

## Will ocean acidification affect marine microbes?

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**Key words:** ocean acidification / rapid pH change / biogeochemical processes

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**Running title:** Ocean acidification and microbes.

**Subject category:** Geomicrobiology and microbial contributions to geochemical  
cycles

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The pH of the surface ocean is changing as a result of increases in atmospheric carbon dioxide (CO<sub>2</sub>) and there are concerns about potential impacts of lower pH and associated alterations in seawater carbonate chemistry on the biogeochemical processes in the ocean. However, it is important to place these changes within the context of pH in the present day ocean, which is not constant; it varies systematically with season, depth and along productivity gradients. Yet this natural variability in pH has rarely been considered in assessments of the effect of ocean acidification on marine microbes. Surface pH can change as a consequence of microbial utilisation and production of carbon dioxide, and to a lesser extent other microbially-mediated processes such as nitrification. Useful comparisons can be made with microbes in other aquatic environments that readily accommodate very large and rapid pH change. For example, in many freshwater lakes, pH changes that are orders of magnitude greater than those projected for the 22<sup>nd</sup> century oceans can occur over periods of hours. Marine and freshwater assemblages have always experienced variable pH conditions. Therefore, an appropriate null hypothesis may be, until evidence is obtained to the contrary, that major biogeochemical processes in the oceans other than calcification will not be fundamentally different under future higher CO<sub>2</sub> / lower pH conditions.

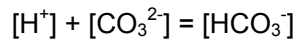
The pH of the oceans will change in the coming decades as the result of anthropogenic emission of carbon dioxide (CO<sub>2</sub>); changes to ocean chemistry are incontrovertible (Doney et al, 2009). The chemistry is straightforward but not simple; as anthropogenic CO<sub>2</sub> increases in the atmosphere, it dissolves in the surface ocean and aqueous carbon dioxide (CO<sub>2</sub>(aq)) reacts with water to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>) – a weak acid:



Carbonic acid dissociates to hydrogen ions (H<sup>+</sup>) and bicarbonate ions (HCO<sub>3</sub><sup>-</sup>):



A large fraction of the additional hydrogen ions combine with carbonate ions (CO<sub>3</sub><sup>2-</sup>) to form bicarbonate ions:



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Therefore, the result of dissolving  $\text{CO}_2$  in seawater is an increase in the concentrations of aqueous carbon dioxide, carbonic acid, hydrogen ions and bicarbonate ions, and a decrease in carbonate ions. Since pH is defined by the negative logarithm of the activity of hydrogen ions, it is clear that an increase in  $\text{H}^+$  must result in a decrease in pH. It is inevitable that the pH of the surface oceans will change with increasing anthropogenic  $\text{CO}_2$ .

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Caldeira & Wickett (2003), in their seminal paper, modelled the possible future changes in pH in the oceans and suggested that surface seawater pH would reduce by 0.77 units by the year 2300 if we burned the bulk of available fossil fuels (mostly coal). They also pointed out that, if  $\text{CO}_2$  emissions continued at the present rate, ocean pH would show larger changes, and faster rates of change, than are detected in the geological record with the possible exception of a few past catastrophic events (Kump et al, 2009). Pearson & Palmer (2000) suggested that the pH of seawater has varied by less than 0.1 units in the last 25 million years. As a result of these and other studies, there has been increasing concern that “ocean acidification” could have severe consequences for marine biota (<http://www.ocean-acidification.net>). Calcifying organisms, such as corals, molluscs, and coccolithophores, will undoubtedly be particularly vulnerable to higher  $\text{CO}_2$  conditions, since under lower pH the saturation levels decline for calcium carbonate minerals, leading to increased difficulty in maintaining calcite and aragonite shells and skeletons. However, there is little firm evidence for how many other organisms will respond.

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All microbes have complex proton pumps that are involved in bioenergetics, but it is not clear how microbes might respond to changes in environmental proton balance. The situation is complicated by the fact that the partial pressure of dissolved  $\text{CO}_2$  ( $\text{pCO}_2$ ) will increase in the future ocean, as well as pH decreasing. Some laboratory studies have shown that higher  $\text{pCO}_2$  has a fertiliser effect for some phytoplankton species under certain conditions. There is even less information about how heterotrophic microbes might respond to the future, coupled  $\text{pCO}_2$ -pH change in the surface ocean – but there is a great deal of relevant information from other environments that can be evaluated. Since ocean warming and increased stratification of the upper water column will also lead to decreases in dissolved oxygen ( $\text{O}_2$ ) concentrations (Keeling et al, 2010), changes in the  $\text{O}_2$ : $\text{CO}_2$  ratios may

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95 impose thermodynamic limitations to aerobic microbial life, especially in regions of  
low ambient O<sub>2</sub> (Brewer & Pelzer, 2009).

### **Surface ocean pH is variable**

Discussions of ocean pH are often made in the context of geological time scales  
100 (Pearson & Palmer, 2000; Caldeira & Wickett, 2003), and they imply a constancy and  
spatial uniformity of pH for the present-day oceans that is inconsistent with field  
observations. Over timescales of decades or longer, it may be appropriate to  
consider average annual values. But in reality, pH of the oceans is not constant and  
there are considerable seasonal, depth and regional variations. Therefore, when  
105 considering the overall consequences of ocean acidification for biogeochemical  
processes, it is necessary to understand that microbes in the present day ocean  
experience variable pH; indeed, most of that variability is a consequence of microbial  
activity. For example, phytoplankton blooms can rapidly reduce pCO<sub>2</sub>, with a  
concomitant increase in pH. Although this is the opposite sign in pH change to that  
110 expected in the future ocean, it demonstrates that pH is naturally variable and that  
marine organisms – particularly microbes – must already be capable of adapting to  
rapid and sometimes large changes in pH. Nevertheless, it is important to know if the  
average surface water decline of ~0.3 pH units that is expected by the current  
century end will present particular challenges for marine microbes or the elemental  
115 cycles that they sustain.

Effects of biological activity on ocean pH were recognised in some of the earliest  
studies of marine chemistry. Atkins (1922) reported that the pH of seawater in  
laboratory reservoirs was highly variable and that in tanks containing a decaying  
120 shrimp, pH fell from 8.27 to 7.2 over a period of 16 days. Although there may be  
doubts about the accuracy and precision of these early measurements, it is clear that  
these changes were a likely consequence of microbial respiration increasing CO<sub>2</sub>.  
Rapid changes in CO<sub>2</sub>, and hence pH, also occur in the present-day ocean. For  
example, a detailed study in Antarctic coastal water involving daily measurements of  
125 pH, clearly demonstrated how pH in the sea is altered by biological activity (Shibca et  
al, 1977). For most of a 10 month period, pH was 7.9, but in-situ acid-base chemistry  
changed rapidly during a phytoplankton bloom to pH 8.8. Shibca et al (1977)  
calculated that this represented pCO<sub>2</sub> saturation of only 15%, at a time when the  
measured O<sub>2</sub> saturation was 120% - clear evidence that phytoplankton  
130 photosynthesis results in large and rapid changes in CO<sub>2</sub> and hence in pH. The  
potential speed of pH change in a phytoplankton bloom is illustrated in Figure 1. In a

mesocosm experiment to investigate ocean acidification (Gilbert et al, 2008), nutrient additions initiated a phytoplankton bloom in an enclosed seawater sample (11 000 L) that had been adjusted to pH 7.8 by bubbling with CO<sub>2</sub>-enriched air (750 µatm).  
135 Within 4 days, through the utilization of CO<sub>2</sub>, the actively growing phytoplankton assemblage had returned pH to present-day conditions – a change of 0.3 pH units.

There are other examples of the importance of biology in changing the surface water pH. Figure 2 compares CO<sub>2</sub> at station ALOHA, off the Hawaiian Islands (Dore et al,  
140 2009), with the atmospheric record from Mauna Loa on the Island of Hawaii. Seasonal variations in the surface water are greater than those in the atmosphere because of biological and physical processes (mostly temperature). The pH can change by up to 0.06 pH units during the year even in the oligotrophic Central Pacific, which does not experience the dramatic phytoplankton blooms of temperate  
145 oceans.

In many environments, bacteria are already experiencing pH as low (or lower) as that projected for the end of the century in the surface ocean. For example, the thermocline at station ALOHA is a region where respiration exceeds photosynthesis  
150 and where sinking organic matter decomposition via aerobic respiration results in the release of CO<sub>2</sub> and a reduction in pH (Figure 3). Water below 350m has a pH less than 7.8, which is projected to be the pH of the surface ocean by the year 2100, yet microbial processes continue. Even in regions of the surface ocean, high respiration:photosynthesis ratios (e.g., high latitude regions in winter; residual signal  
155 in newly upwelled waters) could result in periodic reduced pH, if dissolved CO<sub>2</sub> accumulates. Increases in pH with depth were also noted by Atkins (1922) as a feature of one of the earliest studies of pH in the ocean. Some regions, particularly estuaries and coastal regions, show large seasonal and spatial variations in pH (Salisbury et al, 2008); indeed, they have been among the first regions to  
160 demonstrate significant ecological change as a result of a steep decline in seawater pH over time (e.g. study of benthic algal and invertebrate community by Wootton et al, 2008).

Given their vital role in marine productivity and planetary habitability, it is imperative  
165 to know if microbial assemblages will continue to function at the lower pH values that are projected for the near future. That is, do present day populations have the metabolic and genetic plasticity to compensate for lower pH conditions? Or are marine microbes less able to acclimate because the average pH of the oceans has

changed more slowly over geological time periods, a factor of 30-100 times slower  
170 than projected rates for this century, and perhaps has not varied by more than 0.6 pH  
units for 350 million years (Kump et al, 2009)?

One clue might come from a comparison with freshwater lakes. These have much  
lower buffering capacity than the oceans, so significant daily variations in pH occur  
175 as a result of normal temporal phasing of net photosynthesis and net respiration. For  
example, Maberly et al (1996) showed that diel variations in a lake can be as much  
as 2-3 pH units. In contrast, the pH change in the ocean is expected to be ~0.3 pH  
units over the next 100 years. Variations in pH also occur over very small distances.  
Talling (2006) showed that in some English lakes, pH could change by > 2.5 pH units  
180 over a depth of only 14 m in the water column. Yet phytoplankton, bacteria, archaea  
and metazoans are all present in lakes, and appear to be able to accommodate large  
daily and seasonal changes in pH. Will marine microbes be different, with a lower  
capacity to acclimate and adapt than freshwater microbes? Is it possible that long-  
term exposure to much smaller variations in pH means that marine microbes are less  
185 able to accommodate pH change than freshwater microbes?

This seems unlikely from the evidence available to date, at least from distribution  
studies of marine heterotrophic bacteria. For example, a genomic study at station  
ALOHA (DeLong et al, 2006) investigated the vertical distribution of bacteria.  
190 Although there were large differences with depth, particularly in cyanobacteria and  
numbers of sequences retrieved, a number of taxa were found nevertheless  
throughout the water column to a depth of 4000m. These included representatives of  
the alphaproteobacteria and the gammaproteobacteria with 16S sequences from  
SAR202, SAR324 and SAR406 detected at every depth sampled, including within the  
195 core of the acidified zone (200-4000 m). Although it is likely that there were different  
phenotypes and genetic subpopulations with depth, the 16S sequences were  
identical (as demonstrated for SAR11 by Field et al, 1997). These data demonstrate  
that diverse bacterial assemblages, composed of similar lineages, are present over a  
range of pH (as well as different temperatures and pressures). An important question  
200 that could be readily addressed by sampling a depth profile, or comparing marine,  
coastal and freshwater systems is: Do marine phytoplankton, bacteria and archaea  
have the genetic flexibility for homeostasis of intracellular pH (Booth, 1985) under  
higher CO<sub>2</sub> / lower pH (i.e. they can acclimate)? Or will genetic change be required,  
involving adaptation by the acquisition of genes through lateral gene transfer or  
205 selection of beneficial mutations? Or will small variations in external pH affect

chemical equilibria and kinetics at the cell surface and with membrane transporters, leading to consequences for microbial physiology? We urgently need answers to these questions.

210 **Microbially-mediated processes – will they be sensitive to ocean acidification?**

Table 1 is a brief summary of current knowledge of how key biogeochemical processes may respond in a high CO<sub>2</sub> ocean. Experiments to date do not provide a clear indication of how pH change might affect marine microbes and results are inconsistent and at times conflicting. For example, a number of studies have  
215 investigated the consequences of reduced pH for calcifying phytoplankton (Table 1). Results by a number of laboratories suggest negative effects of higher CO<sub>2</sub> / lower pH on coccolithophore cultures (e.g., Riebesell et al, 2000, 2007) but at least two studies indicate enhanced calcification under elevated CO<sub>2</sub> (Langer et al, 2006; Iglesias-Rodriguez et al, 2008). Ridgwell et al (2009) have suggested that some of  
220 these differences may be due to strain variations in the cultures used for these experiments; or there may be experimental design consequences resulting from the procedures used to adjust the pH (Rost et al, 2008). Clearly, further experiments are required to clarify the effect of ocean acidification on this important group of phytoplankton.

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Neither are there robust, consistent results on the effects of pH on non-calcifying phytoplankton. Increasing photosynthesis with elevated CO<sub>2</sub> is observed for some cyanobacteria (*Synechococcus*) but not others (*Prochlorococcus*) (Fu et al, 2007), and many eukaryotic phytoplankton species, most notably diatoms, have carbon  
230 concentrating mechanisms that diminish almost entirely the sensitivity of photosynthesis to CO<sub>2</sub> variations (e.g., Tortell et al, 1997). Bottle incubations and mesocosm experiments with natural plankton communities indicate only a weak sensitivity of primary production to CO<sub>2</sub> (e.g. Tortell et al, 2000), though limited CO<sub>2</sub> fertilization is observed in some cases (e.g., Tortell et al, 2008; Egge et al, 2009).

235 There are suggestions that the carbon content of phytoplankton cells may increase under high CO<sub>2</sub> conditions (Riebesell et al, 2007), but any physiological changes appear to be quite subtle, and there is conflicting evidence from different studies on how plankton carbon/nitrogen stoichiometry varies with CO<sub>2</sub>. In terms of all of the major biogeochemical processes list in Table 1, there is still a state of considerable  
240 ignorance about how the ocean system will respond to higher CO<sub>2</sub> / lower pH.

**Future priorities**

A number of experimental approaches could be taken that will lead to better understanding. Many of these have been reviewed in the report of a recent expert group of microbial oceanographers that met to consider the consequences of ocean acidification for marine microbes (<http://cmore.soest.hawaii.edu/oceanacidification/>) and will be only briefly summarized here. A combination of approaches varying greatly in scope and scale was recommended. These range from autecological studies on model marine microbes to investigate intracellular pH regulation, to microcosm and mesocosm scale experiments, and possibly open-ocean, mesoscale perturbation experiments. Comparative studies of similar marine, coastal and freshwater microbial communities (such as those dominated by diatoms or dinoflagellates) could help to answer questions about whether there are intrinsic differences in the response to rapid (i.e. hourly or daily) pH change, as occurs in lakes (and to some degree in coastal waters) but not the same extent in open-ocean waters. Much of the present literature on microbe responses to pH/CO<sub>2</sub> perturbations is phenomenological, and more detailed studies are required to assess mechanisms at biochemical and cellular levels. Advances in high-throughput sequencing technology now offers opportunities to define how complex microbial communities might respond to CO<sub>2</sub>/ lower pH – for example using 16S rRNA-tag pyrosequencing of the V6 region (Sogin et al, 2006) or similar approaches. Metagenomics and metatranscriptomics technology has now developed sufficiently to study how whole communities might respond to CO<sub>2</sub> perturbation (Gilbert et al, 2008). Some existing projects to sample long ocean transects, such as the Atlantic Meridional Transect (Robinson et al (2006), offer opportunities to test how the increasingly well-defined microbial assemblages in different ocean provinces (Schattenhofer et al, 2009) might vary with pH. Large-scale surveys and time-series efforts are also crucial for scaling up physiological and genomic studies to ecosystems, with the ultimate goal of understanding impacts on biogeochemical cycling and ecosystem services (Doney et al, 2004).

Laboratory experiments with cultured organisms are invaluable for exploring plankton physiological responses to perturbations in pH and CO<sub>2</sub>. However, it should be recognised and appreciated that most plankton cultures have been maintained in the laboratory for many generations and may not be appropriate model organisms for these investigations on the effects of pH change. Most growth media do not adequately control pH and, at the high cell densities that are common in the maintenance of stock cultures, cells may well have been growing at >pH 9 for phytoplankton, and <pH 6 for heterotrophic bacteria, for many decades in some



280 cases. Existing culture collections may have unwittingly selected for high or low pH  
conditions, respectively, or for organisms less sensitive to rapid pH variations with  
time. It may be necessary to conduct experiments using fresh isolates, rather than  
relying on the more convenient established cultures. Change and selection in  
laboratory cultures is poorly documented but could be readily investigated. Whole  
285 genome sequences are available for an increasing number of phytoplankton and  
bacterial species. It would be very interesting to compare the genomes of fresh  
isolates with long established cultures. For example, the common laboratory diatom,  
*Phaeodactylum*, was isolated from the English Channel in 1910 (Allen & Nelson,  
1910) and has been maintained in culture collections for 100 years –albeit under  
290 poorly defined conditions. How would the genome of this long-term culture compare  
with a fresh isolate from the English Channel?

Long-term experiments lasting many generations may be necessary to establish how  
individual organisms might respond to higher CO<sub>2</sub> / lower pH. Yet these are very  
295 challenging experiments. Collins & Bell (2004) maintained *Chlamydomonas* for 1000  
generations under high CO<sub>2</sub> (1050 µatm) but failed to find evidence for adaptive,  
evolutionary change. Long-term adaptation experiments may need to be maintained  
for decades – much longer than is possible with current funding models (Boyd et al,  
2008). And there is no guarantee that adaptation / evolution would be detected at the  
300 end of the experiment, so there is a large element of risk in the design and conduct of  
these studies.

Finally, ocean acidification is only one aspect of habitat variability and climate  
change. The temperature of the oceans will also increase as a consequence of  
305 higher CO<sub>2</sub> concentrations in the atmosphere, but little is known about how natural  
assemblages will respond to higher temperatures. Higher temperatures will also lead  
to increased stratification that will alter the flux of nutrients from below and the mean  
light levels experienced by microbes throughout the euphotic zone (Steinacher et al,  
2010). Given that ocean acidification will occur at the same time as temperature  
310 increases, nutrient decreases and light flux alterations, to name a few habitat  
variables, there is a strong case for multifactorial experiments to examine the  
possible synergistic or antagonistic effects of multiple stressors on microbial  
assemblage diversity and function.

315 In conclusion, CO<sub>2</sub> and pH in the surface ocean is not, and never has been, constant.  
Microbes in some parts of the present-day ocean already experience average

surface ocean pH that will occur by the end of the century. And freshwater and coastal microbes experience short-term and seasonal changes that are many times greater than those that will occur in the open-ocean. Given these facts, perhaps the most appropriate null hypothesis to test is that marine microbes possess the flexibility to accommodate pH change and there will be no catastrophic changes in marine biogeochemical processes that are driven by phytoplankton, bacteria and archaea. Clearly calcifying organisms are a special case since carbonate minerals will be less saturated - and for the case of aragonite, undersaturated in surface waters in a high-CO<sub>2</sub> ocean. Photosynthetic organisms may also be influenced and it is even possible that higher CO<sub>2</sub> may be beneficial. But the rest of the microbial community should not be assumed to be at risk until evidence to the contrary is obtained.

### 330 *Acknowledgements*

The authors acknowledge stimulating discussions with members of the expert group of microbial oceanographers that met to consider the consequences of ocean acidification for marine microbes (<http://cmore.soest.hawaii.edu/oceanacidification/>). Funding from the Gordon and Betty Moore Foundation, and logistical support from the Plymouth Marine Laboratory and the Center for Microbial Oceanography: Research and Education (National Science Foundation grant EF-0424599) are gratefully acknowledged.

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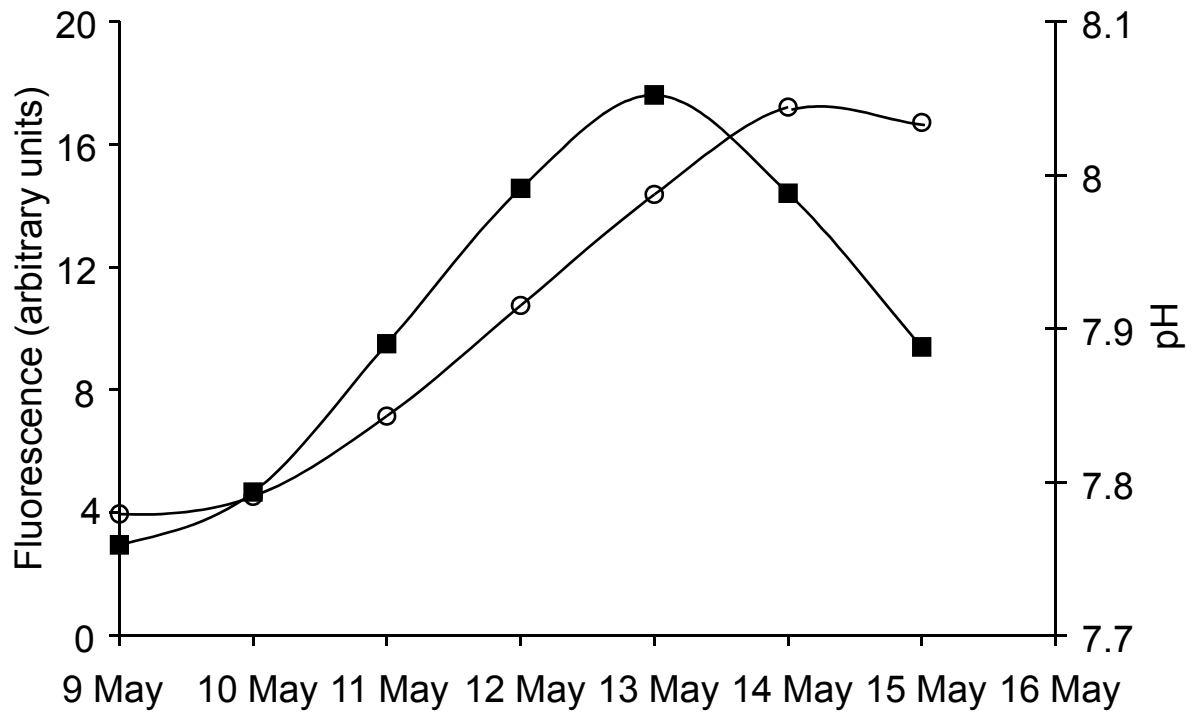
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## Figure legends

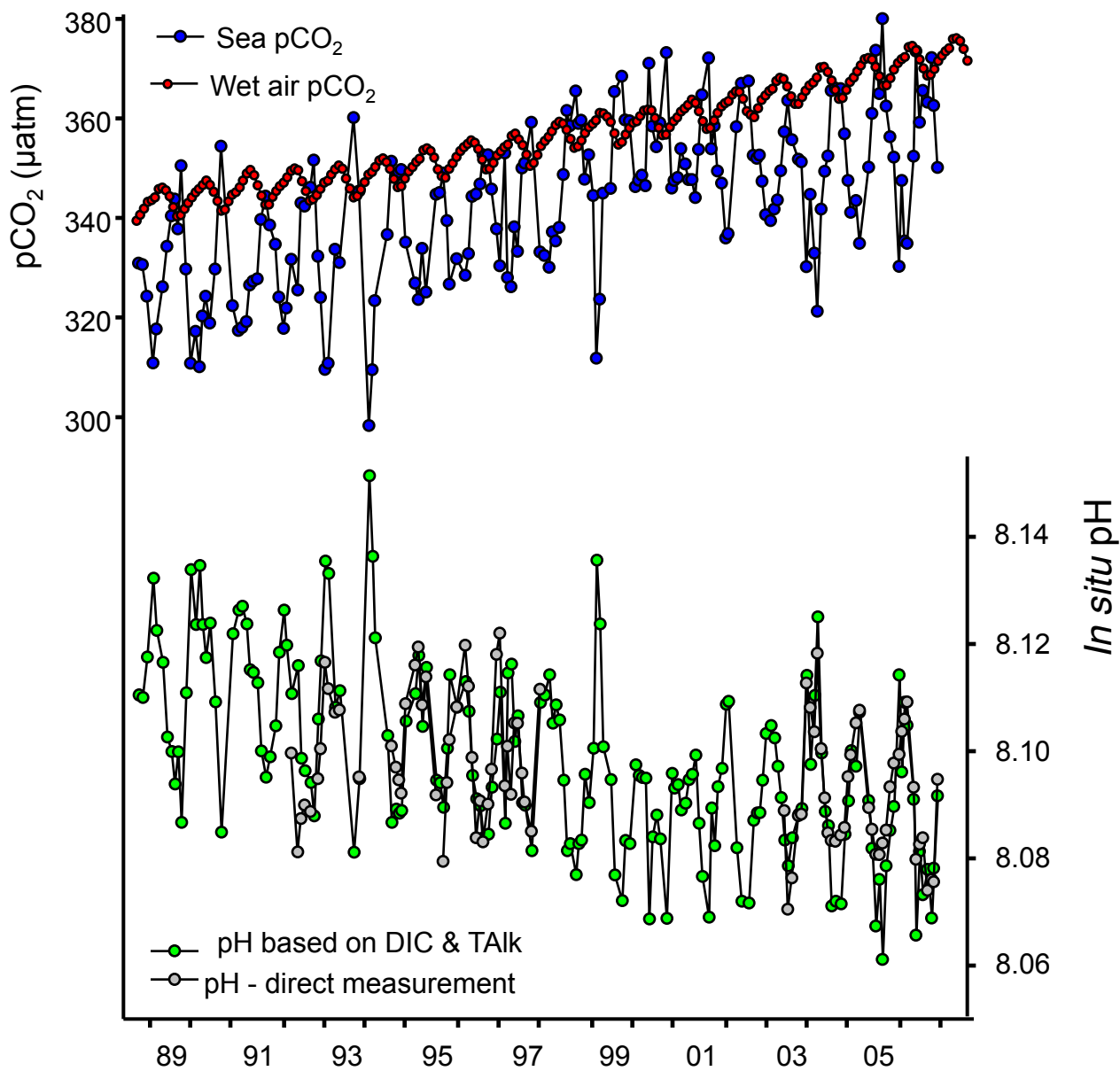
475 Figure 1. Rapid change in pH (○) as phytoplankton biomass (■) increased during a bloom in a mesocosm experiment (Gilbert et al, 2008). Water had been bubbled with CO<sub>2</sub>-enriched air (750 μatm) and the bloom was initiated by the addition of nitrate and phosphate. The mesocosm was covered and there was no exchange of CO<sub>2</sub> with the atmosphere; the observed changes in pH were the result to the utilisation of dissolved CO<sub>2</sub> by the actively growing  
480 phytoplankton assemblage.

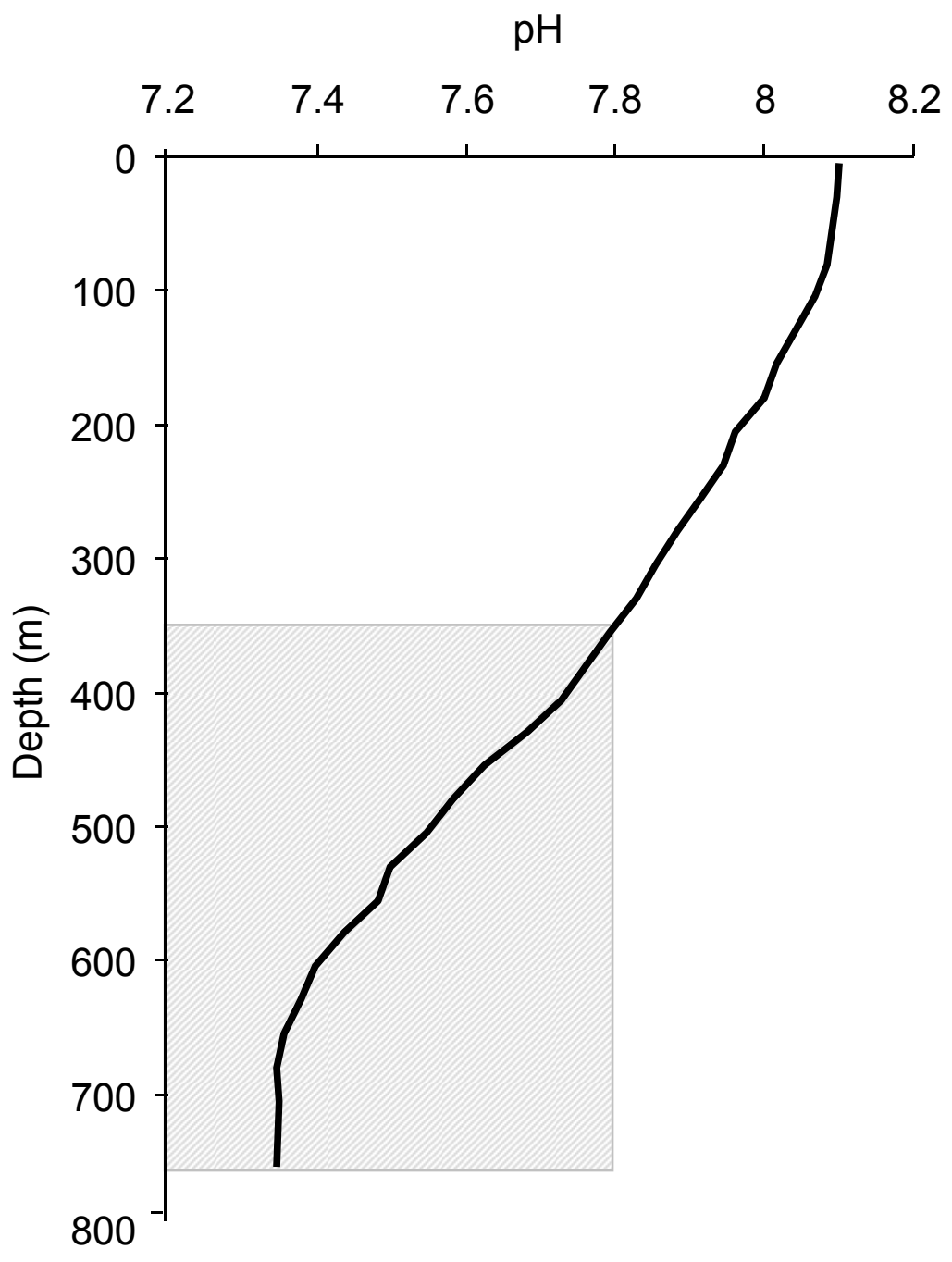
Figure 2 Long-term trends in surface ocean pH and CO<sub>2</sub> at station ALOHA in the Central Pacific, along with atmospheric CO<sub>2</sub> from nearby Mauna Loa. Net CO<sub>2</sub> flux was into the ocean, adding carbonic acid and lowering pH. The  
485 long-term declining pH trend was highly significant, but is overlain by substantial seasonal variability (Dore et al, 2009). Direct pH measurements agreed well with calculated values based on measurements of total dissolved inorganic carbon (DIC) and alkalinity. Surface ocean pH declined by 0.032 pH units from 1989-2006, an approximate 7% increase in H<sup>+</sup> concentration.

490 Figure 3 Depth profile of pH at station ALOHA in the central Pacific. The shaded area indicates water that is more acidic than that projected for surface waters at the end of the 21<sup>st</sup> century.









**Table 1. Current state of knowledge on microbially-mediated process that may be susceptible to ocean acidification.**

PROCESS	SIGNIFICANCE	STATE OF KNOWLEDGE
Primary productivity	Major influence on global carbon cycle, since ocean productivity is equivalent to terrestrial primary production.	Laboratory and field incubations showing both little change or enhanced productivity with elevated CO <sub>2</sub> . No reliable estimates of how global ocean productivity will change in relation to higher pCO <sub>2</sub> / lower pH.
Dominant phytoplankton species	Size of phytoplankton is important, influencing food availability (different grazing efficiencies) and potentially changing population structure of herbivores and carbon export	Little information available; Kim et al, (2006) found only small changes in diatoms populations while Tortell et al. (2008) report increase in chain-forming diatoms.
Phytoplankton biochemical composition	Food quality may change (e.g. protein, lipid and carbohydrate content)	Changes in C:N ratio, with higher C content reported in some studies (Riebesell et al, 2007) but not others (Hutchins et al, 2009); experimental conditions (light and nutrients) are important (Leonardos & Geider, 2005).
Calcification and carbonate dissolution	Calcifying phytoplankton and zooplankton produce particulate inorganic carbon that can alter particle sinking and export flux (ballast hypothesis) and influence bio-optical properties; balance between calcification and carbonate dissolution influences large-scale inorganic carbon cycle and geochemical processes.	Conflicting data on effect of high pCO <sub>2</sub> on coccolithophore calcification with reports of both reduced (Riebesell et al., 2000, 2007) and enhanced (Iglesias-Rodriguez et al, 2008) rates. Ridgwell et al (2009) emphasise importance of strain differences in culture experiments. There is clearer evidence of reduced calcification for foramanifera and pteropods (Fabry et al, 2008)
Particle sinking and export of organic carbon	The biological carbon pump controls carbon sequestration in the deep ocean (linked to species composition since small cells do not sink) and “ballast” – the organic carbon associated with sinking inorganic particles.	Delille et al (2005) suggested vertical flux will increase in higher pCO <sub>2</sub> .
Bacterial respiration and remineralisation	The vast majority of organic carbon in the surface oceans is utilised by heterotrophic bacteria; changes in bacterial activity and growth efficiency could profoundly affect the oceanic carbon balance.	Grossart et al (2006) found changes in bacterial metabolism in high pCO <sub>2</sub> , but it was difficult to distinguish direct effects on bacteria from changes in phytoplankton assemblages at high pCO <sub>2</sub> .

Nitrogen cycle	Microbes control the cycling of nutrients, particularly biologically available nitrogen which limits primary productivity in many oceanic provinces. Key processes are nitrogen fixation, denitrification and nitrification.	Hutchins et al (2007; 2009) and Levitan et al (2007) found increased rates of nitrogen fixation by <i>Trichodesmium</i> at high pCO <sub>2</sub> . Huesemann et al (2002) and Beman et al (2009) report reductions in nitrification under elevated CO <sub>2</sub> . The impact of realistic CO <sub>2</sub> variations on denitrification has not been examined (Hutchins et al, 2009).
Trace gas production	Microbes drive the production and consumption of many other potent climate-active gases (e.g. methane, nitrous oxide) and alter atmospheric chemistry (dimethylsulphide and organohalides).	Hopkins et al (2010) found decreased production of dimethylsulphide and volatile iodocarbon compounds under high pCO <sub>2</sub> conditions.
Trace metal availability	Altered pH may change trace metal availability, for through changes in pH and CO <sub>2</sub> dependent chemical speciation and dust dissolution.	Shi et al (2010) report a reduction in iron bioavailability and phytoplankton uptake under elevated CO <sub>2</sub> .

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