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Interactions of anthropogenic stress factors on marine phytoplankton

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Abstract
Phytoplankton are the main primary producers in aquatic ecosystems. Their biomass production and CO₂ sequestration equals that of all terrestrial plants taken together. Phytoplankton productivity is controlled by a number of environmental factors, many of which currently undergo substantial changes due to anthropogenic global climate change. Most of these factors interact either additively or synergistically. Light availability is an absolute requirement for photosynthesis, but excessive visible and UV radiation impair productivity. Increasing temperatures enhance stratification and decrease the depth of the upper mixing layer exposing the cells to higher solar radiation and reduce nutrient upward transport from upwelling deeper water. At the same time, stratospheric ozone depletion exposes phytoplankton to higher solar UV-B radiation especially in polar and mid latitudes. Terrestrial runoff carrying sediments and dissolved organic matter into coastal waters leads to eutrophication while reducing UV penetration. All these environmental forcings are known to affect physiological and ecological processes. Ocean acidification due to increased atmospheric CO₂ concentrations changes the seawater chemistry; it reduces calcification in phytoplankton, macroalgae and many zoological taxa. Ocean warming results in changing species composition and favors blooms of toxic prokaryotic and eukaryotic
phytoplankton. Increasing pollution from crude oil spills, persistent organic pollutants, heavy metal as well as industrial and household wastewaters affect phytoplankton which is augmented by solar UV radiation. Extensive analyses of the impacts of multiple stressors are scarce. Here, we review reported findings on the impacts of anthropogenic stressors on phytoplankton with an emphasis on their interactive effects and make an effort to provide a prospect for future studies.

**Introduction**

More than two thirds of our planet is covered by water and more than 99 % of the liquid water are constituted by marine waters (Charette and Smith, 2010). Marine ecosystems are responsible for about half of the biomass production on Earth even though their standing crop represents only about 1 % of the terrestrial biomass (Tardent, 2005; Gruber et al., 2009; Häder et al., 2011). Prokaryotic and eukaryotic phytoplankton are the major primary producers in the oceans, contributing more than 90% photosynthetic carbon fixation; while macroalgae and seagrasses occupy less than 1% of oceanic habitats, they also play important roles in carbon cycles in coastal waters. These organisms form the basis of the intricate food webs feeding the subsequent levels of the food web and eventually providing food for the growing human population. The marine ecosystems represent a large carbon sink and sequester an equal amount of CO$_2$ as all terrestrial ecosystems taken together (Falkowski et al., 2000; Zepp et al., 2007), absorbing about 26 million tons of anthropogenically released CO$_2$ per day (Gao et al., 2012b). Therefore, they play a key role in the regulation of the atmospheric CO$_2$ concentration and dampen extreme temperature and weather events (Chester and Jickells, 2012).

Productivity of marine phytoplankton is determined by a number of environmental factors, many of which are influenced by human activities, so that they undergo massive changes due to global climate change, ozone depletion and pollution (Behrenfeld et al., 2006) (Fig. 1). One of the major factors affecting primary productivity in phytoplankton is temperature (Lewandowska
and Sommer, 2010; Thyssen et al., 2011). Only a few organisms show a positive net photosynthetic activity below freezing (Staehr and Sand-Jensen, 2006; Boyd et al., 2013).

Increasing temperatures augment productivity up to a certain optimum beyond which there is a decrease or cessation of active carbon sequestering or death of the organisms, i.e. species are adapted to a thermal window for photosynthetic productivity (Huertas et al., 2011). Water temperatures have increased on a global scale due to anthropogenically caused climate change by about 1°C over the last 112 years (Fischetti, 2013). Increasing temperatures can also be the cause of deoxygenation especially in coastal and estuarine ecosystems which in extreme cases turn into dead zones for most marine organisms (Howarth et al., 2011; Carstensen et al., 2014).

Regionally, temperature increases can be even much higher; e.g. for the Arctic Ocean a temperature increase of 2°C has been recorded over the last 20 years (Forsström, 2006; Economist, 2013), resulting in a substantial loss of ice cover and extension of the growing season (Comiso et al., 2008; Kahru et al., 2010). These dramatic developments caused major changes in species composition and productivity of phytoplankton and the whole food web (Coupel et al., 2012).

Increasing atmospheric CO₂ concentrations result in a higher uptake of CO₂ into surface waters. The atmospheric CO₂ concentration before the onset of the industrial revolution was about 270 ppm (Sigman et al., 2010); today it has exceeded 400 ppm (Solomon, 2007) and a further rapid increase is being predicted (Meinshausen et al., 2011). Even though the marine waters are well buffered, the CO₂ increase has resulted in a global decrease of the pH by about 0.1 units (Gao et al., 2012a). This does not seem dramatic but represents an increase of 30% in the H⁺ concentrations in the surface waters and further increases are expected to decrease the pH value by another 0.3 – 0.4 units (100 - 150% increase in H⁺ concentration) over the next century (Feely et al., 2004). Such progressive ocean acidification is known to affect calcification of
calcifying phytoplankton (Beaufort et al., 2011), growth and photosynthesis of many photosynthetic organisms (Wu et al., 2008). While it is almost certain that ocean acidification decreases biogenic calcification, controversial findings on photosynthesis and growth of algae have been documented (Law et al., 2007; Riebesell and Tortell, 2011).

Stratospheric ozone depletion due to anthropogenic production and emission of chlorinated fluorocarbons (CFCs) and other trace gases resulted in an increasing impact of solar UV-B (280 – 315 nm) radiation on the Earth and water surface especially in Polar Regions and at mid latitudes, while tropical waters are less affected by increasing UV-B radiation scenarios (McKenzie et al., 2011b). However, due to the successful implementation of the Montreal Protocol and the subsequent amendments, further ozone depletion could be limited and the UV-B radiation climate is expected to slowly return to normal (pre-1980) levels over the next decades (Ungar, 2012). This slow remediation is due to the long lifetimes of the CFCs up to over 100 years in the stratosphere. In addition, accumulation in the atmosphere of volatile halocarbons produced and released from algae is known to destroy ozone, resulting in increased incident UVB radiation at the Earth surface (Law et al., 2007). However, how changing conditions in the ocean caused by global climate change will affect productions of these compounds is unknown.

Light availability in the euphotic zone – the lower limit of which is defined by an attenuation of the surface radiation to 1 % – is determined by the concentration of dissolved and particulate constituents in the water (Häder et al., 2011; Pace et al., 2012). In contrast to open oceanic waters, in coastal areas large amounts of silt and organic material attenuate the incoming radiation especially in the UV band. This material is derived from terrestrial runoff carrying organic matter from decaying vegetation (Yamashita et al., 2013). In addition, organic material from decaying macroalgae and seagrasses contribute to the absorbing substances on the continental shelves (Nelson et al., 2013).
One of the decisive factors controlling phytoplankton growth is the concentration of available nutrients. In coastal areas most of the nutrients are derived from terrestrial runoff (Fabricius et al., 2013). Increasing discharge of industrial and household wastes as well as fertilizers used in agriculture enhance the mineral loads of rivers (Qu and Kroeze, 2012). In open ocean ecosystems wind-blown material contributes P, N and Fe among others (Santos, 2010; Biller and Bruland, 2013). Contributions by upwelling waters into the euphotic zone is limited by the lower sharp boundary of the upper mixed layer (UML) called thermocline or pycnocline (Gao et al., 2012b).

Increasing pollution by indiscriminate discharge of heavy metals, pesticides, persistent organic pollutants (POP) and accidental spills of crude oil products affect phytoplankton in both coastal and open ocean waters (Echeveste et al., 2011; Huang et al., 2011). Many of these key factors additively or synergistically affect phytoplankton productivity. Satellite monitoring of phytoplankton densities, which is available since 1979, indicates that phytoplankton levels have been declining at about 1% a year over the past half century. A decline over the prior half century is also likely (Boyce et al., 2010b). According to simulations by Laufkoetter et al. the biomass of phytoplankton has decreased by 8.5% from 1960-2006 (Laufkoetter et al., 2013). In this review we summarize the effects of major factors determining phytoplankton productivity and highlight the interactions between these effects.

**Decreasing stratospheric ozone, UV-B and effects on phytoplankton**

Solar radiation hitting the Earth surface can be distinguished into infrared (>700 nm), visible (400 – 700 nm) and UV, which is subdivided into UV-C (<280 nm), UV-B (280 – 315 nm) and UV-A (315 – 400 nm). UV-C does not penetrate the stratosphere, since ozone absorbs radiation
below 280 nm completely; it also absorbs most of the UV-B radiation (Seckmeyer et al., 2008). Anthropogenic emission of chlorinated fluorocarbons and other gaseous pollutants have partially deteriorated the stratospheric ozone layer due to a catalytic effect starting in the 1970s (Lindley, 1988), resulting in increasing UV-B radiation at the Earth surface. This effect is most pronounced in the polar regions but also noticeable at mid latitudes while in the tropics no significant changes have been measured (McKenzie et al., 2011a). The fast rise of solar UV-B was dampened by the Montreal Protocol and subsequent amendments which virtually stopped the production and emission of ozone depleting substances (Egorova et al., 2013; Barnes et al., 2014), so that solar UV-B reaching the Earth surface peaked in the first decade of the current century and is expected to return to pre-1980 levels by about 2065 (United Nations Environment Programme Environmental Effects Assessment Panel, 2012).

Aquatic primary producers need to dwell in the photic zone in order to harvest sufficient solar radiation for their photosynthesis. There, they are simultaneously exposed to solar UV radiation and photosynthetically active radiation (PAR). Even though UV-B radiation amounts to only 1-3% of UV-A, they are extremely detrimental since they damage more biomolecules and cellular structures (Sinha et al., 2008). UV-B is absorbed by proteins, membranes and other functional and structural elements of the cell (Sinha et al., 2008), interferes with enzymatic reactions (Walczak, 2008) and impairs physiological responses such as motility and orientation (Ekelund, 1994; Richter et al., 2007). UV-A can also damage biomolecules, but moderate levels of it are known to play roles in repairing UV-B induced damages and stimulating photosynthetic carbon fixation by micro-phytoplankton (Gao et al., 2007; Li et al., 2011). The damage can be induced either by direct hits on cellular targets or by the formation of reactive oxygen species (ROS) such as singlet oxygen ($^1$O$_2$) (Heck et al., 2003; Zhang et al., 2013). Increasing water temperatures enhance the formation of ROS (Muller et al., 2012).
Photosynthesis of phytoplankton (and other light-harvesting organisms) is specifically vulnerable to solar UV-B radiation. In addition to other targets, short-wavelength radiation hits D1 and D2 proteins (subunits of PSII) in the electron transport chain (Watanabe and Ikeuchi, 2013; Watanabe et al., 2014), which is kinked and the degraded protein is subsequently removed from its site on the thylakoids (Bouchard et al., 2006). The damage is repaired by replacement with newly synthesized proteins. Increasing water temperatures augment the repair process (Li et al., 2012). In contrast, limiting nutrient availability hampers the repair mechanisms (Doyle et al., 2005). An unexpected finding was that UV-B damages phytoplankton more by impairing the repair mechanism than by the direct damage of the protein (Wu et al., 2012). Decreasing depth of the upper mixing layer (UML) due to stronger stratification induced by increasing temperatures exposes phytoplankton to higher solar UV-B and excessive PAR (photosynthetic active radiation) worsening the effect on the photosynthetic apparatus (Bouchard et al., 2005; Helbling et al., 2005). In contrast, in coastal habitats, increasing concentrations of UV-absorbing substances in the water column, such as dissolved organic material (DOM) and sediment, protect the phytoplankton from excessive exposure to detrimental solar radiation (Fichot et al., 2013a). Likewise, increased mixing and vertical circulation in the UML by wind and waves promotes the repair process: when the cells are exposed to solar radiation close to the surface they experience increased damage (Zhou et al., 2009). This is being repaired when the cells are passively moved to deeper horizons close to the thermocline (lower limit of the UML) (Helbling et al., 2003).

Other targets of solar UV-B in photosynthesis are the accessory pigments which funnel solar energy to the reaction centers. Especially the phycobilins in cyanobacterial cells are affected (Sinha et al., 2005; Six et al., 2007). These pigments are organized in structural elements called phycobilisomes situated on the thylakoids attached to PS II in cyanobacteria and rhodophyta. UV-B was found to first sever the energy transduction to PS II, then disintegrate the phycobilisomes
and finally destroy the proteins carrying the phycobilin chromophores (Sinha et al., 2003; Sinha and Häder, 2003).

The DNA in both prokaryotic and eukaryotic organisms is strongly affected by solar UV-B radiation which can penetrate into the water column to ecologically significant depths especially in open ocean waters (Helbling et al., 2005; Meador et al., 2009). There are a large number of UV-B-induced lesions in DNA including single- and double strand breaks, and formation of 6-4 photoproducts and their Dewar valence isomers; however, the most frequent lesion is the formation of cyclobutane pyrimidine dimers (CPD) (Richa et al., 2014). When the cells are exposed to high levels of solar radiation, CPDs are predominantly induced when they are close to the surface and repaired when mixed downward near the thermocline in the UML (Helbling et al., 2008). In addition to excision repair, mismatch repair and SOS response, mainly the photoactivated CPD photolyase is engaged to break the dimers using the energy of UV-A or blue light photons (Cheng et al., 2007; Ioki et al., 2008).

Other mechanisms to avoid or mitigate UV-afflicted damage include active vertical migration by moving out of zones of excessive radiation using flagella or changes in buoyancy, e.g. by production of gas vacuoles or oil vesicles (Overmann and Pfennig, 1992; Ma and Gao, 2009) or by mat formation (Pelletier et al., 2006). With the exception of very small cells (picoplankton) most prokaryotic and eukaryotic phytoplankton use UV-absorbing pigments to block excessive solar UV-B radiation from reaching central regions of the cell such as the nucleus. Scytonemin is exclusively synthesized by cyanobacteria to reduce the impact of UV (Balskus and Walsh, 2008; Rastogi et al., 2013). In addition, they synthesize a variety of mycosporine-like amino acids (MAAs), which are also employed by eukaryotic phytoplankton and a number of macroalgae for the same purpose (Singh et al., 2008; Llewellyn et al., 2012). In most cases biosynthesis of MAAs is induced by UV-B exposure (Sinha et al., 1999). Enhanced nutrient
availability augments their production and thus provides better protection (Mohovic et al., 2006).

**Light penetration into the water column**

Light is the single most important factor for photosynthesis in phytoplankton. The irradiance of solar radiation in the water column is controlled by many atmospheric factors and components in the water (Boss et al., 2007), which govern the transmission and the wavelength distribution (Häder et al., 2007; Smith and Mobley, 2007). The penetration of light into the water is controlled by dissolved organic matter (DOM) and inorganic substances (DIM) (Vione et al., 2009; Xie et al., 2009) as well as by particulate organic (POM) and inorganic matter (PIM) (Mayer et al., 2006; Vahatalo and Jarvinen, 2007). Phytoplankton cells contribute to POM, so that the light penetration is controlled by their concentration (Bracchini et al., 2006; Sommaruga and Augustin, 2006). The attenuation of the incoming light can be neutral, i.e. the same percentage at all wavelengths, or it can be higher at specific wavelengths. One fraction of DOM absorbs at shorter wavelengths and is called colored or chromophoric DOM (CDOM) (Osburn et al., 2009).

Eutrophic freshwater ecosystems have much higher DOM concentrations than oligotrophic open ocean systems. Also coastal ecosystems can have high DOM concentrations due to terrestrial runoff and discharge of rivers (Day and Faloona, 2009). The plumes of large rivers can be seen extending sometimes tens to hundreds of kilometers into the sea (Fichot et al., 2013b). Most of the DOM is derived from organic material of decaying plant residues (Boyle et al., 2009). High mountain lakes located above the tree line show lower CDOM concentrations because of the lower terrestrial input of organic material and therefore have a higher transparency (Rose et al., 2009). In coastal habitats material from decaying macroalgae which dwell in coastal habitats, contributes to DOM (Hulatt et al., 2007). In open oceanic ecosystems DOM is almost exclusively derived from decaying phytoplankton (Vahatalo and Jarvinen, 2007; Behrenfeld et al., 2009) with the exception of material which stems from decaying swimming macroalgae, as in the
Sargasso Sea (Bailey et al., 2008). Since CDOM is produced by the degradation of organic material of terrestrial or marine origin its concentration varies seasonally depending on the time of degradation as well as on rainfall patterns (Suhett et al., 2007). The concentration of CDOM directly and indirectly affect biogeochemical cycles (Zepp et al., 2007; Fernandes et al., 2008).

Extremely high UV-B and UV-A transmissions have been measured in super-oligotrophic waters such as the South Pacific Gyre, where hardly any DOM exists (Tedetti et al., 2007). At 325 nm 1 % of the surface radiation was found to be transmitted down to 84 m depth. In the oligotrophic Lake Tahoe (California-Nevada, USA) 1 % of the surface radiation at 320 nm was measured at 27 m (Rose et al., 2009) and at 62 m in the extremely clear Crater Lake (Oregon, USA) (Hargreaves et al., 2007). The light penetration into the water column can be measured with broadband radiometers or spectroradiometers. Furthermore, actinometers based on chemical reactions or damages in DNA have been employed (Schouten et al., 2007; 2009).

In Arctic and Antarctic fresh and marine habitats light availability is attenuated by DOM, POM, DIM and PIM originating from runoff during ice melting (Schindler and Smol, 2006). Large changes in the transparency occur because of the massive increases in temperature in the Polar Regions by climate change affecting phytoplankton and the subsequent levels in the food web (Keller et al., 2006; Paterson et al., 2008).

The impact of solar UV radiation on CDOM causes a feedback mechanism (Hayakawa and Sugiyama, 2008); CDOM strongly absorbs UV-B which breaks the double bonds. This photodegradation causes a bleaching of these humic substances (Feng et al., 2006; Tzortziou et al., 2007; Zhang et al., 2008). During a cruise in the Bering Sea CDOM levels were found to decrease by 18 – 55 % when exposed to solar UV-B (Brooks and Lovvorn, 2008). The resulting fragments are easily consumed by bacteria and other microorganisms so that the overall transparency of the water increases resulting in a deeper penetration of UV-B radiation into the
water column (Tedetti et al., 2009), causing detrimental effects on aquatic organisms (Feng et al., 2006). Photodegradation is the key process for mineralization and carbon recycling of DOM (Feng et al., 2006; Anusha and Asaeda, 2008; Wang et al., 2009); the breakdown of plankton and macroalgae biomass releases nutrients such as phosphorus and iron (Bastidas Navarro and Modenutti; Shiller et al., 2006; Navarro et al., 2009). In the presence of DOM dissolved free amino acids are decomposed because of the production of singlet oxygen ($^1$O$_2$) which has been observed both in Antarctica and in several lakes and rivers in the Northern U.S. (Boreen et al., 2008).

Motile phytoplankton can respond to the ambient light conditions by selecting the vertical position in the water column. Some organisms use active motility such as flagellates (Häder and Griebenow, 1988) while others change their buoyancy by producing gas vacuoles (cyanobacteria) or oil vesicles (diatoms) (Walsby, 1987). Often the motility of phytoplankton is directed by light and gravity. At low light intensities cells often move to the surface using positive phototaxis (Matsunaga et al., 2003) and negative gravitaxis (Lebert et al.; Eggersdorfer and Häder, 1991; Roberts, 2006). When exposed to higher PAR and UV irradiances the cells often switch to negative phototaxis and/or sediment to escape into deeper waters (Lenci et al., 1983; Matsuoka, 1983; Josef et al., 2005). In the open ocean these vertical migrations and orientation mechanisms are often overruled by the action of wind and waves (Helbling et al., 2005; Barbieri et al., 2006) which passively transport the phytoplankton within the mixing layer (Yoshiyama and Nakajima, 2002). However, active movement superimposes the passive mixing so that vertical distribution patterns of phytoplankton can be seen in the water column (Piazena and Häder, 1995).

**Effects of ocean acidification and changes in seawater chemistry**

With more and more CO$_2$ building up in the atmosphere, seawater pCO$_2$ increases with increased dissolution of CO$_2$ along with altered seawater carbonate chemistry, as reflected by a drop of pH, increased bicarbonate and decreased carbonate concentrations. Such a progressive ocean
acidification also reduces the CaCO$_3$ saturation state. Therefore, calcifying and non-calcifying phytoplankton populations are being affected directly or indirectly due to changed availability of nutrients or forms of different ions. It is predicted that the pH of the surface oceans will decline by another 0.3-0.4 units (Orr et al., 2005), increasing [H$^+$] by 100%-150% by the end of this century. While large scale ocean acidification is superimposed to regional oscillation of pH, phytoplankton as well as other organisms in both pelagic and coastal waters are being affected (Capone and Hutchins, 2014; Waldbusser and Salisbury, 2014).

Phytoplankton species may benefit from increased availability of CO$_2$ or be harmed by the pH drop, depending on species, levels of other factors or stressors (Gao and Campbell, 2014). Under 800 $\mu$atm pCO$_2$, growth of diatom-dominated phytoplankton assemblages was not enhanced during 2–5 days shipboard incubation under ~30% of incident sunlight (Tortell, 2000). In the diatom Skeletonema costatum, growth was not stimulated by an enriched CO$_2$ concentration (800 $\mu$atm) under laboratory conditions (Burkhardt and Riebesell, 1997; Chen and Gao, 2003; 2004), but was enhanced in a mesocosm at an elevated CO$_2$ concentration of 750 $\mu$atm (Kim, 2006). For the diatoms Phaeodactylum tricornutum (Schippers et al., 2004; Wu et al., 2010), Navicula pelliculosa (Low-Décarie et al., 2011) and Attheya sp. (King et al., 2011), growth was enhanced under elevated CO$_2$ levels under laboratory conditions. When the diatoms S. costatum (CCMA110), P. tricornutum (CCMA 106) and Thallasiora pseudonana (CCMP 1335) were grown under different levels of sunlight and elevated CO$_2$ of 1000 $\mu$atm, their growth was stimulated under lower light levels (5–30% surface daytime mean solar PAR), but inhibited under higher light levels (Gao et al., 2012a). These results show that elevated CO$_2$ and light levels interact affecting diatom growth responses to ocean acidification. However, such interaction was not observed in the coccolithophore Emiliania huxleyi (Jin, Gao et al. unpublished data). Ocean acidification may increase (Wu et al., 2010; Yang and Gao, 2012) or
decrease (Hennon et al., 2014) mitochondrial respiration, increase photorespiration (Gao et al., 2012b; Xu and Gao, 2012) and influence the photophysiology of phytoplankton (Gao and Campbell, 2014). In the South China Sea, surface phytoplankton assemblages were found to assimilate less carbon either based on chl $a$ or based on per volume of seawater under elevated CO$_2$ levels of 800 or 1000 μatm, with their non-photochemical quenching enhanced (Fig. 2)[7], after acclimation to the CO$_2$-induced acidification for about a week. Such a phenomenon reflects the balanced net outcome of photosynthetic carbon fixation and respiratory carbon loss associated with seawater acidification and the enhanced regulation of thermal energy dissipation [7].

Recently, across a CO$_2$/pH gradient off the volcanic island of Vulcano (NE Sicily), periphyton communities altered significantly as CO$_2$ concentrations increased, with significant increases in chl $a$ concentrations and in diatom abundance (Johnson et al., 2013). This implies a possibility that the “winners” could have increased their photosynthetic antenna to capture additional light energy to cope with the extra energy demand (such as enhanced respiration) due to increased seawater acidity. These inconsistent or controversial findings can be attributed to different experimental setups or environmental conditions or species-specific physiology (Langer et al., 2006; Langer et al., 2009; Gao et al., 2012a; Hutchins et al., 2013; Schaum et al., 2013). Molecular changes leading to an altered physiology and subsequently to changed ecology level responses of different phytoplankton taxa are expected to be seen in the near future. At the same time, our present understanding of potential ocean acidification impacts is almost entirely limited to single species responses, species competition and phytoplankton community changes with ocean acidification remain poorly understood (Rossoll et al., 2012; Calbet et al., 2014).

In waters of low pH or hypoxia or upwelling areas, low pH values coincide with low dissolved oxygen (DO), which mirrors acidification and deoxygenation; net photosynthetic production may inversely relate to the ratio of DO to pCO$_2$ (Gao and Campbell, 2014). While the
exchanges of O₂ and CO₂ during photosynthesis or respiration can be affected by the external ratio of DO to pCO₂ (Flynn et al., 2012), thickness of diffusion layers around the phytoplankton cells depends on physical processes, such as mixing or stirring, therefore, effects of increased pCO₂ or decreased DO would be altered by physical processes. Since stratification leads to future shoaling of upper-mixed-layer (UML) depths, phytoplankton cells are exposed to increasing solar irradiances with reduced mixing pathlength; therefore, interaction of physical and chemical environmental forcings will determine phytoplankton physiological performances and their ecological roles. A recent study showed that frequencies of light fluctuation that mimic different mixing regimes affect a coccolithophore’s response to ocean acidification (Jin et al., 2013). In comparison, diatoms grown under fluctuating light decreased their growth and altered their responses to elevated CO₂ in contrast to those under constant light regimes (Hoppe et al., 2014). Obviously, effects of ocean acidification can differ under indoor constant conditions from those under incident solar radiation (Gao et al., 2012b), which could impose an additional interaction on the net species and community responses to increasing pCO₂.

**Effects of ocean warming**

The accumulated CO₂ in the atmosphere traps heat reflected as infrared radiation (>700 nm) and both terrestrial and aquatic ecosystems are thus being warmed. Although global mean sea surface temperatures are increasing at about half the pace of those on land, 0.13 vs. 0.27°C per decade since 1979, ocean warming is one of the strongest anthropogenic forcing on primary and secondary production in marine systems (Halpern et al., 2008). Warming trends are suggested to alter distributions of major phytoplankton groups (Thomas et al., 2012), such as dinoflagellates (Hallegraeff, 2010; Fu et al., 2012), diatoms, coccolithophorides (Mericoa et al., 2004; Hare et al., 2007) and cyanobacteria (Breitbarth et al., 2007; Pittera et al., 2014). It was reported that rising temperatures in this century will cause pole-ward shifts in species’ thermal niches and a sharp
decline in tropical phytoplankton diversity in the absence of an evolutionary response (Thomas et al., 2012). A 50-year (1960 – 2009) time series survey revealed a decline of dinoflagellate abundance in the northeast Atlantic and North Sea, but this was not observed in the abundance of diatoms (Hinder et al., 2012). For a long time marine scientists have hinted that phytoplankton levels were decreasing, but no systematic, worldwide account of their decline had been made. Boyce et al. showed that phytoplankton levels have been declining on a global scale at about 1% a year over the past half century (Boyce et al., 2010a). Ocean warming and consequent stratification as well as other ocean changes (such as ocean acidification, thermocline/nutricline changes) would be responsible for such observed declines.

Pelagic and coastal waters exhibit different scales of day/night temperature changes, being larger in the latter; different latitudes have differences in annual and daily temperature means and ranges. Therefore, marine phytoplankton cells are subjected to substantial changes of temperature on different time scales. These include rapid shifts associated with tides, diurnal and seasonal fluctuations caused by clouds and changes in solar elevation and long-term, inter-annual variability associated with natural climatic cycles and likely, human influence. These changes in temperature can influence the thermal windows of marine organisms by influencing their enzymatic efficiency and heat tolerance (Pörtner and Farrell, 2008).

For photosynthetic organisms, photosynthesis usually increases with increased temperature to reach an optimum and then declines with further warming, while respiration increases with increasing temperatures. Elevated temperature increase growth (Feng et al., 2008; Arnold et al., 2013; Sett et al., 2014), photosynthesis (Feng et al., 2008; Xu et al., 2011b) and calcification (Xu et al., 2011a; Müller et al., 2014) of coccolithophores. There is also growing evidence showed that elevated temperature had a positive impact on the photosynthesis of other phytoplankton taxa, such as diatoms (Li et al., 2012), cyanobacteria (Fu et al., 2007; Fu et al.,
A recent study demonstrated that elevated temperatures (+6°C) increased the phytoplankton biomass in natural plankton communities (Lewandowska et al., 2014). A combination of elevated temperature and increased CO₂ levels acted synergistically to increase the growth of phytoplankton (Connell and Russell, 2010; Fiorini et al., 2011); however, no interactive effects of these two factors were reported in some previous studies (Hutchins et al., 2007; Feng et al., 2008). The combination of elevated temperature and CO₂ levels acted synergistically to raise the repair rate of the PSII machinery damaged by UV radiation (Li et al., 2012). The respiration coefficient (T₁₀, change in respiration rate induced by a 10°C temperature change) may increase under ocean acidification conditions in algae (Zou et al., 2011). While respiration increased by over 30% in the diatoms Phaeodactylum tricornutum and Thallasiora pseudonana at 1000 µatm pCO₂, combined effects of ocean acidification and warming on phytoplankton respiration have little been documented. Since both photosynthesis and respiration will likely be influenced by ocean warming and ocean acidification, but probably to a different extent, the ratio of photosynthetic carbon fixation to respiratory carbon loss within the euphotic zone will vary in future warmer and acidified oceans. Two strains of the marine picocyanobacteria Synechococcus and Prochlorococcus responded differentially to warming as well as to elevated CO₂, with the growth rate of the former increasing and that of the latter not changing under the combined “greenhouse” treatment (Fu et al., 2007). Growth and nitrogen fixation rates of two isolates of the filamentous cyanobacterium Trichodesmium were strongly enhanced by either increasing CO₂ (750 µatm) or a 4°C temperature increase, but synergistic effects between the two variables were not observed (Hutchins et al., 2007). Levitan et al. reported similar findings for these two parameters in one of these same Trichodesmium isolates under the combination of warmer temperature (+6°C) and higher CO₂ (900 µatm) (Levitan et al., 2010). In contrast, in an experiment using a mixed natural North Atlantic bloom community,
cocolithophore cell abundance was greatly increased under the “greenhouse” combination of increased pCO\textsubscript{2} and temperature, but not when either one was increased alone (Feng et al., 2009). Obviously, responses of most phytoplankton species studied under elevated CO\textsubscript{2} are temperature dependent. In the cocolithophorides, Emiliania huxleyi and Gephyrocapsa oceanica, at the optimal levels of CO\textsubscript{2} concentration for growth, calcification and carbon fixation rates are modulated by temperature, with the optimum temperature level increased under elevated CO\textsubscript{2} concentrations (Sett et al., 2014). Recent culture work, however, showed that two strains of the cocolithophore E. huxleyi were unable to adapt to pronounced warming to 30 °C (Huertas et al., 2011). Further studies looking at the potential for long-term adaptation of phytoplankton to higher temperatures is needed, especially in combination with acidification and other global change variables.

**Nutrients**

Phytoplankton productivity in most of the world’s oceans is limited by the availability of nutrients, including nitrogen, iron and phosphorus, in the euphotic zone. Especially large areas of the tropics and subtropics are characterized by nitrogen depletion [135]. Microbial denitrification decreases available stocks of nitrogen from the water column even though cyanobacteria enhance nitrogen resources since these prokaryotes are capable of fixing atmospheric nitrogen, using the enzyme nitrogenase, and make it available to eukaryotic phytoplankton (Bergman, 1999; Mulholland and Capone, 2000). However, in coastal areas, agricultural runoff spikes large phytoplankton blooms stretching over many square kilometers such as in the Gulf of California (Beman et al., 2005; Beman et al., 2011). Also rivers are often subject to increased nutrient concentrations due to runoff of fertilizers from adjacent fields to a point that they must be considered as pollution. This eutrophication deteriorates the water quality and affects consumers higher up in the food web (Hutchins et al., 2010).
Also estuaries are affected by increased nutrient concentrations. Phytoplankton species composition, their abundance and diurnal and spatial distribution were studied in the Changjiang Estuary. Increasing phosphorous concentrations were identified as causing blooms with an average cell density of $6.75 \times 10^5$ cells l$^{-1}$ of dominant phytoplankton species such as *Prorocentrum dentatum* and *Skeletonema costatum* which is much higher than cell counts in previous years.

Increasing temperatures and enhanced nutrient supply support the occurrence of harmful algal blooms, which are often formed by dinoflagellates. El Niño events and changes in the North Atlantic Oscillation promote the cell density of these phytoplankton by altering stratification, ocean currents and nutrient availability as fossil records of dinoflagellate cysts have indicated (Hallegraeff, 2010). Also with limited inorganic nitrogen supply blooms of toxic algae can develop fueled by a large pool of dissolved organic nitrogen (DON) (Bronk et al., 2007). Upwelling of submarine groundwater can be another source of nitrogen (Paytan et al., 2006).

Nitrogen shortage results in reduced biomass synthesis and decreased production of sulfur compounds such as DMS, DMSP and DMSO (Harada et al., 2009). Phosphate limitation affects alkaline phosphatase activity in phytoplankton and bacteria (Labry et al., 2005). Cyanobacteria such as *Microcystis* can also produce harmful and toxic plankton blooms which deteriorate drinking water reservoirs and potentially threaten animal and human health (Oberholster et al., 2004). In some habitats like recreationally used rivers and lakes the occurrence of algal blooms is regarded as a nuisance. Recent discussions how to limit the development of these blooms include reduction of nutrient pollution as opposed to riparian shading (Hutchins et al., 2010).

**Pollutants**

The increasing environmental pollution is another anthropogenic stress factor which affects phytoplankton. Especially coastal areas and freshwater habitats are affected. However, sewage,
even at high concentrations does not seem to negatively affect phytoplankton. A study of the Cauvery river, Tamil Nadu, India, showed high concentrations of phytoplankton and zooplankton even though the river is highly contaminated with household and industrial effluents (Mathivanan et al., 2007). In contrast, a multivariate analysis of one of the most polluted rivers in Latin America (Reconquista River, Buenos Aires Province, Argentina) showed strong inhibition of the phytoplankton populations due to agrochemicals as well as domestic and industrial untreated effluents (Olguín et al., 2004). But the water quality changed seasonally. High concentrations of pollutants and nutrients (ammonium and orthophosphates, high BOD, hardness and conductivity) favored the dominance of a single algal species, while better water quality resulted in higher algal and crustacean diversity. Analysis of the phytoplankton communities can be used to determine the water quality polluted by industrial effluents as shown at the downstream stretch of the Bhadra river (Shashi Shekhar et al., 2008). Heavy metal pollution is often found in lakes and rivers in developing countries affecting phytoplankton productivity (Oberholster et al., 2010). This has recently been summarized for many freshwater ecosystems in Pakistan (Azizullah et al., 2011).

Fluorescence of chl \(a\) was used as an endpoint for monitoring of the ecotoxicological effectiveness of atrazine, which is used as a herbicide since it inhibits the photosynthetic electron transport chain (Seguin et al., 2002). Phytoplankton isolated from Lake Geneva (Switzerland) showed signs of inhibition at atrazine concentrations of 10 \(\mu g/L\). However, the phytoplankton could adapt to the herbicide stress since a 25-days exposure to 30 \(\mu g/L\) of atrazine significantly increased the tolerance of the organisms.

Crude oil spills affect phytoplankton especially in shallow-water marine habitats such as the Arctic Ocean. Water-soluble fractions of heavy oil such as pyrene are the most toxic ones; pyrene accumulates in the sediment. Exposure to solar UV-B and increasing temperatures worsen
the toxicity (Grenvald et al., 2013). Oil exploration will increase in many coastal habitats such as Western Greenland (Hylander et al., 2014). The impact of oil pollution on growth rates and physiological activities have been studied in phytoplankton assemblages in the river Nile (Egypt), comparing a polluted with a non-polluted site (El-Sheekh et al., 2000). Both crude oil and its refinery products affect growth, proteins and nucleic acids: at low concentrations these pollutants augment the physiological performance but at higher concentrations they are inhibitory; however different species show different sensitivities.

Polychlorinated biphenyls (PCBs) are a major problem in both marine and freshwater ecosystems. Since they are lipophilic they can easily penetrate the cell membranes of phytoplankton resulting in substantial bioconcentration as shown in four marine phytoplankton species sampled in the Baltic Sea (Gerofke et al., 2005). PCBs also play a role in the air-water exchange and vertical fluxes. A simulation showed that air-water exchange controls PCB concentrations in phytoplankton in remote environments which are not directly affected by terrigenous pollution (Dachs et al., 2000). Persistent organic pollutants (POP) accumulate in phytoplankton as was found recently during a cruise in the Greenland Current and Arctic Ocean (Galbán-Malagón et al., 2012). Both phytoplankton and bacteria are involved in the biogeochemical cycling of POPs. Being organic, these substances are easily taken up by the cells; this process depends on the ambient temperature, the cell size and the hydrophobicity of POPs. In addition to PCBs, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dioxins and furans (PCDD/Fs), as well as polybrominated diphenyl ethers (PBDEs) are of major concern as pollutants affecting phytoplankton (Del Vento and Dachs, 2002). Marine biogeochemical mechanisms are key in controlling the global dynamics and control the final sink of POPs (Dachs et al., 2002; Niu et al., 2005).

UV exposure increases the toxicity of many pollutants such as PAHs derived from
combustion engines, augmenting the stress in inland and coastal marine environments. This was confirmed by investigations in natural phytoplankton assemblages from the Mediterranean Sea, Atlantic, Arctic and Southern Ocean (Echeveste et al., 2011). Pre-stressed with UV-B, natural phytoplankton communities are more sensitive to pollutants such as atrazine, tributyltin or crude oil which enter coastal waters from terrestrial drainage or maritime traffic than those grown when UV is excluded. Antifouling paints for vessels in aquatic environments contain biocides. More recent developments aim at formulating substances which rapidly degrade when they are released from ship hulls. One of the recently developed components is 4,5-dichloro-2-n-octyl-isothiazoline-3-one (DCOI) (Bérard and Benninghoff, 2001).

Species diversity is a good measure for ecosystem functioning and the stress exerted by environmental pollutants. Model studies of freshwater and brackish ecosystems show a positive relationship between nutrient levels, such as phosphorous, pollutant concentrations and carbon uptake by phytoplankton. Pollution-mediated losses of phytoplankton diversity may have direct detrimental effects on the aquatic primary production (Ptacnik et al., 2008).

Conclusions and future work

The anthropogenic environmental forcings can act interactively to result in harmful, neutral or in some regions stimulating effects on phytoplankton species. Species competition under different environmental settings often differs due to species-specific physiological responses. Nutrients, such as nitrogen, phosphorus and iron, are key elements that limit primary production by marine phytoplankton. The concentrations of these elements usually vary according to regional environmental changes and therefore may affect the physiological and ecological responses of phytoplankton to the anthropogenic stressors, such as ocean acidification and warming and UV-B irradiances. Decreased pH and increased temperature are known to interact with UV radiation to influence photosynthesis and/or growth of typical phytoplankton species (Gao et al., 2012a;Li et
al., 2012). Increased light exposure or fluctuating irradiances of light can also interact with ocean acidification to affect photosynthetic carbon fixation of phytoplankton. How these multivariate feedbacks may change in the oceans under climate change conditions remains speculative.

Ocean warming associated with global warming enhances stratification (reduces the thickness of the upper mixing layer) and decreases nutrient availability due to reduced upward transport of nutrients from deeper layers. Therefore stratification increases UV exposure of phytoplankton cells circulating in a shallower mixed layer (Gao et al., 2012a). Increased UV exposures can lead to more damages to phytoplankton cells, including decreased contents of photosynthetic pigments and increased damages to DNA and proteins of phytoplankton. Therefore, climate change-driven ocean changes may lead to different biogeochemical outcomes.

Which effects these anthropogenic environmental forcings have has to be considered in a holistic context. Most of the studies so far have been conducted under laboratory conditions without considering multiple factors (Gao et al., 2012a). This is one of the main limitations in our knowledge of phytoplankton community transition as well as their nutritious changes under the global change factors in the real oceans. The relations of changes in PAR and temperature to phytoplankton species in the oceans are obvious; however, few phytoplankton studies have addressed their physiological and ecological interactions with high CO₂ and lower pH in the presence of other stressors. Additionally, effects of solar UVR have not been taken into account in laboratory experiments due to the common use of UV-free light sources. Experimental tests of the impacts of anthropogenic stressors under real sunlight or more realistic conditions would allow more reliable predictions of effects of future ocean changes on marine primary production.

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**Figure legends**

Fig. 1. Interactions between various external factors affecting productivity of phytoplankton.

Fig. 2. Primary production in CO$_2$-perturbed microcosms by phytoplankton assemblages collected in the SCS and East China Sea (station PN07). a,b, Per volume of sea water (μg C l$^{-1}$ h$^{-1}$) (a) and per chl a (μg C chl a l$^{-1}$ h$^{-1}$) (b); For the high-pCO$_2$ (HC, 800 μatm, red circle) for all stations except SEATS and C3, where 1,000 μatm pCO$_2$ was applied and low-pCO$_2$ (LC, 385 μatm, blue square) experiments, triplicate microcosms (32 l) were used for each pCO$_2$ level. The phytoplankton assemblages in all microcosms were equally exposed to 91% incident solar visible radiation. Insets: additional 24 h incubations carried out at the two stations. Error bars represent standard deviations of triplicate incubations of samples from triplicate microcosms. Note, higher rates of photosynthetic carbon fixation were found in low-CO$_2$ microcosms. c, The NPQ of phytoplankton assemblages at station E606 grown under low pCO$_2$ (385 μatm, filled blue triangle) and high pCO$_2$ (800 μatm, filled red triangle) in the microcosms on day six. The black line represents the visible light intensity of that day. Note, higher NPQs were always found in the high-CO$_2$ microcosms. (from Gao et al. 2012).
Fig. 2