.
- Jakobsen, H. H. & S. Markager, 2016. Carbon-to-chlorophyll ratio for phytoplankton in temperate coastal waters: Seasonal patterns and relationship to nutrients. *Limnology and Oceanography* 6: 1853–1868.
- Kalenak, D., E. Boss & S. W. Effler, 2013. Inherent optical properties of suspended particulates in four temperate lakes: Application of in situ spectroscopy. *Hydrobiologia* 713: 127–148.
- Krishfield, R., J. Toole, A. Proshutinsky & M. L. Timmermans, 2008. Automated ice-tethered profilers for seawater observations under pack ice in all seasons. *Journal of Atmospheric and Oceanic Technology* 25: 2091–2105.

- Larson, G. L., R. Collier & M. Buktenica, 2007a. Long-term limnological research and monitoring at Crater Lake, Oregon. *Hydrobiologia* 574: 1–11.
- Larson, G. L., R. L. Hoffman, D. C. McIntire, M. W. Buktenica & S. F. Girdner, 2007b. Thermal, chemical, and optical properties of Crater Lake, Oregon. *Hydrobiologia* 574: 69–84.
- Leach, T. H., B. E. Beisner, C. C. Carey, P. Pernica, K. C. Rose, Y. Huot, J. A. Brentrup, I. Domaizon, H. P. Grossart, B. W. Ibelings & S. Jacquet, 2018. Patterns and drivers of deep chlorophyll maxima structure in 100 lakes: The relative importance of light and thermal stratification. *Limnology and Oceanography* 63: 628–646.
- Letelier, R. M., R. R. Bidigare, D. V. Hebel, M. Ondrusek, C. D. Winn & D. M. Karl, 1993. Temporal variability of phytoplankton community structure based on pigment analysis. *Limnology and Oceanography* 38: 1420–1437.
- Lyngsgaard, M. M., S. Markager, K. Richardson, E. F. Møller & H. H. Jakobsen, 2017. How well does chlorophyll explain the seasonal variation in phytoplankton activity? *Estuaries and Coasts* 40: 1263–1275.
- MacIntyre, H. L., T. M. Kana, T. Anning & R. J. Geider, 2002. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria I. *Journal of Phycology* 38: 17–38.
- McIntire, C. D., G. L. Larson & R. E. Truitt, 2007. Seasonal and interannual variability in the taxonomic composition and production dynamics of phytoplankton assemblages in Crater Lake, Oregon. *Hydrobiologia* 574: 179–204.
- Mignot, A., H. Claustre, J. Uitz, A. Poteau, F. D’Ortenzio & X. Xing, 2014. Understanding the seasonal dynamics of phytoplankton biomass and the deep chlorophyll maximum in oligotrophic environments: A Bio-Argo float investigation. *Global Biogeochemical Cycles* 28: 856–876.
- Modenutti, B., E. Balseiro, M. B. Navarro, C. Laspomadere, M. S. Souza & F. Cuassolo, 2013. Environmental changes affecting light climate in oligotrophic mountain lakes: The deep chlorophyll maxima as a sensitive variable. *Aquatic Sciences* 75: 361–371.
- Norman, L., D. J. Cabanesa, S. Blanco-Ameijeiras, S. A. Moisset & C. S. Hassler, 2014. Iron biogeochemistry in aquatic systems: From source to bioavailability. *CHIMIA International Journal for Chemistry* 68: 764–771.
- Pegau, W. S., J. R. V. Zaneveld & K. J. Voss, 1995. Toward closure of the inherent optical properties of natural waters. *Journal of Geophysical Research: Oceans* 100: 13193–13199.
- Schrader, P. S., A. J. Milligan & M. J. Behrenfeld, 2011. Surplus photosynthetic antennae complexes underlie diagnostics of iron limitation in a cyanobacterium. *PLoS ONE* 6: 18753.
- Sullivan, J. M., M. S. Twardowski, J. Ronald, V. Zaneveld & C. C. Moore, 2013. Measuring optical backscattering in water. *Light Scattering Reviews* 7: 189–224.
- Westberry, T. K., P. Schultz, M. J. Behrenfeld, J. P. Dunne, M. R. Hiscock, S. Maritorena, J. L. Sarmiento & D. A. Siegel, 2016. Annual cycles of phytoplankton biomass in the subarctic Atlantic and Pacific Ocean. *Global Biogeochemical Cycles* 30: 175–190.
- White, B. & K. Matsumoto, 2012. Causal mechanisms of the deep chlorophyll maximum in Lake Superior: A numerical modeling investigation. *Journal of Great Lakes Research* 38: 504–513.
- Wood, T.M., Wherry, S.A., Piccolroaz, S., & Girdner, S.F., 2016. Simulation of Deep Ventilation in Crater Lake, Oregon, 1951–2099. No. 2016-5046. US Geological Survey.
- Zhang, X., L. Hu & M. X. He, 2009. Scattering by pure seawater: Effect of salinity. *Optics Express* 17: 5698–5710.

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