Dear Editor,

please find below all responses to the Reviewers‘ comments. At the end of this document you will find the revised version of the manuscript where I have marked all changes in yellow.

Thank you very much for editing this manuscript!

With best regards,

Lennart Bach

Biogeosciences Discuss., 12, C3898–C3900, 2015
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Interactive comment on "Reconsidering the role of carbonate ion concentration in calcification by marine organisms" *by* L. T. Bach

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Received and published: 29 July 2015

I would like to thank Lennart de Nooijer for his comment concerning the importance of CO2 as incorganic carbon substrate for calcification in some taxa. His thoughts are considered in the revised version of the manuscript.

I changed the headline of section 3.6.5 from "Inorganic carbon from respiratory sources" to "CO2 as inorganic carbon source for calcification". The section itself was expanded and addresses Lennart's comments. I wrote:

"Some organisms receive significant amounts of inorganic carbon used for calcification from respiratory sources (Erez, 1978; Furla et al., 2000; Pearse, 1970; Sikes et al., 1981; Tanaka et al., 1986). Here, organisms do not exclusively rely on direct inorganic carbon utilization from seawater but supplement calcification to a variable degree with CO2 gained intracellularly from respired biomass. This CO2 utilization may be further strengthened (1) when metabolic CO2 is 'trapped' inside the organisms through the establishment of pH gradients which limit the diffusive loss of CO2 passively (Bentov et al., 2009; Glas et al., 2012) or (2) when CO2 is transported actively towards the site of calcification (de Nooijer et al., 2014). CO2 which can subsequently react with H2O to form HCO3- and H+ (catalyzed by the enzyme carbonic anhydrase) could therefore be an alternative inorganic carbon source for calcification in particular taxa. Thus, the potential control of seawater [HCO3-]/[H+] on calcification may be weakened by the degree to which calcifiers utilize CO2 as inorganic carbon source."

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Interactive comment on Biogeosciences Discuss., 12, 6689, 2015.

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Interactive comment on "Reconsidering the role of carbonate ion concentration in calcification by marine organisms" *by* L. T. Bach

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Received and published: 29 July 2015

I would like to thank Reviewer #1 for the positive feedback and pointing out that carbonate chemistry is not the sole parameter controlling calcification in the oceans. I fully agree with this statement and emphasized this in the revised version of the manuscript. I updated and restructured section 3.5 (global implications) and pointed out at the very beginning of this section:

"The following paragraphs will address to what extent our view on carbonate chemistry control of calcification in the oceans could be modified when we consider [HCO3-]/[H+] rather than [CO32-] or ΩCaCO3 as the most influential parameter. Before starting the discussion I would like to emphasize, however, that carbonate chemistry patterns discussed here are just one among other abiotic (e.g. temperature or light) or biotic (e.g.

food availability or competition) factors which must also be taken into consideration when trying to understand the patterns of calcification in the oceans."

Reply to minor comments:

1) REVIEWER #1: Pg 6690, Ln 12-13: As well as temperature, salinity and pressure needing to be constant, biological factors will need to be constant in order for unity in the correlation between calcification and [HCO3-]/[H+] as with [CO32-] or CaCO3 saturation state. REPLY: I am not sure if I understood Reviewer #1 correctly but according to equations 9 and 12, salinity, temperature, and pressure are the only factors that need to be constant among treatments in order to establish the proportionality between [HCO3-]/[H+], [CO32-], and ΩCaCO3. Variation of other factors will have no influence on the proportionality.

2) REVIEWER #1: Pg 6691, Ln 18: Remove extra comma after both [Molluscs can have both(,) calcite ...] REPLY: I removed the extra comma.

3) REVIEWER #1: Pg 6692, Ln 23: From which reservoir of CO2 or HCO3-? Cellular or external (boundary layer)? REPLY: From the external reservoir. I added this information to the revised version of the manuscript.

4) REVIEWER #1: Pg 6692, Ln 27: Consider the use of 'success' – in an ecological or physiological sense? Possibly replace with fitness or growth. REPLY: I replaced 'success' with 'fitness' as suggested by Reviewer #1

5) REVIEWER #1: Pg 6693, Ln 21: Replace 'or' with 'and' so that evidence in bivalves and corals support the point. REPLY: I changed 'or' to 'and'.

6) REVIEWER #1: Pg 6698, Ln 21: How variable is the cytosol pH? REPLY: According to the refrerence cited here (Madshus 1988) it is strongly regulated and is typically in the range of 7.0 – 7.4. I added the given range to the revised version of the manuscript.

7) REVIEWER #1: Pg 6698, Ln 23: Add "be" to the line ". . . in the cytosol and <be> transported as HCO3-. . .". REPLY: I thank Reviewer #1 for spotting this mistake and

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changed accordingly.

8) REVIEWER #1: Pg 6702, Ln 7: Extra comma(s)? ". . . start in the pCO2(,) range below 250-500 μatm, where . . ." REPLY: I removed the extra comma.

9) REVIEWER #1: Pg 6704, Ln 25: Maybe a good place to state other factors which influence marine calcification that show strong latitudinal patters (e.g., availability of nutrients, food, light, etc.) REPLY: I addressed this concern at the beginning of section 3.5 (see major comment).

19) REVIEWER #1: Pg 6707, Ln 16: Extra semi-colon ". . .Allemand et al., 2004(;)). . ." REPLY: I removed the extra semi-colon.

REFERENCE: Madshus, I. H. 1988. Regulation of intracellular pH in eukaryotic cells. Biochem. J. 250: 1–8.

Interactive comment on Biogeosciences Discuss., 12, 6689, 2015.

Biogeosciences Discuss., 12, C3905–C3912, 2015
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Interactive comment on "Reconsidering the role of carbonate ion concentration in calcification by marine organisms" *by* L. T. Bach

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Received and published: 29 July 2015

I thank Reviewer #2 for the motivating comments and the interesting perspectives she/he raised.

In general, I think that the questions raised by Reviewer 2 can be answered when strengthening the necessary distinction between gross calcification ($CaCO_3$ precipitation) and net calcification ($CaCO_3$ precipitation + dissolution). $[HCO_3^-]/[H^+]$ would only be applicable to explain gross calcification (which usually equals net calcification if $\Omega CaCO_3>1$). I emphasized the distinction between gross and net calcification in the revised version of the manuscript. In section 3.6.1, for example, I changed the headline and wrote:

“3.6.1. Corrosive conditions - accounting for the difference between gross and net calcification

When discussing the influence of carbonate chemistry on calcification, distinction needs to be made between formation and dissolution of CaCO3 since these two processes are possibly controlled by different carbonate chemistry parameters. The roles of CaCO3 formation and dissolution are incorporated into the terms gross and net calcification. The former exclusively refers to the precipitation of CaCO3 whereas the latter accounts for both precipitation and dissolution. The ratio of [HCO3-] and [H+] can potentially be very useful to determine gross calcification which equals net calcification under non-corrosive conditions (i.e. ΩCaCO3 > 1). When ΩCaCO3 falls below 1, however, the control of [HCO3-]/[H+] on gross calcification would be obscured by the abiotic influence of ΩCaCO3 on dissolution. Accordingly, corrosive conditions would require consideration of both [HCO3-]/[H+] and ΩCaCO3 in order to correctly estimate the impact of carbonate chemistry on net biotic CaCO3 formation.”

Comment 1 by REVIEWER 2: *Firstly, there is the question of the conditions required for the organisms to usefully precipitation calcium carbonate. (The following thoughts are arguably more applicable to hypercalcifiers such as tropical corals and maybe less so to e.g. coccolithophores.) While the precipitation of carbonate minerals is thermodynamically favourable under conditions of > 1, the kinetics are not favourable for only relatively small degrees of supersaturation (although in the case of coccolithophores, presumably the kinetic barrier is considerably reduced by charged / functional groups the organic scaffolding that is layed down?). Given that surface ocean seawater is generally everywhere supersaturated (with respect to both calcite and aragonite) today, modification of the interior calcification space by e.g. corals is not done because calcium carbonate cannot be precipitated (although CO2 released through dark respiration could potentially create under-saturated conditions) but because calcification would not otherwise occur on a biologically/ecologically useful timescale to the organism. The faster the rate of carbonate production required, the higher the degree of* *super-saturation (or potentially faster the rate of seawater movement into the calcifying space) required. A thermodynamic energy difference can be assigned to the difference in modified vs. unmodified seawater, and higher super-saturation equates to a greater energy difference. Conversely, for the same internal degree of super-saturtaion and hence required precipitation rate, a lower saturation environment also equates to a greater external vs. internal energy difference. I appreciate the argument that proton transport may be the physiological relevant mechanism for maintaining internal saturation and under ocean acidification, enhancing the difference between internal and external, but saturation is still a useful concept in quantifying the thermodynamic budget. Indeed, ultimately, the thermodynamic driver for ambient seawater [CO32-] and [Ca2+] to precipitate as CaCO3(s), declines with ocean acidification and reduced ambient [CO32-]. This increased energetic cost (or reduced benefit) can be encapsulated in the saturation concept regardless of whether this represents the full physiological/energetic cost to the organism or not. In summary: I see* Ω *as a thermodynamically relevant measure that changes in an mechanistic interpretable way with ocean acidification, even if it is not the entire story, and hence should not be discarded in its entirety from usage.*

REPLY: I agree with Reviewer #2 that ΩCaCO3 can be a useful parameter for correlations with calcification, when considering net calcification and not only gross calcification. The term ‘meaningless’ was used in a context where I addressed gross calcification (see section 3.2). To avoid confusion, I changed ‘meaningless’ to ‘not useful’ to account for the fact that (1) ΩCaCO3 co-correlates with [HCO3-]/[H+] under constant T, S, and P and may therefore often (indirectly) explain gross calcification (section 3.2) and (2) that ΩCaCO3 contains information on the corrosiveness of seawater and is therefore the key parameter to correlate calcification with when dissolution processes are much more important than precipitation. This has been addressed in sections 3.6.1 and 3.6.2 of the revised version of the manuscript.

Reviewer 2 argues that ΩCaCO3 could be an appropriate measure for the carbonate

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chemistry control on calcification as it addresses calcification rates from a thermodynamic point of view. I agree with this statement as long as it refers to abiotic dissolution processes under corrosive conditions (see above). I disagree with this statement when arguing for CaCO3 formation, because how would we be able to explain that many calcifiers are able to precipitate CaCO3 when ΩCaCO3 is below 1 and conditions therefore thermodynamically unfavorable? In the coccolithophore Emiliania huxleyi, for example, we detected calcification at Ωcalcite of as low as 0.16 in case there was sufficient HCO3- substrate available but could not detect any signs of calcification at Ωcalcite of 4.3 in case [HCO3-] was low (Bach et al., 2015).

Comment 2 by REVIEWER #2: *There are important past implications of how impacts/controls on calcification are described and understood. Consider the Cenozoic projections of seawater carbonate chemistry of Tyrrell and Zeebe (2004) and Ridgwell (2005). At higher past (earlier Cenozoic) atmospheric pCO2 concentrations (as all proxies generally agree on), ocean surface [H+] is rather higher. Multiple proxies also suggest higher [Ca2+] (and lower [Mg2+]) earlier in the Cenozoic. Now given an 'adequate' global biogenic production rate of calcium carbonate, carbonate dissolution in marine sediments will control the mean saturation state of the ocean and in fact, regulate it to be relatively similar to modern (given that preservation is much more sensitive to changes in ocean saturation than global rates of weathering and hence global burial are likely to vary through time). At high [Ca2+], similar-to-modern Ω requires lower [CO32-]. The upshot is that earlier in the Cenozoic (and e.g. later Cretaceous), pH would have been lower, [H+] much higher, but [HCO3-] similar to modern. The implications are: assuming that the proxies are even vaguely 'correct' (they need be only 'correct' in the signs of their respective trends with time through the Cenozoic for the argument to hold) and these carbonate chemistry/carbon cycle model assumptions and calculations are also not fundamentally flawed:*

1)That saturation is an important concept because it helps explain abundant global calcification under conditions of much lower past [HCO3-]/[H+] (but similar to modern *surface Ω).*

2)[HCO3-]/[H+] is the only game in town (in terms of dictating the cost of calcification), but given the higher projected past [HCO3-]/[H+] ratios as compared to may occur in the future (end of century), future ocean acidification will have no impact on calcifying organisms. i.e. past high [HCO3-]/[H+] plus abundant calcification implies not future impact.

3)[HCO3-]/[H+] is the only game in town, but the physiology of past calcificers was very different and they have undergone fundamental evolutionary changes since, and have lost their ability to calcify well at low [HCO3-]/[H+]. (This then requires some suggestion as to how their calcification physiological was so fundamentally different.) or some combination, of none of the above.

The point being to explain that if the [HCO3-]/[H+] ratio is the sole determining factor to consider, how can we reconcile low past [HCO3-]/[H+] ratios with abundant calcification, with concerns for the future?

REPLY: Reviewer 2 argues that the Cenozoic was characterized by lower than modern [HCO3-]/[H+] but still had abundant calcification which would argue against the relevance of [HCO3-]/[H+] to explain calcification rates. I was struggling a bit with the term ‘abundant’ in this context because it could either refer to the relatively high diversity of calcifiers during this time (Martin, 1995) or relatively high CaCO3 accumulation rates (Ridgwell, 2005).

Diversity: [HCO3-]/[H+] would probably not be a very useful measure to assess the diversity of calcifiers as it determines a rate. Diversity can be independent of the calcification rate and is probably related to paleogeography rather than carbonate chemistry (Martin, 1995).

CaCO3 accumulation rate: The amount of CaCO3 accumulating on the sea floor is balanced by CaCO3 precipitation, post-production dissolution, and alterations during

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diagenesis. Accordingly, CaCO3 accumulation can be relatively high even if precipitation is low in case dissolution is also low. Thus, [HCO3-]/[H+] cannot be used to explain CaCO3 accumulation in the geological record as it would be only applicable for biogenic precipitation rates (gross calcification).

Reviewer #2 addressed an important question in comment 2 and I therefore added a new section (3.6.2, see below) to the manuscript which explicitly addresses the applicability of [HCO3-]/[H+] in the geological record.

“3.6.2 The Applicability of [HCO3-]/[H+] in the geological record

The restriction of [HCO3-]/[H+] to gross calcification rates (see previous section) limits its applicability in the geological record because the information on CaCO3 accumulation conserved in the sediments is not only affected by gross calcification but also by post-production dissolution and abiotic modifications of CaCO3 during diagenesis which are both controlled by ΩCaCO3. Thus, in order to verify the substrate-inhibitor concept for the geological record we would need a reliable proxy for biogenic gross calcification exclusively. Conversely, the application of ΩCaCO3 to investigate trends in CaCO3 sedimentation (e.g. Ridgwell 2005; Hönisch et al. 2012) would be reasonable because sedimentation involves both precipitation and dissolution and ΩCaCO3 is a good indicator for the former (under constant T, S, P) and the key parameter for the latter.”

Comment 3a by REVIEWER #2: *Lastly, it is worth considering what is the barrier to globally abundant calcification occurring by e.g. benthic foraminifera (or other calcifiers) at great depth in the ocean. [HCO3-]/[H+] may not be drastically different on the abyssal seafloor compared to the surface, and given a protective membrane, dissolution would not be an issue. This tends to suggest to me that low external saturation (even undersaturation) and at great depth is indeed a relevant and mechanistically (even if not physiologically) based factor in understanding calcification and calcification rates.*

REPLY: Although the vertical decline is more pronounced in ΩCaCO3, it is also present in [HCO3-]/[H+] (Fig. 7). I think on this basis it cannot be determined whether carbonate chemistry conditions for calcification deteriorate because of ΩCaCO3 or [HCO3-]/[H+].

Comment 3b by REVIEWER #2 *My overall summary comment would be: Do we not need to take into consideration and appreciate both facets of the carbonate chemistry system and both physiological and thermodynamic energy costs? The challenge might then be to combine their different facets in understanding ocean acidification controls on biogenic calcification, particularly as Lennart so eloquently argues and illustrates as with their (experimental) co-variance(?)*

REPLY: From my point of view (but see also papers by Jokiel), we would need to take into consideration that CaCO3 precipitation is controlled by other carbonate chemistry parameters than dissolution. The common application of ΩCaCO3 as carbonate chemistry indicator for calcification would only be useful to elucidate dissolution processes but its application would be limited with respect to the biotic formation of CaCO3 due to the differences in latitudinal and vertical gradients (section 3.5.1). With respect to ocean acidification research it should then be distinguished more clearly whether a measured (or computed) response to changing carbonate chemistry was driven by impacts on the formation or dissolution of CaCO3. So I agree with Reviewer 2 that we need to appreciate the different roles of different carbonate chemistry parameters on biotic calcification. This aspect has been strengthened in section 3.6.1 in the revised version of the manuscript.

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Interactive comment on Biogeosciences Discuss., 12, 6689, 2015.

Review by Paul L. Jokiel:

Reconsidering the role of carbonate ion concentration in calcification by marine organisms

By L. T. Bach

Correlation does not prove cause and effect, yet the correlation between calcification in marine organisms and [CO_3^{2-}] has led to the widespread paradigm that [CO_3^{2-}] limits calcification. Adopting this idea creates conflicts because other observations indicate that HCO_3^- rather than CO_3^{2-} is the substrate used for calcification as well as photosynthesis. Bach has made a major contribution in resolving this dilemma through a rearrangement of the carbonate system equations to show the proportional relationship between [CO_3^{2-}] and the ratio of [HCO_3^-] to [H^+]. Due to this proportionality rule, calcification rates will always correlate well with the ratio of [HCO_3^-] to [H^+] and equally well with the ratio of [CO_3^{2-}] to Ω when temperature, salinity, and pressure are constant. Thus [CO_3^{2-}] and Ω are simply good proxies for the control of calcification by [HCO_3^-] / [H+] where [HCO_3^-] is the inorganic carbon substrate and [H^+] functions as a calcification inhibitor. The proportionalities between [CO_3^{2-}] or Ω and the derived [HCO_3^-] / [H^+] relationship provide the chemical basis to reconcile conflicting results. Every correlation between calcification and [CO_3^{2-}] or Ω will be identical to the corresponding correlation with [HCO_3^-] / [H^+] when *T* , *S*, and *P* are stable. Thus, the correlations between calcification and [CO_3^{2-}] and Ω that have previously been reported are more likely attributed to the combined influence of [HCO_3^-] and [H^+]which are the more meaningful physiological parameters.

If this "substrate-inhibitor ratio" rather than [CO_3^{2-}] or Ω controls $CaCO_3$ formation then basic paradigms in ocean acidification research need to be reconsidered. For example, the absence of a latitudinal gradient in [HCO_3^-] / [H^+] in contrast to strong gradients in [CO_3^{2-}] and Ω negates the common assumption that high latitudes will be affected most severely by ocean acidification. Bach's comparison of present and future [CO_3^{2-}], Ω, and [HCO_3^-]/[H^+] patterns in the surface ocean revealed a strong poleward decline in [CO_3^{2-}] and Ω but no decline in [HCO_3^-] / [H^+]. These highly different latitudinal patterns are conserved in models of future climate change. Thus ocean acidification may be a more uniform problem at all latitudes for biotic $CaCO_3$ formation than previously thought.

There has been a long struggle to determine the calcification-relevant inorganic carbon species taken from seawater in order to understand the calcification response of marine organisms to changing carbonate chemistry. Many studies assume that HCO_3^- rather than CO_3^{2-} is the primary inorganic source. However, studies undertaken to determine the inorganic carbon molecule transported by molecular transport systems have not been successful. Bach approached this question using a carbonate chemistry approach to determine whether HCO_3^- or CO_3^{2-} would be the more suitable inorganic carbon substrate for calcification. Three different lines of analysis led to his conclusion that HCO_3^- would be favored:

(1) *Abundance*. HCO_3^- is the most abundant DIC species in seawater. It makes sense for an organism to rely on the largest inorganic carbon pool.

(2) *Homeostasis*. The hydration time of CO_2 is slow while the hydrolysis of HCO_3^{2-} is fast. Thus CO_3^{2-} transported through cytosol with a typical pH of 7.2 would quickly turn into HCO_3^- and bind a proton in the cytosol. The resulting HCO_3^- would be transported to the calcification site where the proton would be released back to the cytosol. Hence, the cytosolic pH would remain stable in case of selective CO_3^{2-} uptake only when CO_3^- uptake and $CaCO_3$ precipitation occur at

the same rate. However, both processes probably run out of equilibrium on occasion, especially in a highly variable diurnal environment. In these cases, the utilization of CO_3^{2-} as the inorganic carbon source would constitute a substantial risk for the pH homeostasis. Excess CO_3^{2-} uptake would elevate cytosolic pH while excess $CaCO_3$ precipitation would reduce it. In contrast, a selective uptake of HCO_3^- from seawater would not perturb the cytosolic pH as much under these conditions because HCO_3^- has a relatively low potential to accept or donate H^+ at pH 7.2. It may therefore be easier for calcifiers to keep cytosolic pH stable at 7.2 by using HCO_3^-.

(3) *Stability*. Seawater pH fluctuate substantially in a diurnal and seasonal timescale with HCO_3^- having a dominant stable concentration over the entire pH range encountered by marine organisms, while $[CO_3^{2-}]$ will show extreme variation. Thus HCO_3^- is a much more reliable inorganic carbon source for calcification.

In sum, this paper makes major contributions to our understanding of the process of calcification in marine organisms, reconciles differences in results from various studies and provides new insights into predicting future changes in marine communities due to ocean acidification.

REPLY to comments by Paul Jokiel: I thank Paul Jokiel for his motivating comments on the manuscript. His effort on reviewing the manuscript will be acknowledged in the revised version.

Lennart Bach

Reconsidering the role of carbonate ion concentration in calcification by marine organisms

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Abstract

Marine organisms precipitate 0.5-2.0 gigaton of carbon as calcium carbonate ($CaCO_3$) every year with a profound impact on global biogeochemical element cycles. Biotic calcification relies on calcium ions (Ca^{2+}) and generally on bicarbonate ions (HCO_3^-) as $CaCO_3$ substrates and can be inhibited by high proton (H^+) concentrations. The seawater concentration of carbonate ions (CO_3^{2-}) and the CO_3^{2-}-dependent $CaCO_3$ saturation state (Ω_{CaCO3}) seem to be irrelevant in this production process. Nevertheless, calcification rates and the success of calcifying organisms in the oceans often correlate surprisingly well with these two carbonate system parameters. This study addresses this dilemma through rearrangement of carbonate system equations which revealed an important proportionality between $[CO_3^{2-}]$ or Ω_{CaCO3} and the ratio of $[HCO_3^-]$ to $[H^+]$. Due to this proportionality, calcification rates will always correlate equally well with $[HCO_3^-]/[H^+]$ as with $[CO_3^{2-}]$ or Ω_{CaCO3} when temperature, salinity, and pressure are constant. Hence, $[CO_3^{2-}]$ and Ω_{CaCO3} may simply be very good proxies for the control by $[HCO_3^-]/[H^+]$ where $[HCO_3^-]$ would be the inorganic carbon substrate and $[H^+]$ would function as calcification inhibitor. If the "substrate-inhibitor ratio" (i.e. $[HCO_3^-]/[H^+]$) rather than $[CO_3^{2-}]$ or Ω_{CaCO3} controls biotic $CaCO_3$ formation then some of the most common paradigms in ocean acidification research need to be reviewed. For example, the absence of a latitudinal gradient in $[HCO_3^-]/[H^+]$ in contrast to $[CO_3^{2-}]$ and Ω_{CaCO3} could modify the common assumption that high latitudes are affected most severely by ocean acidification.

1 Introduction

Calcium carbonate ($CaCO_3$) is used by a large variety of marine organisms as structural material for their exo-and endoskeletons. Calcification requires calcium ions (Ca^{2+}) and dissolved inorganic carbon (DIC) substrate, which is present in seawater as carbon dioxide (CO_2), bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}). From a purely chemical point of view, calcium reacts with inorganic carbon as:

$$\mathrm{Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3} \qquad (1)$$

Precipitation is thermodynamically favored when $[Ca^{2+}]$ and $[CO_3^{2-}]$ reach or exceed the solubility of $CaCO_3$ in seawater. The stoichiometric solubility product is defined as:

$$\mathrm{K_{sp}^{*} = [Ca^{2+}]_{saturated}\ [CO_3^{2-}]_{saturated}} \qquad (2)$$

and is a function of temperature, salinity, and pressure (Mucci, 1983; Zeebe and Wolf-Gladrow, 2001). The saturation state of $CaCO_3$ (Ω_{CaCO3}) is calculated with seawater concentrations of Ca^{2+} and CO_3^{2-} and K_{sp}^* as:

$$\Omega_{\mathrm{CaCO_3}} = \frac{\mathrm{[Ca^{2+}]_{seawater}\ [CO_3^{2-}]_{seawater}}}{\mathrm{K_{sp}^{*}}} \qquad (3)$$

Accordingly, $CaCO_3$ precipitation is thermodynamically favored when the product of $[Ca^{2+}]_{seawater}$ and $[CO_3^{2-}]_{seawater}$ reaches or exceeds K_{sp}^* or $\Omega_{CaCO3} \geq 1$. In the oceans, Ω_{CaCO3} is largely determined by $[CO_3^{2-}]$ because $[Ca^{2+}]$ is rather constant in seawater (Kleypas et al., 1999).

Biogenic $CaCO_3$ is mainly present as calcite or aragonite, which have different crystal structures and solubility. Calcite is predominantly formed by coccolithophores, foraminifera, and some crustaceans while aragonite is typically found in scleractinian corals. Molluscs can have both calcite and aragonite. Echinoderms and octocorals build calcite with a large fraction of magnesiumn (Mg) included in the crystal lattice (Mann, 2001). Aragonite is more soluble than calcite which is expressed in an offset between their individual K_{sp}^* values (Mucci, 1983). This offset, however, is the only major difference in their solubility and changes in $\Omega_{aragonite}$ are very similar to changes in $\Omega_{calcite}$ (Zeebe and Wolf-Gladrow, 2001). They are therefore summarized in the term Ω_{CaCO3} in this study since changes in the saturation state rather than absolute numbers are addressed here.

On the biological level, chemical precipitation of $CaCO_3$ as defined in Eq. (1) is just the final step in the calcification process. Before precipitation, calcium and inorganic have to be transported in a series of active and/or passive processes until they reach the site of calcification which is usually located in specialized cellular compartments, tissues, or tissue interfaces. Transport mechanisms and pathways are highly diverse among the various calcifying taxa which rules out the possibility to formulate a generally applicable calcification model (Mann, 2001). What all calcifiers have in common, however, is their dependency on calcium and inorganic carbon availability in seawater as this is the ultimate source medium (Weiner and Addadi, 2011). Thus, biotic calcification will respond to changes in seawater calcium and inorganic carbon when concentrations cross species-specific thresholds.

Calcium is present in seawater in high concentrations (~10 mmol kg^{-1}) as Ca^{2+} (Zeebe and Wolf-Gladrow, 2001). As this ion is also the form used in the final precipitation reaction (Eq. (1)), calcium does not need to be chemically transformed while being transported from seawater to the site of calcification (Allemand et al., 2004; Bentov et al., 2009; Mackinder et al., 2011). This is in clear contrast to CO_3^{2-} where, the relation between ion source from seawater and ion sink during crystallization is considerably more complex since CO_3^{2-} is in constant exchange with HCO_3^- and CO_2. Thus, CO_3^{2-} used for $CaCO_3$ crystallization (Eq. (1)) at the site of calcification does not have to be taken from the seawater CO_3^{2-} pool but could equally well originate from the seawater CO_2 or HCO_3^- reservoir and be transformed to CO_3^{2-} shortly before reacting with Ca^{2+}.

Despite the unknown seawater DIC source for $CaCO_3$ precipitation, $[CO_3^{2-}]$ or the CO_3^{2-}-dependent Ω_{CaCO3} are often considered *a priori* as the key carbonate system parameters determining calcification rates or the fitness of calcifying organisms in the oceans (Kleypas et al., 1999; Beaufort et al., 2011). This assumption is reasonable under corrosive conditions (i.e. Ω_{CaCO3} <1) where $[CO_3^{2-}]$ controls the dissolution of $CaCO_3$ (Eq. (3)). The relevance of $[CO_3^{2-}]$ for the formation of $CaCO_3$ is, however, poorly constrained because very little is known about a molecular uptake and transport system that can take CO_3^{2-} from seawater and transfer it to the site of calcification. This uncertainty leads to the key questions: Which inorganic carbon species in seawater is/are utilized and which other carbonate system parameter(s) could be relevant for calcification?

Several physiological studies with different calcifying taxa have addressed these questions by setting up experiments where the influence of individual carbonate system parameters could be studied in isolation. Some of these studies found the best correlations of calcification rates with [CO_3^{2-}] and Ω_{CaCO3} (e.g. Schneider and Erez, 2006; Gazeau et al., 2011; de Putron et al., 2011; Keul et al., 2013; Waldbusser et al., 2014) while others highlighted the importance of [HCO_3^-] (e.g. Buitenhuis et al., 1999; Jury et al., 2010). Still others found that the response is not controlled by a single carbonate system parameter but the interplay of two or more. In coccolithophores, for example, calcification rates were repeatedly shown to increase from low to intermediate DIC but decrease again above certain thresholds (Langer et al., 2006; Bach et al., 2011; 2015; Sett et al., 2014). This optimum-curve response pattern was explained by the interaction between HCO_3^- and protons (H^+) where HCO_3^- stimulates calcification as substrate and H^+ functions as inhibitor (Bach et al., 2011, 2013). Similar conclusions have also been made in studies with bivalves (Thomsen et al., 2015) and corals (Jury et al., 2010) where it has been noted that the calcification response to changing carbonate chemistry could be the result of the opposing effects of [HCO_3^-] and [H^+].

Jokiel (2011a, 2011b, 2013) went one step further. Based on the results of his work on coral reef calcification he argued that single carbonate chemistry parameters such as [CO_3^{2-}] or Ω_{CaCO3} have no basic physiological meaning for calcification. Instead, calcification is controlled by the interaction of a “reactant” (i.e. DIC) and an “inhibitor” (i.e. H^+) and that calcification rates only correlate with [CO_3^{2-}] because [CO_3^{2-}] itself is linearly correlated with the ratio of reactant to inhibitor (i.e. DIC/[H+]) (Jokiel, 2013; Jokiel et al., 2014).

The present study builds up on these previous findings and aims to refine the thought that calcification is not controlled by a single carbonate chemistry parameter but reacts to a combination of two or more. Therefore, attention will be drawn to a potentially important proportionality between [CO_3^{2-}], or Ω_{CaCO3}, and the [HCO_3^-]/[H^+] ratio which was uncovered by rearranging carbonate chemistry equations. I will discuss (1) how this proportionality could help to understand carbonate chemistry induced changes in $CaCO_3$ precipitation by marine organisms; (2) how this proportionality could modify the paradigm that high latitude calcifiers are more susceptible to ocean acidification than species living in low latitudes.

2 Material and Methods

2.1 Uncovering the proportionality between $[CO_3^{2-}]$, or Ω_{CaCO3}, and the $[HCO_3^-]/[H^+]$ ratio

The carbonate system is an equilibrium reaction of the form:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2\,H^+ \qquad (4)$$

which can be simplified to:

$$CO_2 + H_2O \overset{K_1}{\Leftrightarrow} HCO_3^- + H^+ \overset{K_2}{\Leftrightarrow} CO_3^{2-} + 2\,H^+ \qquad (5)$$

because $[H_2CO_3]$ is only about 1/1000 of $[CO_2]$ and has no special significance to the acid-base equilibria since both species are uncharged (Butler, 1998; Dickson 2010). Hence, $[CO_2]$ is summarized in the following as:

$$[CO_2] = [CO_2]_{aq} + [H_2CO_3] \qquad (6)$$

where aq denotes for gaseous CO_2 dissolved in seawater.

For the description of the carbonate system, the first and second dissociation constants (K_1 and K_2) are expressed in terms of concentrations - i.e. as stoichiometric dissociation constants:

$$K_1^* = \frac{[HCO_3^-]\,[H^+]}{[CO_2]} \qquad (7)$$

and

$$K_2^* = \frac{[CO_3^{2-}]\,[H^+]}{[HCO_3^-]} \tag{8}$$

which are a function of temperature, salinity, and pressure (Zeebe and Wolf-Gladrow, 2001). Solving Eq. (8) for $[HCO_3^-]/[H^+]$ as:

$$\frac{[HCO_3^-]}{[H^+]} = \frac{1}{K_2^*}\,[CO_3^{2-}] \tag{9}$$

shows that $[CO_3^{2-}]$ is proportional to $[HCO_3^-]/[H^+]$ when temperature, salinity, and pressure remain unchanged because K_2^* is constant under these circumstances.

The $CaCO_3$ saturation state of seawater has been defined in Eq. (3). Solving Eq. (3) and Eq. (9) for $[CO_3^{2-}]$ yields:

$$[CO_3^{2-}] = \frac{\Omega_{CaCO_3}\,K_{sp}^*}{[Ca^{2+}]} \tag{10}$$

and

$$[CO_3^{2-}] = \frac{[HCO_3^-]K_2^*}{[H^+]} \tag{11}$$

Combining these equations and subsequently solving them for $[HCO_3^-]/[H^+]$ yields:

$$\frac{[HCO_3^-]}{[H^+]} = \frac{\Omega_{CaCO_3}\,K_{sp}^*}{[Ca^{2+}]\,K_2^*} \tag{12}$$

It follows that $[HCO_3^-]/[H^+]$ and Ω_{CaCO3} are proportional, under constant T, S, and P. Note that $[Ca^{2+}]$ is conservative in seawater and therefore scales with salinity.

2.2 Carbonate chemistry calculations

Carbonate chemistry data presented in Figs. 1-7 were calculated with the MATLAB (the Mathworks) version of CO2SYS (van Heuven et al., 2011) using K_1^* and K_2^* determined by (Millero, 2010), K_{HSO4} by Dickson (1990), and K_{sp}^* determined by Mucci (1983). $[H^+]$ (free scale) was subsequently calculated from pH_{free}:

$$\mathrm{pH_{free}} = -\log[\mathrm{H^+}]_{\mathrm{free}} \qquad (13)$$

as given in (Zeebe and Wolf-Gladrow, 2001).

Surface ocean (0-50 m) carbonate chemistry (DIC, TA), nutrient (PO_4^{3-}), salinity, and temperature data used for calculations presented in Figs. 1, 5B, D, F, and 6 were extracted from a model simulation with the University of Victoria (UVic) Earth System model performed by Taucher and Oschlies (2011). In their study, the model was spun up for 4000 years with pre-industrial boundary conditions and then forced with reconstructed CO_2 emissions and aerosol dynamics for the period from 1765-2000 (Schmittner et al., 2008). Thereafter, the model was forced with anthropogenic CO_2 emissions as predicted in the IPCC A2 ("business-as-usual") scenario. Note that the data was taken from their reference run ("TEMP"). For further details on the model setup, please refer to the original description by Taucher and Oschlies (2011).

The ratios $[CO_3^{2-}]/([HCO_3^-]/[H^+])$ and $[CO_3^{2-}]/(DIC/[H^+])$ shown in Fig. 3 were calculated with CO2SYS assuming increasing pCO_2 at constant total alkalinity (2350 µmol kg^{-1}), phosphate and silicate concentrations to be zero, and T, S, and P of 15°C, 35, and 0 dbar, respectively.

Sensitivities of $[CO_3^{2-}]$, Ω_{CaCO3}, and $[HCO_3^-]/[H^+]$ to changing P, S, or T (Fig. 4) were calculated with CO2SYS assuming phosphate and silicate concentrations to be zero, a constant pCO_2 of 400 µatm, and a constant TA of ~2350 µmol kg^{-1}. The parameters that were not varied within the particular calculation were set to constant values of 15°C, 35, and 0 dbar for T, S, and P, respectively.

Calcification related measurements and corresponding DIC, TA, T, S, and nutrient data of experiments with different species (Schneider and Erez, 2006; Gazeau et al., 2011; Keul et al., 2013, Fig. 2) were downloaded from the PANGAEA data server (www.pangaea.de).

Surface ocean (0 - 100 m) carbonate chemistry, physical, and nutrient data of the meridional Atlantic transect measured during CLIVAR cruises in 2003 (Peltola et al., 2003) and 2005 (Wanninkhof et al., 2006) (Fig. 5A, C, E) were downloaded from the CARINA data synthesis homepage (http://cdiac.ornl.gov/oceans/CARINA/). Water column carbonate chemistry, physical, and nutrient data between 1988 and 2012 from the ALOHA time-series station (Fig. 7) in the central Pacific (22° 45’ N 158° 00’ W) were downloaded from the ALOHA website (http://aco-ssds.soest.hawaii.edu/ALOHA/).

3 Results and discussion

3.1 Is HCO_3^- or CO_3^{2-} the more suitable inorganic carbon substrate for calcification?

It is important to determine the calcification-relevant inorganic carbon species taken from seawater in order to understand the calcification response of marine organisms to changing carbonate chemistry. Most studies assume that HCO_3^- rather than CO_3^{2-} is the key inorganic carbon ion (e.g. Allemand et al., 2004; Mackinder et al., 2010; Stumpp et al., 2012; Taylor et al., 2012). However, proof of this assumption on a physiological level is still missing because attempts to unequivocally determine the inorganic carbon molecule transported by molecular transport systems were not successful so far (Pushkin and Kurtz, 2006; Mackinder et al., 2010; Lee et al., 2013; Romero et al., 2013). Furthermore, the uptake mechanisms for inorganic carbon are highly diverse among the various calcifying taxa so that generalization of physiological principles would be difficult (see section 3.6 for a discussion on this topic). It may therefore be helpful to approach this question differently and ask more generally whether HCO_3^- or CO_3^{2-} would be the more suitable inorganic carbon substrate for calcification. Three different perspectives will be addressed in the following.

3.1.1 Abundance

HCO_3^- is usually the most abundant DIC species in seawater. At pH_f (free scale) of 8.1 it contributes ~90% to the total DIC pool while CO_3^{2-} contributes less than 10%. Thus, molecular CO_3^{2-} transporters would require a nine times higher affinity to their substrate than

HCO_3^- transporters. It may therefore make more sense for an organism to rely on the largest inorganic carbon pool if molecular transporters take the ions directly from seawater (Mackinder et al., 2010).

3.1.2 Homeostasis

The hydration timescale of CO_2 ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) is comparatively slow (~10 seconds), while the hydrolysis of HCO_3^- ($HCO_3^- \leftrightarrow CO_3^{2-} + H^+$) is fast (~10^{-7} seconds; Zeebe and Wolf-Gladrow, 2001; Schulz et al., 2006). Assuming a transcellular pathway, selectively incorporated CO_3^{2-} that is transported through cytosol with a typical pH around ~7.0 - 7.4 (Madshus, 1988) would quickly turn into HCO_3^- unless the transfer is faster than 10^{-7} seconds. In the likely case that the transfer takes longer, CO_3^{2-} would bind a proton in the cytosol and be transported as HCO_3^- to the site of calcification where the proton would subsequently be released back to the cytosol during $CaCO_3$ precipitation. Hence, the cytosolic pH would remain stable in case of selective CO_3^{2-} uptake as long as CO_3^{2-} uptake and $CaCO_3$ precipitation occur at the same rate. However, both processes may occasionally run out of equilibrium for short periods. In these cases, the utilization of CO_3^{2-} as inorganic carbon source would constitute a substantial risk for the orgamisms' pH homeostasis. Excess CO_3^{2-} uptake would elevate cytosolic pH while excess $CaCO_3$ precipitation would reduce it. In contrast, a selective uptake of HCO_3^- from seawater would perturb the cytosolic pH to a much smaller extent when HCO_3^- uptake and $CaCO_3$ precipitation are not entirely balanced because HCO_3^- has a relatively low potential to accept or donate H^+ at a typical pH of 7.2. It may therefore be easier for calcifiers to keep cytosolic pH stable at ~7.2 when using HCO_3^-.

3.1.3 Stability

Seawater carbonate chemistry conditions are relatively stable on longer timescales but can fluctuate substantially on a seasonal and diurnal cycle (Takahashi et al., 1993; Thomsen et al., 2010; Shaw et al., 2013), especially in diffusive boundary layers of organisms (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012; Glas et al., 2012a; Agostini et al., 2013). Fluctuations are mostly induced by photosynthetic or respiratory turnover of CO_2. The magnitude of fluctuation scales with the productivity of the ecosystem (Schulz and Riebesell, 2013) but fluctuations should usually stay within the ~6.5 - 9 pH range. HCO_3^- is dominant and has a relatively stable concentration in this entire pH range, while [CO_3^{2-}] is highly variable. In the habitat of a temperate coraline algae, for example, typical diurnal pH fluctuations can range from ~8.4 at day to ~7.6 at night (Cornwall et al., 2013). These changes would translate in a moderate day/night difference of ~30% in [HCO_3^-] but a pronounced difference of more than 450% in [CO_3^{2-}]. Hence, HCO_3^- is the much more reliable inorganic carbon source for calcification as it shows significantly smaller variability.

3.2 Relationship between calcification and [CO_3^{2-}] or [HCO_3^-]/[H^+] in existing datasets

If the common assumption holds and marine calcifiers primarily utilize HCO_3^- instead of CO_3^{2-} as $CaCO_3$ substrate (see previous section) then correlations between calcification and [CO_3^{2-}] or Ω_{CaCO3} are not useful under non-corrosive conditions. Still, these correlations often yield high coefficients of determination (R^2) even if conditions are super-saturated (Schneider and Erez, 2006; Marubini et al., 2008; de Putron et al., 2011; Gazeau et al., 2011; Keul et al., 2013; Waldbusser et al., 2014). This dilemma can be resolved when considering the proportionality between [CO_3^{2-}], $\Omega_{CaCO3,}$ and [HCO_3^-]/[H^+] derived above. Every correlation between calcification and [CO_3^{2-}] or Ω_{CaCO3} will be identical to the corresponding correlation with [HCO_3^-]/[H^+] when T, S, and P are stable (see Eqs. (9), (12)).

This is illustrated with data from three publications where the influence different carbonate chemistry parameters on calcification rates was disentangled (Schneider and Erez, 2006; Gazeau et al., 2011; Keul et al., 2013) (Fig. 2). All three studies conclude that [CO_3^{2-}] determines calcification rates although calcification rates or calcification related measurements of the hermatypic coral *Acropora eurystoma* (Schneider and Erez, 2006), the benthic foraminifer *Ammonia* spec. (Keul et al., 2013), and larvae of the Pacific oyster *Crassostrea gigas* (Gazeau et al., 2011) correlate equally well to [HCO_3^-]/[H^+].

3.3 Conceptual basis for the calcification control of [HCO_3^-]/[H^+]

Implicit in the [HCO_3^-]/[H^+] ratio is the thought that biotic $CaCO_3$ precipitation is balanced by the stimulating influence of an inorganic carbon substrate and the negative influence of an inhibitor (Bach et al., 2011; 2013; 2015; Jokiel, 2011a, 2011b, 2013; Jokiel et al., 2014). Higher [HCO_3^-] would stimulate calcification rates as a substrate whereas high seawater [H^+] would inhibit them.

When both, [HCO_3^-] and [H^+] increase, calcification would be balanced by the degree of change of these two ions. [H^+] increases profoundly under ocean acidification while [HCO_3^-] increases only marginally (Fig. 1, see also Schulz et al., 2009). Here, changing [H^+] should be of dominant control (Bach et al., 2011). When increasing [H^+] is paralleled by a significant increase of [HCO_3^-], then the additional bicarbonate could potentially compensate the inhibitory proton effect to some extent (Bach et al. 2015). Indeed, HCO_3^- uptake is a common mechanism to buffer acidosis and might be facilitated at higher HCO_3^- availability (Boron, 2004; Melzner et al., 2009; Stumpp et al., 2012).

The inhibition by high seawater [H^+] is tightly linked to the production of $CaCO_3$ from Ca^{2+} and HCO_3^- because calcification is then a source of H^+ ($Ca^{2+} + HCO_3 \rightarrow CaCO_3 + H^+$). H^+ generated that way would subsequently have to be released back into seawater to avoid acidification at the site of calcification. This removal should be relatively easy when seawater [H^+] is low (i.e. pH is high). It could become more difficult, however, when seawater [H^+] rises and the inside-out [H^+] gradient shrinks (Cyronak et al., 2015; Jokiel, 2011b; Taylor et al., 2011; Stumpp et al., 2012; Venn et al., 2013).

3.4 Similarities and differences between the DIC/[H^+] and the [HCO_3^-]/[H^+] ratio

In a series of papers Jokiel (2011a, 2011b, 2013) proposed that carbonate chemistry controls calcification rates in corals through the combined influence of DIC (“reactant”) and H^+ (“inhibitor”) and noted that favorable carbonate chemistry conditions are established when the ratio of DIC to [H^+] is high. Hence, the underlying thought implicit in the DIC/[H^+] ratio is identical to that implemented in the “substrate-inhibitor-ratio” defined here as [HCO_3^-]/[H^+].

Jokiel (2011a, 2011b, 2013) also noted that there is a linear correlation between DIC/[H^+] and [CO_3^{2-}]. The correlation observed by Jokiel exists because the DIC pool is dominated by HCO_3^- ions under normal pH conditions (see section 3.1.1) and therefore typically follows the same rules as the proportionality between [HCO_3^-]/[H^+] and [CO_3^{2-}] uncovered in section 2.1. However, when the fraction of HCO_3^- in the DIC pool declines, the DIC/[H^+] vs. [CO_3^{2-}] correlation starts to increasingly deviate from linearity. In the oceans, noticeable deviations start in the pCO_2 range below 250-500 µatm, where an exponentially increasing fraction of the DIC pool is present as CO_3^{2-} (Fig. 3). Thus, exchanging DIC/[H^+] with [CO_3^{2-}] to explain the calcification response to carbonate chemistry (comparable to what has been done in Fig. 2) is not meaningful when pCO_2 is below this range. This problem does not exist for the [HCO_3^-]/[H^+] ratio where the linear relation holds under all carbonate chemistry conditions as long as T, S, and P are constant (Fig. 3).

Whether the DIC/[H^+] ratio proposed by Jokiel (2011a, 2011b, 2013) or the [HCO_3^-]/[H^+] ratio could be the more meaningful parameter to explain the carbonate chemistry response of calcification depends on the investigated organism. The DIC/[H^+] ratio would be more meaningful if the organism takes up all DIC species in the same proportion as present in seawater while the [HCO_3^-]/[H^+] ratio would be more appropriate when selective uptake on HCO_3^- occurs (see also section 3.6.4).

3.5 Global implications

The following paragraphs will address to what extent our view on carbonate chemistry control of calcification in the oceans could be modified when we consider [HCO_3^-]/[H^+] rather than [CO_3^{2-}] or Ω_{CaCO3} as the most influential parameter. Before starting the discussion I would like to emphasize, however, that carbonate chemistry patterns discussed here are just one among other abiotic (e.g. temperature or light) or biotic (e.g. food availability or competition) factors which must also be taken into consideration when trying to understand the patterns of calcification in the oceans.

3.5.1 Latitudinal and vertical gradients in [HCO_3^-]/[H^+], [CO_3^{2-}], and Ω_{CaCO3}

The proportionality between [CO_3^{2-}], Ω_{CaCO3}, and [HCO_3^-]/[H^+] derived in section 2.1 is only valid as long temperature, salinity, and pressure are constant and do not alter K_2^* and K_{sp}^*. If these parameters change, then the proportionality no longer holds and the response of calcification would be different, depending on whether calcifiers react to [CO_3^{2-}], Ω_{CaCO3}, or [HCO_3^-]/[H^+].

The influence of temperature, salinity, and pressure is illustrated in Fig. 4. Increasing pressure has a negative effect on all three carbonate system components. It is most pronounced on Ω_{CaCO3} and weakest on [CO_3^{2-}] (Fig. 4A). Increasing salinity has a positive influence on [CO_3^{2-}] and Ω_{CaCO3} but a negative one on [HCO_3^-]/[H^+] (Fig. 4B). Its influence, however, is low since salinity gradients in the oceans are generally too weak to be of high relevance in this context. Increasing temperature has a profound positive impact on [CO_3^{2-}] and Ω_{CaCO3} but almost no influence on [HCO_3^-]/[H^+] (Fig. 4C).

In combination, these factors cause a highly interesting difference of [CO_3^{2-}], Ω_{CaCO3}, and [HCO_3^-]/[H^+] on a latitudinal gradient. While Ω_{CaCO3} and [CO_3^{2-}] decrease 2-3 fold towards the poles, [HCO_3^-]/[H^+] is almost constant over the same range (Figs. 5, 6). This inconsistency is mostly due to different temperature sensitivities among the three parameters. Cooler temperatures in high latitudes lead to higher solubility of CO_2 which results in an equilibrium shift away from [CO_3^{2-}] towards [CO_2] and [HCO_3^-] (Eq.(4)). Accordingly, [CO_3^{2-}] declines towards the poles. Ω_{CaCO3} follows the concentration of CO_3^{2-} since [Ca^{2+}] is too stable to be of any relevance. The slight poleward increase of [HCO_3^-] is balanced by the concomitant increase in [H^+] which explains the stability of [HCO_3^-]/[H^+] over the latitudinal gradient. Thus, carbonate chemistry conditions for biotic $CaCO_3$ production would be fairly constant over the entire surface ocean if controlled by [HCO_3^-]/[H^+], whereas they would show a profound poleward deterioration if determined by [CO_3^{2-}] or Ω_{CaCO3} (Figs. 5, 6).

Vertically, [CO_3^{2-}] and Ω_{CaCO3} decrease more severely than [HCO_3^-]/[H^+] from surface to about a thousand meters depth (~5-fold vs. ~3-fold decrease, Fig. 7). This has two reasons. First, the temperature decline, which is strongest in the upper few hundred meters, negatively affects [CO_3^{2-}] and Ω_{CaCO3} whereas [HCO_3^-]/[H^+] remains unaffected (Fig. 4C). Second, the increase of [H^+] from 0-1000 m due to respiratory CO_2 release is paralleled by ~30% increases of [HCO_3^-] which mitigates the decline of [HCO_3^-]/[H^+]. Below a thousand meters, all three carbonate system parameters are relatively stable. Of the three carbonate system parameters considered here, [HCO_3^-]/[H^+] is the most homogeneous when comparing the development over the entire water column. Thus, the deterioration of carbonate chemistry conditions for biotic $CaCO_3$ formation with depth would be less pronounced if it was controlled by [HCO_3^-]/[H^+] (Fig. 7).

3.5.2 Implications for ocean acidification research

The ongoing perturbation of the surface ocean by anthropogenic CO_2 causes a decline of Ω_{CaCO3}, [CO_3^{2-}], and [HCO_3^-]/[H^+]. The magnitude of change in all three parameters is very similar to each other on global average (Fig. 1). Until 2100 they decrease to about half the value in the year 2000 (Figs. 1, 5). The two different latitudinal patterns for Ω_{CaCO3}, [CO_3^{2-}], or [HCO_3^-]/[H^+] are, however, conserved in the course of climate change (Fig. 6). Hence, latitudinal differences among the three parameters are prevailing for longer timescales and are not restricted to the currents status of the carbonate system.

The presence of two highly different latitudinal patterns has important implications for ocean acidification research. Polar regions have been identified as the most severely acidification-affected regions on Earth as they are the first to experience corrosive conditions (i.e. Ω_{CaCO3} <1; Orr et al., 2005; Fabry et al., 2009; Hofmann et al., 2010). The vulnerability of high latitudes is therefore explained by an abiotic process - i.e. $CaCO_3$ dissolution. The study presented here deals with controls on the opposite process - i.e. biotic $CaCO_3$ formation. Dissolution is determined by Ω_{CaCO3} and there is no doubt that polar regions are most severely affected (see also Fig. 6). From the production perspective, however, this is not necessarily the case. Ocean acidification would be equally harmful in warm-water habitats as in the polar realm in case $CaCO_3$ formation is controlled by $[HCO_3^-]/[H^+]$ and not $[CO_3^{2-}]$ or Ω_{CaCO3}. Thus, when high latitude organisms find a way to efficiently protect their crystal skeletons from corrosive seawater, then they may not be more vulnerable to ocean acidification than their warm water counterparts.

3.6 Limitations and uncertainties

This study has argued that a substrate-inhibitor ratio like $[HCO_3^-]/[H^+]$ (or DIC/[H+]; Jokiel 2011a, 2011b, 2013) could be a useful measure to assess which carbonate chemistry conditions could be favorable for calcification. However, it must also be recognized that such a rather general view on carbonate chemistry control of calcification has its limitations when it comes to a more detailed physiological level. Calcification follows an enormous variety of pathways among the different taxa, with distinct inorganic carbon uptake mechanisms, and distinct sensitivities to H^+. Thus, the capability of the substrate-inhibitor ratio to predict calcification rates can always be confounded by taxon-specific physiological features. The most important limitations and uncertainties for the ability of $[HCO_3^-]/[H^+]$ to serve as predictor variable for the carbonate chemistry control of calcification will be discussed in the following.

3.6.1 Corrosive conditions - accounting for the difference between gross and net calcification

When discussing the influence of carbonate chemistry on calcification, distinction needs to be made between formation and dissolution of $CaCO_3$ since these two processes are possibly controlled by different carbonate chemistry parameters. The roles of $CaCO_3$ formation and dissolution are incorporated into the terms gross and net calcification. The former exclusively refers to the precipitation of $CaCO_3$ whereas the latter accounts for both precipitation and dissolution. The ratio of $[HCO_3^-]$ and $[H^+]$ can potentially be very useful to determine gross calcification which equals net calcification under non-corrosive conditions (i.e. $\Omega_{CaCO3} > 1$). When Ω_{CaCO3} falls below 1, however, the control of $[HCO_3^-]/[H^+]$ on gross calcification would be obscured by the abiotic influence of Ω_{CaCO3} on dissolution. Accordingly, corrosive conditions would require consideration of both $[HCO_3^-]/[H^+]$ and Ω_{CaCO3} in order to correctly estimate the impact of carbonate chemistry on net biotic $CaCO_3$ formation.

Areas with corrosive conditions will expand under ocean acidification (Orr et al., 2005) so that $CaCO_3$ dissolution becomes more widespread problem for future calcifiers. However, dealing with dissolution of $CaCO_3$ is only of secondary relevance for living organisms as everything that dissolves needs to be formed in the first place. Hence, although dissolution processes cannot be left unconsidered, it is reasonable from a biological point of view to focus on the processes that control formation of $CaCO_3$.

3.6.2 Applicability of $[HCO_3^-]/[H^+]$ in the geological record

The restriction of $[HCO_3^-]/[H^+]$ to gross calcification rates (see previous section) limits its applicability in the geological record because the information on $CaCO_3$ accumulation conserved in the sediments is not only affected by gross calcification but also by post-production dissolution and abiotic modifications of $CaCO_3$ during diagenesis which are both controlled by Ω_{CaCO3}. Thus, in order to verify the substrate-inhibitor concept for the geological record we would need a reliable proxy for exclusively biotic gross calcification. Conversely, the application of Ω_{CaCO3} to explain trends in $CaCO_3$ sedimentation (e.g. Hönisch et al., 2012; Ridgwell, 2005) would be reasonable because sedimentation involves both precipitation and dissolution and Ω_{CaCO3} is a good indicator for the former (under constant T, S, P) and the key parameter for the latter.

3.6.3 Extreme concentrations of HCO_3^- and H^+

In some studies, calcification rates correlated better with $[HCO_3^-]$ than with $[CO_3^{2-}]$ (and thus $[HCO_3^-]/[H^+]$) (Bach et al., 2013; Jury et al., 2010), which challenges the potential of $[HCO_3^-]/[H^+]$ to serve as predictor variable for $CaCO_3$ production. However, the dominant control of HCO_3^- in these particular studies can be easily understood when considering the low HCO_3^- concentrations at which its dominance actually occurred. The influence of $[HCO_3^-]$ relative to $[H^+]$ will become more and more influential under conditions where HCO_3^- becomes increasingly limiting. Conversely, any influence of $[HCO_3^-]$ will become negligible when the organism is fully saturated with it. Under these circumstances calcification is most likely primarily controlled by $[H^+]$ (Bach et al., 2011; Jokiel 2011b; Taylor et al., 2011). Thus, the potential of $[HCO_3^-]/[H^+]$ in predicting calcification is low at rather 'extreme' carbonate chemistry conditions where the influence of either HCO_3^- or H^+ strongly outweighs the other. Its potential should be high, however, under conditions where the investigated organism is responsive to both $[HCO_3^-]$ and $[H^+]$.

3.6.4 Transport of seawater

Some foraminifera and coral species have been reported to transfer calcification-relevant ions to the site of $CaCO_3$ precipitation by means of seawater transport (Bentov et al., 2009; de Nooijer et al., 2009; Gagnon et al., 2012; Tambutté et al., 2012). In the case of foraminifera, seawater is engulfed in membrane vesicles, transported to the site of calcification, and on its way alkalized to increase $[CO_3^{2-}]$ (Bentov et al., 2009; de Nooijer et al., 2009). In the case of corals, seawater (or at least its constituents smaller 20 nm; Tambutté et al., 2012) may pass epithelia and reach the $CaCO_3$ skeleton via the paracellular pathway. For both processes (seawater endocytosis and seawater leakage along the intercellular space), all DIC species are potential inorganic carbon sources to fuel calcification. Hence, for organisms which purely rely on seawater endocytosis/leakage, the DIC:[H+] ratio proposed by Jokiel (2011a, 2011b, 2013) could be more appropriate to explain the calcification response to carbonate chemistry than $[HCO_3^-]/[H^+]$.

3.6.5 CO_2 as inorganic carbon source for calcification

Some organisms receive significant amounts of inorganic carbon used for calcification from respiratory sources (Pearse, 1970; Erez, 1978; Sikes et al., 1981; Tanaka et al., 1986; Furla et al., 2000). Here, organisms do not exclusively rely on direct inorganic carbon utilization from seawater but supplement calcification to a variable degree with CO_2 gained intracellularly from respired biomass. This CO_2 utilization may be further strengthened (1) when metabolic CO_2 is ‘trapped’ inside the organisms through the establishment of pH gradients which limit the diffusive loss of CO_2 (Bentov et al., 2009, Glas et al., 2012b) or (2) when CO_2 is transported actively towards the site of calcification (de Nooijer et al., 2014). Thus, CO_2 reacting with H_2O to form HCO_3^- and H^+ (catalyzed by the ubiquitous enzyme carbonic anhydrase) could be an alternative inorganic carbon source for calcification in particular taxa. The potential control of seawater $[HCO_3^-]/[H^+]$ on $CaCO_3$ precipitaiton may therefore be weakened by the degree to which calcifiers utilize CO_2 as inorganic carbon source.

3.6.6 Photoautotrophic calcifiers

Photoautotrophic calcifiers such as coccolithophores or zooxanthellate corals not only interact with HCO_3^- and H^+ but also with CO_2. Photosynthetic and calcification-related processes are physiologically coupled within photoautotrophs (Paasche, 2002; Allemand et al., 2004). Accordingly, calcification rates will be affected indirectly when photosynthesis is

CO_2 limited (Bach et al., 2015). A valuable measure to determine the potential of CO_2 to limit growth and photosynthesis is $K_{1/2}$ which denotes the CO_2 concentration where the process runs at half of its maximum. Available $K_{1/2}$ measurements suggest that CO_2 limitation mostly occurs well below CO_2 concentrations typically encountered by the organisms in their respective habitats (Rost et al., 2003; Sett et al., 2014). Thus, its influence should rarely interfere with the influence of $[HCO_3^-]/[H^+]$ under natural conditions.

4 Conclusions

A variety of studies highlighted that carbonate chemistry controls calcification through the balance of stimulation by an inorganic carbon substrate (HCO_3^- or DIC) and inhibition by protons (e.g. Bach et al., 2011; Jokiel 2011a; Thomsen et al., 2015). Other studies found that $[CO_3^{2-}]$ or Ω_{CaCO3} are the carbonate chemistry parameters which best predict calcification (e.g. Schneider and Erez, 2006; de Putron et al., 2011; Gazeau et al., 2011; Waldbusser et al., 2014). The proportionalities between $[CO_3^{2-}]$ or Ω_{CaCO3} and the $[HCO_3^-]/[H^+]$ ratio derived in Eqs. (9) and (12) provide the chemical basis to reconcile these conflicting results. Every correlation between calcification and $[CO_3^{2-}]$ or Ω_{CaCO3} will be identical to the corresponding correlation with $[HCO_3^-]/[H^+]$ when T, S, and P are stable. Thus, the good correlations to $[CO_3^{2-}]$ and Ω_{CaCO3} that have previously been reported may have simply masked the combined influence of $[HCO_3^-]$ and $[H^+]$ (see also findings by Jokiel, 2011a, 2011b, 2013; Jokiel et al., 2014) which are arguably the physiologically more meaningful parameters to correlate gross calcification with (Bach et al., 2013; Jokiel, 2013; Thomsen et al., 2015; section 3.1 and 3.3).

Accounting for the influence of $[HCO_3^-]/[H^+]$ in controlling $CaCO_3$ formation would also have interesting implications for how we assess carbonate chemistry conditions and calcification along a latitudinal gradient. A comparison of present and future $[CO_3^{2-}]$, Ω_{CaCO3}, and $[HCO_3^-]/[H^+]$ patterns in the surface ocean revealed a strong poleward decline in $[CO_3^{2-}]$ and Ω_{CaCO3} but no decline in $[HCO_3^-]/[H^+]$. These highly different latitudinal patterns are conserved during climate change. Thus, it may turn out that ocean acidification is globally a more uniform problem for biotic $CaCO_3$ formation than previously thought.

Acknowledgements

I thank Kai Schulz and Ulf Riebesell for our frequent discussions about carbonate chemistry and calcification which led to the preparation of this manuscript. I am also grateful to Toste

Tanhua for support on CARINA data, Jan Taucher for sharing the UVic model output, as well as Allanah Paul and Jan Taucher for proof-reading the manuscript. This study profited from the motivating and constructive reviews by Lennart de Nooijer, Paul Jokiel, and two anonymous reviewers. It was funded by the Federal Ministry of education and research (BMBF) in the framework of the Biological Impacts of Ocean Acidification II (BIOACID II) project (W.P. 1.3).

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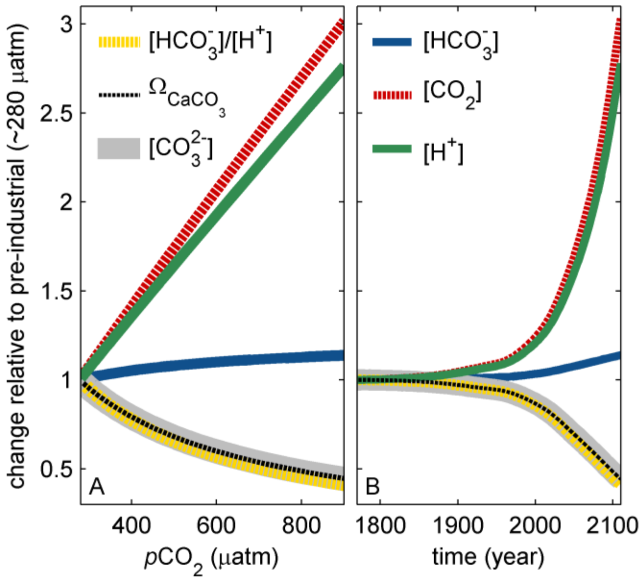

Figure 1. Change of different surface ocean carbonate chemistry parameters under "business-as-usual" climate change scenario SRES A2 with (A) increasing atmospheric pCO_2 and (B) over time. All changes are relative to the pre-industrial CO_2 partial pressure of ~280 µatm. Note that the slight deviations from proportionality between [CO_3^{2-}], Ω_{CaCO3}, and [HCO_3^-]/[H^+] are the result of changes in global average temperature and salinity in the course of climate change which affect K_2^* and K_{sp}^* (see section 3.5 for further details).

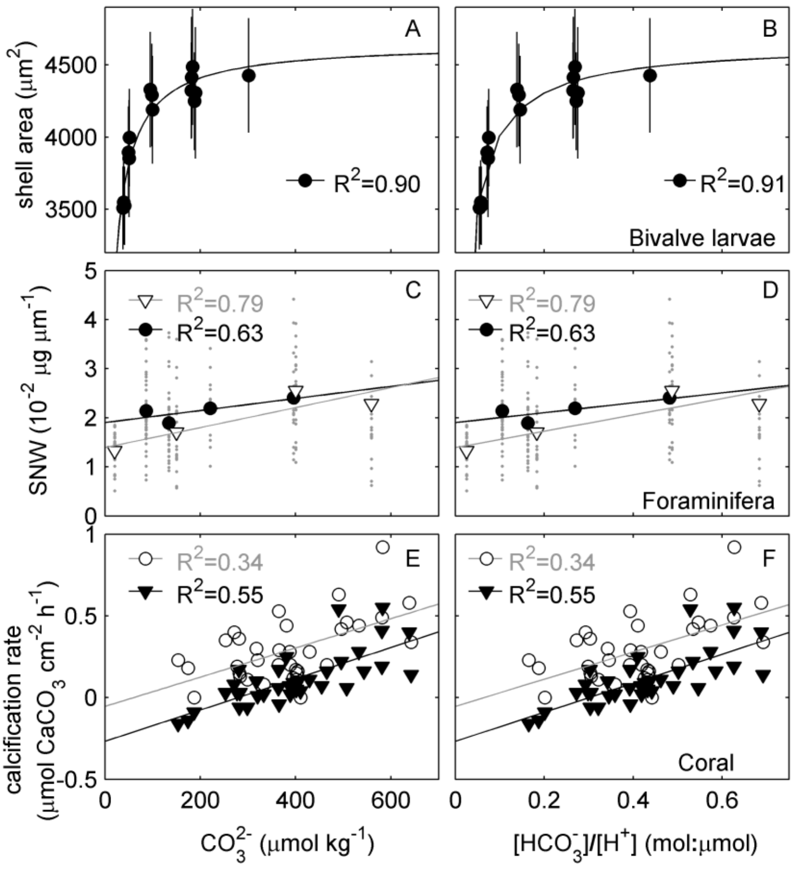

Figure 2. Correlations between calcification related measurements and [CO_3^{2-}] (left panel) or [HCO_3^-]/[H^+] (right panel). (A, B) Shell areas of D-veliger larvae reached 3 days after incubating embryos of the Pacific oyster *Crassostrea gigas* (Gazeau et al., 2011). (C, D) Size normalized weight (SNW) of the foraminifer *Ammonia* spec. in its asexually reproducing life cycle stage (Keul et al., 2013). Black dots: constant DIC, variable CO_2. White triangles: constant pH, variable CO_2. (E, F) Calcification rates of the symbiont-bearing hermatypic coral *Acropora eurystoma* (Schneider and Erez, 2006). White dots and black triangles refer to incubations at light and dark, respectively.

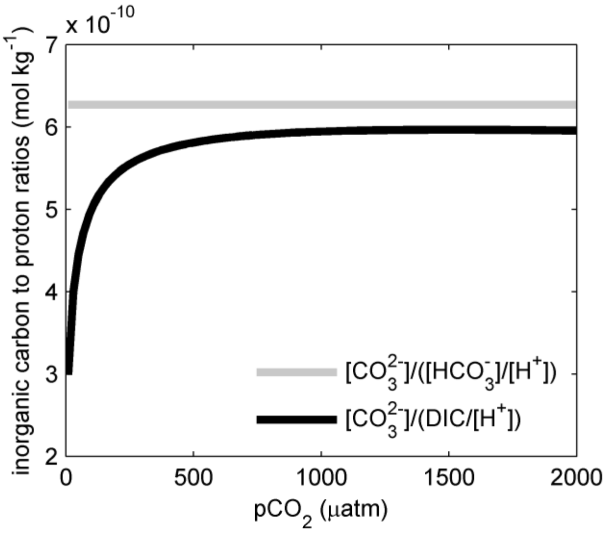

Figure 3. Ratio of [CO_3^{2-}] and [HCO_3^-]/[H^+] compared to the ratio of [CO_3^{2-}] and DIC/[H^+]. [CO_3^{2-}]/([HCO_3^-]/[H^+]) is constant, since CO_3^{2-} and [HCO_3^-]/[H^+] are proportional to each other under constant temperature, salinity and pressure (Eq. (9)). In contrast, the correlation between [CO_3^{2-}] and DIC/[H^+] only works well when the majority of DIC is found in the HCO_3^- pool. This is the case for pCO$_2$ values larger than ~250-500 µatm, where [CO_3^{2-}]/(DIC/[H^+]) shows very little change. Below this threshold, however, the correlation starts deviating from linearity since an exponentially increasing proportion of the DIC pool is present as CO_3^{2-}.

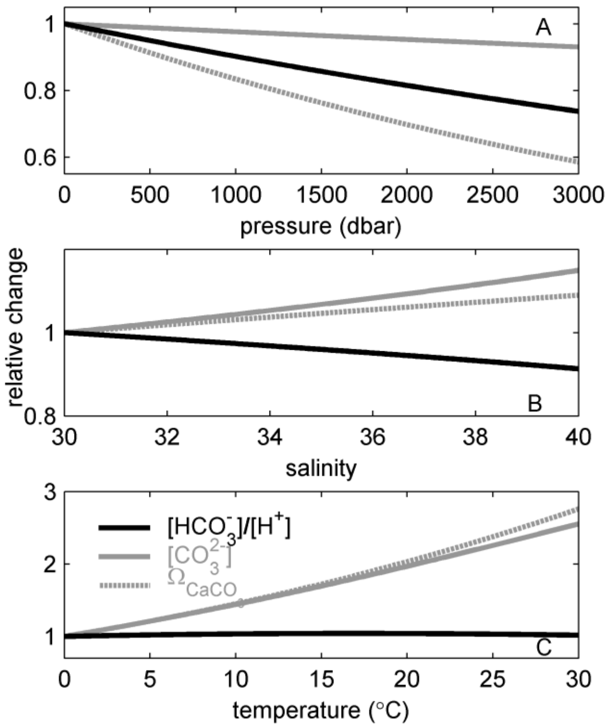

Figure 4. Relative change of $[CO_3^{2-}]$, Ω_{CaCO3}, and $[HCO_3^-]/[H^+]$ on (A) a pressure gradient, (B) a temperature gradient, and (C) a salinity gradient.

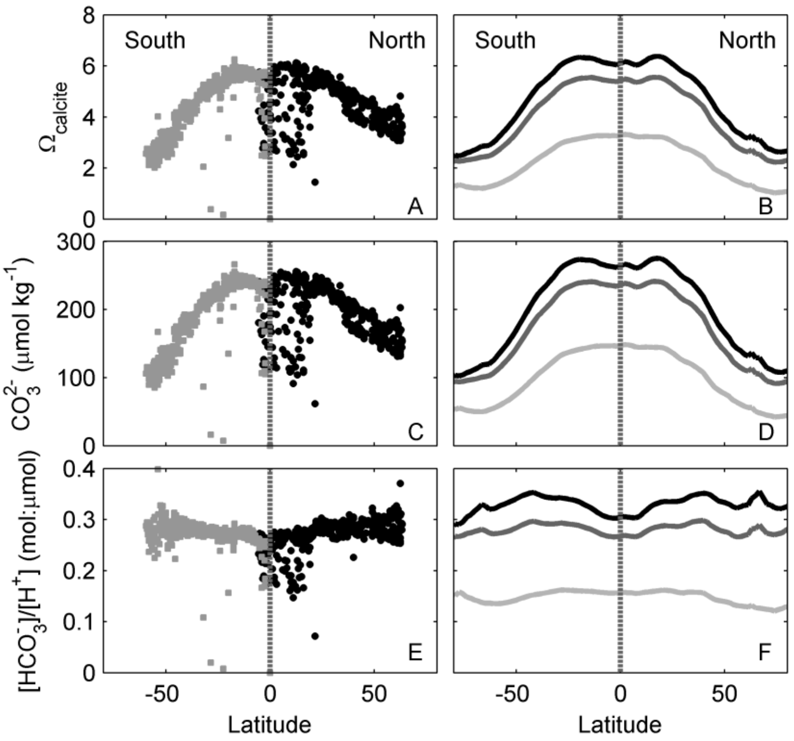

Figure 5. Change of Ω_{CaCO3}, $[CO_3^{2-}]$, and $[HCO_3^-]/[H^+]$ in the surface ocean along a meridional gradient. The left panel (A, C, and E) shows compiled surface ocean (0 - 100 m) data from two north to south transects in the Atlantic, measured during CLIVAR CO_2 cruises in 2003 (black dots) and 2005 (grey squares), respectively. The right panel (B, D, and F) shows the latitudinal surface ocean (0 -50 m) average calculated with the UVic model for the years 1770 (black line), 2000 (grey line), and 2100 (light grey line). The dotted vertical line illustrates the equator.

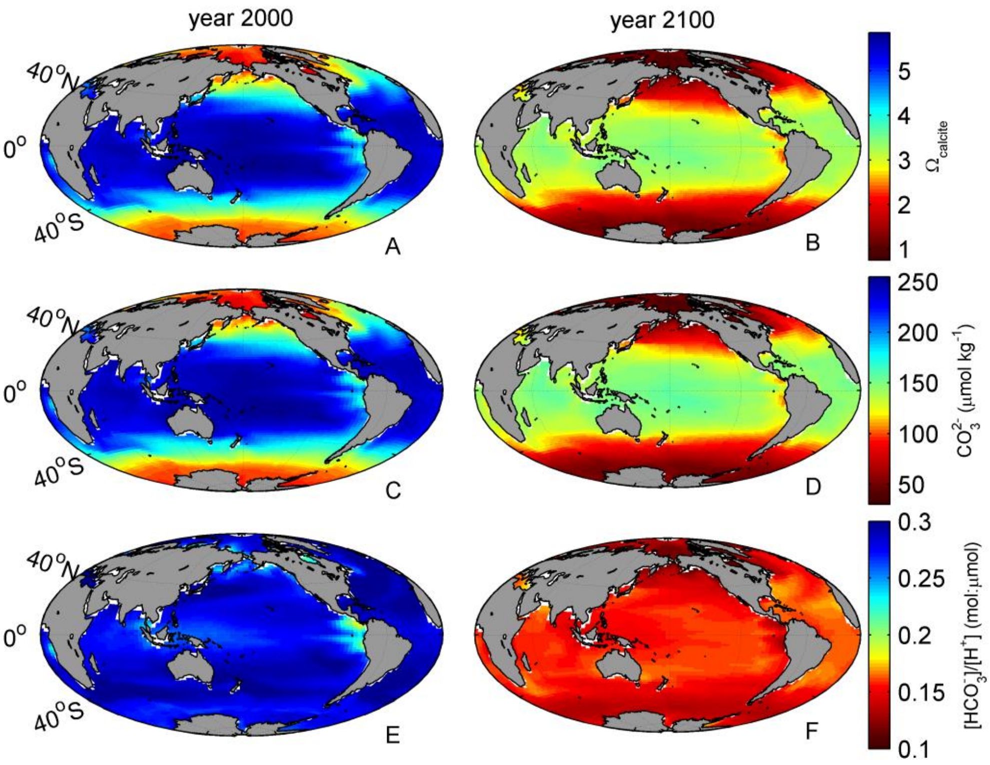

Figure 6. Surface ocean (0 – 50 m) Ω_{CaCO3} (A, B), $[CO_3^{2-}]$ (C, D), and $[HCO_3^-]/[H^+]$ (E, F) in the year 2000 (left panel) compared to 2100 (right panel) calculated with the UVic model. The pronounced latitudinal gradient of Ω_{CaCO3} and $[CO_3^{2-}]$ is absent in $[HCO_3^-]/[H^+]$ (see also Fig. 5). $[HCO_3^-]/[H^+]$ is quite homogeneous in 2000 and 2100 in all major ocean basins, with only some regional anomalies. These are found in some coastal areas (e.g. in the Bering Sea) and in eastern boundary upwelling systems, most noticeably off the west coast of South and Central America.

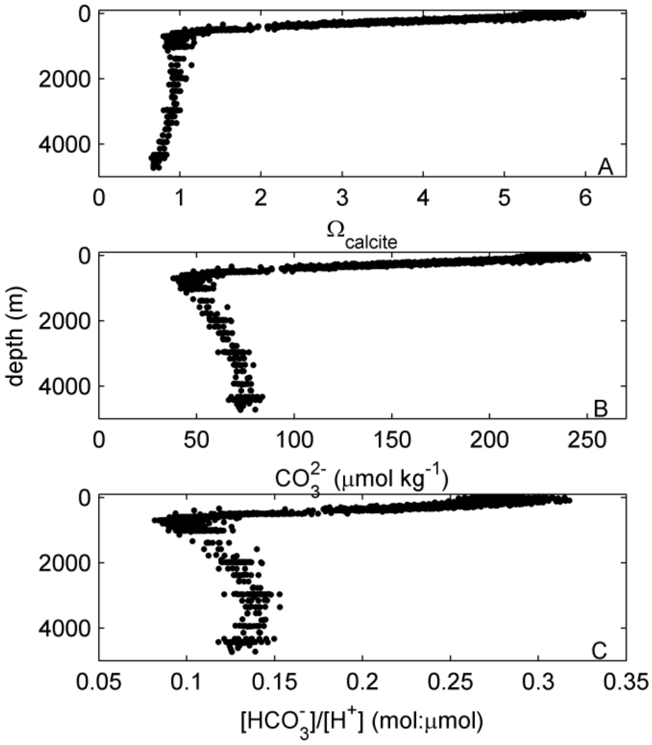

Figure 7. Change of (A) Ω_{CaCO3}, (B) [CO_3^{2-}], and (C) [HCO_3^-]/[H^+] on a depth gradient at ALOHA time-series station near Hawaii (22° 45’ N 158° 00’ W). Compiled data from 1988 - 2012 which was downloaded from the ALOHA website (http://aco-ssds.soest.hawaii.edu/ALOHA/).