

University of Dundee

Dynamic CO₂ and pH levels in coastal, estuarine, and inland waters

Raven, John A.; Gobler, Christopher S.; Hansen, Per Juel

Published in:
Harmful Algae

DOI:
[10.1016/j.hal.2019.03.012](https://doi.org/10.1016/j.hal.2019.03.012)

Publication date:
2020

Licence:
CC BY

Document Version
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Raven, J. A., Gobler, C. S., & Hansen, P. J. (2020). Dynamic CO₂ and pH levels in coastal, estuarine, and inland waters: Theoretical and observed effects on harmful algal blooms. *Harmful Algae*, 91, [101594].
<https://doi.org/10.1016/j.hal.2019.03.012>

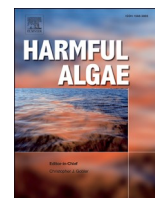
General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Dynamic CO₂ and pH levels in coastal, estuarine, and inland waters: Theoretical and observed effects on harmful algal blooms

John A. Raven^{a,b,c,*}, Christopher J. Gobler^{d,**}, Per Juel Hansen^e

^a Division of Plant Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

^b Climate Change Cluster, University of Technology Sydney, Ultimo, NSW, 2007, Australia

^c School of Biological Science, University of Western Australia, Crawley, WA, 6009, Australia

^d School of Marine and Atmospheric Sciences, Stony Brook University, Southampton NY, 11968, USA

^e University of Copenhagen, Marine Biological Section, Strandpromenaden 5, DK 3000 Helsingør, Denmark

ARTICLE INFO

Keywords:

Acclimation
Adaptation
Algal toxins
Eutrophication
Experimental evolution
Inorganic carbon
Global warming
Harmful algae
Ocean acidification

ABSTRACT

Rising concentrations of atmospheric CO₂ results in higher equilibrium concentrations of dissolved CO₂ in natural waters, with corresponding increases in hydrogen ion and bicarbonate concentrations and decreases in hydroxyl ion and carbonate concentrations. Superimposed on these climate change effects is the dynamic nature of carbon cycling in coastal zones, which can lead to seasonal and diel changes in pH and CO₂ concentrations that can exceed changes expected for open ocean ecosystems by the end of the century. Among harmful algae, i.e. some species and/or strains of Cyanobacteria, Dinophyceae, Prymnesiophyceae, Bacillariophyceae, and Ulvophyceae, the occurrence of a CO₂ concentrating mechanisms (CCMs) is the most frequent mechanism of inorganic carbon acquisition in natural waters in equilibrium with the present atmosphere (400 μmol CO₂ mol⁻¹ total gas), with varying phenotypic modification of the CCM. No data on CCMs are available for Raphidophyceae or the brown tide Pelagophyceae. Several HAB species and/or strains respond to increased CO₂ concentrations with increases in growth rate and/or cellular toxin content, however, others are unaffected. Beyond the effects of altered C concentrations and speciation on HABs, changes in pH in natural waters are likely to have profound effects on algal physiology. This review outlines the implications of changes in inorganic cycling for HABs in coastal zones, and reviews the knowns and unknowns with regard to how HABs can be expected to ocean acidification. We further point to the large regions of uncertainty with regard to this evolving field.

1. Introduction

“Harmful algae” include toxin-producing marine phytoplankton which are mainly comprised of dinoflagellates, many of which are mixotrophic, including some that are kleptoplastidic (e.g. [Medlin and Cembella, 2013](#); [Stoecker et al., 2017](#)) and the diatom *Pseudo-nitzschia* (e.g. [Brunson et al., 2018](#)). Also toxin-producing are the marine phytoplanktonic prymnesiophycean *Prymnesium parvum* ([Manning and La Claire, 2010](#)), marine phytoplanktonic raphidophyceans ([Khan et al., 1987](#)) and freshwater (and estuarine, coastal and open ocean) toxin-producing cyanobacteria (e.g. [Cox et al., 2005](#); [Schock et al., 2011](#); [O’Neil et al., 2012](#); [Codd et al., 2015](#); [Huisma et al., 2018](#); [Lines and Beardall, 2018](#)). “Harmful algae” also include ecosystem-disruptive marine microalgae (e.g. members of the Pelagophyceae; [Marshall and](#)

[Hallegraeff, 1999](#); [Gobler and Sunda, 2012](#)) and macroalgae such as *Ulva* (‘green tides’) and *Sargassum* (‘golden tides’) ([Hayden et al., 2002](#); [Smetacek and Zingame, 2013](#); [Xu et al., 2017](#)). Harmful algal blooms represent an expanding threat to human health, aquatic life, and economies in marine and freshwater ecosystems across the globe ([Anderson et al., 2012](#); [O’Neill et al., 2012](#)). As HABs have expanded across during the past half-century ([Anderson et al., 2015](#); [Gobler et al., 2017](#)), levels of atmospheric and surface water CO₂ concentrations have concurrently increased by more than 25% ([Doney et al., 2012](#)). Changing levels of dissolved inorganic carbon in surface waters may impact phytoplankton inorganic carbon fixation ([Badger et al., 1998](#); [Giordano et al., 2005](#); [Raven et al., 2017](#)). Thus, rising CO₂ concentrations in surface waters may potentially contribute to the global expansion of HABs (i.e. fertilization effect; [Hallegraeff, 2010](#); [Fu et al., 2012](#); [Flynn et al., 2015](#)). The

* Corresponding author at: Division of Plant Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK.

** Corresponding author.

E-mail addresses: j.a.raven@dundee.ac.uk (J.A. Raven), christopher.gobler@stonybrook.edu (C.J. Gobler).

<https://doi.org/10.1016/j.hal.2019.03.012>

Received 7 March 2019; Accepted 8 March 2019

Available online 6 April 2019

1568-9883/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

extent to which any phytoplankton may benefit from rising dissolved CO₂ concentrations associated with climate change, however, will be dependent upon a complex myriad of physiological, ecological, and biogeochemical factors. The goal of this review is to describe the dissolved inorganic carbon (DIC) system in aquatic systems as it relates to photosynthesis, to contextualize the dynamics of DIC in coastal ecosystems where HABs occur, and to assess how changing dissolved CO₂ concentrations and pH levels may influence harmful algae and the occurrence of HABs.

2. Natural variation of inorganic carbon and pH, and the role of climate change

Understanding of the impact of the dissolved inorganic carbon system, and its short- and long-term variations on phytoplankton, in general, and harmful algae, in particular, involves consideration of the dissolved inorganic carbon system in marine and freshwater habitats (Zeebe and Wolf-Gladrow, 2001; Falkowski and Raven, 2007; Riebesell et al., 2010). The molecular and ionic species involved are atmospheric and dissolved CO₂ (carbon dioxide), and dissolved H₂CO₃ (carbonic acid), dissolved HCO₃⁻ (bicarbonate) and CO₃²⁻ (carbonate), and solid CaCO₃ (calcium carbonate) (Zeebe and Wolf-Gladrow, 2001; Falkowski and Raven, 2007; Riebesell et al., 2010) (Table 1). Net conversion among the dissolved inorganic carbon species involves changes in H⁺/OH⁻, i.e. pH. It is important to recognise that the pH changes are an outcome of biologically induced and other changes in the dissolved inorganic carbon system and, to a lesser extent in other solutes. H₂CO₃ is the least well-characterised of these components of the inorganic carbon system (Reddy and Balasubramanian, 2004; Adamczyk et al., 2009; Bucher and Sander, 2014; Jakubowska and Szlag-Wasilewska, 2015; Pines et al., 2016), but the available evidence indicates that H₂CO₃ is not directly involved in algal metabolism. The available evidence shows for the green tide alga, *Ulva*, CO₃²⁻ does not inhibit HCO₃⁻ use in photosynthesis, and is not used (in the sense of entering photosynthetic cells) in photosynthesis (Maberly, 1992). There seem to be no recent attempts to examine the influence of CO₃²⁻ on photosynthesis by phytoplankton; inhibitory effects are difficult to distinguish from those of high pH, discussed below. In contrast, HCO₃⁻ and CO₂ are both involved in various versions of carbon transport and metabolism in photosynthesis.

The IPCC (Intergovernmental Panel for Climate Change) AE4 2007 projected that the pH in surface waters in the open ocean will decrease ('ocean acidification': see below) from the present 8.05 to 7.75–7.95 as a result of rising atmospheric CO₂ concentrations, with surface ocean CO₂ concentrations increasing from 400 to 550–950 μmol mol⁻¹ by year 2100 (Figure 10.23 of IPCC, 2007; see also The Royal Society, 2005). IPCC, 2013 AR5 (Technical Summary TS20) suggests Ocean surface pH of 8.19 in 1850 and predicts pH 7.78–8.05 by the year 2100). The projections of the future CO₂ concentration and pH are, however, based

Table 1

Inorganic carbon speciation in seawater in equilibrium with extant and future CO₂ mole fractions at 20 °C (Gao et al., 2018a,b). In freshwater, for the same gas phase CO₂ mol fraction, the greater CO₂ solubility, the higher pK_{a1} and, especially, the higher pK_{a2} of the inorganic carbon system means that, at a given temperature and pH, the concentration of dissolved CO₂ is higher, and HCO₃⁻ and, especially, CO₃²⁻ is lower (Falkowski and Raven, 2007).

| | Atmospheric CO ₂ 400 μmol CO ₂ mol ⁻¹ | Atmospheric CO ₂ 1000 μmol CO ₂ mol ⁻¹ |
|---|---|--|
| Dissolved inorganic carbon μmol kg ⁻¹ | 2030 | 2118 |
| CO ₂ μmol kg ⁻¹ | 12.6 | 33.7 |
| HCO ₃ ⁻ μmol kg ⁻¹ | 1827 | 2005 |
| CO ₃ ²⁻ μmol kg ⁻¹ | 175 | 79 |
| Titrateable alkalinity μmol kg ⁻¹ | 2263 | 2199 |
| pH | 8.19 | 7.82 |

on changes in the open tropical ocean (present day data on atmospheric CO₂ from the Mauna Loa Observatory, Hawaii). Open ocean waters contain 2.2–2.4 mol m⁻³ DIC (dissolved inorganic carbon) although levels can be lower in the upper photic zone due to uptake by the primary producers (e.g. Marchal et al., 1996). pH affects the speciation of inorganic carbon species and at present ocean pH values, very little is available as CO₂ (typically 7–20 mmol m⁻³). CO₂ per volume is similar in the sea level atmosphere and dissolved in the surface ocean (with more dissolved CO₂ at lower temperatures), but the diffusion coefficient for CO₂, the immediate inorganic carbon substrate for RuBisCO (Ribulose biphosphate carboxylase-oxygenase), the carboxylase involved in the Calvin-Benson cycle in seawater is 10⁴ times slower than that in the atmosphere (Falkowski and Raven, 2007). This is probably the evolutionary reason for the occurrence of CO₂ concentrating mechanisms (CCMs) in many marine (and freshwater) algae (Badger et al., 1998; Raven et al., 2017). CCMs actively transport HCO₃⁻ and/or CO₂ from the surrounding water to the site of Rubisco, raising the steady-state CO₂ concentration there to values higher than those in the water, and increasing the extent to which the carboxylase activity of Rubisco is saturated with CO₂, and decreasing the oxygenase activity (Badger et al., 1998; Giordano et al., 2005; Raven et al., 2017). When active transport involves HCO₃⁻, the inorganic species present at highest concentrations in seawater, intracellular carbonic anhydrase is essential to convert HCO₃⁻ to CO₂ sufficiently rapidly. Diffusive CO₂ entry occurs in a minority of marine, and a rather greater fraction of freshwater, photosynthetic organisms (Badger et al., 1998; Giordano et al., 2005; Raven et al., 2017; Shen et al., 2017). The larger fraction of freshwater algae dependent on diffusive CO₂ entry could be related to the greater solubility of CO₂ in lower-salinity waters, as well as the relatively greater input of inorganic carbon resulting from terrestrial productivity (Maberly, 1996; Falkowski and Raven, 2007). Less than a third of terrestrial primary productivity is carried out by organisms expressing CCMs (Still et al., 2003); most of these CCMs involve C₄ photosynthesis (Raven et al., 2017). For harmful algae, active transport of inorganic carbon for fixation by Rubisco involves uptake of HCO₃⁻ as well as CO₂ in red tide dinoflagellates (Rost et al., 2006), toxic diatoms (Trimborn et al., 2008), and all (i.e. including toxic) cyanobacteria (Raven et al., 2017); this is discussed in more detail below in the section, *Effects of variations in the inorganic carbon system on algal photosynthesis and growth*.

The dissolved inorganic carbon system functions as a buffer against pH changes so variations in the upper water column of open ocean waters are minimal (< 0.1 pH units; Doney et al., 2009; Duarte et al., 2013), at least in tropical and subtropical waters. However, the variations may be considerably higher in systems with seasonality as in temperate waters (the North Sea; 0.2 pH units; Salt et al., 2013) and Arctic waters with an ice cover (and hence no CO₂ exchange with the atmosphere) during the boreal winter (West coast of Greenland, 0.8 pH units; Thoisen et al., 2016). A further influence on CO₂ in high latitude waters is the impact of fresh water from glacial melting which increases (at a given temperature) CO₂ solubility; however, the meltwater is significantly undersaturated with CO₂, so in coastal waters continuous melting maintains undersaturation (Meire et al., 2015). Similarly, a series of biogeochemical processes in coastal zones lead to large pH variation on a seasonal and even daily basis or across a vertically stratified water column (>1 pH unit; Fig. 1; Wallace et al., 2014; Baumann et al., 2015; Baumann and Smith, 2017).

While marine HABs can occur anywhere in the World's Oceans, chemical (e.g. enhanced nutrient loading) and physical (e.g. shallow water, low flushing rates) factors makes them predominantly features of coastal zones. Within these regions, inorganic carbon pools can be highly dynamic (Cai, 2011). It is well-known that the progressive rise in atmospheric CO₂ and its equilibration with the oceans is increasing the concentration of dissolved CO₂ and HCO₃⁻ and H⁺ and decreasing the concentration of OH⁻ and CO₃²⁻, the phenomenon known as ocean acidification (Doney et al., 2009, 2012). Within coastal regions, there are a myriad of other significant sources of inorganic carbon that can

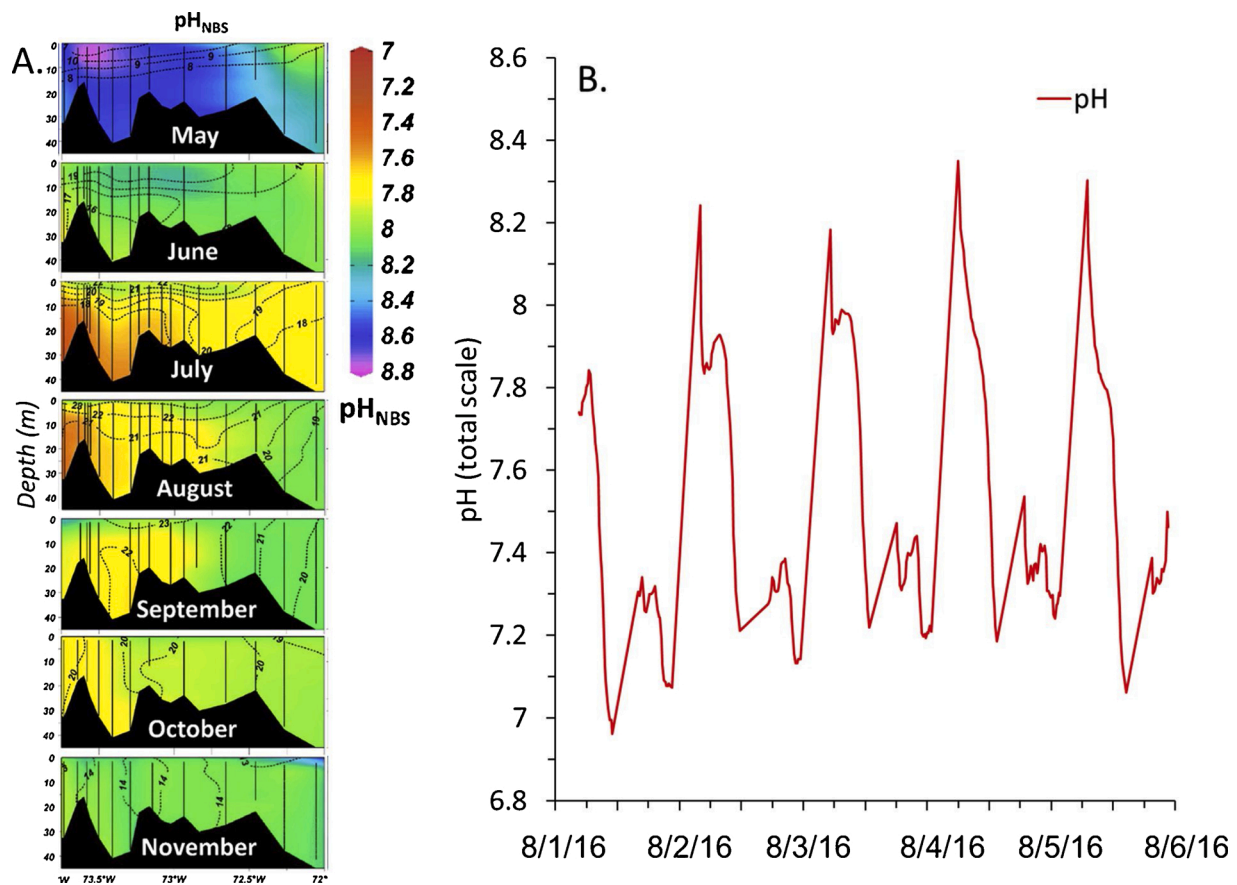


Fig. 1. Monthly changes in pH measured on the NBS scale in Long Island Sound, 2011, the third largest estuary in the USA. Vertical section plots extend from New York City in the western extreme to Block Island to the east (adopted from Wallace et al., 2014).

Diurnal changes in pH on the total scale as measured in Shinnecock Bay, NY, USA, August 2016, which hosts HABs caused by *Alexandrium catenella*, *Aureococcus anophagefferens*, and *Cochlodinium polykrikoides*.

also promote acidification. Along many coastlines, rivers are acidic and capable of significantly depressing calcium carbonate saturation and increasing concentrations of CO_2 within marine coastal zones (Cai et al., 2008; Salisbury et al., 2008; Waldbusser and Salisbury, 2014). Cai et al. (2009) demonstrated that only two of the 25 largest (by discharge) rivers have significantly higher HCO_3^- concentrations than does open ocean seawater. An extreme case is the Baltic Sea, dominated by riverine inputs rather than exchange with the North Atlantic, where values as low as 1.2 mol m^{-3} or 40% lower than ocean water can be found (Thomas and Schneider, 1999). These low HCO_3^- concentrations in riverine inputs to coastal waters are despite significant anthropogenic increases in the inorganic carbon content of lakes (Perga et al., 2016) and rivers (Jarvie et al., 2017). Such low DIC concentrations lead to a much lower pH buffering capacity, making such coastal zones more vulnerable to pH changes as biological processes removes or adds CO_2 (Cai, 2011; Waldbusser and Salisbury, 2014; Gledhill et al., 2015).

Upwelled waters, originating from the deep ocean where biological mineralisation of sinking organic matter enriches the water in CO_2 , in coastal zones are typically highly enriched in dissolved CO_2 (Feely et al., 2008, 2010). Furthermore, most estuaries are net heterotrophic ecosystems where rates of respiration exceed rates of gross photosynthesis (Caffery, 2004; Del Giorgio and Williams, 2005), leading to super-saturated concentrations of CO_2 resulting in decreased pH (Melzner et al., 2013; Wallace et al., 2014; Cai et al., 2011, 2017). Such coastal acidification is becoming increasingly recognized as seasonal increases in the concentrations of CO_2 and H^+ , and decreases in calcium carbonate saturation in some coastal zones ($\text{pH} < 7.7_T$, CO_2 in equilibrium with an atmosphere of more than $1000 \mu\text{mol mol}^{-1}$, $\Omega_{\text{aragonite}} < 1$; Melzner et al., 2013; Wallace et al., 2014; Cai et al., 2011, 2017) already

exceed the predicted extremes in the future open ocean associated with projected climate change (Doney et al., 2012; Duarte et al., 2013).

An ecological niche of many HABs is turbid estuaries where light levels are low, but organic matter is enriched (Sunda et al., 2006; Gobler et al., 2011). Beyond the natural turbidity present in estuaries, the pigmented biomass found in many HABs can create extreme turbidity and thus low light conditions in the water column (Gobler et al., 2005; Sunda et al., 2006). These conditions decrease photosynthesis, thus increasing the relative influence of respiration by the HAB algae, and decreasing or reversing the role of HAB algae in decreasing the external CO_2 concentration. Many HAB species, e.g. many dinoflagellates, and including those that are low light adapted, are mixotrophic. Such algae, in addition to photosynthesis, catalyse the entry, metabolism, and use for growth of dissolved organic compounds (osmotrophy) or particulate organic carbon (phagotrophy) (Burkholder et al., 2008; Stoecker et al., 2017; Flynn et al., 2018). As a consequence of these trophic modes, external pH will not increase as much per unit growth of HAB as would occur with purely autotrophic growth. Furthermore, Eberlein et al. (2014) demonstrated that dark respiration increases while net photosynthesis decreases in *Alexandrium tamarense* under elevated CO_2 concentrations, changes that would contribute to increasing CO_2 concentrations over the course of *Alexandrium* blooms, a phenomenon observed and reported by Hattenrath-Lehman et al. (2015).

While many factors have been attributed to the global expansion of HABs, excessive nutrient loading to coastal waters is frequently cited as a primary factor promoting many of these events (Anderson et al., 2002; Heisler et al., 2008). Interestingly, some of the coastal processes that promote acidification in coastal zones also deliver high levels of inorganic nutrients, thus potentially creating a synergistic opportunity for

the intensification of HABs. Rivers are major sources of nutrients such as phosphorus and combined nitrogen (Tappin, 2002), as well as CO₂ (Cai et al., 2008; Salisbury et al., 2008), and some HABs are known to develop near riverine outflow (Heil et al., 2007; Paerl et al., 2008; Zhou et al., 2008). Upwelling zones involve advection from the deep, mineralising waters to the surface ocean; this upwelled seawater is enriched in CO₂ as well as HPO₄²⁻ and NO₃⁻, and a diversity of HABs develop within upwelling zones (Pitcher and Weeks, 2006, 2018). Some develop within the advected CO₂ water while others develop after an initial upwelling-stimulated bloom subsides, and thus occurs in water pre-conditioned with high levels of DOM (Pitcher and Weeks, 2006, 2018). Nutrients from remineralization of organic matter within estuaries are thought to specifically select for the growth of many species of harmful alga (Anderson et al., 2002; Heisler et al., 2008; Glibert, 2017; Glibert and Burford, 2017) and the process of utilizing these remineralized nutrients may promote acidification. For example, NH₄⁺ uptake and assimilation results in the excretion of one H⁺ molecule per NH₄⁺ molecule to maintain the acid-base balance of the cells and subsequently decreases external pH and increases CO₂ (Brewer and Goldman, 1976). Given the Redfield atomic ratio, the inorganic C influx will be 6.6 times that of NH₄⁺ (Brewer and Goldman, 1976) and thus the acidifying effects of the assimilation of NH₄⁺ only partly offsets the alkalising effect of inorganic carbon assimilation. In contrast, assimilation of NO₃⁻ yields one OH⁻ molecule effluxed per NO₃⁻ molecule assimilated (Brewer and Goldman, 1976), thus increasing external pH. The intensity of acidification within estuaries may be associated with multiple factors including their degree of eutrophication with higher nutrient loading rates promoting more organic enrichment and seasonal acidification, and flushing rates with well-flushed estuaries being less likely to retain acidified water. Expanses of salt marshes and/or mangroves may also influence acidification as these ecosystems are well-known for their high respiration rates in the substrate and overlying water (Caffery, 2004; Baumann et al., 2015). These complex interactions deserve further investigation.

The timing of some HABs also suggests they may develop in acidified environments, at least in shallow regions. During the spring bloom of diatoms, rapid rates of primary production can decrease dissolved CO₂ concentration and create a subsequent basification (increased pH) of the water column (Nixon et al., 2015). In some temperate zones, however, HABs do not co-occur with the spring bloom, but rather develop after its demise, which releases large stocks of organic matter (Sunda et al., 2006) that enhance microbial respiration and the production of dissolved CO₂ (Wootton et al., 2008). The peak of annual acidification may appear during warmer months when respiration rates are maximal (Fig. 1; Melzner et al., 2013; Wallace et al., 2014), thermal stratification is most likely, and several temperate HABs commonly occur (Sunda et al., 2006; Heil et al., 2007; Kudela and Gobler, 2012). Alternatively, as explained below, some coastal zones with HABs are prone to basification.

The persistence of acidification in coastal zones can vary from hours to months, depending on the rate of respiration, the geomorphology of the region, tidal flushing, the depth and structure of the water column, and other factors related to the hydrodynamics of a given water body. In shallow, well-mixed coastal zones with high rates of respiration, acidification can occur on a diel basis, because maximal photosynthetic rates during the day result in high dissolved oxygen and pH levels, while the cumulative effects of respiration during night decrease dissolved oxygen concentration and pH values to predawn minima (Wootton et al., 2008; Baumann et al., 2015; Baumann and Smith, 2017). The extent of this process can be related to the depth of the water column, given that sediments are known to be major sources of CO₂ (Green and Allen, 1998) and their influence is likely to be inversely proportional to the depth of the water column. Given the propensity of many HAB algae to undergo diel vertical migration to assimilate nutrients (Doblin et al., 2006), it seems likely that such algae will be exposed to lower pH and elevated concentrations of dissolved CO₂ at night. NH₄⁺ (or NO₃⁻) assimilation

needs anaplerotic inorganic carbon assimilation but this would be saturated in the dark by air-equilibrium seawater (Amory et al., 1991; Falkowski and Raven, 2007). Superimposed upon diel changes in net metabolism in coastal zones are the actions of tides. In general, low tides are likely to maximize the influence of local metabolic rates on carbon dioxide concentration while high tides may bring less acidified water if the incoming water mass originates from a region further from land with lower concentrations of dissolved CO₂ and dissolved and particulate organic matter, less benthic influence, and lower rates of respiration (Waldbusser and Salisbury, 2014; Baumann et al., 2015). The precise dynamics of dissolved CO₂ in coastal zones are highly complex and will differ among coastal regions. It is unlikely that they will be fully characterized in the near future, but are likely to influence the growth and photosynthesis of some HABs.

As climate change progresses, the effects of atmospheric CO₂ on coastal acidification will increase in a non-linear fashion, leading to acidification becoming even more significant in temperate and, especially, tropical coastal zones (Sunda and Cai, 2012). Modelling efforts have shown that under future climate change scenarios, synergistic interactions may occur between CO₂ from atmospheric sources and from the respiration of organic matter, especially at higher temperatures (Sunda and Cai, 2012). As a consequence, the buffering capacity of some ocean regions may be overwhelmed, resulting in degree of acidification that is non-additive and greater than would have been predicted from the CO₂ loading by each individual source (Sunda and Cai, 2012). This may make temperate and tropical estuaries even more vulnerable to coastal acidification, as they warm and experience the synergistic effects of acidification driven by both respiration and increased atmospheric CO₂, with the latter decreasing the outgassing of respired CO₂. At high latitudes the larger increases in temperature, but still lower overall temperatures, suggests that the overall impact on HABs may be smaller. It is important to re-emphasize that environmental drivers may differ among global regions.

In addition to acidification, high nutrient levels in coastal zone that drive high rates of primary production can also lead to basification, with high pH and low pCO₂ (Nixon et al., 2015). As a consequence, in some eutrophicated estuaries, semi-enclosed fjords and lagoons the pH values vary from 7.2 to 9.75 as a function of season, latitude, and depth (e.g. Fig. 3; Hinga, 2002; Macedo et al., 2001; Hansen, 2002). During summer at high latitudes, extended and even 24 h photoperiods will maximize the influence of photosynthesis and keep pH levels high (>9), especially within surface waters (Fig. 3; Hansen, 2002). Thus, to properly evaluate the consequences of climate changes for a given location, the natural variations in pH that occur during period of low respiration and productivity and the variations in the inorganic carbon system that underlie it, should be taken into account.

For inland waters where many toxic cyanobacterial blooms occur, there is a latitudinal trend in large lakes from net autotrophic (atmospheric CO₂ sinks) at low latitudes to net heterotrophic at higher latitudes with greater dependence of food chains on allochthonous organic carbon input (Alin and Johnson, 2007). There are large diel and seasonal variation in pH and CO₂ concentration in some lakes; Maberly (1996) found diel variations in pH of up to 1.8 pH units and an annual change from 7.3 to nearly 10.1 pH units near the surface of Esthwaite Water in the English Lake District. Global anthropogenic effects increasing lake surface CO₂ has been shown for the smaller lakes studied (Perga et al., 2016). Trophic status can also strongly effect the dynamics of CO₂ in lakes with eutrophic lakes being more likely to be undersaturated in CO₂, at least during summer, due to rapid rates of photosynthesis (Maberly, 1996; Trolle et al., 2012).

3. Effects of variations in the inorganic carbon system on algal photosynthesis and growth

All oxygenic photosynthetic organisms have Rubisco as the carboxylase underlying their autotrophy (Raven et al., 2017; Bathellier et al.,

2018). Organisms with CCMs (Fig. 2) have higher in vivo CO_2 affinity than is the case for organisms relying on CO_2 diffusion to Rubisco, assuming identical Rubisco kinetics and Rubisco content (Raven et al., 2017). Only a small fraction of the work on the effects of CO_2 availability on algal metabolism and growth has focussed on HAB-forming algae. From what is known of the phylogeny of HAB-forming algae and of the occurrence of CCMs it appears that HAB-forming algae generally have CCMs. More work is needed to determine if this prediction is correct. The cyanobacterial Form IA or IB Rubiscos, and especially the Form II Rubisco of the phylogenetically basal (peridinin-containing) dinoflagellates, have very low CO_2 affinities and $\text{CO}_2:\text{O}_2$ selectivities; the affinity (expressed in terms of external CO_2) of cyanobacterial CCMs is, however, much higher than those of dinoflagellates (Griffiths et al., 2017; Raven et al., 2017). Other eukaryotic algal Rubiscos (Form IB in the 'green' line of evolution and Form ID in the 'red' line of evolution, including fucoxanthin-containing dinoflagellates such as *Karenia*) have higher CO_2 affinities and $\text{CO}_2:\text{O}_2$ selectivities than both cyanobacteria and (basal) dinoflagellate Rubiscos (Griffiths et al., 2017; Raven et al., 2017; Bercel and Kranz, 2019; Shen et al., 2017). However, in cases where the algae with these Rubiscos also have CCMs, the CO_2 affinities of their CCMs are generally lower than is the case for the CCMs of cyanobacteria (Griffiths et al., 2017; Raven et al., 2017; Lines and Beardall, 2018; Bercel and Kranz, 2019). Ji et al. (2017) and Beardall and Raven (2017) discuss the extent to which the affinity of CCMs for inorganic carbon among phytoplankton organisms could influence evolutionary fitness among the range of other relevant environmental factors; these data are further discussed below. Among harmful algae, all cyanobacteria and dinoflagellates have and express CCMs, as do most, if not all, prymnesiophyceans, diatoms and ulvophyceans, in natural waters at equilibrium with the present atmosphere ($400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ total gas), with varying phenotypic modification of the CCM (Hall-Spencer and Allen, 2015; Griffiths et al., 2017; Raven and Giordano, 2017; Raven et al., 2017; Wilkes et al., 2017).

Ji et al. (2018) suggested that a harmful raphidophycean has a CCM, based on the up-regulation of transcription of three carbonic anhydrase genes in the photoperiod; this is not adequate for demonstration of a CCM. Ji et al. (2018) provide no data on the occurrence of a mechanism for energized (other than by energized Rubisco and other Calvin Benson Bassham cycle activity generating gradient for diffusive entry of CO_2)

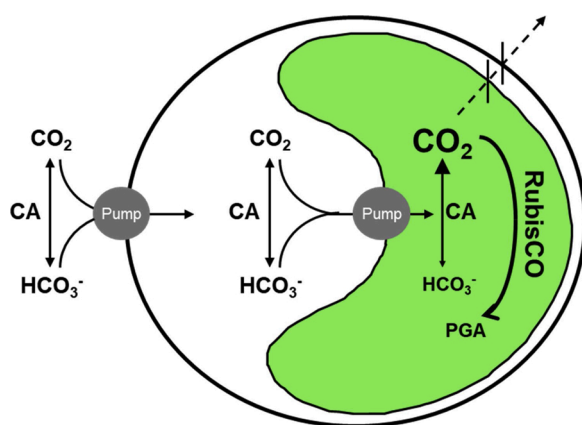


Fig. 2. Generalised diagram of inorganic carbon acquisition by eukaryotic algal cells using a CO_2 concentrating mechanism (denoted by 'Pump'). CO_2 concentrating mechanism actively transporting CO_2 and/or HCO_3^- into the cytoplasm and chloroplast (denoted in green). Active (= energized, uphill) HCO_3^- occurs at the plasmalemma and/or the chloroplast, with carbonic anhydrase (CA) in the chloroplast compartment containing Rubisco, i.e. the chloroplast stroma or the pyrenoid (a sub-compartment of the stroma). CA may also be present on the cell surface outside the plasmalemma (outer black circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

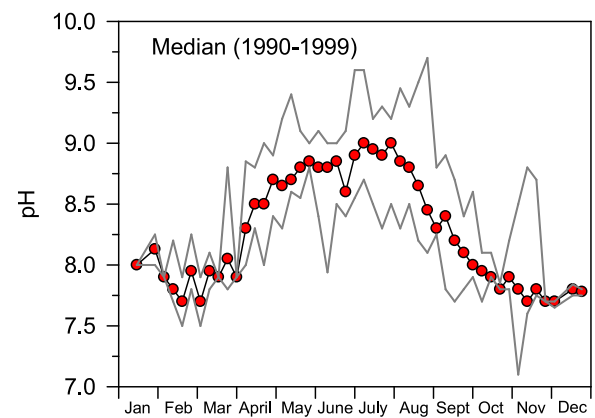


Fig. 3. Surface pH values within the Mariager Fjord, Denmark, as reported in Hansen (2002). pH can reach very high values in coastal eutrophic waters during summer due to

the photosynthetic activity of algal blooms and near-constant sunlight, maximizing the influence of photosynthesis. pH was measured using a glass electrode calibrated with buffers at pH 7.0 and at pH 10.0.

transport of inorganic carbon, an essential feature of CCMs (Raven and Giordano, 2017; Raven et al., 2017). The study of Clark and Flynn (2000) on the photosynthetic affinity for inorganic carbon of marine phytoplankton shows that the raphidophycean *Heterosigma akashiwo* had the lowest inorganic carbon affinity of any of the ten species of phytoplankton examined, and the investigation of the in vitro CO_2 affinity of Rubisco of *Olisthodiscus luteus* showed a moderate affinity (Newman et al., 1989). Neither of these data sets give conclusive evidence on the occurrence of a CCM in these raphidophyceans. The data on the toxic marine prymnesiophycean *Prymnesium parvum* (Lysgaard et al., 2018) is also inconclusive with regard to the possession of a CCM. No data seem to be available for the brown tide pelagophyceans (Gobler and Sunda, 2012; Raven and Giordano, 2017). More work on the Raphidophyceae, Pelagophyceae and *Prymnesium* to determine if they have CCMs.

The high in vivo CO_2 affinities discussed above are for organisms grown in media in equilibrium with the extant atmospheric CO_2 of $400 \mu\text{mol CO}_2$ per mol total gas. Much of the work on variations in CO_2 on the physiology of algae has focussed on the effects of anthropogenic increase in CO_2 on specific growth rates (Ratti et al., 2007; De Paula Silva et al., 2013; Lapointe et al., 2008; Trimborn et al., 2013; Sandrini et al., 2014; Hattenrath-Lehman et al., 2015; Hoins et al., 2015, 2016a,b, Thaisen et al., 2015; Richier et al., 2018; Zhu et al., 2017; Lysgaard et al., 2018; Brunson et al., 2018). Doney et al. (2012) and Raven et al. (2017) point out that global environmental change involves increased temperature of the upper mixed layer, shoaling of the thermocline and increasing temperature difference across the thermocline. These global effects result, for phytoplankton, in an increased mean flux of photosynthetically active radiation and UVB, and increased temperature, and decreased availability of iron, phosphorus and combined nitrogen (Doney et al., 2012; Raven et al., 2017); there are also local changes in DIC and nutrients.

There are many studies on algae, some on HAB species, involving the interaction of increased DIC with changes in other factors that often occur with increased DIC. The interaction of DIC with increased temperature has been studied by, for example, Fu et al. (2012); Tatters et al. (2013); Elera et al. (2014); Kübler and Dudgeon (2015); Guanyong et al. (2017); Wells et al. (2015); Raven et al. (2017); Mardones et al. (2017); Boyd et al. (2018) and Roth-Schulze et al. (2018). There have also been investigations of the interaction of increased DIC with local anthropogenically increased availability of combined nitrogen and of phosphorus and ocean warming-related decreased combined nitrogen and phosphorus by shoaling and steepening of the thermocline (e.g. Sun et al.,

2011; Tatters et al., 2012a,b; Eberlein et al., 2016; Raven et al., 2017; Reidenbach et al., 2017). Finally, there have been studies of increased DIC with changed photosynthetically active radiation and increased UV-B (e.g. Gao and Campbell, 2014; Raven et al., 2017).

Some of these studies of interactions of increased DIC with other environmental factors have involved toxin-producing HAB algae (Macintyre et al., 2011; Tatters et al., 2012a,b; Fu et al., 2008; Visser et al., 2016; Guanyong et al., 2017; Glibert, 2019). Other studies of the interaction of increased DIC with other environmental factors have involved nuisance green tide algae (Raven and Taylor, 2003; Gao et al., 2016; Young and Gobler, 2016; Reidenbach et al., 2017; Gao et al., 2018a,b) algae, as well as the introduced 'Killer Alga' *Caulerpa taxifolia* (Roth-Schulze et al., 2018; see also Kevekordes et al., 2006).

Ji et al. (2017; see commentary by Beardall and Raven, 2017) showed that the freshwater toxin-producing *Microcystis* was less competitive with green microalgae at low CO₂ than at high CO₂; the competitive order among four algae studied at low CO₂ was *Scenedesmus* > *Chlorella* > *Microcystis* > *Monoraphidium*, while at high CO₂ the order was *Microcystis* ≈ *Scenedesmus* > *Chlorella* > *Monoraphidium*. There are also reports of increased cellular toxin content in several algae under increased CO₂ and interactions of CO₂ availability with availability of other nutrients (Van de Waal et al., 2009; Fu et al., 2010; Sun et al., 2011; Tatters et al., 2012a,b; Hattenrath-Lehman et al., 2015; Bercel and Kranz, 2019). Van de Waal et al. (2011a,b) stoichiometric argument for toxin synthesis suggests that the synthesis of C-rich toxins (e.g. domoic acid) might be promoted under high CO₂ availability as a means to provide internal elemental balance and there have been several studies to support this concept (e.g. Van de Waal et al., 2009; Fu et al., 2010; Sun et al., 2011; Brunson et al., 2018). In contrast, there have also been studies documenting an increased saxitoxin content in *Alexandrium* spp., despite the N-rich nature of this molecule (Tatters et al., 2013; Hattenrath-Lehman et al., 2015). Given excess nitrogen was available in the medium during these experiments, there may be an increase in cell size leading to an increase in cellular saxitoxin content (Tatters et al., 2013; Hattenrath-Lehman et al., 2015). The data discussed here are consistent with an interaction between increased CO₂ and increased toxin production in toxic algae; more work is needed.

High algal productivities resulting from environmental factors other than increased CO₂ lead to increased hydroxyl ion and carbonate concentrations, and decreased hydrogen ion, bicarbonate and CO₂ concentrations. Several studies have involved freshwaters (Maberly, 1996; Talling, 2006; Van de Waal et al., 2009, Van de Waal et al., 2011a,b; Sandrini et al., 2015a, 2015b, 2016). Other studies have involved marine habitats (Poole and Raven, 1997; Raffaelli et al., 1998; Raven and Taylor, 2003; Middelboe and Hansen, 2007; Trimborn et al., 2008; Semesi et al., 2009; Van de Waal et al., 2009, Van de Waal et al., 2011a, b; Berge et al., 2012; Flores-Moya et al., 2012; Saderme et al., 2013).

Some of these studies were specifically in the context of harmful algae (Poole and Raven, 1997; Raffaelli et al., 1998; Raven and Taylor, 2003; Trimborn et al., 2008; Van de Waal et al., 2009, Van de Waal et al., 2011a,b; Berge et al., 2012; Flores-Moya et al., 2012; Sandrini et al., 2015a, 2015b, 2016). The environmental factors other than increased CO₂ that allow high algal productivities include high (but not inhibitory) concentrations of bioavailable nutrient elements other than C, high (but not inhibitory) fluxes of photosynthetically active radiation, low fluxes of UVB, limited impact of herbivores, and viruses and limited mixing with water bodies permitting lower productivity (Smayda, 1997a, b). Extended residence times of water bodies can also promote algal blooms. Examples of very limited exchange are rock pools in marine habitats with a small tidal range, e.g. within the Mediterranean (Calabetti et al., 2017).

In considering the effects of CO₂ variations on the photosynthesis, growth, and metabolism of harmful algae it is well-known that many harmful microalgae have mixotrophic potential using osmotrophy and/or phagotrophy (Burkholder et al., 2008; Jeong, 2011; Lim et al., 2018). Osmotrophy, involving the uptake and assimilation of dissolved organic

carbon, does not always result in net organic carbon entry since there is also dissolved organic carbon loss from photosynthetic algae (López-Sandorval et al., 2013; Thornton, 2014); however, it is important in the brown tide pelagophycean alga *Aureococcus anophagefferens* (Lomas et al., 2001; Gobler and Sunda, 2012), toxin-producing cyanobacteria (Dai et al., 2009), and other harmful algae (Burkholder et al., 2008). Phagotrophy is only an option for eukaryotes without a complete cell wall, e.g. many toxic dinoflagellates (e.g. Burkholder et al., 2008; Carvalho et al., 2008; Lim et al., 2018), Prymnesiophyceae (Burkholder et al., 2008; Carvalho and Granéli, 2010; Brutemark and Granéli, 2011; Carvalho and Granéli, 2011; Vidyarthana et al., 2014) and Raphidophyceae (Burkholder et al., 2008; Jeong, 2011), but not toxic diatoms. For harmful macroalgae, bacterial associations (not phagotrophy) influence morphogenesis of *Ulva* (Hayden et al., 2002; Wichard et al., 2015) into morphologies that are involved in 'green tides' and also alter the external diffusion boundary layers that partly determine the capacity to acquire inorganic carbon.

4. Strain- and species-dependent response to high CO₂

Studies often focus on differences between genera or species when the effects of CO₂ levels on physiological rates and toxin contents are considered. However, an increasing body of evidence suggest that considerable variations exist within and among genera, species, and even strains. Examples of this can be found in a range of experiments on toxic and non-toxic strains of *Microcystis aeruginosa* in relation to rising CO₂ and the expression of components of the CCM (Jähnichen et al., 2007; Van de Waal et al., 2009, Van de Waal et al., 2011a,b; Sandrini et al., 2015a, 2015b, 2016; Yu et al., 2015; Liu et al., 2016; Visser et al., 2016). Sandrini et al. (2016) cultured five strains of *Microcystis aeruginosa* in chemostats, initially with equal numbers of each for 175 days under either 100 μmol CO₂ mol⁻¹ total gas or 1000 μmol CO₂ mol⁻¹. Strains with both CCM components were favoured in low CO₂, but were partially replaced by strains with only the low affinity CCM component, one of which produced the cyanotoxin microcystin in high CO₂. It is of interest that microcystin may be involved in the acclimation of *Microcystis* to variations in external CO₂ concentration (Jähnichen et al., 2007). Sandrini et al. (2016) did not determine growth rates as a function of inorganic carbon concentration of the individual strains at the beginning and end of the 175 days to determine if genetic change had taken place.

The studies above are relatively short-term and hence the responses are acclimatory, i.e. involving variations in expression of a constant genome through differential transcriptions. There have also been long-term experiments on phytoplankton comparing, over times (10–33 months for the required 300–1000 generations for unicellular algae dividing about once a day) allowing genetic (and epigenetic change) controls at present-day CO₂ with high-CO₂ treatments (Collins and Bell, 2004; Reusch and Boyd, 2013; Kronholm et al., 2017; Raven et al., 2017). There are also studies on the filamentous toxic diazotroph *Trichodesmium* showing an irreversible (by return to control CO₂) increase in growth rate and N₂ fixation after >500 generations growth in high CO₂ (Hutchins et al., 2015). While in some of these experiments at least some of the high-CO₂ genotypes showed phenotypes that can be interpreted as adapted to high CO₂ (e.g. faster growth a high CO₂ than the control genotypes), this has not been the case for all experiments (Low-Décarie et al., 2013).

While most longer-term experiments have not focussed on harmful algae, Tatters et al. (2013) isolated four potentially harmful dinoflagellates from a coastal algal bloom and grew them under high or low pCO₂ for one year and found no strong evidence for fitness increases attributable to the conditioning dissolved CO₂ concentrations. In culture, Flores-Moya et al. (2012) grew two clonal strains of the toxic dinoflagellate *Alexandrium minutum* for 200 generations at two temperature (20 and 25 °C) and pH (7.5 and 8.0) levels to explore genetic changes associated with increased CO₂ (Flores-Moya et al., 2012). The

differences in growth rate among treatments were statistically significant for both strains with specific growth rates decreasing in the order: (1) grown and measured at pH 7.5 and 25 °C > (2) grown at pH 8.0 and 20 °C = measured at pH 7.5 and 25 °C > (3) grown and measured at pH 8.0 and 20 °C. The difference between (2) and (3) was attributed to phenotypic acclimation and the difference between (2) and (1) was attributed to genetic adaptation meaning that 32% of the difference between (1) and (3) was due to acclimation and 68% was due to adaptation.

The findings of Flores-Moya et al. (2012) do not necessarily mean that harmful blooms of *Alexandrium minutum* will increase in a warmer and lower pH ocean, since there are many other biotic and abiotic factors that influence the development of harmful (and other) blooms. Flores-Moya et al. (2012) also measured the toxin content per cell under the different treatments, although content did not vary among treatments. Collectively, *Alexandrium* species from Europe (*A. minutum*, Flores-Moya et al., 2012; *A. ostenfeldii*, Kremp et al., 2012), the west coast of North America (*A. catenella*; Fu et al., 2012; Tatters et al., 2013a), and the east coast of North America (*A. catenella*; Hattenrath-Lehman et al., 2015) have displayed strain-specific and mainly acclimatory increases in growth and/or cellular toxin content when exposed to increased dissolved CO₂ concentrations.

While the presence of CCMs has yet to be confirmed among raphidophytes, there is evidence that high CO₂ environments promote the occurrence of HABs formed by this class of algae. For example, in a field experiment the toxic microalga *Vicicitus globosus* had a selective advantage under ocean acidification, increasing its abundance in natural plankton communities at CO₂ levels higher than 600 µatm and developing blooms above 800 µatm CO₂ (Riebesell et al., 2018). Separate studies using two strains of *Heterosigma akashiwo* (CCMP 2393 and CCMP 2809) isolated from Delaware Bay (USA) and Puget Sound (WA; USA), respectively, demonstrated increased growth rates when cultures were provided high CO₂ (750 ppm CO₂; Fu et al., 2008; Kim et al., 2013). In addition to growth, there were effects of pH level on swimming behaviour of *H. akashiwo* as cells provided with high CO₂ displayed downward swimming behaviour more so than cells grown at ambient CO₂ levels (Kim et al., 2013).

Strain-specific differences have been reported with regard to changes in toxin production associated with high CO₂ by *P. multiseriis* with some groups reporting an increase in growth and toxin production at low pH/high dissolved CO₂ (Sun et al., 2011; Tatters et al., 2012a,b, Brunson et al., 2018) and others reporting enhanced toxin production at high pH (low dissolved CO₂; Lundholm et al., 2004; Trimbom et al., 2008). Culture methods, however, varied between the two groups with some groups (Lundholm et al., 2004; Trimbom et al., 2008) adjusting culture pH via direct additions of acid/base and others injecting CO₂ into cultures (Sun et al., 2011; Tatters et al., 2012a,b), suggesting the enhanced toxin production as associated with excess carbon from high CO₂, an outcome consistent with Van de Waal's stoichiometric hypothesis for toxin production (Van de Waal et al., 2011a,b). There are also differences in the response to low pH/high dissolved CO₂ among three strains of the toxic prymnesiophycean *Prymnesium parvum* (Lysgaard et al., 2018). The effect on target organisms of any increased toxin production under ocean acidification would be exacerbated if the increased effect of paralytic shellfish toxins on the fitness of the edible mussel *Mytilus chilensis* (Mellado et al., 2019).

The response of harmful marine macroalgae to elevated dissolved CO₂ has been explored on a limited basis. Koch et al. (2013) performed a meta-analysis of >100 species of marine macroalgae to determine that 85% have C₃ biochemistry and are capable of using HCO₃⁻ and mostly have CCMs. They concluded that most species are not saturated at current ocean DIC and that the photosynthetic and growth rates of marine macro-autotrophs are likely to increase under elevated dissolved CO₂ concentrations (Koch et al., 2013). *Ulva* is well-known for the formation of harmful (nuisance) green tides along eutrophied coastlines of North America, Europe, and China (Valiela et al., 1992; Smetacek and

Zingame, 2013; Zhao et al., 2013; Perrot et al., 2014) and has a CCM (Maberly, 1990). Consistent with the conclusions of Koch et al. (2013), several species of *Ulva* have been shown to experience increased growth rate under elevated CO₂ concentrations (Björk et al., 1993; Olischläger et al., 2013; Young and Gobler, 2016, 2017; Ober and Thornber, 2017; Young et al., 2018; Gao et al., 2018b); however, some other studies showed no increase in growth rate with dissolved CO₂ above the present air-equilibrium concentration (Rautenberger et al., 2015; Gao et al., 2016; Reidenbach et al., 2017; Gao et al., 2018a).

Young and Gobler (2016) specifically examined the effect of increased CO₂, increased P, and increased combined N, and of their interaction, on the growth of the bloom-forming macroalgae *Gracilaria* and *Ulva* on the US east coast. The growth rate of *Gracilaria* was increased by elevated CO₂ but not by elevated combined N or P, while the growth rate of *Ulva* was increased by elevated CO₂, and by elevated combined N or P, and, in two experiments, synergistically increased growth rate with elevated CO₂ combined with elevated combined N and P (Young and Gobler, 2016), a finding consistent with Ober and Thornber's (2017) investigation of *Ulva* from the northeast US. Young and Gobler (2017) extended this work to investigate competition between the two macroalgae and natural phytoplankton. Growth of *Gracilaria* was unaffected by the presence of *Ulva* or phytoplankton at either ambient or elevated CO₂, while growth of *Ulva* was inhibited by the presence of *Gracilaria* or phytoplankton (Young and Gobler, 2017). The conclusion was that, under increased CO₂, *Gracilaria* outcompetes *Ulva*, and dinoflagellates outcompete diatoms under these conditions. Reidenbach et al. (2017) and Gao et al. (2018a,2018b) also investigated the interaction of increased dissolved CO₂ with bioavailable N and P on *Ulva* spp. and found significant biochemical and physiological changes under higher CO₂ conditions.

While it would be desirable to extend adaptation studies to harmful macroalgae, even the fastest-growing of the macroalgae (e.g. *Ulva*) has a generation time of three to five weeks (Wichard et al., 2015), meaning the required 300-1000 generations would take at least 210-700 months (18-58 years). Possible 'natural experiments' of marine macroalgae in enhanced CO₂ occur at different distances from CO₂ vents in the Mediterranean (Hall-Spencer et al., 2008; Porzio et al., 2011; Cornwall et al., 2017). These studies show significant differences in macroalgal distribution as a function of CO₂ concentration, with imperfect correlation of the genotypic or phenotypic absence of CCMs and closeness of the sampling sites to the vent (Hall-Spencer et al., 2008; Porzio et al., 2011; Cornwall et al., 2017). Interpretation of these data in terms of (genotypic) adaptation requires molecular genetic evidence; complications of interpretation include the generally unknown age of the vents, unrestricted genotype loss to, and gain from, water uninfluenced by the vent, and the role of herbivory in the region.

5. Distinguishing effects of CO₂ from pH

pH can affect the rates of photosynthesis and growth of algae directly by altering acid-base balance or via the effects on speciation of DIC (e.g. Maberly, 1990). In most cases, published studies cannot differentiate direct pH effects from changes in levels of DIC and the speciation of DIC (CO₂, HCO₃⁻ and CO₃²⁻). Attempts to differentiate direct pH effects and inorganic carbon limitation on growth rates in HAB species have been limited. Studies of *Ulva* spp. have examined a pH range of 5.6-10.6 and a range of CO₂ concentrations of 2000 - 0.001 mmol m⁻³; photosynthesis occurred at pH 5.6 but was not measured at pH 10.6 (Maberly, 1990; Drechsler and Beer, 1991). The effects of dissolved inorganic carbon (DIC) on the growth of three red-tide dinoflagellates (*Ceratium* (= *Tripes*) *lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum*) were studied at pH 8.0 and at higher pH values, depending upon the pH tolerance of the individual species (Hansen et al., 2007). The higher pH levels chosen for experiments were 8.55 for *C. lineatum* and 9.2 for the other two species, as *C. lineatum* is a more pH sensitive species. At pH 8.0, which approximates the pH found in the open sea, the maximum growth in all

species was maintained until the total DIC concentration was reduced below 0.4 and 0.2 mol m⁻³ for *C. lineatum* and the other two species, respectively. Growth compensation points (concentration of inorganic carbon needed for maintenance of cells) were reached at 0.18 and 0.05 mol DIC m⁻³ for *C. lineatum* and the other two species, respectively. At higher pH levels, maximum growth rates were lower compared to growth at pH 8, even at very high DIC concentrations, indicating a direct pH effect on the growth rate. Moreover, the concentration of bio-available inorganic carbon (CO₂ + HCO₃⁻) required for maintenance of biomass, as well as the half-saturation constants for inorganic carbon, were increased considerably at high pH compared to pH 8.0. Experiments with pH-drift were carried out at initial concentrations of 2.4 and 1.2 mol DIC m⁻³ to test whether pH or DIC was the main limiting factor at a natural range of DIC. Independent of the initial DIC concentrations, growth rates were similar in both incubations until pH had increased considerably, consistent with operation of CCMs. Thus, these results demonstrated that growth rates of these three species were mainly limited by pH, while inorganic carbon limitation played a minor role only at very high pH levels and low DIC concentrations. The extent to which these results can be extrapolated to cover red tide dinoflagellates in general is unknown and clearly more studies are needed on this in the future. It would also be useful to extend studies to the lower pH limit.

Little is known of how external pH affects the physiology of algae, but several possibilities have been suggested. High pH may affect the availability of some macronutrients and micronutrients; the ratio NH₃:NH₄⁺ increases with increasing pH, and limitation by trace metals, and metal toxicity, can increase at high pH (Raven, 1990) and low pH (Hoffmann et al., 2012). A possible explanation for the observed effects of external pH is that increased extracellular pH increases intracellular, cytoplasmic, pH (normally near pH 7.4: Smith and Raven, 1979; Raven and Smith, 1980; Lines and Beardall, 2018) in algae by 0.05 – 0.5 pH units per unit external pH (Smith and Raven, 1979; Raven, 1980; Raven and Smith, 1980; Kallasi and Castenholz, 1982; Raven, 1993; Nimer et al., 1994; Giraldez-Ruiz et al., 1997; Dason and Colman, 2004; Hervé et al., 2012). Intracellular pH regulates many cellular processes including enzyme activity such that changes in intracellular pH could affect cell growth (Smith and Raven, 1979; Raven, 1980, 1993). Such an effect on growth is demonstrated for the marine planktonic diatom *Thalassiosira weissflogii* in Fig. 1A and Fig. 2 of Hervé et al. (2012). In this diatom, the highest growth rate is at an external pH of 7.8, with a uniform increase in internal pH of 0.94 units as external pH is increased by 2.1 units from pH 6.4 to pH 8.5 (Hervé et al. (2012). What is needed to test the hypothesis that it is the internal pH rather than external pH that is related to changes in growth rate is altering internal pH with constant external pH or vice versa and determining the effects on growth rate; such experiments would be technically very difficult. Experiments on *Skeletonema costatum* at increasing external pH, for instance, showed changes in cellular amino acid content that were related to metabolic changes and leakage of organic material (Taraldsvik and Mykkestad, 2000); however, this does not directly address the role of changes in external pH. Studies of two dinoflagellate species showed that external pH changes from 8 to 7 were associated with a lowering of internal pH, which was suggested to be the cause of the observed decrease in cell growth rate (Dason and Colman, 2004; see also Kallasi and Castenholz, 1982 and Giraldez-Ruiz et al., 1997).

Therefore, changes in external pH may affect processes involved in growth that may not be directly associated with photosynthesis. Maintenance of a relatively stable intracellular pH is important for microalgal and macroalgal cells. In spite of changes in external pH, maintenance of a relatively stable internal pH is associated with energy expenditure that relates, in the algae examined, to H⁺/OH⁻ fluxes across the plasmalemma (Smith and Raven, 1979; Raven, 1980; Pucéat, 1999; Gerloff-Elias et al., 2006; Smith et al., 2011; Taylor et al., 2011, 2012; DeCoursey and Hosler, 2015; DeCoursey, 2018). The best investigated algal plasmalemma H⁺ transport mechanism is a voltage gated H⁺ channel that is outward rectifying, i.e. only transports protons when the proton

electrochemical gradient favours passive H⁺ efflux (Smith et al., 2011; Taylor et al., 2011, 2012; DeCoursey and Hosler, 2015; DeCoursey, 2018); one of the algae examined is the toxin-producing dinoflagellate *Karlodinium veneficum* (Smith et al., 2011). Even though there is no direct energization of the proton efflux through the channel from biochemical (e.g. ATP) or biophysical (e.g. coupling to Na⁺ influx) sources, energy is required to produce the transplasmalemma electrochemical potential difference driving passive H⁺ efflux. Such energy expenses may increase at elevated extracellular pH, diverting energy from cell growth. Nevertheless, re-emphasising what was stated earlier, how extracellular pH affects intracellular pH, and how they together affect growth and photosynthesis certainly deserves more attention in the future.

6. Co-stressors

Our use of the term 'stress' is in the context of the definition of Grime (1974) and the discussion by Borowitzka (2018). Anthropogenic atmospheric CO₂ increase is associated globally with increased temperature and hence shoaling of the thermocline. This is globally associated with increased mean photosynthetic photon flux density and UV fluxes, and, especially at lower latitudes, and with decreased nutrient (other than inorganic carbon) availability (Doney et al., 2012; Walworth et al., 2016; Raven et al., 2017; Keys et al., 2018). In addition, increased CO₂ is often associated locally with environmental changes other than the global changes such as heightened nutrient loading (Cai et al., 2011; Wallace et al., 2014). Glibert (2019) Hence, prediction of the response of HABs to changing CO₂ levels must be considered in the light of other, co-occurring changes in the ocean including temperatures, nutrients such as combined nitrogen, phosphorus, iron, and silicon, and photosynthetically available radiation and UVB. These interactions have been explored for some phytoplankton species, but have been rarely considered for HABs. Boyd et al. (2015, 2016, 2018) explored the co-effects of varying levels of light, nutrients, CO₂, temperature, and iron on a strain of *Pseudo-nitzschia multiseries* isolated from the Southern Ocean and found that warming and iron enrichment led to significant growth enhancement but also concluded that future predictions from experimental outcomes can be biased if only a subset of multi-stressors is considered (Boyd et al., 2015, 2016, 2018). Boyd et al. (2015, 2016, 2018) did not report on domoic acid production by this strain, although given the strong effect of high CO₂ (Sun et al., 2011; Tatters et al., 2012a,b; Brunson et al., 2018) it seems likely that trends for toxin production under these stressors could differ from the growth response. In an experimentally induced autumn phytoplankton bloom in the western English Channel, the biomass of the toxin-producing dinoflagellate *Prorocentrum cordatum* significantly increased by combination of elevated CO₂ and increased temperature (Keys et al., 2018). Clearly, the study of multiple stressors on the growth and toxicity of HABs is in its infancy.

7. Future directions

As this review has demonstrated, there are many aspects of the effects of ocean acidification on HABs that are unknown (Table 2), ;1; as are the effect of HABs, as altered by environmental change, as co-stressors on aquatic ecosystems (Griffith and Gobler, 2019). Inorganic carbon concentrations and speciation is highly dynamic in coastal zones, changing horizontally, vertically, seasonally, and diurnally. While there have been many studies that have examined how HABs respond to static levels of high or low pCO₂ virtually nothing is known with regard to the implications of dynamic changes in dissolved CO₂ on HABs. Given the strong temporal and spatial gradients in pH and dissolved CO₂ in estuaries (Fig. 1; Mosley et al., 2010) and the propensity for HABs to vertically migrate and horizontally aggregate, an understanding of the effects of dynamic CO₂ levels on HABs is needed.

While there have been dozens of studies of the effects of differing CO₂ levels on HABs, it is clear than many more investigations are

Table 2

Summary of some properties of HAB algae See text for references.

| Higher Taxonomy | Examples of genera | Occurrence of CCM | Increased photosynthetic and/or growth rate at high CO ₂ | Mixotrophy |
|-----------------------------|-------------------------------|-------------------|---|-----------------|
| Cyanobacteria | <i>Microcystis</i> | Yes | – | – |
| Chlorophyta Ulvophyceae | <i>Ulva</i> | Yes | +/- | – |
| Dinophyta Dinophyceae | <i>Alexandrium</i> | Yes | +/- | +/(phagotrophy) |
| Haptophyta Prymnesiophyceae | <i>Prymnesium</i> | ? | +/- | +(phagotrophy) |
| Ochrista Pelagophyceae | <i>Aureococcus Aureaumbra</i> | ? | ? | +(osmotrophy) |
| Ochrista Raphidophyceae | <i>Chattonella</i> | ? | ? | +(phagotrophy) |

needed. While several strains of HABs experience enhanced growth and/or toxicity under high CO₂, many others do not. “Within genera” studies have found that different species respond differently to high CO₂, e.g. *Alexandrium* spp from Europe (*A. minutum*, from Europe (Flores-Moya et al., 2012) *A. ostenfeldii* from Europe (Kremp et al., 2012), *A. catenella* from the west coast of North America; Fu et al., 2012; Tatters et al., 2013a), and *A. catenella* from the east coast of North America (Hattenrath-Lehman et al., 2015). Similar differences have been found across *Ulva* spp. (Björk et al., 1993; Olischläger et al., 2013; Rautenberger et al., 2015; Gao et al., 2016; Young and Gobler, 2016, 2017; Reidenbach et al., 2017; Thornber et al., 2017; Young et al., 2018; Gao et al., 2018a, b), and *Trichodesmium* species (Hutchins et al., 2013). Even within a given species, different strains may have opposite responses to high and low CO₂ for both prokaryotic HABs (e.g. *Microcystis aeruginosa*; Sandrini et al., 2015a, 2015b, 2016) and eukaryotic HABs (Lundholm et al., 2004; Trimborn et al., 2008; Sun et al., 2011; Tatters et al., 2012a,b; Brunson et al., 2018). Furthermore, almost nothing is known for some classes of algae (e.g. the Pelagophyceae and Raphidophyceae). Hence, additional studies on the effects of differing CO₂ levels on HABs are needed for broader conclusions to be drawn regarding how changing CO₂ levels may influence HABs. Such studies must be carefully designed, accounting for many experimental design factors including the method by which CO₂ is delivered to experimental vessels, the time frame of experiments, the proper characterization of the DIC pool including the careful measurements of at least two of the suite of pH, DIC, dissolved CO₂ concentration, alkalinity (Dickson et al., 2007). Crucially, standardisation among investigators are needed (Dickson et al., 2007). Importantly, ‘-omics’ should be incorporated when appropriate into predictions of the effects of environmental change on HABs (Hennon and Dyhrman, 2019).

Finally, much more effort should be devoted to differentiating direct pH effects from effects of DIC/CO₂ limitation on physiological rates and toxin contents. Given that accurate and precise quantification of these parameters can be a challenge, particularly in high biomass cultures that are reflective of HABs, collaborative efforts between scientists with expertise in experimentally culturing HABs and the chemistry of inorganic carbon may be the most fruitful in generating high quality data sets.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

The University of Dundee is a registered Scottish charity, No 015096. CJG was supported by New York Sea Grant R-FMB-38 and grants from the Chicago Community Foundation, the Laurie Landaeu Foundation, and the Pritchard Foundation. PJH was supported by a grant from the Danish Research Council for Independent Research, grant no 4181-00484.[CG]

References

- Adamczyk, K., Prémont-Schwarz, M., Pines, D., Pines, E., Nibbering, E.J., 2009. Real-time observation of carbonic acid formation in aqueous solution. *Science* 326, 1690–1694.
- Alin, S.R., Johnson, T.C., 2007. Carbon cycling in large lakes of the world: a synthesis of production, burial and lake-atmospheric exchange estimates. *Global Biogeochem. Cycles* 21, GB3002.
- Amory, A.M., Vanleberghe, G.C., Turpin, D.H., 1991. Demonstration of both a photosynthetic and a non-photosynthetic CO₂ requirement for NH₄⁺ assimilation in the green alga *Selenastrum minutum*. *Plant Physiol.* 95, 192–196.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 704–726.
- Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2012. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring and management. *Annu. Rev. Mar. Sci.* 4, 143–176.
- Anderson, C.R., Moore, S.K., Tomlinson, H.C., Silke, J., Cusack, C.K., 2015. Living with harmful algal blooms in a changing world: strategies for modelling and mitigating their effects in coastal marine ecosystems. In: Shroder, J.F., Ed, Series, Ellis, J.T., Sherman, D.J. (Eds.), *Coastal and Marine Hazards, Risks and Disasters*, Volume. Elsevier, Amsterdam, pp. 495–561.
- Badger, M.R., Andrews, T.J., Whitney, S.M., Ludwig, M., Yellowlees, D.C., Leggat, W., Price, G.D., 1998. The diversity and coevolution of Rubisco, plastids, pyrenoids and chloroplast-based CO₂-concentrating mechanisms in algae. *Can. J. Bot.* 76, 1052–1071.
- Bathellier, C., Tcherkez, G., Lorimer, G.H., Farquhar, G.D., 2018. Rubisco is not really so bad. *Plant Cell Environ.* 4, 705–716.
- Baumann, H., Wallace, R.B., Tagliaferri, T., Gobler, C.J., 2015. Large natural pH, CO₂ and O₂ fluctuations in a temperate salt marsh on diel, seasonal and interannual time scales. *Estuaries Coasts* 38, 220–231.
- Beardall, J., Raven, J.A., 2017. Cyanobacteria vs green algae: which group has the edge. *J. Exp. Bot.* 68, 3697–3699.
- Bercel, T.L., Kranz, S.A., 2019. Insights into carbon acquisition and photosynthesis in *Karenia brevis* under a range of CO₂ concentrations. *Progr. Oceanogr.* 172, 65–76.
- Berge, T., Daughbjerg, N., Hansen, P.J., 2012. Isolation and cultivation of microalgae selected for low growth rate and tolerance of high pH. *Harmful Algae* 20, 101–110.
- Björk, M., Ramazanov, Z., Pedersen, M., 1993. Inducible Mechanisms for HCO₃⁻ utilization and repression of photorespiration in protoplasts and thalli of three species of *Ulva* (Chlorophyta). *J. Phycol.* 29, 166–173.
- Borowitzka, M.A., 2018. The ‘stress’ concept in microalgal biology – homeostasis, acclimation and adaptation. *J. Appl. Phycol.* 30, 2815–2825.
- Boyd, P.W., Lennatz, S.T., Glover, D.M., Doney, S.C., 2015. Biological ramifications of climate-change-mediated oceanic multi-stressors. *Nat. Clim. Chang.* 5, 71–79.
- Boyd, P.W., Dillingham, P.W., McGraw, C.M., Armstrong, E.A., Cornwall, C.E., Feng, Y.-y., Hurd, C.L., Gault-Ringold, M., Roleda, H.Y., Timmins-Schiffman, E., Nunn, B.L., 2016. Physiological responses of a Southern Ocean diatom to complex future ocean conditions. *Nat. Clim. Chang.* 6, 207–2.
- Boyd, P., Collins, S., Dupont, S., Fabricius, K., Gattuso, J.P., Havenhand, J., Hutchins, D. A., Riebesell, U., Rintoul, M., Vichi, M., Biswas, H., Ciottis, A., Gao, K., Gehlen, M., Hurd, C.L., Kurihawa, H., McGraw, C.M., Navarro, J., Nilsson, G.E., Passow, U., Portner, H.O., 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change – a review. *Glob. Change Biol. Bioenergy* 24, 2239–2261.
- Brewer, P.G., Goldman, J.C., 1976. Alkalinity changes generated by phytoplankton growth. *Limnol. Oceanogr.* 21, 108–117.
- Brunson, J.K., McKinnie, S.M.K., Chekan, J.R., McCrow, J.P.M., Miles, Z.D., Bertrand, E. M., Bielinski, V.A., Luhavaya, H., Obornik, M., Smith, G.J., Hutchins, D.A., Allen, A. E., Moore, B.S., 2018. Biosynthesis of the neurotoxin domoic acid in a bloom-forming diatom. *Science* 361, 1356–1358.
- Brutemark, A., Granéli, E., 2011. Role of mixotrophy and light for growth and survival of the toxic haptophyte *Prymnesium parvum*. *Harmful Algae* 10, 388–394.
- Bucher, G., Sander, W., 2014. Clarifying the structure of carbonic acid. *Science* 346, 503–545.
- Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8, 77–93.

- Caffery, J.M., 2004. Factors affecting net ecosystem metabolism in US estuaries. *Estuaries* 27, 90–101.
- Cai, W.-J., 2011. Estuarine and coastal ocean carbon paradox: CO₂ sinks or sites of terrestrial carbon incineration. *Annu. Rev. Mar. Sci.* 3, 123–145.
- Cai, W.-J., Guo, X., Chen, C.-T.A., Dai, M., Zhang, L., Zhai, W., Lohrenz, S.E., Yin, K., Harrison, P.J., Wang, Y., 2008. A comparative overview of weathering intensity and HCO₃⁻ flux in the world's major rivers with emphasis on the Changjiang, Huanghe, Zhujiang (Pearl) and Mississippi rivers. *Cont. Shelf Res.* 28, 1538–1549.
- Cai, W.-J., Hu, X., Huang, W.J., Murrell, M.C., Lehrter, J.C., Lohrenz, S.E., Chon, W.C., Zhai, W., Hollibaugh, J.T., Wang, Y., Zhao, P., Gup, X., Gundersen, K., Dai, M., Gong, G.-C., 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nat. Geosci.* 4 article 766.
- Cai, W.-J., Huang, W.-J., Luther, G.W., Pierrot, D., Li, M., Testa, J., Xue, M., Joesoef, A., Mann, R., Brodeur, J., Xu, Y.-Y., Chen, B., Hussain, N., Waldbusser, G.G., Cornwell, J., Kemp, W.M., 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nat. Commun.* 8 article 369.
- Calabetti, C., Citterio, S., Delaria, M.A., Gertilli, R., Montagnani, C., Navon, A., Caronni, S., 2017. First record of two potentially toxic dinoflagellates in tide pools along the Sardinian coast. *Biodiversity* 18, 2–7.
- Carvalho, W.F., Granéli, E., 2010. Contribution of phagotrophy versus autotrophy to *Prymnesium parvum* growth under nitrogen and phosphorus sufficiency and deficiency. *Harmful Algae* 9, 105–115.
- Carvalho, W.F., Minnhagen, S., Granéli, E., 2008. *Dinophysis norvegica* (Dinophyceae), more a predator than a producer? *Harmful Algae* 7, 174–283.
- Clark, D.R., Flynn, K.J., 2000. The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton. *Proc. R. Soc. B* 267, 953–950.
- Codd, G.A., Morton, H., Baker, P.D., 2015. George Francis: a pioneer in the investigation of the quality of South Australia's drinking water source (1978–1883). *Trans. R. Soc. S. Aust.* 139, 164–170.
- Collins, S.L., Bell, G., 2004. Phenotypic consequences of 1,000 generations of selection at elevated CO₂ in a green alga. *Nature* 431, 736–739.
- Cornwall, C.E., Revill, A.T., Hall-Spencer, J.M., Milazzo, M., Raven, J.A., Hurd, C.L., 2017. Inorganic carbon physiology underpins macroalgal responses to elevated CO₂. *Sci. Rep.* 7, 46297.
- Cox, P.A., Banack, S.A., Murch, S.J., Rasmussen, U., Rier, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., Bergman, B., 2005. Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. *Proc. Nat. Acad. Sci. U. S. A.* 102, 5074–5078.
- Dai, R., Liu, H., Qu, J., Zhao, X., Hou, Y., 2009. Effects of amino acids on microcystin production of the *Microcystis aeruginosa*. *J. Hazard. Mater.* 161 (2–3), 730–736.
- Dason, J.S., Colman, B., 2004. Inhibition of growth in two dinoflagellates by rapid changes in external pH. *Can. J. Bot.* 82, 515–520.
- De Paula Silva, P.H., Paul, N.A., de Nys, R., Mata, L., 2013. Enhanced production of green tide algal biomass through additional carbon supply. *PLoS One* 8, e81164.
- DeCoursey, T.E., 2018. Voltage and pH sensing by the H⁺ proton-gated channel, Hv1. *J. R. Soc. Interface* 11, 20130799.
- DeCoursey, T.E., Hosler, J., 2015. Philosophy of voltage gated proton channels. *J. R. Soc. Interface* 15, 20180108.
- Del Giorgio, P.A., Williams, P.J.Le B. (Eds.), 2005. *Respiration in Aquatic Ecosystems*. Oxford University Press, Oxford, UK, p. 328.
- Dickson, A.G., Sabine, C.L., Christian, J.R. (Eds.), 2007. *Guide to Best Practice for Ocean CO₂ Measurements*. PICES Special Publication, 3. North Pacific Marine Science Organisation, Sidney, Canada, 191pp.
- Doblin, M.S., Thompson, P.A., Revill, A.T., Butler, E.C.V., Blackburn, S.I., Hallegraef, C. M., 2006. Vertical migration of the toxic dinoflagellate *Cymonodinium catenatum* under different concentrations of nutrients and humic substances in culture. *Harmful Algae* 5, 665–677.
- Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO₂ problem. *Annu. Rev. Mar. Sci.* 1, 169–192.
- Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, F., English, C.V.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., 2012. Climate change impacts on marine ecosystems. *Annu. Rev. Mar. Sci.* 4, 11–37.
- Drechsler, Z., Beer, S., 1991. Utilization of inorganic carbon by *Ulva lactuca*. *Plant Physiol.* 97, 1439–1444.
- Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J.A., McCulloch, M., 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coasts* 36, 221–236.
- Eberlein, T., Van de Waal, D.B., Rost, B., 2014. Differential effects of ocean acidification in two bloom-forming dinoflagellate species. *Physiol. Plant.* 151, 468–479.
- Eberlein, T., Van de Waal, D.B., Brandenburg, K.M., John, U., Voss, M., Achterberg, E.P., Rost, B., 2016. Interactive effects of ocean acidification and nitrogen limitation on two bloom-forming dinoflagellate species. *Mar. Ecol. Progr. Ser.* 543, 127–140.
- Erera, R.M., Yvon-Lewis, S., Kessler, J.D., Campbell, L., 2014. Responses of the dinoflagellate *Karenia brevis* to climate change: pCO₂ and sea surface temperature. *Harmful Algae* 37, 110–116.
- Falkowski, P.G., Raven, J.A., 2007. *Aquatic Photosynthesis*, second edition. Princeton University Press, Princeton, NJ, USA.
- Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Jenson, D., Hales, B., 2008. Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science* 320, 1490–1492.
- Feely, R.A., Alin, S.R., Newton, J., Sabine, C.L., Warner, M., Devol, A., Krembs, C., Maloy, C., 2010. The combined effects of ocean acidification, mixing and respiration on pH and carbonate saturation in an urbanised estuary. *Estuar. Coast. Shelf Sci.* 88, 442–449.
- Flores-Moya, A., Rouco, M., Garcia-Sánchez, M.J., Garcia-Balboa, C., González, R., Costas, E., López-Rodas, V., 2012. Effects of adaptation, chance, and history on the evolution of the toxic dinoflagellate *Alexandrium minutum* under selection of increased temperature and acidification. *Ecol. Evol.* 2, 1251–1259.
- Flynn, K., Clark, D., Mitra, A., Heiner, F., Hansen, P.J., Glibert, P.M., Wheeler, G., Stoecker, D., Blackford, J., Brownlee, C., 2015. Ocean acidification with (de) eutrophication alters phytoplankton growth and succession. *Proc. R. Soc. B* 282, 20142604.
- Flynn, K.J., Mitra, A., Glibert, P.M., Burkholder, J.M., 2018. Mixotrophy in harmful algal blooms: by whom, on whom, when, why and what next. In: Glibert, P., Berdalet, E., Burford, M., Pitcher, G., Zhou, M. (Eds.), *Global Ecology and Oceanography of Harmful Algal Blooms*, Vol. 232. Global Ecology and Oceanography of Harmful Algal Blooms. Ecological Studies (Analysis and Synthesis). Springer, Cham, pp. 113–132.
- Fu, F.-X., Zhang, T., Warner, M.E., Feng, Y., Sun, J., Hutchins, D.A., 2008. A comparison of future increased CO₂ and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. *Harmful Algae* 7, 76–90.
- Fu, F.-X., Place, A.R., Garcia, N.S., Hutchins, D.A., 2010. CO₂ and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium verficium*. *Aquat. Microb. Ecol.* 59, 55–65.
- Fu, F.-X., Tatters, A.O., Hutchins, D.A., 2012. Global change and the future of harmful algal blooms in the ocean. *Mar. Ecol. Progr. Ser.* 470, 207–233.
- Gao, K., Campbell, D.A., 2014. Photophysiological responses of marine diatoms to elevated CO₂ and decreased pH: a review. *Funct. Plant Biol.* 41, 449–459.
- Gao, G., Liu, Y., Li, X., Feng, Z., Xu, J., 2016. An ocean acidification acclimated green tide alga is robust to changes in seawater chemistry and vulnerable to light stress. *PLoS One* 11, e0169040.
- Gao, G., Beardall, J., Bao, M., Wang, C., Ren, W., Xu, J., 2018a. Ocean acidification and nutrient limitation synergistically reduce growth and photosynthetic performance of a green tide alga *Ulva linza*. *Biogeosciences* 15, 3409–3420.
- Gao, G., Clare, A.S., Rose, C., Caldwell, G.S., 2018b. *Ulva rigida* in the future ocean: potential for carbon capture, bioremediation and biomethane production. *Gcb Bioenergy* 10, 39–51.
- Gerloff-Elias, A., Barua, D., Mólích, A., Spijkerman, E., 2006. Temperature- and pH-dependent accumulation of heat-shock proteins in the acidophilic green alga *Chlamydomonas acidophila*. *FEMS Microb. Ecol.* 56, 345–354.
- Giordano, M., Beardall, J., Raven, J.A., 2005. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annu. Rev. Plant Biol.* 68, 3701–3716.
- Giraldez-Ruiz, N., Mateo, P., Bonilla, I., Fernandez-Piñas, F., 1997. The relationship between intracellular pH, growth characteristics and calcium in the cyanobacterium *Anabaena* sp. Strain PCC7120 exposed to low pH. *New Phytol.* 137, 599–605.
- Gledhill, D.K., White, M.M., Salisbury, J., Thomas, H., Mlsna, I., Liebman, M., Mook, B., Grear, J., Candelmo, A.C., Chambers, R.C., Gobler, C.J., 2015. Ocean and coastal acidification off New England and Nova Scotia. *Oceanography* 28, 182–197.
- Glibert, P.M., 2017. Eutrophication, harmful algae and biodiversity – challenging paradigms in a world of complex nutritional challenges. *Mar. Pollut. Bull.* 124, 591–606.
- Glibert, P.M., Burford, M.A., 2017. Globally changing nutrient loads and harmful algal blooms: recent advances, new paradigms, and continuing challenges. *Oceanography* 30, 58–69.
- Gobler, C.J., Sunda, W.G., 2012. Ecosystem disruptive algal blooms of the brown tide species, *Aureococcus anophagefferens* and *Aureocoumbra lagunensis*. *Harmful Algae* 14, 36–45.
- Gobler, C.J., Lonsdale, D.J., Boyer, G.L., 2005. A review of the causes, effects and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargraves et Sieburth). *Estuaries* 28, 726–749.
- Gobler, C.J., Berry, D.L., Dyrman, S.T., Wilhelm, S.W., Salomov, A., Lobanov, A.V., Xiang, Y., Collier, J.L., Wurch, L.L., Kustka, A.B., Dill, B.D., Dhah, M., VerBerkmoes, N.C., Kuo, A., Terry, A., Pangilian, J., Lindquist, E.A., Lucas, S., Paulsen, I.T., Hattenrath-Lehman, T.K., Talmage, S.C., Walker, E.A., Kochain, F., Burson, A.M., Marcoval, M.A., Tang, Y.-Z., ClCclair, G.R., Coyne, K.J., Berg, G.M., Bertrand, E.M., Saito, M.A., Gladyshev, V.N., Grigoriev, I.V., 2011. Niche of harmful *Aureococcus anophagefferens* revealed through ecogenomics. *Proc. Nat. Acad. Sci. U. S. A.* 108, 4352–4357.
- Gobler, C.J., Doherty, O.M., Hattenrath-Lehman, T.K., Griffith, A.W., Kang, Y., Litaker, R.W., 2017. Ocean warming since 1982 has expanded the niche of harmful algal blooms in the North Atlantic and North Pacific. *Proc. Nat. Acad. Sci. U. S. A.* 114, 4975–4980.
- Green, M.A., Allen, R.C., 1998. Seasonal patterns of carbonate diagenesis in nearshore terrigenous muds relation to spring phytoplankton bloom and temperature. *J. Mar. Res.* 56, 1097–1123.
- Griffiths, H., Meyer, M.T., Rickaby, R.E.M., 2017. Overcoming adversity through diversity: aquatic carbon concentrating mechanisms. *J. Exp. Bot.* 68, 3689–3695.
- Griffith, A.W., Gobler, C.J., 2019. Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae*, 91, 101590.
- Grime, J.P., 1974. Vegetation classification by reference to strategies. *Nature* 250, 26–31.
- Guanyong, O., Hong, W., Ranran, S., Wanchun, G., 2017. The dinoflagellate *Akashiwo sanguinea* will benefit from future climate change: The interactive effects of ocean acidification, warming and high irradiance on photophysiology and haemolytic activity. *Harmful Algae* 68, 118–127.
- Hallegraef, G., 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235.

- Hall-Spencer, J.M., Allen, R., 2015. The impact of CO₂ emissions on 'nuisance' marine species. *Res. Rep. Biodivers. Stud.* 4, 33–46.
- Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.-C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454, 96–99.
- Hansen, P.J., 2002. Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquat. Microb. Ecol.* 28, 279–288.
- Hansen, P.J., Lundholm, N., Rost, B., 2007. Growth limitation in marine red-tide dinoflagellates: effects of pH versus inorganic carbon availability. *Mar. Ecol. Progr. Ser.* 334, 63–71.
- Hattenrath-Lehman, T.K., Smith, J.L., Wallace, R.B., Merlo, L.R., Koch, F., Mittelsdorf, H., Goleski, J.A., Anderson, D.M., Gobler, C.J., 2015. The effects of elevated CO₂ on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*. *Limnol. Oceanogr.* 60, 198–214.
- Hayden, H.S., Blomster, L., Maggs, C.A., Silva, P.C., Stanhope, M.J., Waaland, J.R., 2002. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* 39, 277–294.
- Heil, C.A., Revilla, M., Glibert, P.M., Murasko, S., 2007. Nutrient quality driven differential phytoplankton community composition on the southwest Florida shelf. *Limnol. Oceanogr.* 53, 1067–1068.
- Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C.J., Hell, C.A., Humphries, E., Lewitus, A., Magnier, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13.
- Hennon, G.M.M., Dyhrman, S.T., 2019. Progress and promise of -omics for predicting the impacts of climate change on harmful algal blooms. *Harmful Algae*, 91, 101587.
- Hervé, V., Derr, J., Douady, S., Quinet, M., Moisan, L., Lopez, P.J., 2012. Multiparametric analyses reveal the pH-dependence of silicon biomineralization in diatoms. *PLoS One* 7, e46722.
- Hinga, K.R., 2002. Effects of pH on coastal marine phytoplankton. *Mar. Ecol. Progr. Ser.* 238, 281–300.
- Hoffmann, L.J., Breitbarth, E., Boyd, P.W., Hunter, K.A., 2012. Influence of ocean warming and acidification on trace metal biogeochemistry. *Mar. Ecol. Progr. Ser.* 470, 191–205.
- Hoins, M., Van de Waal, D.B., Eberlein, T., Rost, B., Sluijs, A., 2015. Stable carbon isotope proxy. *Geochim. Cosmochim. Acta* 160, 267–276.
- Hoins, M., Eberlein, T., Großmann, C.H., Brandenberg, K., Reichart, G.-J., Rost, B., Sluijs, A., Van de Waal, D.B., 2016a. Combined effect of ocean acidification and light or nitrogen availability on ¹³C fractionation in marine dinoflagellates. *PLoS One* 11, e0154370.
- Hoins, M., Eberlein, T., Van de Waal, D.B., Slijs, A., Reichart, G.-J., Rost, B., 2016b. CO₂-dependent carbon isotope fractionation in dinoflagellates relates to their inorganic carbon fluxes. *J. Exp. Mar. Biol. Ecol.* 481, 9–14.
- Huisma, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. *Nat. Rev. Microbiol.* 16, 471–483.
- Hutchins, D.A., Fu, F.-X., Webb, E.A., Walworth, N., Tagliabue, A., 2013. Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. *Nature Geosci.* 6, 790–795.
- Hutchins, D.A., Walworth, N.G., Webb, E.A., Saito, M.A., McIlvinn, M.R., Gale, J., Fu, F.-X., 2015. Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide. *Nature Comm* 6, 8155.
- IPCC, 2007. Climate change 2007: the physical science basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- IPCC, 2013. Climate change 2013: the physical basis. Contributions of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Jähnichen, S., Ihle, T., Petzoldt, T., Benndorf, J., 2007. Impact of the inorganic carbon availability on microcystin production by *Microcystis aeruginosa* PCC 7806. *Appl. Environ. Microbiol.* 73, 6994–7002.
- Jakubowska, N., Szelag-Wasilewska, E., 2015. Toxic picoplanktonic cyanobacteria – a review. *Mar. Drugs* 13, 1497–1518.
- Jarvie, H.P., King, S.M., Neal, C., 2017. Inorganic carbon dominates total dissolved carbon concentrations in British rivers: application of the THINCARB model – Thermodynamic modelling of inorganic carbon in freshwaters. *Sci. Total Environ.* 575, 496–512.
- Jeong, H.J., 2011. Mixotrophy in red tide algae Raphidophytes. *J. Euk. Microbiol.* 58, 215–222.
- Ji, X., Verspagen, J.M.H., Stomp, M., Huisman, J., 2017. Competition between cyanobacteria and green algae at low versus elevated CO₂: who will win, and why? *J. Exp. Bot.* 68, 3815–3828.
- Ji, N., Lin, L., Li, L., Yu, L., Zhang, Y., Luo, H., Li, M., Shi, X., Wang, D.-Z., Lin, S., 2018. Metatranscriptome analysis reveals environmental and diel regulation of a *Heterosigma akashiwo* (Raphidophyceae) bloom. *Environ. Microbiol. Rep.* 20, 1078–1094.
- Kallasi, T., Castenholz, R.W., 1982. Internal pH and ATP-ADP pools in the cyanobacterium *Synechococcus* sp. during exposure to growth-inhibiting low pH. *J. Bact.* 149, 229–236.
- Kevekordes, K., Holland, D., Häubner, N., Jenkins, S., Koss, R., Roberts, S., Raven, J.A., Scrimgeour, C.M., Shelly, K., Stojkovic, S., Beardall, J., 2006. Inorganic carbon acquisition by eight species of *Caulerpa* (Caulerpaceae, Chlorophyta). *Phycologia* 45, 442–449.
- Keys, M., Tilstone, G., Findlay, H.S., Widdicombe, C.E., Lawson, T., 2018. Effects of elevated CO₂ and temperature on the phytoplankton community biomass, species composition and photosynthesis during an experimentally induced autumn bloom in the western English Channel. *Biogeosciences* 15, 3203–3222.
- Khan, S., Arakawa, O., Onoue, Y., 1987. Neurotoxins in a red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. *J. Aquac. Res. Dev.* 28, 9–14.
- Kim, H., Spivack, A.J., Menden-Deuer, S., 2013. pH alters the swimming behaviors on the raphidophyta *Heterosigma akashiwo*: implications for bloom formation in an acidified ocean. *Harmful Algae* 26, 1–11.
- Koch, M., Boves, G., Ross, C., Zhang, X.-H., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob. Change Biol. Bioenergy* 19, 103–132.
- Kremp, A., Godhe, A., Egarat, J., Dupont, S., Suikkanen, S., Casabianca, S., Penna, A., 2012. Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. *Ecol. Evol.* 2, 1185–1207.
- Kronholm, I., Bassett, A., Baulcombe, D., Collins, S., 2017. Epigenetic and genetic contributions to adaptation in *Chlamydomonas*. *Mol. Biol. Evol.* 34, 2285–2306.
- Kübler, J.E., Dudgeon, S.R., 2015. Predicting effects of ocean acidification and warming on algae lacking carbon concentrating mechanisms. *PLoS One* 10, e0132806.
- Lapointe, M., MacKenzie, T.D.B., Morse, D., 2008. The external δ-carbonic anhydrase in a free-living marine dinoflagellate may circumvent diffusion-limited carbon acquisition. *Plant Physiol.* 147, 1427–1436.
- Lim, A.S., Jeong, H.J., Ok, J.H., Kim, S.J., 2018. Feeding by harmful phototrophic dinoflagellate *Takayama tasmanica* (Family Kareniaceae). *Harmful Algae* 74, 19–29.
- Lines, T., Beardall, J., 2018. Carbon acquisition characteristics of six microalgal species isolated from a subtropical reservoir: potential implications for species succession. *J. Phycol.* 54, 599–607.
- Liu, J., Van Ossterhout, E., Faasen, E.J., Lüring, M., Helmingsen, N.R., Van de Waal, D. B., 2016. Elevated pCO₂ causes a shift towards microcystin variants in nitrogen-limited *Microcystis aeruginosa*. *FEMS Microbiol. Ecol.* 92, fiv159.
- Lomas, M.W., Glibert, P.M., Clougherty, D.A., Huber, D.R., Jones, J., Alexander, J., Haramoto, E., 2001. Elevated organic nutrient ratios associated with brown tide blooms of *Aureococcus anophagefferens* (Pelagophyceae). *J. Plankton Res.* 23, 1339–1344.
- López-Sandorval, D.C., Rodríguez-Ramos, T., Cermeño, P., Marañón, E., 2013. Exudation of organic carbon by marine phytoplankton: dependence on axon and size. *Mar. Ecol. Progr. Ser.* 477, 53–60.
- Low-Décarie, E., Jewell, M.D., Fussmann, G.F., Bell, G., 2013. Low-term culture at elevated atmospheric fails to evoke specific adaptation in seven freshwater phytoplankton species. *Proc. R. Soc. B* 280, 20122598.
- Lundholm, N., Hansen, P.J., Kotaki, Y., 2004. Effect of pH on growth and domoic acid production by potentially toxic diatoms of the genera *Pseudo-nitzschia* and *Nitzschia*. *Mar. Ecol. Progr. Ser.* 275, 1–5.
- Lysgaard, M.L., Eckford-Soper, L., Daugbjerg, N., 2018. Growth rates of three geographically separated strains of the ichthyotoxic *Prymnesium parvum* (Prymnesiophyceae) in response to six different pH levels. *Estuarine Coast. Shelf Sci.* 204, 98–102.
- Maberly, S.C., 1990. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *J. Phycol.* 26, 439–449.
- Maberly, S.C., 1992. Carbonate ions appear to neither inhibit nor stimulate use of bicarbonate by *Ulva lactuca*. *Plant Cell Environ.* 15, 255–260.
- Maberly, S.C., 1996. Diel, episodic and seasonal changes in pH and concentration of inorganic carbon in a productive lake. *Freshw. Res.* 35, 579–598.
- Macedo, M.F., Duarte, P., Mendes, P., Ferreira, J.G., 2001. Annual variation of environmental variables, phytoplankton species composition and photosynthetic parameters in a coastal lagoon. *J. Plankton Res.* 23, 719–732.
- MacIntyre, H.L., Stutes, A.L., Smith, W.L., Dorsey, C.P., Abraham, A., Dickey, R.W., 2011. Environmental correlates of community composition and toxicity during a bloom of *Pseudo-nitzschia* spp. in the northern Gulf of Mexico. *J. Plankton Res.* 33, 273–295.
- Manning, S.R., La Claire II, J.W., 2010. Prymnesins: toxic metabolites of the Golden Alga, *Prymnesium parvum* Carter (Haptophyta). *Mar. Drugs* 8, 678–704.
- Marchal, O., Monfray, P., Bates, N.R., 1996. Spring-summer imbalance of dissolved inorganic carbon in the mixed layer of the north-western Sargasso Sea. *Tellus B Chem. Phys. Meteorol.* 48, 115–134.
- Mardones, J.I., Müller, M.N., Hallegraeff, G.M., 2017. Toxic dinoflagellate blooms of *Alexandrium catenella* in Chilean fjords: a resilient winner from climate change. *ICES J. Mar. Sci.* 74, 988–995.
- Marshall, J.A., Hallegraeff, G.M., 1999. Comparative ecophysiology of the harmful alga *Chattonella marina* (Raphidophyceae) from South Australia and Japanese waters. *J. Plankton Res.* 21, 1809–1822.
- Medlin, L.K., Cembella, A.D., 2013. Biodiversity of harmful marine algae. In: Levin, S.A. (Ed.), *Encyclopedia of Biodiversity*, second edition, Volume 1. Academic Press, Waltham, MA, USA, pp. 470–484.
- Meire, L., Sogaard, D.H., Meusman, F.J.R., Soetaert, K., Arendt, K.E., Juul-Pedersen, T., Blicher, M.E., Rysgaard, S., 2015. Glacial meltwater and primary production are drivers of strong CO₂ uptake in fjord and coastal waters adjacent to the Greenland Ice-Sheet. *Biogeosciences* 12, 2347–2363.
- Mellado, C., Chaparro, O.R., Duarte, C., Villanueva, P.A., Ortiz, A., Valdivia, N., Torres, R., Navarro, J.M., 2019. Ocean acidification exacerbates the effects of paralytic shellfish poisoning on the fitness of the edible mussel *Mytilus edulis*. *Sci. Total Environ.* 653, 455–464.
- Melzner, F., Thomson, J., Koeve, W., Oeschlies, A., Gutowska, M.A., Bange, H.W., Hansen, H.P., Körtzner, A., 2013. Future ocean acidification will be amplified by anoxia in coastal habitats. *Mar. Biol.* 160, 1875–1888.
- Middelboe, A.L., Hansen, P.J., 2007. High pH in shallow-water macroalgal habitats. *Mar. Ecol. Progr. Ser.* 338, 107–117.

- Newman, S.M., Derocher, J., Cattolico, R.A., 1989. Analysis and chromophyte and rhodophytic ribulose-1,5-bisphosphate carboxylase indicates extensive structural and functional similarities among evolutionary diverse algae. *Plant Physiol.* 91, 939–946.
- Nimer, N.A., Brownlee, C., Merrett, M.J., 1994. Carbon dioxide availability, intracellular pH and growth of the coccolithophore *Emiliania huxleyi*. *Mar. Ecol. Prog. Ser.* 109, 257–262.
- Nixon, S.W., Oczkowski, A.J., Pilson, M.E.Q., Fields, L., Oviatt, C.A., Hunt, S.W., 2015. On the response of pH to inorganic nutrient enrichment in well-mixed coastal marine waters. *Estuaries Coasts* 38, 232–241.
- O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate changes. *Harmful Algae* 14, 313–334.
- Ober, G.T., Thornber, C.S., 2017. Divergent responses in growth and nutritional quality of coastal macroalgae to the combination of increased pCO₂ and nutrients. *Mar. Environ. Res.* 131, 69–79.
- Olischläger, M., Bartsch, I., Gutow, L., Wiencke, C., 2013. Effects of ocean acidification on growth and physiology of *Ulva lactuca* (Chlorophyta) in a rockpool-scenario. *Phycol. Res.* 61, 180–190.
- Pael, H.W., Joyner, J.J., Joyner, A.R., Arthur, K., Paul, V., O'Neil, J.M., Heil, C.A., 2008. Co-occurrence of dinoflagellate and cyanobacterial harmful algal blooms in southwest Florida coastal waters: dual nutrient (N and P) input controls. *Mar. Ecol. Progr. Ser.* 371, 143–153.
- Perga, M.-E., Maberly, S.C., Jenny, J.-P., Alric, B., Pignol, C., Neffrechoux, E., 2016. A century of climate-driven changes in the carbon dioxide concentration of lakes. *Global Biogeochem. Cycles* 30, 93–104.
- Perrot, T., Rossi, N., Ménesguen, A., Dumas, F., 2014. Modelling green macroalgal blooms on the coasts of Brittany, France to enhance water quality management. *J. Mar. Syst.* 132, 38–53.
- Pines, D., Dikovich, J.T., Mukra, Miller, Y., Kiefer, P.M., Daschakraborty, S., Hynes, J.T., Pines, E., 2016. How acidic is carbonic acid? *J. Phys. Chem. B* 120, 2440–2451.
- Pitcher, G.C., Weeks, S.J., 2006. The variability and potential for prediction of harmful algal blooms in the southern Benguela ecosystem. *Large Mar. Ecosyst.* 14, 125–146.
- Pitcher, G.C., Figueras, F.G., Kudela, P.M., Moitas, T., Reghera, B., Ruiz-Villareal, M., 2018. Key questions and recent advances on harmful algal blooms in Eastern Boundary upwelling systems. In: Glibert, P.E., Berdalet, E., Pitcher, G., Zhou, M. (Eds.), *Global Ecology and Oceanography of Harmful Algal Blooms*, Vol. 232. *Ecological Studies (Studies and Analysis)*, Springer, Cham, pp. 205–227.
- Poole, L.J., Raven, J.A., 1997. The biology of *Enteromorpha*. *Progr. Phycol. Res.* 12, 1–147.
- Porzio, L., Buia, M.C., Hall-Spencer, J.M., 2011. Effects of ocean acidification on macroalgal communities. *J. Exp. Mar. Biol. Ecol.* 400, 278–287.
- Pucéat, M., 1999. pH regulatory ion transporters: an update on structure, regulation and cell function. *Cell. Mol. Life Sci.* 55, 1216–1229.
- Raffaelli, D., Raven, J.A., Poole, L.J., 1998. Ecological impact of mass blooms of benthic algae. *Mar. Biol. Oceanogr. Annu. Rev.* 36, 97–125.
- Ratti, S., Giordano, M., Morse, D., 2007. CO₂-concentrating mechanisms of the potentially toxic dinoflagellate *Protoceratium reticulatum* (Dinophyceae, Gonyaulacales). *J. Phycol.* 43, 693–701.
- Rautenberger, R., Fernández, P.A., Strittmatter, M., Heesch, S., Cornwall, C.E., Hurd, C.L., Roleda, M.Y., 2015. Saturating light and not increased carbon dioxide under ocean acidification drive photosynthesis and growth of *Ulva rigida* (Chlorophyta). *Ecol. Evol.* 5, 864–885.
- Raven, J.A., 1980. Nutrient transport in microalgae. *Adv. Microb. Physiol.* 21, 47–226.
- Raven, J.A., 1990. Sensing pH? *Plant Cell Environ.* 13, 721–729.
- Raven, J.A., 1993. Limits on growth rate. *Nature* 361, 209–210.
- Raven, J.A., Giordano, M., 2017. Acquisition and metabolism of carbon in the Ochrophyta other than diatoms. *Phil. Trans. Roy. Soc. Lond. B* 372, 20160400.
- Raven, J.A., Smith, F.A., 1980. Intracellular pH regulation in the giant-celled marine alga *Chaetomorpha darwinii*. *J. Exp. Bot.* 31, 1357–1369.
- Raven, J.A., Taylor, R., 2003. Macroalgal growth in nutrient-enriched estuaries: a biogeochemical perspective. *Water Air Soil Pollut. Focus* 3, 7–26.
- Raven, J.A., Beardall, J., Sánchez-Baracaldo, P., 2017. The possible evolution, and future, of CO₂ concentrating mechanisms. *J. Exp. Bot.* 68, 3701–3716.
- Reddy, S.K., Balasubramanian, S., 2004. Carbonic acid: molecule, crystal aqueous solution. *Chem. Commun.* 50, 503–514.
- Reidenbach, L.B., Fernandez, P.A., Leal, P.P., Noisette, F., McGraw, C.M., Revill, A.T., Hurd, C.L., Kübler, J.E., 2017. Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. *PLoS One* 12, e0188389.
- Reusch, T.B.H., Boyd, P.W., 2013. Experimental evolution meets marine phytoplankton. *Evolution* 67, 1849–1859.
- Richier, S., Achterberg, E.P., Humphreys, M.P., Poulton, A.J., Suggett, D.J., Tyrell, T., Moore, C.M., 2018. Geographical CO₂ sensitivity of phytoplankton correlates with ocean buffer capacity. *Glob. Change Biol. Bioenergy* 24, 4438–4452.
- Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), 2010. *Guide to Best Practices for Ocean Acidification Research and Data Reporting*. Office for Official Publications of the European Union, Luxembourg, pp. 41–52.
- Riebesell, U., Aberle-Malzahn, N., Achterberg, E.P., Algueró-Muñoz, M., Alvarez-Fernandez, S., Aristigui, J., Bach, L.T., Boersma, M., Boxhaum, T., Guan, W., Hanost, J., Horn, H.G., Löscher, C.K., Ludwig, A., Spisla, C., Sswat, M., Strahne, P., Toucher, J., 2018. Toxic algal bloom induced by ocean acidification disrupts the pelagic food web. *Nat. Clim. Change* 8, 1082–1086.
- Rost, B., Richter, U.-W., Riebesell, U., Hansen, P.J., 2006. Inorganic carbon acquisition in red tide dinoflagellates. *Plant Cell Environ.* 29, 810–822.
- Roth-Schulze, A.J., Thomas, T., Steinberg, P., Deveney, M.R., Tanner, J.E., Wiltshire, K. H., Papantonou, S., Runcie, J.W., Gurgel, C.F.D., 2018. The effects of warming and ocean acidification on growth, photosynthesis, and bacterial communities for the marine invasive macroalga *Caulerpa taxifolia*. *Limnol. Oceanogr.* 63, 459–471.
- Saderme, V., Fietzek, P., Herman, P.M.J., 2013. Extreme variations of pCO₂ and pH in a macrophyte meadow of the Baltic Sea in summer: evidence of the effect of photosynthesis and local upwelling. *PLoS One* 8, 62689.
- Salisbury, J., Green, M., Hunt, C., Campbell, J., 2008. Coastal acidification by rivers: a threat to shellfish? *Eos* 89, 513–514.
- Salt, L.A., Thomas, H., Prowe, A.E.F., Borges, A.V., Bozec, Y., de Baar, H.J.W., 2013. Variability of North Sea pH and CO₂ in response to North Atlantic Oscillation forcing. *J. Geophysical Res.: Biogeosciences* 118, 1–9.
- Sandrini, G., Matthijs, H.C.P., Verspagen, J.M.H., Muzyer, G., Huisman, J., 2014. Genetic diversity of inorganic carbon uptake systems causes variation in CO₂ response of the cyanobacterium *Microcystis*. *ISME J.* 8, 589–600.
- Sandrini, G., Cunsolo, S., Schuurmans, J.M., Matthijs, H.C.P., Huisman, J., 2015a. Changes in gene expression, cell physiology and toxicity of the harmful cyanobacterium *Microcystis aeruginosa* at elevated CO₂. *Front. Microbiol.* 6, 401.
- Sandrini, G., Jakupovic, D., Matthijs, H.C.P., Huisman, J., 2015b. Strains of the harmful cyanobacterium *Microcystis aeruginosa* differ in gene expression and activity of inorganic carbon uptake systems at elevated CO₂ levels. *Appl. Environ. Microbiol.* 81, 7730–7739.
- Sandrini, G., Ji, X., Verspagen, J.M.H., Tann, R.P., Slot, P.C., Luimstra, V.M., Schuurmans, J.M., Matthijs, H.C.P., Huisman, J., 2016. Rapid adaptation of harmful cyanobacteria to rising CO₂. *Proc. Nat. Acad. Sci. U. S. A.* 113, 9315–9320.
- Schock, T.B., Huncik, K., Beauchesne, K.R., Villareal, T.A., Moeller, P.D.R., 2011. Identification of trichotoxin, a novel chlorinated compound associated with the bloom-forming cyanobacterium, *Trichodesmium thiebautii*. *Environ. Sci. Technol.* 45, 7503–7509.
- Semesi, I.S., Beer, S., Bjork, M., 2009. Seagrass photosynthesis controls rate of calcification and photosynthesis of calcareous macroalgae in a tropical seagrass meadow. *Mar. Ecol. Progr. Ser.* 382, 41–88.
- Shen, C., Dupont, C.L., Hopkinson, B.M., 2017. The diversity of CO₂-concentrating mechanisms in marine diatoms as inferred from their genetic content. *J. Exper. Bot.* 68, 3937–3948.
- Smayda, T.J., 1997a. What is a bloom? A commentary. *Limnol. Oceanogr.* 42, 1132–1136.
- Smayda, T.J., 1997b. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton in the sea. *Limnol. Oceanogr.* 42, 1137–1153.
- Smetacek, V., Zingame, A., 2013. Green and golden seaweeds on the rise. *Nature* 504, 84–88.
- Smith, F.A., Raven, J.A., 1979. Intracellular pH and its regulation. *Annu. Rev. Plant Physiol.* 30, 289–311.
- Smith, S.M.E., Morgan, D., Musset, B., Cherny, V.V., Place, A.R., Hastings, J.W., DeCoursey, T.E., 2011. Voltage-gated proton channel in a dinoflagellate. *Proc. Nat. Acad. Sci. U. S. A.* 108, 18162–18167.
- Still, C.J., Berg, J.A., Collatz, G.J., DeFries, R.S., 2003. Global distribution of C₃ and C₄ vegetation: carbon cycle implications. *Glob. Biogeochem. Cycles* 17, 1006. <https://doi.org/10.1029/2001GB001807>.
- Stoecker, F.K., Hansen, P.J., Caron, D.A., Mitra, A., 2017. Mixotrophy in the marine plankton. *Annu. Rev. Mar. Sci.* 9, 311–335.
- Sun, J., Hutchins, D.A., Feng, Y., Seubert, E.L., Caron, D.A., Fu, F.-X., 2011. Effects of changing pCO₂ and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. *Limnol. Oceanogr.* 56, 828–840.
- Sunda, W.G., Cai, W.-J., 2012. Eutrophication induced CO₂ acidification of subsurface coastal waters: interactive effects of temperature, salinity and atmospheric P_{CO2}. *Environ. Sci. Technol.* 46, 10651–10659.
- Sunda, W.G., Graneli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystems and disruptive algal blooms. *J. Phycol.* 42, 963–974.
- Talling, J.F., 2006. Interrelated shifts in acid-base and oxidation-reduction systems that determine chemical stratification in three dissimilar English lake basins. *Hydrobiologia* 568, 275–286.
- Tappin, A.D., 2002. An examination of the fluxes of nitrogen and phosphorus in temperate and tropical estuaries: current estimates and uncertainties. *Estuar. Coast. Shelf Sci.* 55, 885–901.
- Taraldsvik, M., Mykkestad, S.M., 2000. The effect of pH on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom *Skeletonema costatum*. *Eur. J. Phycol.* 35, 189–194.
- Tatters, A.O., Fu, F.-X., Hutchins, D.A., 2012a. High CO₂ and silicate limitation synergistically increase the toxicity of *Pseudo-nitzschia fraudulenta*. *PLoS One* 7, e32116.
- Tatters, A.O., Flewelling, L.J., Fu, F.-X., Granolm, A.A., Hutchins, D.A., 2012b. High CO₂ promotes the production of paralytic shellfish poisoning toxins by *Alexandrium catenella* from Southern California water. *Harmful Algae* 30, 37–43.
- Tatters, A.O., Roleda, M.Y., Schnetzer, A., Fu, F.-X., Hurd, C.L., Boyd, P.W., Caron, P.A., Li, A.A.Y., Hoffman, L.J., Hutchins, D.A., 2013. Short and long-term conditioning of temperate marine diatom community to acidification and warming. *Philos. Trans. Biol. Sci.* 368, 20120437.
- Taylor, A.R., Chrachri, A., Wheeler, G., Goddard, H., Brownlee, C., 2011. A voltage-gated H⁺ channel underlying pH homeostasis in calcifying coccolithophores. *PLoS Biol.* 9, e1001085.
- Taylor, A.R., Brownlee, C., Wheeler, G.L., 2012. Proton channels in algae: reasons to be excited. *Trends Plant Sci.* 17, 675–684.
- The Royal Society of London Report 12/05, 2005. Ocean acidification due to increasing carbon dioxide. (J A Raven, K Caldeira, H Elderfield, O Hoegh-Guldberg, P Liss, U

- Riebesell, J. Shepherd, C. Turley, A. Watson, R. Heap, R. Banes, R. Quinn). The Royal Society, London pp. vii + 60.
- Thoisen, C., Riisgaard, K., Lundholm, N., Nielsen, T.G., Hansen, P.J., 2015. Effect of acidification on an Arctic phytoplankton community from Disko Bay, West Greenland. *Mar. Ecol. Progr. Ser.* 520, 21–34.
- Thomas, H., Schneider, B., 1999. The seasonal cycle of carbon dioxide in the Baltic Sea surface waters. *J. Mar. Syst.* 22, 53–67.
- Thorner, C.S., Guidame, M., Deacatis, C., Green, L., Ramsay, C.N., Palmisiano, M., 2017. Spatial and temporal variability in macroalgal blooms in a eutrophied coastal estuary. *Harmful Algae* 68, 92–96.
- Thornton, D.C.O., 2014. Dissolved organic carbon release by phytoplankton in the contemporary and future ocean. *Eur. J. Phycol.* 49, 20–46.
- Trimborn, S., Lundholm, N., Thoms, S., Richter, K.U., Krock, B., Hansen, P.J., Rost, B., 2008. Inorganic carbon acquisition in potentially toxic and non-toxic diatoms: the effects of pH-induced changes in seawater carbonate chemistry. *Physiol. Plant.* 133, 92–105.
- Trimborn, S., Brenneis, T., Sweet, E., Rost, B., 2013. Sensitivity of Antarctic phytoplankton species to ocean acidification: growth, carbon acquisition, and species interaction. *Limnol. Oceanogr.* 58, 997–1007.
- Trolle, D., Staehr, P.A., Davidson, T.A., Bjerring, R., Lauridsen, T.L., Søndergaard, M., Jeppesen, E., 2012. Seasonal dynamics of CO₂ flux across the surface of shallow temperate lakes. *Ecosystems* 15, 336–347.
- Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-Andreson, M., D'Avanzo, C., Babione, M., Sham, C.-H., Brawley, J., Lajtha, K., 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries* 15, 443–457.
- Van de Waal, D.B., Verspagen, J.M.H., Lürling, M., van Dank, E., Visser, P.H., Huisman, J., 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient hypothesis. *Ecol. Lett.* 12, 1326–1335.
- Van de Waal, D.B., Verspagen, J.M.H., Finke, J.F., Vournazou, V., Immers, A.K., Kardinaal, W.E.A., Tonk, L., Becker, S., Van Donk, E., Visser, P.M., Huisman, J., 2011a. Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO₂. *ISME J.* 5, 1438–1450.
- Van de Waal, D.B., Smith, V.H., Declerck, S.A.J., Stam, E.C.M., Elser, J.J., 2011b. Stoichiometric regulation of phytoplankton in toxins. *Ecol. Lett.* 17, 736–742.
- Vidyarathna, N.K., Fiori, E., Lundgren, V.M., Granéli, E., 2014. The effects of aeration on growth and toxicity of *Prymnesium parvum* grown with and without algal prey. *Harmful Algae* 39, 55–63.
- Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* 54, 145–159.
- Waldbusser, G.G., Salisbury, J.E., 2014. Ocean acidification in the coastal zone from an organism's perspective: multiple system parameters, frequency domains, and habitats. *Annu. Rev. Mar. Sci.* 6, 221–247.
- Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal ocean acidification: the other eutrophication problem. *Estuar. Coast. Shelf Sci.* 148, 1–13.
- Walworth, N.G., Fu, F.-X., Webb, E.A., Saito, M.A., Moran, D., McIlvin, M.R., Lee, M.D., Hutchins, D.A., 2016. Mechanisms of increased *Trichodesmium* fitness under iron and phosphorus colimitation in the present and future ocean. *Nat. Commun.* 7, 12081.
- Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.N., Ishikawa, A., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: learning from the past to forecast the future. *Harmful Algae* 49, 68–93.
- Wichard, T., Charrier, B., Mineur, F., Bothwell, J.H., De Clerck, O., Coates, J.C., 2015. The green seaweed *Ulva*: a model system to study morphogenesis. *Front. Plant Sci.* 6, 72.
- Wilkes, E.B., Carter, S.J., Pearson, A., 2017. CO₂ dependent carbon isotope discrimination in the dinoflagellate *Alexandrium tamarense*. *Geochim. Cosmochim. Acta* 212, 48–61.
- Wootton, J.T., Dfister, C.A., Forester, J.D., 2008. Dynamic patterns of declining ocean pH in a high-resolution multi-year dataset. *Proc. Nat. Acad. Sci. U. S. A.* 105, 18848–18853.
- Xu, Z., Gao, G., Xu, J., Wu, H., 2017. Physiological response of the golden tide alga (*Sargassum muticum*) to the interaction of ocean acidification and phosphorus enrichment. *Biogeosciences* 14, 671–681.
- Young, C.S., Gobler, C.J., 2016. Ocean acidification accelerates the growth of two bloom-forming macroalgae. *PLoS One* 11, e0155152.
- Young, C.S., Gobler, C.J., 2017. The organising effect of elevated CO₂ on competition among estuarine primary producers. *Sci. Rep.* 7, 7667.
- Young, C.S., Peterson, B.J., Gobler, C.J., 2018. The bloom-forming macroalgae, *Ulva*, outcompetes the seagrass, *Zostera marina*, under high CO₂ conditions. *Estuaries Coasts* 1–16.
- Yu, L., Kong, F., Shi, X., Yang, Z., Zhang, M., Yu, Y., 2015. Effects of elevated CO₂ on dynamics of microcystin-producing and non-microcystin-producing strains during *Microcystis* blooms. *J. Environ. Sci.* 27, 251–258.
- Zeebe, R., Wolf-Gladrow, D., 2001. CO₂ in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Oceanography Series 65. Elsevier, Amsterdam. ISBN 044509461.
- Zhao, J., Jiang, P., Liu, Z.Y., Wei, W., Lin, H.Z., Li, F.C., et al., 2013. The Yellow Sea green tides were dominated by one species, *Ulva (Enteromorpha) prolifera*, from 2007 to 2011. *Chin. Sci. Bull.* 58, 2298–2302.
- Zhou, M.J., Shen, Z.L., Yu, R.C., 2008. Responses of a coastal phytoplankton community to increased nutrient input from the Changjiang (Yangtze) River. *Continental Shelf Res.* 28, 1483–1489.
- Zhu, Z., Qu, P., Gale, J., Fu, F.-X., Hutchins, D.A., 2017. Individual and interactive effects of warming and CO₂ on *Pseudo-nitzschia subcurvata* and *Phaeocystis Antarctica*, two dominant phytoplankton from the Ross Sea, Antarctica. *Biogeosciences* 14, 5281–5295.