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Dynamic CO_2 and pH levels in coastal, estuarine, and inland waters: Theoretical and observed effects on harmful algal blooms

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ABSTRACT

Rising concentrations of atmospheric CO_2 results in higher equilibrium concentrations of dissolved CO_2 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 CO_2 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 CO_2 concentrating mechanisms (CCMs) is the most frequent mechanism of inorganic carbon acquisition in natural waters in equilibrium with the present atmosphere (400 μmol CO_2 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 CO_2 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 raphidiophyceans (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 CO_2 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 CO_2 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

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extent to which any phytoplankton may benefit from rising dissolved CO_2 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 CO_2 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 CO_2 (carbon dioxide), and dissolved H_2CO_3 (carbonic acid), dissolved HCO_3^- (bicarbonate) and CO_3^{2-} (carbonate), and solid $CaCO_3$ (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. H_2CO_3 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 Szelag-Wasilewska, 2015; Pines et al., 2016), but the available evidence indicates that H_2CO_3 is not directly involved in algal metabolism. The available evidence shows for the green tide alga, *Ulva*, CO_3^{2-} does not inhibit HCO_3^- 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 CO_3^{2-} on photosynthesis by phytoplankton; inhibitory effects are difficult to distinguish from those of high pH, discussed below. In contrast, HCO_3- and CO_2 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 CO_2 concentrations, with surface ocean CO_2 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 CO_2 concentration and pH are, however, based on changes in the open tropical ocean (present day data on atmospheric CO_2 from the Mauna Loa Observatory, Hawai'i). 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 CO_2 (typically ~7–20 mmol m^{-3}). CO_2 per volume is similar in the sea level atmosphere and dissolved in the surface ocean (with more dissolved CO_2 at lower temperatures), but the diffusion coefficient for CO_2, the immediate inorganic carbon substrate for RuBisCO (Ribulose bisphosphate carboxylase-oxygenase), the carboxylase involved in the Calvin-Benson cycle in seawater is 10^4 times slower than that in the atmosphere (Falkowski and Raven, 2007). This is probably the evolutionary reason for the occurrence of CO_2 concentrating mechanisms (CCMs) in many marine (and freshwater) algae (Badger et al., 1998; Raven et al., 2017). CCMs actively transport HCO_3- and/or CO_2 from the surrounding water to the site of Rubisco, raising the steady-state CO_2 concentration there to values higher than those in the water, and increasing the extent to which the carboxylase activity of Rubisco is saturated with CO_2, and decreasing the oxygenase activity (Badger et al., 1998; Giordano et al., 2005; Raven et al., 2017). When active transport involves HCO_3-, the inorganic species present at highest concentrations in seawater, intracellular carbonic anhydrase is essential to convert HCO_3- to CO_2 sufficiently rapidly. Diffusive CO_2 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 CO_2 entry could20 be related to the greater solubility of CO_2 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 C_4 photosynthesis (Raven et al., 2017). For harmful algae, active transport of inorganic carbon for fixation by Rubisco involves uptake of HCO_3- as well as CO_2 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 CO_2 exchange with the atmosphere) during the boreal winter (West coast of Greenland, 0.8 pH units; Thoisen et al., 2016). A further influence on CO_2 in high latitude waters is the impact of fresh water from glacial melting which increases (at a given temperature) CO_2 solubility; however, the meltwater is significantly undersaturated with CO_2, 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 CO_2 and its equilibration with the oceans is increasing the concentration of dissolved CO_2 and HCO_3^- and H^+ and decreasing the concentration of OH^- and CO_3^{2-}, 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

Table 1
Inorganic carbon speciation in seawater in equilibrium with extant and future CO_2 mole fractions at 20 °C (Gao et al., 2018a,b). In freshwater, for the same gas phase CO_2 mol fraction, the greater CO_2 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 CO_2 is higher, and HCO_3^- and, especially, CO_3^{2-} 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
Titratable alkalinity μmol kg^{-1}	2263	2199
pH	8.19	7.82

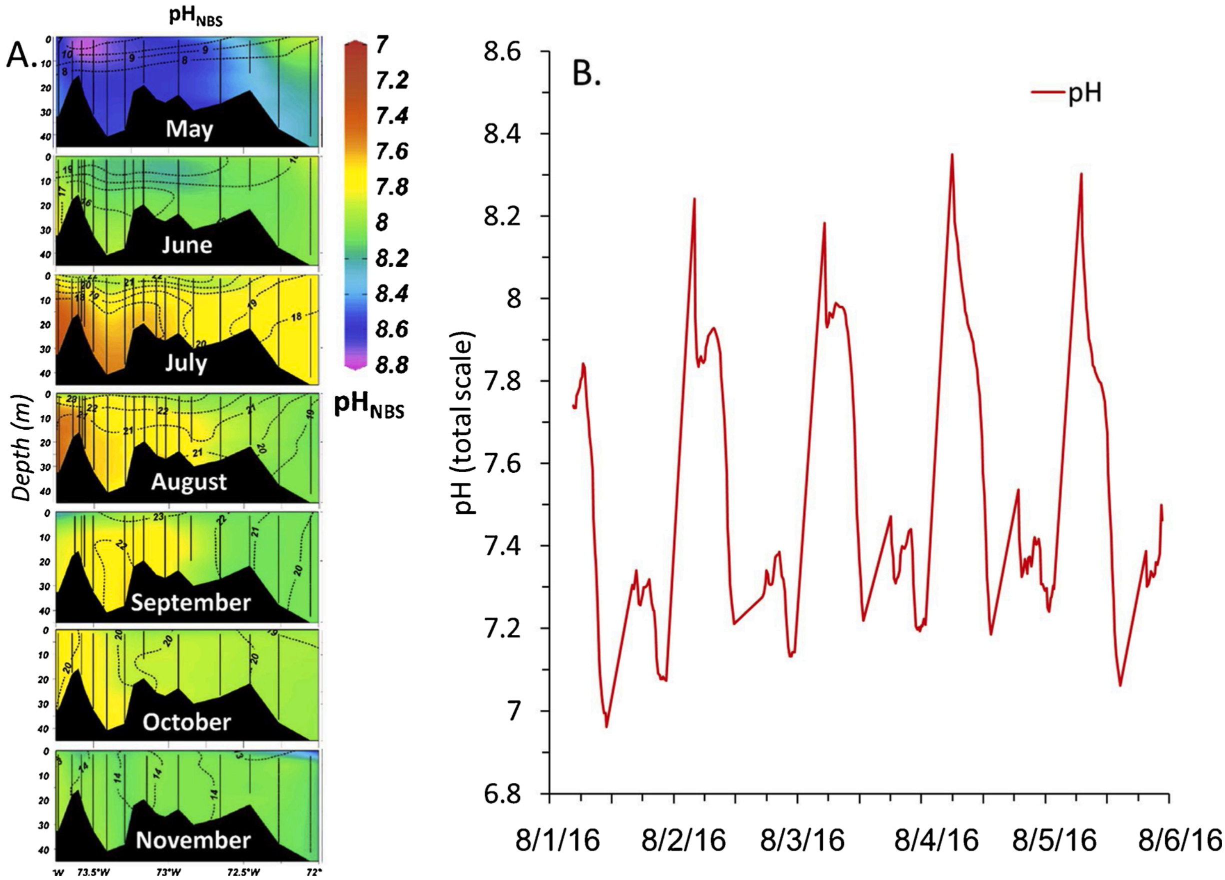

Fig. 1. Monthly changes in pH measured on the NBS scale in Long Island Sound, 2011, the third largest estuary in the USA. Vertical section plots extend from New York City in the western extreme to Block Island to the east (adopted from Wallace et al., 2014).
Diurnal changes in pH on the total scale as 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 anthoropogenic 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 (pH $< 7.7_T$, CO_2 in equilibrium with an atmosphere of more than 1000 μmol mol^{-1}, $\Omega_{aragonite} < 1$; 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 CO_2 (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 CO_2 as well as HPO_4^{2-} and NO_3-, and a diversity of HABs develop within upwelling zones (Pitcher and Weeks, 2006, 2018). Some develop within the advected CO_2 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, NH_4^+ uptake and assimilation results in the excretion of one H^+ molecule per NH_4^+ molecule to maintain the acid-base balance of the cells and subsequently decreases external pH and increases CO_2 (Brewer and Goldman, 1976). Given the Redfield atomic ratio, the inorganic C influx will be 6.6 times that of NH_4^+ (Brewer and Goldman, 1976) and thus the acidifying effects of the assimilation of NH_4^+ only partly offsets the alkalinising effect of inorganic carbon assimilation. In contrast, assimilation of NO_3- yields one OH- molecule effluxed per NO_3- 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 CO_2 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 CO_2 (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 CO_2 (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 CO_2 at night. NH_4^+ (or NO_3^-) 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2, with the latter decreasing the outgassing of respired CO_2. 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-emphasis 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 pCO_2 (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 CO_2 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 CO_2 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 CO_2 has been shown for the smaller lakes studied (Perga et al., 2016). Trophic status can also strongly effect the dynamics of CO_2 in lakes with eutrophic lakes being more likely to be undersaturated in CO_2, 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 CO_2: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 CO_2: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 µmol CO_2 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)

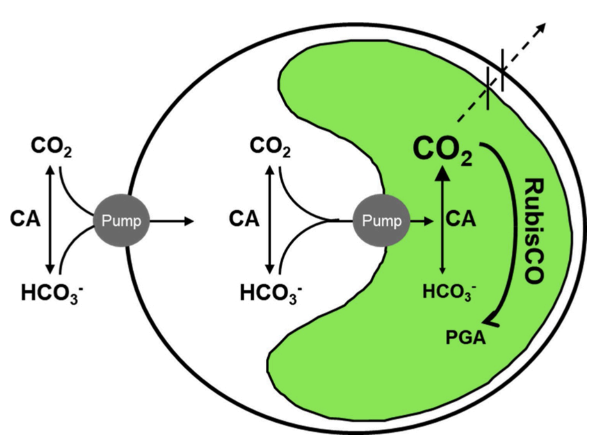

Fig. 2. Generalised diagram of inorganic carbon acquisition by eukaryotic algal cells using a CO_2 concentrating mechanism (denoted by 'Pump'). CO_2 concentrating mechanism actively transporting CO_2 and/or HCO_3^- into the cytoplasm and chloroplast (denoted in green). Active (= energized, uphill) HCO_3^- 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).

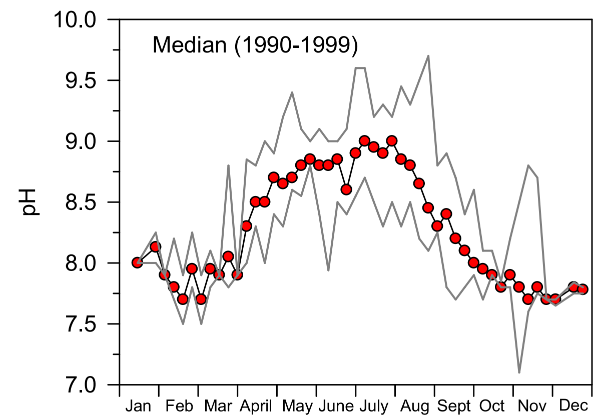

Fig. 3. Surface pH values within the Mariager Fjord, Denmark, as reported in Hansen (2002). pH can reach very high values in coastal eutrophic waters during summer due to.
the photosynthetic activity of algal blooms and near-constant sunlight, maximizing the influence of photosynthesis. pH was measured usinga glass eletrode 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 µmol 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, Thoisen 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 co-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); Erera 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 CO_2 than at high CO_2; the competitive order among four algae studied at low CO_2 was *Scenedesmus* > *Chlorella* > *Microcystis* > *Monoraphidium*, while at high CO_2 the order was *Microcystis* ≈ *Scenedesmus* > *Chlorella* > *Monoraphidium*. There are also reports of increased cellular toxin content in several algae under increased CO_2 and interactions of CO_2 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 CO_2 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 CO_2 and increased toxin production in toxic algae; more work is needed.

High algal productivities resulting from environmental factors other than increased CO_2 lead to increased hydroxyl ion and carbonate concentrations, and decreased hydrogen ion, bicarbonate and CO_2 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 CO_2 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 CO_2 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; Vidyarathna 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 CO_2

Studies often focus on differences between genera or species when the effects of CO_2 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 CO_2 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 CO_2 mol^{-1} total gas or 1000 μmol CO_2 mol^{-1}. Strains with both CCM components were favoured in low CO_2, but were partially replaced by strains with only the low affinity CCM component, one of which produced the cyanotoxin microcystin in high CO_2. It is of interest that microcystin may be involved in the acclimation of *Microcystis* to variations in external CO_2 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 CO_2 with high−CO_2 treatments (Collins and Bell, 2004; Reusch and Boyd, 2013; Kronholm et al., 2017; Raven et al., 2017). There area also studies on the filamentous toxic diazotroph *Trichodesmium* showing an irreversible (by return to control CO_2) increase in growth rate and N_2 fixation after >500 generations growth in high CO_2 (Hutchins et al., 2015). While in some of these experiments at least some of the high-CO_2 genotypes showed phenotypes that can be interpreted as adapted to high CO_2 (e.g. faster growth a high CO_2 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 pCO_2 for one year and found no strong evidence for fitness increases attributable to the conditioning dissolved CO_2 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 CO_2 (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., 2013*a*), 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 CO_2 concentrations.

While the presence of CCMs has yet to be confirmed among raphidophytes, there is evidence that high CO_2 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 CO_2 levels higher than 600 µatm and developing blooms above 800 µatm CO_2 (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 CO_2 (750 ppm CO_2; 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 CO_2 displayed downward swimming behaviour more so than cells grown at ambient CO_2 levels (Kim et al., 2013).

Strain-specific differences have been reported with regard to changes in toxin production associated with high CO_2 by *P. multiseries* with some groups reporting an increase in growth and toxin production at low pH/high dissolved CO_2 (Sun et al., 2011; Tatters et al., 2012a,b, Brunson et al., 2018) and others reporting enhanced toxin production at high pH (low dissolved CO_2; Lundholm et al., 2004; Trimborn et al., 2008). Culture methods, however, varied between the two groups with some groups (Lundholm et al., 2004; Trimborn et al., 2008) adjusting culture pH via direct additions of acid/base and others injecting CO_2 into cultures (Sun et al., 2011; Tatters et al., 2012a,b), suggesting the enhanced toxin production as associated with excess carbon from high CO_2, 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 CO_2 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 CO_2 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 C_3 biochemistry and are capable of using HCO_3^- 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 CO_2 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 CO_2 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 CO_2 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 CO_2, 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 CO_2 but not by elevated combined N or P, while the growth rate of *Ulva* was increased by elevated CO_2, and by elevated combined N or P, and, in two experiments, synergistically increased growth rate with elevated CO_2 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 CO_2, while growth of *Ulva* was inhibited by the presence of *Gracilaria* or phytoplankton (Young and Gobler, 2017). The conclusion was that, under increased CO_2, *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 CO_2 with bioavailable N and P on *Ulva* spp. and found significant biochemical and physiological changes under higher CO_2 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 CO_2 occur at different distances from CO_2 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 CO_2 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 CO_2 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 (CO_2, HCO_3^- and CO_3^{2-}). 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 CO_2 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 (=Tripos) 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 ($CO_2 + HCO_3^-$) 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 NH_3: NH_4^+ 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 Myklestad, 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 veneficium* (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 CO_2 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 CO_2 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 CO_2 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, CO_2, 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 CO_2 (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 CO_2 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 pCO_2 virtually nothing is known with regard to the implications of dynamic changes in dissolved CO_2 on HABs. Given the strong temporal and spatial gradients in pH and dissolved CO_2 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 CO_2 levels on HABs is needed.

While there have been dozens of studies of the effects of differing CO_2 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	*Microcystis*	Yes	–	–
Chlorophyta Ulvophyceae	*Ulva*	Yes	+/-	–
Dinophyta Dinophyceae	*Alexandrium*	Yes	+/-	+/-(phagotrophy)
Haptophyta Prymnesiophyceae	*Prymnesium*	?	+/-	+(phagotrophy)
Ochrista Pelagophyceae	*Aureococcus Aureaumbra*	?	?	+ (osmotrophy)
Ochrista Raphidophyceae	*Chattonella*	?	?	+(phagotrophy)

needed. While several strains of HABs experience enhanced growth and/or toxicity under high CO_2, many others do not. "Within genera" studies have found that different species respond differently to high CO_2, 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., 2013*a*), and *A. catanella* 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 CO_2 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 CO_2 levels on HABs are needed for broader conclusions to be drawn regarding how changing CO_2 levels may influence HABs. Such studies must be carefully designed, accounting for many experimental design factors including the method by which CO_2 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 CO_2 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 devoted to differentiating direct pH effects from effects of DIC/CO_2 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.

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