Reconsideration of the phytoplankton seasonality in the open Black Sea

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Scientific Significance Statement
Phytoplankton production supports the entire food web of the open Black Sea, and it is believed that new production is sustained by inputs of nitrate from deeper waters during winter convective mixing. However, this current assertion is primarily based on analyses of chlorophyll a (Chl a) data, disregarding the large seasonal variations in cell-specific Chl a depending on the species, light intensity and nutrient concentrations. Our study shows that phytoplankton biomass in the open Black Sea primarily accumulates during late summer and autumn, when nitrate is depleted in the surface layer. The enhanced productivity could be driven by large diatoms harvesting nutrients from depths and nitrogen fixation by cyanobacteria, living in symbiotic relationship with diatoms.

Abstract
Chlorophyll a (Chl a) observations from satellites exhibit a unimodal seasonal variation in the open Black Sea that peaks during winter, which has led to the hypothesis that phytoplankton production is sustained by convective mixing of nitrate from deeper layers. We compiled in situ carbon-to-Chl a ratios for the entire year, displaying an expected seasonal pattern ranging from 46 in February to 195 mg C mg Chl a−1 in September. We combined monthly ratios with satellite Chl a to obtain a comprehensive proxy data set for phytoplankton carbon biomass, and used this to examine the seasonal variation in phytoplankton biomass and bloom occurrences. Contrary to current understanding, our results showed that phytoplankton accumulation is predominantly taking place from July to September, when biomass increased threefold despite nitrate transport from below being negligible. We hypothesize that nitrate harvesting at depths and endosymbiotic nitrogen fixation by large diatoms could be important, albeit unexplored mechanisms.

Over the last two decades, the Black Sea has experienced reduced nutrients inputs and increasing temperatures (Oguz et al. 2003). The deep open regions (> 1000 m) has entered a new low-nutrient regime with dissolved inorganic nitrogen almost depleted in surface waters (~ 1 μmol L−1) throughout the year (Pakhomova et al. 2014) and subsurface

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Data Availability Statement: Chlsat, Csat, in situ C : Chl a ratio data, and Metadata template for data set are available in the Zenodo repository at http://doi.org/10.5281/zenodo.4447830.

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peak nitrate concentrations halved since the early 1990s (Yunev et al. 2005). Unfortunately, our knowledge on the response of the phytoplankton community to these changes is limited due to sparse and scattered information from in situ samples. Consequently, satellite-derived chlorophyll a (Chl a) concentrations (Chlsat) have provided convenient information to investigate seasonal and long-term changes in phytoplankton biomass and trace algae blooms in the open Black Sea (e.g., Finenko et al. 2014; Mikaelyan et al. 2017a,b; Vostokov et al. 2019). These studies led to the recognition of two characteristic Chlsat maxima: a main winter–spring peak in February–March and a less pronounced autumn peak in November, based on the implicit assumption that Chlsat peaks also represented phytoplankton biomass maxima.

Different mechanisms supporting the intense phytoplankton development and algae blooms have been suggested based on this tenet. Finenko et al. (2014) proposed that the winter peak develops over the domes of the eastern and western cyclonic gyres due to the shallower position of the main pycnocline, the upper boundary of which is located near the euphotic zone, where nutrients transported from deeper layers support algae development. Mikaelyan et al. (2017b) proposed the Pulsing-Bloom hypothesis for the winter–spring bloom, based on the idea of alternations between deep-mixing and low-turbulence regimes. Moreover, Mikaelyan et al. (2017a) proposed that the autumn bloom is driven by deepening of the upper mixed layer through erosion of the seasonal thermocline, which alleviates nutrient limitation and promotes phytoplankton growth.

However, we propose to reconsider the current perception of the phytoplankton seasonality and mechanisms supporting phytoplankton blooms in the open Black Sea. Our proposition is based, above all, on studies demonstrating substantial seasonal changes in cell-specific Chl a depending on the species, light intensity and nutrient concentrations (Bellaccioco et al. 2016; Jakobsen and Markager 2016). A recent study has shown that the carbon-to-Chl a ratio (C : Chl a; weight : weight) on the Black Sea shelf is 3–6 times lower in winter than in summer (Stelmakh and Gorbunova 2018), and it is reasonable to assume similar seasonal changes in the open sea as well. Consequently, using Chl a or Chlsat as a proxy of phytoplankton biomass could potentially lead to a misperception of the seasonal variation in phytoplankton biomass, including the actual timing and mechanisms sustaining phytoplankton blooms.

We have compiled C : Chl a data from the open Black Sea and combined it with the Chlsat to obtain a better proxy of phytoplankton biomass. Our objective was to describe the seasonal variation in phytoplankton biomass and occurrences of blooms using the algorithm of Carstensen et al. (2015).

Material and methods

Selection of study areas

We selected six regions (Fig. 1) representing the main elements of the Black Sea cyclonic circulation system (Supporting Information Fig. S1). In addition to the Rim Current, a cyclonically meandering peripheral current, there are a number of anti-cyclonic eddies along the periphery and two large-scale cyclonic gyres (so-called western and eastern halistatics) (Oguz et al. 1993). These gyres and eddies are characterized by different types of vertical water movements (Ivanov and Belokopitov 2013). In our study, Regions 2 and 5 represent the central areas of halistatics, characterized by upwelling of deeper water, and Regions 1, 3, 4 and 6 represent the northern and southern peripheral regions, characterized by downwelling.

Satellite-derived Chl a concentration

In the absence of regular in situ phytoplankton monitoring data, we used Chlsat data (1998–2018) derived from the regional algorithm of the Marine Hydrophysical Institute in Sevastopol (Suslin and Churilova 2016). It combines data from three satellite instruments (SeaWiFS, 1997–2010; MODIS [Aqua], 2002–2018; and MODIS [Terra], 2000–2018) and has substantially lower relative error of Chlsat recovery (30–50%) compared to the standard NASA algorithm (200–400%) (Suslin et al. 2018). 5-d composite Chlsat for rectangular areas of 0.3° longitude and latitude were aggregated into the six regions (Fig. 1) by averaging (estimated from about 250–250,000 pixels). The loss of satellite information due to cloud cover (~5% of 5-d composites per year, primarily in December–March) was minimized by combining data from three satellite instruments with multiple overflights per day (Suslin and Churilova 2016).
**Carbon-to-Chl \( a \) ratio**

In contrast to Chlsat, there is no regional algorithm for the Black Sea for obtaining phytoplankton biomass from satellite data. Therefore, we used the C : Chl \( a \) ratio, calculated from surface samples in the open Black Sea (Fig. 1) taken during various research cruises (1988–2013; \( n = 78 \)), where both phytoplankton biomass and Chl \( a \) concentration were analyzed (Black Sea Data Base 2003; Stelmakh 2018). Microscopic analysis of phytoplankton biomass included only nano- and microphytoplankton, but these cells (> 2 \( \mu m \)) account for the major share of Chl \( a \) and phytoplankton biomass in the surface layer throughout the year. Picophytoplankton (< 2 \( \mu m \)) accounts for less than 10% as measured by epifluorescent microscopy in the 1980s (Ratkova et al. 1989) and less than 5% as measured by flow cytometry in the 2000s (Churilova et al. 2017). Phytoplankton species identification was carried out using Tomas (1997), and cell counts were converted into volume using measured dimensions and species-specific geometric shapes. Phytoplankton carbon biomass was calculated from cell volume using Menden-Deuer and Lessard (2000) for diatoms and dino-bi英格物 was calculated from cell volume using Menden-Montagnes et al. (1994) for the coccolithophore Emiliania huxleyi, and Strathmann (1967) for other algae. Monthly means of C : Chl \( a \) were computed and used for converting Chlsat to carbon biomass (Csat).

**Statistical analysis**

Time series of the average 5-d composite Chlsat and Csat for each of the six regions (Fig. 1) were separated into a base seasonal pattern and outliers. Outliers were defined as observations exceeding the 99\(^{th}\) percentile of the normal distribution using a periodic spline function (gam [bs = “cp”] in R-package mgcv) to describe the expected seasonal variation (Carstensen et al. 2015). The algorithm iteratively sorts out outliers until all remaining observations follow a normal distribution with a seasonal mean. In the case of Csat, outliers were defined as phytoplankton blooms.

**Results**

The 78 C : Chl \( a \) observations were reasonably distributed over all months of the year, with slightly fewer samples in autumn and more samples in early summer (Fig. 2). The C : Chl \( a \) ratios (15–275) followed an expected unimodal seasonal variation with monthly averages ranging from 46 in February to 195 in September (Fig. 2). After employing this seasonal variation to the satellite observations (Chlsat), we observed a marked shift in the base seasonal pattern (non-bloom observations) from December–January for Chlsat to September–November for Csat (Fig. 3), and this difference between Chlsat and Csat was consistent across all regions and years (Fig. 3 and Supporting Information Figs. S2–S7), allowing us to describe the general seasonal variation by means of monthly averages (Fig. 4). Importantly, Csat displayed a seasonal variation markedly different from the sinusoidal pattern of Chlsat, increasing rapidly from July to September, keeping high values in October and November, with a gradual decrease over the remainder of the annual cycle.

The majority of the phytoplankton blooms, which were observed only during August–November and most pronounced in September (Fig. 5), were short-lived, lasting only 5 d (69% of Csat bloom observations), whereas 14% and 12% of the blooms extended 10 d and 15 d, respectively (Fig. 3 and Supporting Information Figs. S2–S7). The phytoplankton biomass of blooms typically exceeded the base seasonal variation by 1.3–3 times. Region 2 experienced more blooms (7.1% of the observations) than any other region (between 2.5% and 4.5%).

**Discussion**

The phytoplankton community of the open Black Sea is understudied because of a severe lack of in situ measurements. Consequently, remote sensing of Chl \( a \) from satellites has delivered a convenient alternative for describing phytoplankton biomass and productivity, which are important for understanding the basic functioning of the ecosystem. The observed seasonality both in Chl \( a \) (Yunev et al. 2002) and Chlsat (Finenko et al. 2014; Mikaelyan et al. 2017a,b; Vostokov et al. 2019) have led to the perception that most of the annual phytoplankton production occurs during the colder months (November–March), driven by inputs of new nutrients from convective mixing and deepening of the upper mixed layer (Mikaelyan et al. 2017a,b). According to these studies, phytoplankton exhibits a Pulsing-Bloom behavior with loopholes of high productivity occurring with stabilization of the water column subsequent to periods of high nutrient entrainment from below. However, such proposed pulsing events were not apparent in our Csat data (Fig. 3 and Supporting Information Figs. S2–S7). On the contrary, our analysis suggests that peak phytoplankton biomass

![Fig 2. Seasonal patterns for C : Chl \( a \) ratio in the open Black Sea. Standard deviations are marked by the error bars with the number of samples for calculating the monthly means inserted.](image-url)
biomass occurs much earlier in the season, implying that the conception of phytoplankton seasonality and the pervasive Pulsing-Bloom hypothesis needs to be revisited.

Our study is the first to assemble C : Chl a ratios from the open Black Sea covering the entire seasonal cycle, and combine it with satellite imagery to obtain a comprehensive proxy.
data set for phytoplankton carbon biomass in the surface layer. We found seasonal variations in C : Chl $a$ (Fig. 2) that match those for the Black Sea shelf ranging from 56 in winter to 234 in summer (Stelmakh and Gorbunova 2018). The seasonal pattern and range of variation in C : Chl $a$ in the open Black Sea are also in accordance with published full annual cycles of C : Chl $a$, based on in situ and satellite data, for other temperate open sea ecosystems (Varquez-Dominguez et al. 2013; Bellacicco et al. 2016; Jakobsen and Markager 2016; Supporting Information Fig. S8). Thus, the combination of satellite chlorophyll with C : Chl $a$ has changed our understanding of the seasonal distribution of phytoplankton biomass in the open Black Sea.

The relatively few phytoplankton investigations since 2000 suggest that small cellular diatoms Chaetoceros curvisetus and Pseudo-nitzschia pseudodelicatissima as well as dinoflagellates Scrippsiella trochoidea and Akashiwo sanguinea dominate in spring, switching to the predominance of the coccolithophore E. huxleyi in early summer, followed by large diatoms Pseudosolenia calcari-avis and Proboscia alata in summer and autumn months, and finally shifting towards smaller diatoms such as Skeletonema costatum, Chaetoceros socialis, and Chaetoceros curvisetus during winter (Silkin et al. 2013; Churilova et al. 2017; Mikaelyan et al. 2018; Stelmakh and Gorbunova 2018). Noteworthy, the summer and autumn large diatom species are characterized by relatively low chlorophyll concentrations in their cells and high C : Chl $a$ ratios reaching 590 (e.g., Moor and Villareal 1996). Thus, it is likely that the threefold increase in $C_{sat}$ from July to September is due to enhanced growth of these large diatoms (LDs), but this leads to the question: Which mechanisms can sustain this new discovery?

In theory, the increase in $C_{sat}$ may result from enhanced phytoplankton growth or released grazing pressure or a combination of both. Considering the relatively low productivity (Yunev et al. 2017) and the strong nitrogen limitation (Yayla et al. 2001) of the open Black Sea, it is most likely that the phytoplankton biomass accumulation is sustained by inputs of “new” nitrogen. For the open Black Sea, the two most likely sources of “new” nitrogen to the euphotic surface water are the nitrate-rich deeper water and the atmosphere.

**Deep-water mixing into the surface**

Entrainment of nutrient-rich deeper water to the surface layer largely depends on the vertical structure of the water column, the depth location of the nitrate peak, meteorological conditions, and season (Yunev et al. 2005). From April until October, a seasonal thermocline forms with maximum vertical gradient at around 15–20 m (Ivanov and Belokopitov 2013), which effectively seals off the surface layer from nitrate-rich waters found at $>/=24$ m and deeper (Yunev et al. 2005). Typically, nitrate transport to the surface layer by convective mixing does not start until second half of October or November when the thermocline breaks down due to cooling and increasing winds (Ivanov and Belokopitov 2013). Importantly, this transport by convective mixing has decreased substantially since the early 1990s, because (1) nitrate concentrations at the halocline have approximately halved and (2) the location of the nitrate-rich deep water has deepened by 10–15 m, resulting in a twofold to threefold decrease of the nitrate gradient between the nitrate maximum and the euphotic zone (Yunev et al. 2005), as well as...
enhanced thermal stratification and diminished surface circulation due to warming, thereby reducing upward mixing and nutrient supply to the euphotic zone (Oguz et al. 2003).

As a result, nitrate transport from deeper water is essentially not realized, even in winter, when convective mixing is much more intense than in November (Ivanov and Belokopitov 2013). This supports the absence of biomass increases and blooms in winter and early spring months (Fig. 3 and Supporting Information Figs. S2–S7). The absence of significant upward nitrate transport is also evidenced by the lack of differences in the seasonal patterns among regions (Fig. 3 and Supporting Information Figs. S2–S7). Hence, if the phytoplankton growth was sustained by “new” nitrogen from the deeper layers, we would expect higher biomass in the halistatic regions than in the periphery regions, particularly in the cold years.

Nitrogen from the atmosphere

Atmospheric deposition of inorganic fixed nitrogen (IFN) is predominantly through wet deposition, when droplets scavenge nitrogen-containing compounds from the atmosphere, but most of the precipitation in the Black Sea region occurs during winter (Ivanov and Belokopitov 2013). Consequently, IFN deposition is largest in winter and lowest in summer (Varenik et al. 2015). Hence, it is unlikely that atmospheric deposition could sustain the increase in phytoplankton biomass from July to September.

Atmospheric nitrogen can also be fixed by diazotrophic cyanobacteria, which supply fixed nitrogen to their partner, typically LDs, so-called Diatom Diazotroph Associations (DDAs) (Villareal 1992). Nitrogen fixed by diazotroph endosymbionts is effectively transferred to the host (Mulholland et al. 2004; Foster et al. 2011) and DDAs have been observed in tropical and subtropical waters of all the major oceans (see review, Foster and O’Mullan 2008). For example, the symbiotic heterocystous cells of the cyanobacteria Richelia intracellularis and Calothrix rhizosoleniae have an average N2 fixation rate 170–420 times higher than the rate estimated for free-living cyanobacteria cells (Foster et al. 2011). Moreover, cyanobacteria symbionts have rapid transfer (within ~ 30 min) of fixed N and fix substantially more N than required for their own growth; up to 97.3% of the total fixed N is transferred to their diatom partners and not assimilated by the symbionts themselves nor simply released to the environment (Mulholland et al. 2004; Foster et al. 2011).

Although DDAs have not been specifically investigated in the Black Sea yet, there is indirect evidence suggesting that they may have been involved in the autumn C4at increase. Abundance of free cyanobacteria (up to 2 x 10^3 cells L^-1) in the open regions peaks in September (Zaika et al. 1993), and the most important symbionts (R. intracellularis and Calothrix rhizosoleniae) are known to partner with several diatom genera, including R. rhizosolenia and Chaetoceros (Foster et al. 2011), which are also abundant in the open Black Sea (Nesterova et al. 2008). Moreover, DDAs have been found in the eastern Mediterranean (Zeev et al. 2008), which shares features of the seasonal thermocline and nutricline with the open Black Sea. Given the above evidences, it is not unrealistic to assume that the autumn LDs bloom in the open Black Sea could be supported by nitrogen supplied by endosymbiotic diazotrophs as well.

Large diatom adaptations to nutrient depleted conditions

LDs are unique among marine phytoplankton, having a large central vacuole that allow for stockpiling nutrients, particularly nitrate (Raven 1987). LDs can maintain high intracellular nitrate concentrations for several days when exposed to nutrient starvation in the external environment (Dortch et al. 1984). In fact, the stockpiling mechanism was demonstrated for the two dominant Black Sea LDs in an experiment, where Pseudosolenia calcar-avis and Proboscia alata did not respond to nitrate and phosphate additions, unlike the smaller species in the mixed culture (Silkin et al. 2015). Furthermore, LDs possess the ability to regulate their buoyancy in response to intracellular nutrient condition, which allow them to obtain nutrients from depths and migrate upward to higher light levels (Kemp and Villareal 2018 and references therein).

Our results (Fig. 4B) testify that early autumn in the open Black Sea is the most favorable season for LDs to utilize the stockpiling mechanism. Beginning September, temperature decreases and wind intensifies, which weakens the seasonal thermocline (Ivanov and Belokopitov 2013), enabling LDs to migrate across the thermocline and harvest nutrients from the deeper nutricline before returning to the euphotic zone. The intracellular nutrient storage allows LDs to carry out multiday migrations without nutrient exhaustion (Kemp and Villareal 2018). In addition to supporting growth of LDs, the vertical migration also enriches the upper mixed layer through nitrate release (Singler and Villareal 2005), thereby sustaining higher biomass of the overall phytoplankton community (Fig. 4B).

Top-down control of phytoplankton biomass

Top-down control of phytoplankton is achieved mainly through zooplankton grazing and lysis caused by exogenous viral infection (Kuipers and Witte 1999; Baudoux et al. 2008). Stelmakh et al. (2009) showed that microzooplankton consumes up to 70% of the annual primary production in the Black Sea, similar to most coastal and oceanic systems (e.g., Calbet and Landry 2004). The grazing rate of both micro- and mesozooplankton is size-dependent and decreases with larger phytoplankton cells (Strom et al. 2007). LDs like Pseudosolenia calcar-avis are poorly grazed by micro- and mesozooplankton (Stelmakh et al. 2009). Furthermore, nutrient-deplete conditions reduce fecundity and growth of copepods (Kierboe 1989), which are probably the main grazers of large diatoms. Thus, the high phytoplankton biomass and blooms during whole autumn (Fig. 3 and Supporting
new research avenues are needed for understanding today’s Black Sea ecosystem.

Information Figs. S2—S7 were probably also supported by reduced grazing of LDs.

At the same time, high virus abundance can cause a rather rapid bloom termination through viral lysis of plant cells (e.g., Bratbak et al. 1993). Experiments on Black Sea algae cultures of diatom *Phaeodactylum tricornutum* and chlorophyte *Tetraselmis viridis* showed that virus-infected cells changed their shape and increased in volume after 2–3 d, hereafter cell numbers sharply declined (Stelmakh and Stepnova 2020). Consequently, the relatively short bloom duration (typically not exceeding 5 d) could be related to the rapid termination by viruses due to their fast reproduction.

In summary, our results challenge current paradigms and new research avenues are needed for understanding today’s Black Sea ecosystem.

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