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Key Points:

- Alternative indicators of biological calcification have implications for how carbonate chemistry variations are interpreted in OA studies
- The design of many CO_2 manipulation experiments gives rise to Ω_{Ar} correlations that can be misleading
- Coastal carbonate system dynamics may complicate accurate assessments of ocean acidification impacts on some species

Supporting Information:

- Supporting Information S1

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Consideration of coastal carbonate chemistry in understanding biological calcification

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Abstract Correlations between aragonite saturation state (Ω_{Ar}) and calcification have been identified in many laboratory manipulation experiments aiming to assess biological responses to ocean acidification (OA). These relationships have been used with projections of Ω_{Ar} under continued OA to evaluate potential impacts on marine calcifiers. Recent work suggests, however, that calcification in some species may be controlled by the ratio of bicarbonate to hydrogen ion, or the substrate-to-inhibitor ratio (SIR), rather than Ω_{Ar}. SIR and Ω_{Ar} are not always positively correlated in the natural environment, which means that Ω_{Ar} can be a poor indicator of the calcifying environment when Ω_{Ar}->1. Highly variable carbonate chemistry in the coastal zone challenges our ability to monitor fluctuations in Ω_{Ar}, SIR, and the Ω_{Ar}-SIR relationship making it difficult to assess biological OA exposures and vulnerability. Careful consideration of natural variability throughout ocean environments is required to accurately determine the influence of OA on biological calcification.

1. Introduction

The declining pH of ocean surface waters caused by anthropogenic carbon dioxide (CO_2) absorption, commonly referred to as ocean acidification [*Doney et al.*, 2009; *Ciais et al.*, 2013], is observable throughout the global open ocean [e.g., *Bates et al.*, 2014; *Sutton et al.*, 2014; *Lauvset et al.*, 2015]. The dramatic rate and spatial scale over which ocean acidification (OA) is occurring has led to an eruption of scientific inquiry into how biological organisms will be impacted by changing marine chemistry [e.g., *Fabry et al.*, 2008; *Guinotte and Fabry*, 2008; *Hoegh-Guldberg et al.*, 2008; *Doney et al.*, 2009; *Hofmann et al.*, 2010; *Kroeker et al.*, 2010, 2013; *Gattuso and Hansson*, 2011; see *Browman*, 2016]. Over the past decade, the first direct links between OA and biological calcification in the natural environment were made in the Pacific Northwest, spurring local action to address the issue [*Adelsman and Binder*, 2012; *Barton et al.*, 2012, 2015; *Waldbusser et al.*, 2014]. As more scientists engage in biological OA research and adaptation strategies are pursued, there is a critical need to develop consistent approaches and outline key parameters that must be monitored, particularly in the coastal zone where most biological OA manipulations studies take place.

Perhaps the most confounding area of OA research at present is the dissimilarity in perspectives between (and among) biologists and chemists regarding which inorganic carbon parameter most accurately reflects the influence of OA on marine calcifiers. From the biological perspective, recently summarized by *Roleda et al.* [2012] and *Cyronak et al.*, 2015a, it is recognized that most calcifying marine organisms use CO_2 and bicarbonate ion (HCO_3^-) as substrates for calcification rather than carbonate ion (CO_3^{2-}). In the perspective dominated by chemists, however, CO_3^{2-} is almost exclusively relied upon as a proxy to infer environmental calcification conditions [e.g., *Kleypas et al.*, 1999; *Orr et al.*, 2005; *Hoegh-Guldberg et al.*, 2008; *Doney et al.*, 2009; *Barton et al.*, 2012; *Waldbusser and Salisbury*, 2014; *Waldbusser et al.*, 2014]. This nuanced disparity in viewpoints has largely gone unnoticed in the OA literature until recently and is currently being debated through journal forums [*Cyronak et al.*, 2015a, 2015b; *Waldbusser et al.*, 2015a]. Weighing heavily in this discussion is a recent publication by *Bach* [2015], which unveils more complexity to the biological calcification arena and calls into question the presently held, chemical view of how ongoing OA may impact some calcifying organisms in the future.

Here we expand upon the insights of *Bach* [2015], projecting the open ocean implications of his framework onto the coastal zone where we identify dramatically different characteristics of the chemical environment. In particular, we expose extreme challenges in conducting and accurately interpreting biological OA calcification experiments that rely upon seawater taken from near shore where the magnitude and frequency of carbonate system variations are high, causing unpredictable chemical conditions that may explain discrepancies across experimental results. Finally, we discuss limitations in using metrics that include only partial information about seawater carbonate chemistry as indicators of the calcification environment and encourage the use of more complete chemical information to promote better understanding of how calcifiers may be impacted by ongoing and future OA.

2. Background and Theory

Calcium carbonate ($CaCO_3$) is a mineral excreted by calcifying marine organisms to build rigid structures or shells, commonly precipitated as the calcite or aragonite mineral phase [*Fabry et al.*, 2008; *Roleda et al.*, 2012]. When the mineral phase is in equilibrium with surrounding seawater, the concentration product of calcium (Ca^{2+}) and $CO_3{}^{2-}$ ions in solution is equal to the temperature-, pressure-, and salinity-dependent apparent solubility product (K_{sp}') [*Mucci*, 1983]:

$$K_{sp}' = \left[Ca^{2+}\right]\left[CO_3{}^{2-}\right] \tag{1}$$

K_{sp}' is empirically derived and unique to each mineral phase, with aragonite being more soluble than calcite. The concentration of calcium ($[Ca^{2+}]$) is generally conservative in seawater and therefore scales with salinity, so the solubility of calcite and aragonite is primarily dependent on $[CO_3{}^{2-}]$ in the ocean. Because declining surface ocean pH results in declining $[CO_3{}^{2-}]$ and numerous organisms make their shells out of $CaCO_3$ [*Orr et al.*, 2005; *Doney et al.*, 2009], the solubility of this mineral is of interest to the OA and shellfish grower communities [*Barton et al.*, 2015]. In these communities, saturation state (Ω) is a commonly used parameter to summarize the stability of each $CaCO_3$ mineral phase in the environment:

$$\Omega = \frac{\left[Ca^{2+}\right]\left[CO_3{}^{2-}\right]}{K_{sp}'} \tag{2}$$

Here $[CO_3{}^{2-}]$ and $[Ca^{2+}]$ represent the in situ concentrations of carbonate and calcium ions in seawater. If the in situ concentration product is equal to or larger than the apparent solubility product, Ω is ≥ 1 and the specific mineral phase will be stable in the environment. If the concentration product is smaller than the apparent solubility product, Ω is < 1 for the specific mineral phase and environmental conditions will be conducive to $CaCO_3$ dissolution. When $\Omega < 1$, the term "net calcification" is used to describe the sum of simultaneous calcification and dissolution. When Ω is ≥ 1 and dissolution is not thermodynamically favorable, net calcification is equivalent to calcification, though observations of dissolution occurring at Ω values slightly above 1 do exist [e.g., *Bednaršek et al.*, 2014; *Kwiatkowski et al.*, 2016].

From a purely chemical perspective, $CaCO_3$ is produced from the reaction of Ca^{2+} with $CO_3{}^{2-}$; however, marine organisms play an active role in calcification and often facilitate transport of $HCO_3{}^{-}$ or CO_2 to the internal site of calcification, rather than $CO_3{}^{2-}$ [e.g., *Nicol*, 1967; *Roleda et al.*, 2012; *Cyronak et al.*, 2015b]. Nevertheless, the strong positive correlation observed between $[CO_3{}^{2-}]$ and various metrics of calcification and net calcification in numerous biological OA manipulation experiments [e.g., *Langdon et al.*, 2000; *Riebesell et al.*, 2000; *Leclercq et al.*, 2002; *Marubini et al.*, 2003; *Schneider and Erez*, 2006; *Gazeau et al.*, 2007, 2011; *Comeau et al.*, 2010; *Barton et al.*, 2012; *Keul et al.*, 2013; *Waldbusser et al.*, 2014] has led to the use of $[CO_3{}^{2-}]$ (or Ω) as an indicator of future OA impacts on marine calcifiers [e.g., *Kleypas et al.*, 1999; *Gattuso and Buddemeier*, 2000; *Feely et al.*, 2004; *Orr et al.*, 2005; *Hoegh-Guldberg et al.*, 2008; *Albright et al.*, 2016; see *Jokiel*, 2016; *Mongin et al.*, 2016]. In most cases, these studies suggest or project negative impacts on calcification at Ω values > 1, which is not consistent with the concept of mineral stability as a threshold. In particular, the perceived implications of this empirical relationship have now been extrapolated to the entire United States coastline where projections of sea surface Ω values under future OA conditions were used to estimate vulnerabilities of coastal communities that depend on shellfish economies [*Ekstrom et al.*, 2015]. It is possible, however, that the relationship between $[CO_3{}^{2-}]$ and calcification does not reflect causation and is instead a correlation artifact [*Jokiel*, 2011, 2013; *Bach*, 2015; *Thomsen et al.*, 2015]. *Bach* [2015] recently pointed out that for many biological OA manipulation experiments where strong positive correlations were found between $[CO_3{}^{2-}]$ and various metrics of calcification [*Schneider and Erez*, 2006; *Gazeau et al.*, 2011; *Keul et al.*, 2013], equal or

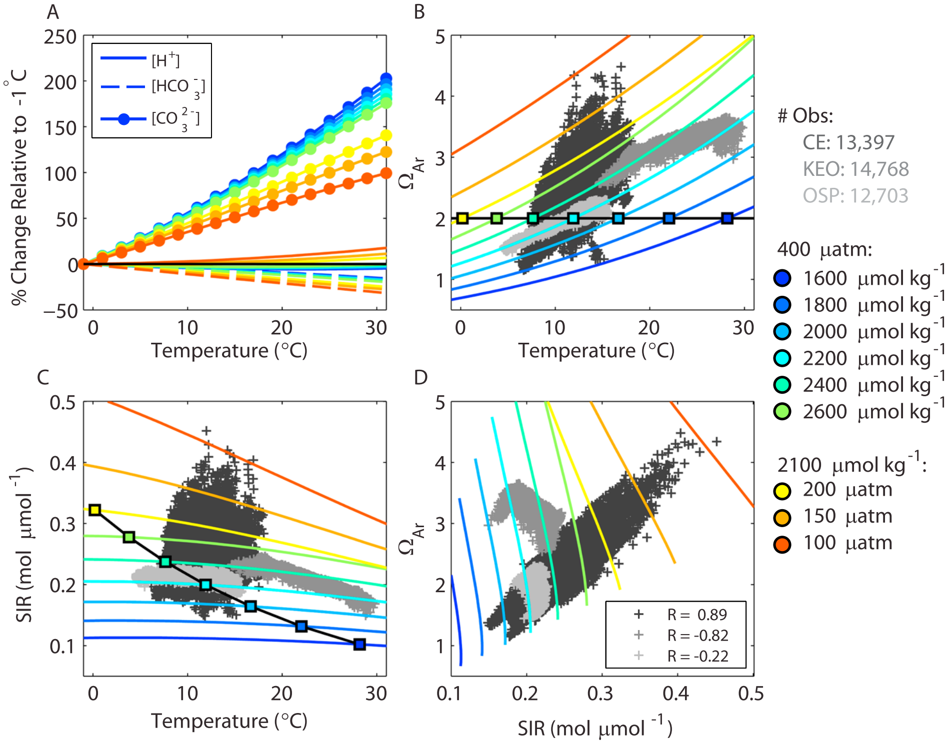

Figure 1. Temperature dependencies of (a) $[H^+]$, $[HCO_3^-]$, and $[CO_3^{2-}]$ in percent change relative to −1°C, (b) Ω_{Ar}, and (c) SIR in units of mol µmol^{-1}. Colored lines represent the carbonate chemistry conditions noted to the right of the figure where pCO$_2$ was held at 400 µatm and TA was varied from 1600 to 2600 µmol kg^{-1}, then TA was held at 2100 µmol kg^{-1}, and pCO$_2$ was varied from 200 to 100 µatm. Boxes in Figure 1c highlight SIR values that correspond to boxes in Figure 1b, where $\Omega_{Ar} = 2$ across the temperature range. (d) Ω_{Ar} versus SIR with regression R values given. Gray symbols in each plot are data from three NOAA surface moorings: the Kuroshio Extension Observatory (KEO) and Ocean Station Papa (OSP) in the open North Pacific Ocean, as well the Cape Elizabeth (CE) mooring on the outer coast of Washington State. The number of observations (# Obs) from each mooring is noted to the right of the figure.

stronger correlations could be achieved using the ratio of $[HCO_3^-]$ to hydrogen ion concentration ($[H^+]$), referred to as the substrate-to-inhibitor ratio, or SIR:

$$\mathrm{SIR} = \frac{[HCO_3^-]}{[H^+]} \tag{3}$$

where $[HCO_3^-]$ reflects the abundance of substrate in external seawater that is available for transport to the internal site of calcification and $[H^+]$ reflects the proton gradient against which internal H^+ by-products of calcification must be extruded, hence inhibitor [*Bach et al.*, 2011, 2013, 2015; *Jokiel*, 2011, 2013; *Taylor et al.*, 2011; *Bach*, 2015; *Thomsen et al.*, 2015]. Changes in the ratio of the substrate-to-inhibitor (SIR) influence the organism's ability to precipitate $CaCO_3$—in addition to other environmental variables such as temperature, nutrient concentrations, and light [*Bach et al.*, 2015]. Under this construct, higher SIR values suggest more favorable calcification conditions and, since CO_3^{2-} is not a substrate for calcification, Ω may only provide pertinent information when $\Omega < 1$ and conditions are conducive to shell dissolution [e.g., *Cyronak et al.*, 2015a]. While some of the details regarding the SIR hypothesis are actively being debated in regard to bivalve sensitivities [*Cyronak et al.*, 2015a, 2015b; *Thomsen et al.*, 2015; *Waldbusser et al.*, 2015a] and the theory will require further investigation across a range of environmental conditions and on organisms other than bivalves, corals [*Jokiel*, 2011, 2013, 2016], and coccolithophores [*Bach et al.*, 2011, 2013, 2015; *Bach*, 2015], this paradigm challenges the canonical view that a lower Ω categorically equates to a less ideal calcification environment when $\Omega > 1$.

The importance of this new perspective lies in the interpretation of how future OA will impact marine calcifiers. For most marine surface waters, $[CO_3^{2-}]$ and Ω are strongly temperature dependent [*Jiang et al.*, 2015], while SIR is only minimally influenced by temperature (Figures 1a–1c). This results in a large latitudinal gradient in $[CO_3^{2-}]$ and Ω at the ocean surface, with lower values near the poles, and a very small latitudinal gradient in SIR [*Bach*, 2015, Figures 5 and 6]. As the ocean continues to absorb anthropogenic CO_2, the relative increase in [HCO_3^-] will be minimal compared to that of [H^+] [e.g., *Bach*, 2015, Figure 1A], causing SIR to decrease slowly with time. SIR changes will be nearly uniform across latitudes due to the global nature of OA, with minor differences that arise from the meridional gradient in ocean buffer capacity [*Revelle and Suess*, 1957; *Sabine et al*., 2004]. According to *Bach's* [2015] theory, we would not expect to see significant reductions in calcification at the high latitudes relative to the low latitudes. On the other hand, [CO_3^{2-}] and Ω will maintain a strong meridional gradient as they decline under future OA, with the high latitudes reaching $\Omega < 1$ long before the tropics [*Orr et al*., 2005]. Thus, the earliest, definitive signs of OA impacts on surface-dwelling, calcifying organisms may come from enhanced dissolution at high latitudes, rather than reduced calcification, and may only be observable once $\Omega < 1$ [*Bach*, 2015].

3. Carbonate System Dynamics in the Coastal Zone

In addition to the global-scale implications of the SIR theory, there is another layer of complexity that could greatly influence interpretations of biological OA manipulation experiments attempting to assess calcification responses. The carbonate system is principally defined by four commonly measured parameters: dissolved inorganic carbon (DIC), total alkalinity (TA), partial pressure of carbon dioxide (pCO_2), and pH [*Millero*, 2007]. Two of these parameters must be measured simultaneously, in addition to temperature, salinity, pressure, and ideally silicate and phosphate in order to adequately characterize the carbonate system [*Dickson*, 2010]. In the open ocean, once two parameters have been measured, the system is constrained and changes in seawater carbonate chemistry can often be rationalized by our understanding of how various processes (e.g., gas exchange, biology, physical transport and mixing, and evaporation and precipitation) influence DIC and TA. In the coastal zone, the additional input of freshwater from rivers coupled with higher-frequency and higher-magnitude natural variability complicates our ability to understand and predict how carbonate chemistry will vary in space and time.

Near-shore, rapid, unpredictable, and large variations in carbonate chemistry caused by changes in the influence of river discharge can cause the same Ω to arise from widely different SIRs and multiple Ω values to reflect the same SIR over short timescales (less than a day). A visual representation of this concept is given in Figure 1. Boxes in Figure 1c highlight SIR values for different seawater chemistries that correspond to an aragonite saturation state (Ω_{Ar}) of 2. These SIR values span 1.02 to 3.22 mol μmol^{-1}, which is equivalent to a ~215% range in SIR (ΔSIR) for the same Ω_{Ar} value over ~30°C of temperature. Underlain in Figures 1b–1d (gray symbols) are Ω_{Ar} and SIR values from three National Oceanic and Atmospheric Administration (NOAA) surface moorings (data: http://cdiac.ornl.gov/oceans/). Two of these moorings are located in the open ocean, one at Ocean Station Papa (OSP) in the eastern subarctic Pacific and the other at the Kuroshio Extension Observatory (KEO) in the western subtropical Pacific. The third mooring is located in the coastal zone near Cape Elizabeth (CE), ~37 km from the Washington State shoreline. Autonomous sensors that measure sea surface pressure, temperature, salinity, pCO_2, and a suite of other parameters every 3 h have been maintained for ~9 years at each of these locations [*Sutton et al*., 2014]. Previously identified empirical relationships between TA and salinity at these sites [*Fassbender*, 2014; *Fassbender et al*., 2016, A. J. Fassbender et al., Estimating total alkalinity in the coastal zone: Considerations, complexities, and the surprising utility for ocean acidification research, submitted to *Estuaries and Coasts*, 2016.] were used to calculate time series of TA from the mooring salinity records. Estimates of TA were then paired with in situ pCO_2 observations to calculate Ω_{Ar} and SIR (see supporting information) using the program CO_2SYS [*Lewis and Wallace*, 1998; *van Heuven et al*., 2011] applying the constants of *Lueker et al*. [2000] and *Dickson* [1990] (no nutrient data included).

Figure 1 displays the dramatically larger range of carbonate chemistry conditions that can occur in the coastal zone (CE) relative to the open ocean (OSP and KEO). At OSP, Ω_{Ar} follows the temperature dependence shown for waters with a pCO_2 of 400 µatm and TA concentration of 2200 $\mu mol\ kg^{-1}$—similar to the mean conditions observed at this location [*Wong et al*., 2002; *Fassbender et al*., 2016]. This is interesting because the lines in Figure 1 are constructed such that the same pCO_2 and TA are maintained across a temperature gradient from −1 to 31°C. Normally, as surface waters warm or cool, the distribution of carbonate species (e.g., CO_2, HCO_3^-, and CO_3^{2-}) changes due to the temperature dependence of carbonate system equilibrium constants [*Millero*, 2007]. This primarily alters the sea surface [CO_2], [H^+], and [CO_3^{2-}] but does not influence TA and, therefore, should cause Ω_{Ar} *and* SIR to deviate from the temperature dependence lines. However, seasonal temperature changes in the ocean are often accompanied by biological and physical processes that also influence SIR and Ω_{Ar} in unique ways.

At all three mooring locations, seawater [CO_3^{2-}] variations are primarily controlled by changes in the DIC to TA ratio (they are anticorrelated; supporting information), with temperature playing a much smaller role. The timing of seasonal DIC:TA variations is similar across all three mooring sites, with the highest values in winter and the lowest values in summer. Unique to each site is the timing of seasonal SIR variations. SIR is strongly anticorrelated with pCO_2 due to the tight relationship between pCO_2 and [H^+], and differences in the timing and efficiency at which temperature, physics, and biology influence pCO_2 expression in each region result in dissimilar SIR seasonalities (supporting information). At OSP, pCO_2 is nearly constant year round due to the counteracting effects of physics and biology on seasonal CO_2 solubility changes [*Takahashi et al.*, 1993; *Fassbender*, 2014]. In addition, the seasonal TA cycle at OSP is on the order of only 10 μmol kg^{-1}. With nearly constant pCO_2 and TA at OSP, SIR is tightly constrained throughout the year (ΔSIR ~25%; Figure 1c) and Ω_{Ar} follows the theoretical temperature dependence line. In contrast, at KEO the seasonal pCO_2 cycle is large (~100 μatm) due to temperature-driven changes in CO_2 solubility that are not counterbalanced by physics and biology [*Fassbender*, 2014]. In addition, the seasonal TA cycle at KEO is about 6 times larger (~60 μmol kg^{-1}) than at OSP due to the entrainment of high-TA waters in winter and heavy precipitation in summer. These characteristics give rise to a more negative relationship between SIR and temperature than would occur from seasonal temperature changes alone, and a wider range of SIR variability (ΔSIR ~50%). Ω_{Ar} at KEO maintains a positive relationship with temperature, even during the summer when TA is low and pCO_2 is high, due to the low DIC:TA (high [CO_3^{2-}]) at this time of year. The slight bend in KEO data near 18°C (Figure 1) results from different salinity and temperature seasonalities, which are the dominant controls on TA and pCO_2 at this location, respectively [*Fassbender*, 2014]. Conditions are much different at the CE mooring. In the coastal zone, the temperature relationships for SIR and Ω_{Ar} are obscured by the wide range of carbonate system conditions that arise from more variable physical and biological processes as well as sporadic freshwater input. These multiple sources of variability cause SIR to span a range of ~200% at the CE mooring.

Perhaps most importantly, Figure 1 exemplifies that Ω and SIR are predominantly influenced by different factors, and relationships between Ω and SIR can be unique to specific ocean regions (Figure 1d). Discovery of positive, negative, and insignificant Ω-SIR relationships at CE, KEO, and OSP, respectively, suggests that the complexity of the calcifying environment cannot be adequately surmised from Ω alone. For example, it is often assumed that elevated Ω values are associated with more ideal calcification conditions [e.g., *Kleypas et al.*, 1999; *Gattuso and Buddemeier*, 2000; *Feely et al.*, 2004; *Orr et al.*, 2005; *Hoegh-Guldberg et al.*, 2008; *Albright et al.*, 2016; see *Jokiel*, 2016; *Mongin et al.*, 2016]; however, the highest SIR values at KEO are observed during winter when Ω_{Ar} is lowest, and the lowest SIR values are observed during summer when Ω_{Ar} is highest. This illustrates that Ω (or [CO_3^{2-}]) may not always provide useful information about the calcification conditions organisms experience in the environment, particularly when $\Omega > 1$ and $CaCO_3$ dissolution is not expected to occur. As a result, the use of Ω to infer calcifying conditions could lead to misinterpretations of OA vulnerability for some organisms. Although long-term ocean CO_2 uptake will lead to a strong positive correlation between Ω and SIR as both parameters decline throughout the global ocean, the seasonal relationship between these parameters is spatially variant and will likely lead to regional differences in the time of emergence for biological impacts resulting from OA.

4. Implications for Biological OA Manipulation Experiments

Carbonate chemistry fluctuations in the coastal zone occur on smaller space and timescales than in the open ocean and may impede our ability to accurately interpret biological calcification responses to OA using common methodologies. Many biological OA manipulation experiments are conducted using seawater collected near shore that is then placed in a closed system where chemical manipulations occur. The chemical conditions at the time of seawater collection set the baseline constraints for the manipulation experiment. The subsequent addition of CO_2 to alter the seawater chemistry causes Ω and SIR to decline in a predictable, linear fashion. Although there should be no $CaCO_3$ dissolution provided $\Omega > 1$, a negative calcification response caused by the declining SIR would also be highly correlated with declining Ω in the closed system environment—making it difficult to determine whether the calcification response mechanism was related to changes in Ω (despite being >1) or SIR [*Bach*, 2015; *Bach et al.*, 2015; *Cyronak et al.*, 2015a, 2015b].

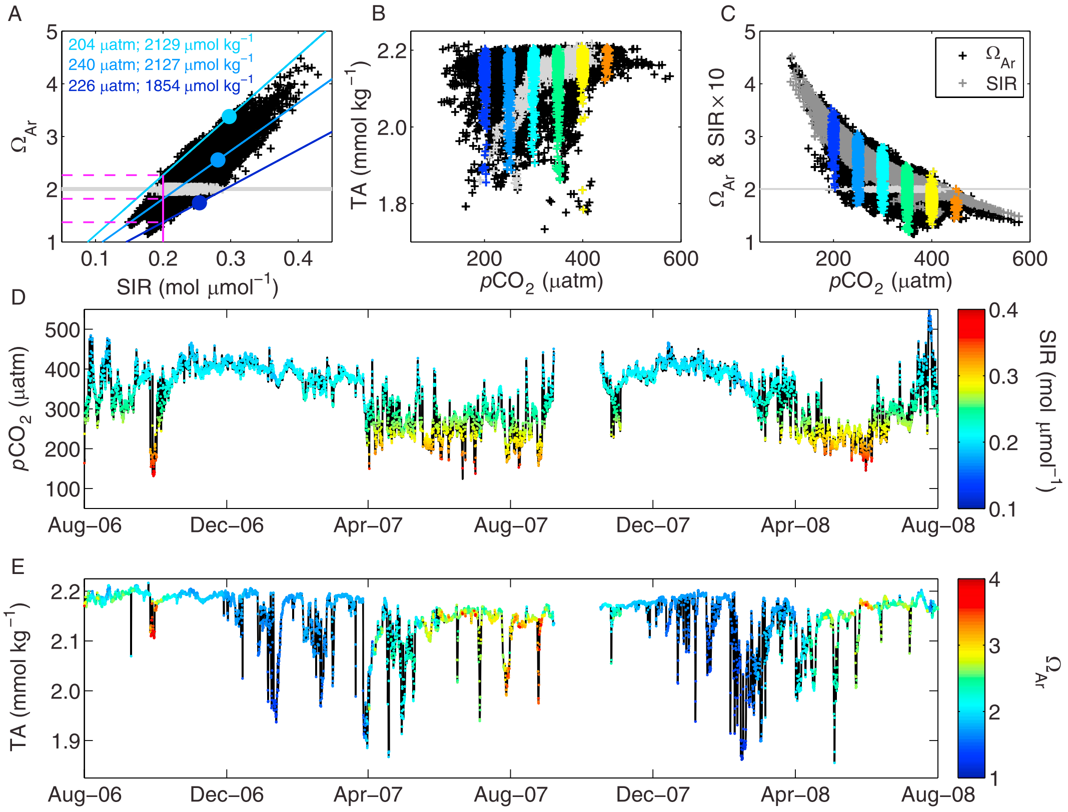

Figure 2. Observations from the NOAA CE mooring. (a) Ω_{Ar} versus SIR, (b) TA versus pCO$_2$, and (c) Ω_{Ar} and SIR × 10 versus pCO$_2$. Lines in Figure 2a represent hypothetical OA manipulation experiments in which CO$_2$ is added or removed from seawater samples with initial chemical conditions indicated by the circles. Pink lines show the range of Ω_{Ar} values that correspond to a SIR value of 0.2 mol µmol^{-1}. Colored markers in Figures 2b and 2c highlight pCO$_2$ values that can correspond to $\Omega_{Ar} = 2$ (plotted in light gray). Sea surface (d) pCO$_2$ observations and (e) TA estimates from August 2006 to 2008 colored by SIR and Ω_{Ar}, respectively. Data are missing from October and November of 2007.

In the natural environment, seawater conditions are not restricted by a closed system and can vary widely and often unpredictably in the coastal zone [*Hofmann et al*., 2011; *Takeshita et al*., 2015]. The wide range in Ω and SIR values observed across all temperatures at the CE mooring indicates that these parameters, and the relationship between them, can vary significantly on timescales much shorter than the seasonal cycle (supporting information). Therefore, someone attempting to repeat an experiment using seawater collected at a later time or in a different place than the original experiment could end up with dissimilar results. This concept is displayed in Figure 2a using data from the coastal CE mooring, where SIR is plotted against Ω_{Ar} and three initial water chemistries are selected for hypothetical CO$_2$ manipulation experiments. A linear relationship develops between Ω_{Ar} and SIR as CO$_2$ is added to the water, which is expected under closed system conditions. The slope of each line is unique, however, such that each line intersects $\Omega_{Ar} = 1$ at a different SIR value. In addition, the different slopes cause equivalent SIRs to correspond to dissimilar Ω_{Ar} values across the three hypothetical experiments. This is shown in Figure 2a for a SIR of 0.2 mol µmol^{-1}, which corresponds to Ω_{Ar} values of 1.4, 1.8, and 2.3 ($\Delta\Omega_{Ar}$ ~65%). Thus, as SIR and calcification decline with added CO$_2$, the associated decrease in Ω_{Ar} values will be different for each experiment, which could be used to address whether Ω_{Ar} or SIR is a more appropriate calcification indicator. This type of scenario may also help explain apparent discrepancies across coastal OA manipulation experiments carried out on the same species under similar experimental conditions [e.g., *Gazeau et al*., 2011; *Barton et al*., 2012; *Waldbusser et al*., 2014].

In the past few years, biological OA manipulation experiments have become more complex and flow-through systems are now being used to evaluate multiple stressors [e.g., *Andersson and MacKenzie*, 2012; *McElhany and Shallin Busch*, 2013; *Reum et al*., 2014]. If the intake water for these systems comes from a coastal environment similar to the NOAA CE mooring site, accurately interpreting experimental results will be challenging.

In many flow-through OA experimental systems, pCO_2 is monitored and manipulated to achieve a projected future seawater concentration using a feedback system where CO_2 bubbling is initiated when the sample concentration falls below the target value. Large changes in TA caused by riverine inputs result in a wide range of TA values that can occur for a given pCO_2 level (Figure 2b). This means that the same intake seawater pCO_2 value can represent a wide range of Ω_{Ar} values as well as a somewhat smaller range of SIR values (Figure 2c). Flow-through systems that rely on pCO_2 alone to assess and manipulate carbonate chemistry conditions may, therefore, experience unanticipated changes in SIR and Ω_{Ar} within experimental pCO_2 treatments over time. In addition, these TA variations may alter the slope of the SIR-Ω_{Ar} relationship (e.g., Figure 2a) throughout the experiment such that calcification effects driven by SIR could show no discernable relationship with Ω_{Ar}. It is important to acknowledge that the organism may not be able to respond rapidly enough to SIR (or Ω_{Ar}) changes to record the environmental signal. However, if the organism does respond to the environmental variability, then it will be critical that all experimental treatments be run simultaneously to ensure internal consistency and the ability to accurately interpret differences in the calcification responses across treatments [see *Cornwall and Hurd*, 2015].

Observations from the coastal NOAA CE mooring indicate that changes in SIR, Ω_{Ar}, and the SIR-Ω_{Ar} relationship occur on timescales relevant for OA manipulations experiments (supporting information). At the coastal CE mooring, pCO_2 varies seasonally and in the opposite direction of its temperature sensitivity [*Takahashi et al.*, 1993], suggesting that biology and physics play an important role in the pCO_2 seasonality (Figure 2d). TA does not display a clear seasonal cycle but often declines by ~100 μmol kg^{-1} between subsequent samples, showing even larger decreases over consecutive sample intervals that can be maintained for multiple weeks (Figure 2e). These large, sporadic decreases in TA (and DIC) are primarily caused by changes in the influence of river discharge at the mooring site (A. J. Fassbender et al., submitted manuscript, 2016). In particular, the Columbia River dominates freshwater input along the outer coast of Washington and the river plume is often found north of the river mouth [*Hickey et al.*, 2005; *Hickey and Banas*, 2008], occasionally crossing the CE mooring path. Different controls on pCO_2 and TA at the CE mooring are what allow the SIR-Ω_{Ar} relationship to vary unpredictably at this location (see *Bach* [2015, Figure 4] for salinity influence on SIR and Ω_A). Notably, the NOAA CE mooring is ~37 km from land and may be a conservative analog for the magnitude and intermittency of chemical variability found closer to shore and in estuaries where numerous tributaries drain. Observations from the CE mooring suggest that multiple frequencies of variability can occur in river-influenced coastal regions and that fluctuations in the SIR-Ω_{Ar} relationship must be considered during the interpretation and design of biological OA manipulation experiments conducted in areas with similarly dynamic carbonate system variability.

It has been acknowledged for some time that the range of natural variability in multiple parameters should be incorporated into OA manipulation experiments and used to guide experimental design [e.g., *Reum et al.*, 2014; *Breitberg et al.*, 2015], but this challenging task is not yet commonplace [*Reum et al.*, 2015; *Yang et al.*, 2015; *Hauser et al.*, 2016]. While we support this holistic approach, critical to any biological OA experiment is the ability to fully and accurately characterize the carbonate chemistry conditions of the treatments [*Cornwall and Hurd*, 2015] and frame the results in the context of the natural environment [*Hofmann et al.*, 2011; *Andersson and MacKenzie*, 2012; *McElhany and Shallin Busch*, 2013; *Wahl et al.*, 2016]. The difficulty in monitoring and predicting carbonate chemistry conditions in the coastal zone challenges our ability to easily assess organism exposures and identify biological calcification responses to OA in these environments. Continuous observations may be required to adequately characterize carbonate chemistry near shore, which might explain why intermittent shifting of carbonate system relationships in the coastal zone has only been alluded to in OA literature thus far [*Waldbusser and Salisbury*, 2014; *Thomsen et al.*, 2015; *Waldbusser et al.*, 2015b].

The CE mooring site displays a positive correlation between SIR and Ω_{Ar}, albeit with a wide range of variability, making it a moderate example of the challenges associated with carbonate chemistry dynamics in the coastal zone. In other regions where biological OA manipulation experiments take place, SIR and Ω_{Ar} may not be positively correlated, making it even more critical to identify and use the most appropriate calcification indicator when interpreting results. In addition, the fact that positive, negative, and insignificant correlations between Ω and SIR were found within the North Pacific Ocean suggests that extrapolating results from regionally based biological OA manipulation experiments to the basin or global scale will likely result in incorrect assessments of exposure and vulnerability for some organisms. Thus, in order to determine where, when, and how a diversity of marine calcifiers may be influenced by OA, further research is needed to identify SIR-Ω_{Ar} relationships globally and to assess which species rely on calcification mechanisms modulated by SIR.

5. Conclusions

It is widely accepted that saturation state (Ω) reflects the environmental $CaCO_3$ dissolution potential; however, recent insights from *Bach* [2015] and others [*Jokiel*, 2011, 2013, 2016; *Bach et al.*, 2015; *Cyronak et al.*, 2015a, 2015b; *Thomsen et al.*, 2015] suggest that Ω may not always be an appropriate indicator of the calcifying environment when $\Omega > 1$. Instead, the substrate-to-inhibitor ratio (SIR) may more accurately reflect the calcifying environment for some species. In order to better understand how ocean acidification (OA) will influence the ability of organisms to calcify in waters with $\Omega > 1$, it is important to consider the full carbonate chemistry and how it varies, rather than rely on a single carbonate system parameter. This work explores the issues with using Ω as the sole indicator of calcification potential in real-world, ocean environments where Ω is not tightly coupled with SIR and where fluctuating relationships between SIR and Ω may help to explain discrepancies across biological OA manipulation experiments. Although the SIR hypothesis requires further examination across a diversity of marine calcifying species, emerging evidence and mature conceptual theory on calcification are now aligning for corals, coccolithophores, and some bivalves, suggesting that SIR warrants the attention of the broader OA community.

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References

Adelsman, H., and L. W. Binder (2012), *Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response*, Olympia.

Albright, R., et al. (2016), Reversal of ocean acidification enhances net coral reef calcification, *Nature*, doi:10.1038/nature17155.

Andersson, A. J., and F. T. MacKenzie (2012), Revisiting four scientific debates in ocean acidification research, *Biogeosciences*, *9*(3), 893–905, doi:10.5194/bg-9-893-2012.

Bach, L. T. (2015), Reconsidering the role of carbonate ion concentration in calcification by marine organisms, *Biogeosciences*, *12*(16), 4939–4951, doi:10.5194/bg-12-4939-2015.

Bach, L. T., U. Riebesell, and K. G. Schulz (2011), Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, *Limnol. Oceanogr.*, *56*(6), 2040–2050, doi:10.4319/lo.2011.56.6.2040.

Bach, L. T., L. C. M. MacKinder, K. G. Schulz, G. Wheeler, D. C. Schroeder, C. Brownlee, and U. Riebesell (2013), Dissecting the impact of CO_2 and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliania huxleyi*, *New Phytol.*, *199*(1), 121–134, doi:10.1111/nph.12225.

Bach, L. T., U. Riebesell, M. A. Gutowska, L. Federwisch, and K. G. Schulz (2015), A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework, *Prog. Oceanogr.*, *135*, 125–138, doi:10.1016/j.pocean.2015.04.012.

Barton, A., B. Hales, G. G. Waldbusser, C. Langdon, and R. A. Feely (2012), The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects, *Limnol. Oceanogr.*, *57*(3), 698–710, doi:10.4319/lo.2012.57.3.0698.

Barton, A., et al. (2015), Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response, *Oceanography*, *25*(2), 146–159, doi:10.5670/oceanog.2015.38.

Bates, N. R., Y. M. Astor, M. J. Church, K. Currie, J. E. Dore, M. González-Dávila, L. Lorenzoni, F. Muller-Karger, J. Olafsson, and J. M. Santana-Casiano (2014), A time-series view of changing surface ocean chemistry due to ocean uptake of anthropogenic CO_2 and ocean acidification, *Oceanography*, *27*(1), 126–141, doi:10.5670/oceanog.2014.16.

Bednaršek, N., G. A. Tarling, D. C. E. Bakker, S. Fielding, and R. A. Feely (2014), Dissolution dominating calcification process in polar pteropods close to the point of aragonite undersaturation edited by P. Ross, *PLoS One*, *9*(10), e109183, doi:10.1371/journal.pone.0109183.

Breitberg, D., et al. (2015), And on top of all that… Coping with ocean acidification in the midst of many stressors, *Oceanography*, *25*(2), 48–61, doi:10.5670/oceanog.2015.31.

Browman, H. I. (2016), Applying organized scepticism to ocean acidification research, *ICES J. Mar. Sci. J. du Cons.*, *73*(3), 529.1–529.536, doi:10.1093/icesjms/fsw010.

Ciais, P., et al. (2013), Carbon and Other Biogeochemical Cycles, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by T. F. Stocker et al, Cambridge Univ. Press, Cambridge, U. K., and New York.

Comeau, S., R. Jeffree, J. L. Teyssie, and J. P. Gattuso (2010), Response of the arctic pteropod *Limacina helicina* to projected future environmental conditions, *PLoS One*, *5*(6), 1–7, doi:10.1371/journal.pone.0011362.

Cornwall, C. E., and C. L. Hurd (2015), Experimental design in ocean acidification research: Problems and solutions, *ICES J. Mar. Sci.*, doi:10.1093/icesjms/fsv118.

Cyronak, T., K. G. Schulz, and P. L. Jokiel (2015a), Response to Waldbusser et al. (2015c): "Calcium carbonate saturation state: on myths and this or that stories," *ICES J. Mar. Sci.*, doi:10.1093/icesjms/fsv224.

Cyronak, T., K. G. Schulz, and P. L. Jokiel (2015b), The Omega myth: What really drives lower calcification rates in an acidifying ocean, *ICES J. Mar. Sci.*, doi:10.1093/icesjms/fsv075.

Dickson, A. G. (1990), Standard potential of the reaction: AgCl(s) + $1/2H_2$(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K, *J. Chem. Thermodyn.*, *22*, 113–127.

Dickson, A. G. (2010), Part 1: Seawater carbonate chemistry. The carbon dioxide system in seawater: Equilibrium chemistry and measurements, in *Guide to Best Practices for Ocean Acidification Research and Data Reporting*, pp. 17–40, Publications Office of the European Union, Luxembourg.

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas (2009), Ocean acidification: The other CO_2 problem, *Ann. Rev. Mar. Sci.*, *1*(1), 169–192, doi:10.1146/annurev.marine.010908.163834.

Ekstrom, J. A., et al. (2015), Vulnerability and adaptation of US shellfisheries to ocean acidification, *Nat. Clim. Chang.*, *5*(3), 207–214, doi:10.1038/nclimate2508.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr (2008), Impacts of ocean acidification on marine fauna and ecosystem processes, *ICES J. Mar. Sci.*, *65*(3), 414–432, doi:10.1093/icesjms/fsn048.

Fassbender, A. J. (2014), New approaches to study the marine carbon cycle PhD dissertation, Univ. of Washington. Proquest, 1/11/2016. [Available at http://hdl.handle.net/1773/27552.]

Fassbender, A. J., C. L. Sabine, and M. F. Cronin (2016), Net community production and calcification from 7 years of NOAA Station Papa Mooring measurements, *Global Biogeochem. Cycles*, *30*, 250–267, doi:10.1002/2015GB005205.

Feely, R. A., C. L. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, and F. J. Millero (2004), Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans, *Science*(80-.)., *305*(5682), 362–366, doi:10.1126/science.1097329.

Gattuso, J., and R. W. Buddemeier (2000), Calcification and CO_2, *Nature*, *407*(6802), 311–313, doi:10.1038/35030280.

Gattuso, J.-P., and L. Hansson (Eds.) (2011), *Ocean Acidification*, Oxford Univ. Press, Oxford, U. K.

Gazeau, F., C. Quiblier, J. M. Jansen, J.-P. Gattuso, J. J. Middelburg, and C. H. R. Heip (2007), Impact of elevated CO_2 on shellfish calcification, *Geophys. Res. Lett.*, *34*, L07603, doi:10.1029/2006GL028554.

Gazeau, F., J.-P. Gattuso, M. Greaves, H. Elderfield, J. Peene, C. H. R. Heip, and J. J. Middelburg (2011), Effect of carbonate chemistry alteration on the early embryonic development of the Pacific oyster (*Crassostrea gigas*), *PLoS One*, *6*(8), e23010, doi:10.1371/journal.pone.0023010.

Guinotte, J. M., and V. J. Fabry (2008), Ocean acidification and its potential effects on marine ecosystems, *Ann. N. Y. Acad. Sci.*, *1134*, 320–342, doi:10.1196/annals.1439.013.

Hauser, D. D. W., E. D. Tobin, K. M. Feifel, V. Shah, and D. M. Pietri (2016), Disciplinary reporting affects the interpretation of climate change impacts in global oceans, *Glob. Chang. Biol.*, *22*(1), 25–43, doi:10.1111/gcb.12978.

Hickey, B. M., and N. S. Banas (2008), Why is the northern end of the California Current system so productive?, *Oceanography*, *21*(4), 90–107.

Hickey, B., S. Geier, N. Kachel, and A. MacFadyen (2005), A bi-directional river plume: The Columbia in summer, *Cont. Shelf Res.*, *25*(14), 1631–1656, doi:10.1016/j.csr.2005.04.010.

Hoegh-Guldberg, O., et al. (2008), Coral reefs under rapid climate change and ocean acidification, *Science*, *318*(5857), 1737–1742, doi:10.1126/science.1152509.

Hofmann, G. E., J. P. Barry, P. J. Edmunds, R. D. Gates, D. A. Hutchins, T. Klinger, and M. A. Sewell (2010), The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective, *Annu. Rev. Ecol. Evol. Syst.*, *41*(1), 127–147, doi:10.1146/annurev.ecolsys.110308.120227.

Hofmann, G. E., et al. (2011), High-frequency dynamics of ocean pH: A multi-ecosystem comparison, *PLoS One*, *6*(12), e28983, doi:10.1371/journal.pone.0028983.

Jiang, L., R. A. Feely, B. R. Carter, D. J. Greeley, D. K. Gledhill, and K. M. Arzayus (2015), Climatological distribution of aragonite saturation state in the global oceans, *Global Biogeochem. Cycles*, *29*, 1656–1673, doi:10.1002/2015GB005198.

Jokiel, P. L. (2011), Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux, *Bull. Mar. Sci.*, *87*(3), 639–657, doi:10.5343/bms.2010.1107.

Jokiel, P. L. (2013), Coral reef calcification: Carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification, *Proc. R. Soc. B Biol. Sci.*, *280*(1764), 20,130,031–20,130,031, doi:10.1098/rspb.2013.0031.

Jokiel, P. L. (2016), Predicting the impact of ocean acidification on coral reefs: Evaluating the assumptions involved, *ICES J. Mar. Sci. J. du Cons.*, *73*(3), 550–557, doi:10.1093/icesjms/fsv091.

Keul, N., G. Langer, L. J. De Nooijer, and J. Bijma (2013), Effect of ocean acidification on the benthic foraminifera *Ammonia* sp. is caused by a decrease in carbonate ion concentration, *Biogeosciences*, *10*(10), 6185–6198, doi:10.5194/bg-10-6185-2013.

Kleypas, J. A., R. W. Buddemeier, D. Archer, J. Gattuso, C. Langdon, and B. N. Opdyke (1999), Geochemical consequences of increased atmospheric carbon dioxide on coral reefs, *Science*, *118*, 10–13, doi:10.1126/science.284.5411.118.

Kroeker, K. J., R. L. Kordas, R. N. Crim, and G. G. Singh (2010), Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, *Ecol. Lett.*, *13*(11), 1419–1434, doi:10.1111/j.1461-0248.2010.01518.x.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso (2013), Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming, *Global Change Biol.*, *19*(6), 1884–1896, doi:10.1111/gcb.12179.

Kwiatkowski, L., et al. (2016), Nighttime dissolution in a temperate coastal ocean ecosystem increases under acidification, *Nat. Publ. Gr.*, 1–9, doi:10.1038/srep22984.

Langdon, C., T. Takahashi, C. Sweeney, D. Chipman, J. Goddard, F. Marubini, H. Aceves, H. Barnett, and J. Atkinson (2000), Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef, *Global Biogeochem. Cycles*, *14*(2), 639–654, doi:10.1029/1999GB001195.

Lauvset, S. K., N. Gruber, P. Landschützer, A. Olsen, and J. Tjiputra (2015), Trends and drivers in global surface ocean pH over the past 3 decades, *Biogeosciences*, *12*(5), 1285–1298, doi:10.5194/bg-12-1285-2015.

Leclercq, N., J.-P. Gattuso, and J. Jaubert (2002), Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure, *Limnol. Oceanogr.*, *47*(2), 558–564, doi:10.4319/lo.2002.47.2.0558.

Lewis, E., and D. W. R. Wallace (1998), Program developed for CO_2 system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge Natl. Lab., U.S. Department of Energy, Oak Ridge, Tenn., doi:10.3334/CDIAC/otg.CO2SYS_DOS_CDIAC105.

Lueker, T. J., A. G. Dickson, and C. D. Keeling (2000), Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2: Validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium, *Mar. Chem.*, *70*, 105–119, doi:10.1016/S0304-4203(00)00022-0.

Marubini, F., C. Ferrier-Pages, and J.-P. Cuif (2003), Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: A cross-family comparison, *Proc. R. Soc. B Biol. Sci.*, *270*, 179–184, doi:10.1098/rspb.2002.2212.

McElhany, P., and D. Shallin Busch (2013), Appropriate pCO_2 treatments in ocean acidification experiments, *Mar. Biol.*, *160*(8), 1807–1812, doi:10.1007/s00227-012-2052-0.

Millero, F. J. (2007), The marine inorganic carbon cycle, *Chem. Rev.*, *107*, 308–341, doi:10.1021/cr0503557.

Mongin, M., et al. (2016), The exposure of the Great Barrier Reef to ocean acidification, *Nat. Commun.*, *7*, 10,732, doi:10.1038/ncomms10732.

Mucci, A. (1983), The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure, *Am. J. Sci.*, *283*(7), 780–799, doi:10.2475/ajs.283.7.780.

Nicol, J. A. C. (1967), Skeletons, shelters and special defences, in *The Biology of Marine Animals*, pp. 620, Sir Isaac Pitman, London.

Orr, J. C., et al. (2005), Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, *Nature*, *437*(7059), 681–686, doi:10.1038/nature04095.

Reum, J. C. P., S. R. Alin, R. A. Feely, J. Newton, M. Warner, and P. McElhany (2014), Seasonal carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary: Implications for the design of ocean acidification experiments, *PLoS One*, *9*(2), doi:10.1371/journal.pone.0089619.

Reum, J. C. P., et al. (2015), Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen, *ICES J. Mar. Sci.*, doi:10.1093/icesjms/fsu231.

Revelle, R., and H. E. Suess (1957), Carbon dioxide exchange between atmosphere and ocean and the question of an increase of atmospheric CO_2 during the past decades, *Tellus*, *9*(1), 18–27, doi:10.1111/j.2153-3490.1957.tb01849.x.

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, and F. M. M. Morel (2000), Reduced calcification of marine plankton in response to increased atmospheric CO_2, *Nature*, *407*(6802), 364–367, doi:10.1038/35030078.

Roleda, M. Y., P. W. Boyd, and C. L. Hurd (2012), Before ocean acidification: Calcifier chemistry lessons, *J. Phycol.*, *48*(4), 840–843, doi:10.1111/j.1529-8817.2012.01195.x.

Sabine, C. L., et al. (2004), The oceanic sink for anthropogenic CO_2, *Science*, *305*(5682), 367–371, doi:10.1126/science.1097403.

Schneider, K., and J. Erez (2006), The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*, *Limnol. Oceanogr.*, *51*(3), 1284–1293, doi:10.4319/lo.2006.51.3.1284.

Sutton, A. J., et al. (2014), A high-frequency atmospheric and seawater $p\mathrm{CO}_2$ data set from 14 open ocean sites using a moored autonomous system, *Earth Sst. Sci. Data*, *6*, 353–366, doi:10.5194/essdd-7-385-2014.

Takahashi, T., J. G. Goddard, and D. W. Chipman (1993), Seasonal variation of CO_2 and nutrients in the high-latitude surface oceans: A comparative study, *Global Biogeochem. Cycles*, *7*(4), 843–878, doi:10.1029/93GB02263.

Takeshita, Y., C. A. Frieder, T. R. Martz, J. R. Ballard, R. A. Feely, S. Kram, S. Nam, M. O. Navarro, N. N. Price, and J. E. Smith (2015), Including high-frequency variability in coastal ocean acidification projections, *Biogeosciences*, *12*(19), 5853–5870, doi:10.5194/bg-12-5853-2015.

Taylor, A. R., A. Chrachri, G. Wheeler, H. Goddard, and C. Brownlee (2011), A voltage-gated H+ channel underlying pH homeostasis in calcifying coccolithophores edited by P. G. Falkowski, *PLoS Biol.*, *9*(6), e1001085, doi:10.1371/journal.pbio.1001085.

Thomsen, J., K. Haynert, K. M. Wegner, and F. Melzner (2015), Impact of seawater carbonate chemistry on the calcification of marine bivalves, *Biogeosciences*, *12*(14), 4209–4220, doi:10.5194/bg-12-4209-2015.

van Heuven, S., D. Pierrot, J. W. B. Rae, E. Lewis, and D. W. R. Wallace (2011), MATLAB program developed for CO_2 system calculations. ORNL/CDIAC-105b, Carbon Dioxide Inf. Anal. Center, Oak Ridge Natl. Lab. U.S. Dep. Energy, Oak Ridge, Tenn., doi:10.3334/CDIAC/otg.CO2SYS_MATLAB_v1.1.

Wahl, M., V. Saderne, and Y. Sawall (2016), How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations, *Mar. Freshw. Res.*, *67*(1), 25–36, doi:10.1071/MF14154.

Waldbusser, G. G., and J. E. Salisbury (2014), Ocean acidification in the coastal zone from an organism's perspective: Multiple system parameters, frequency domains, and habitats, *Ann. Rev. Mar. Sci.*, *6*, 221–247, doi:10.1146/annurev-marine-121211-172238.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, and I. Gimenez (2014), Saturation-state sensitivity of marine bivalve larvae to ocean acidification, *Nat. Clim. Chang.*, *5*(3), 273–280, doi:10.1038/nclimate2479.

Waldbusser, G. G., B. Hales, and B. A. Haley (2015a), Calcium carbonate saturation state: On myths and this or that stories, *ICES J. Mar. Sci.*, doi:10.1093/icesjms/fsv174.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, I. Gimenez, and G. Hutchinson (2015b), Ocean acidification has multiple modes of action on bivalve larvae edited by P. Ross, *PLoS One*, *10*(6), e0128376, doi:10.1371/journal.pone.0128376.

Wong, C. S., N. A. D. Waser, Y. Nojiri, F. A. Whitney, J. S. Page, and J. Zeng (2002), Seasonal cycles of nutrients and dissolved inorganic carbon at high and mid latitudes in the North Pacific Ocean during the Skaugran cruises: Determination of new production and nutrient uptake ratios, *Deep. Res. II*, *49*(24–25), 5317–5338, doi:10.1016/S0967-0645(02)00193-5.

Yang, Y., L. Hansson, and J.-P. Gattuso (2015), Data compilation on the biological response to ocean acidification: An update, *Earth Syst. Sci. Data Discuss.*, *8*, 889–912, doi:10.5194/essdd-8-889-2015.