Geochemical Proxies for
Estimating Faunal Exposure
to Ocean Acidification

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A Flabellum coral situated within its biomineralized skeleton.
Photo credit: Greg Rouse
ABSTRACT. Growing concern over the impacts of modern ocean acidification (OA) and interest in historical pH excursions have intensified the development of geochemical proxies for organism exposure to acidification and other components of the carbonate system. The use of carbonate structures produced by foraminifers, coccolithophores, corals, mollusks, brachiopods, echinoderms, ostracods, and fish for paleoreconstructions is an active area of study, and the resulting proxy development offers new opportunities for studying modern faunal exposures. Here we review information from field studies and laboratory experiments on carbonate system geochemical proxies in protists and metazoa. Geochemical proxy development for foraminifers and corals is most advanced; studies of fish and echinoderms are in their infancy. The most promising geochemical proxies are those with a mechanistic link to changes in seawater carbonate chemistry, such as boron isotopes ($\delta^{11}B$), B/Ca, and U/Ca ratios recorded in skeletal hard parts. We also discuss indirect geochemical proxies (other trace elements and carbonate polymorphs) along with their potential uses and limitations due to modification by physiological processes, precipitation rate, and degree of calcification. Proxy measurements in modern skeletal structures, otoliths, statoliths, and other hard parts could reveal environmental exposures of organisms from larval through adult stages, and could advance inferences about effects of OA (and other stressors) on survival, growth, population connectivity, and other ecological attributes. Use of geochemical proxies in live, field-collected organisms is an underutilized and underdeveloped approach to studying OA consequences, but it may offer a powerful, complementary approach to laboratory observations.

INTRODUCTION
Ocean acidification (OA) is the term used to describe the reduction in ocean pH and changes in other components of the carbonate system that result primarily from ocean uptake of excess carbon dioxide (CO$_2$) from the atmosphere (Caldeira and Wickett, 2003). Anthropogenic OA is documented in all ocean basins (Takahashi et al., 2014) and has raised a host of questions about the biological responses in different ecosystems, among different taxa, at different life stages (Doney et al., 2009; Duarte et al., 2013), and under different OA variability regimes (Hofmann et al., 2011). The quest is to understand OA impacts mechanistically, from molecular and physiological levels to population and ecosystem levels, which often requires knowledge of organism exposure to OA.

The growing interest in anthropogenic OA has led to an intensive search for geochemical proxies that reflect environmental conditions and organism exposure to different aspects of marine carbonate chemistry such as pH, alkalinity, carbonate ion concentration, the partial pressure of CO$_2$ (pCO$_2$), and the saturation state of CaCO$_3$ minerals in seawater. Geochemical proxies include chemical and isotopic compositions of biological structures that allow estimation of environmental conditions that cannot or can no longer be measured directly. Over the past 60 years, the study of proxies in foraminiferal remains has flourished, and many of the proxies now studied in animal fossils such as corals, mollusks, brachiopods, bryozoans, and fish were originally observed and validated in foraminifers. Paleoceanographic objectives are to reconstruct past variations in, for example, climate, productivity, and carbon and nutrient cycling and their effects on the evolution or extinction of life forms. Application of geochemical proxies to modern, live-collected organisms attempts estimates of organismal exposure to varying environmental conditions in order to infer, for example, migration routes, stock structure, natal origin, population connectivity, or water mass affinities (Campana, 1999; Thorrold et al., 2007). Analysis of ontogenetic (sequential life stage) exposure to carbonate system parameters could potentially inform researchers about OA-induced sources of population variability and carry-over (legacy) effects on subsequent settlement, growth, survival, reproduction, and demographic fitness. When combined with other proxies, it may eventually be possible to evaluate the interactive effects of OA with co-occurring stressors such as warming and deoxygenation (Breitburg et al., 2015, in this issue).

Many metazoans exhibit ontogenetic changes in motility and habitat that affect their OA exposures. The early stages of some coastal fish species occupy wetland, seagrass, or estuarine nursery habitats with highly variable carbonate chemistry. In contrast, adults may occupy coastal and offshore habitats with less variable conditions. Reducing conditions within sediments can expose bivalves to corrosive pore waters sufficient to increase vulnerability due to dissolution (Green et al., 2009). Planktonic organisms are exposed to changes over time within a given water mass or in different water masses as they migrate, whereas benthic forms experience changes in the water masses overlying their habitats. These differences must be considered when interpreting proxy signals and could affect vulnerability to OA.

In this paper, we review efforts to develop geochemical proxies of OA. Progress and challenges are highlighted, focusing mainly on foraminifers, corals, mollusks, echinoderms, and fishes. We emphasize the potential application of proxies developed for the geological record to work on modern faunas, and we discuss future applications that will likely allow novel inference about marine population response to OA.

TAXON SUITABILITY FOR OA PROXY DEVELOPMENT
Foraminiferan Protists
The presence and abundance of planktonic and benthic foraminifers in sea-floor sediments has been studied since the Challenger expeditions (1872–1876),
and their predominance in paleoceanographic reconstructions, based largely on their remnant carbonate structure called a test, dates back to Emiliani’s (1955) iconic Pleistocene temperature study using oxygen isotopes.

Metazoans
A large number of marine invertebrates and vertebrates produce calcified structures that may record environmental conditions. Preservation of molluscan statoliths (Arkhipkin, 2005), echinoderm spines, ossicles, and other skeletal elements (Kroh and Nebelsick, 2010), and fish otoliths (Nolf, 2013) in the sediment record have made them reasonable targets for use in OA paleoceanographic reconstructions.

Among metazoans, coral CaCO₃ skeletons are in the most advanced state of geochemical proxy development for OA. Because coral colonies can live for decades to centuries, and even thousands of years in deep water, they can provide a view of environmental changes over time (e.g., Druffel, 1997). Shallow-water corals typically secrete aragonite and are restricted to tropical conditions, whereas deepwater coral species secrete either aragonite or calcite, and occur at a broader range of latitudes and depths. Mollusks occupy a very broad range of ocean environments, in both pelagic and benthic realms, from intertidal and estuarine waters to the deep ocean. Shells, statoliths, and beaks offer carbonate structures precipitated under varying degrees of organism regulation. Echinoderms produce skeletal elements that are commonly preserved in marine deposits and make them attractive for paleoceanographic reconstructions. Geochemical analysis of fish otoliths has a long history (Campana and Thorrold, 2001), but only recently has the influence of carbonate chemistry on otolith size or geochemistry been considered (Checkley et al., 2009; Munday et al., 2011a).

Bivalve and gastropod shells as well as molluscan statoliths and fish otoliths are initially precipitated during the larval phase, then retained during metamorphosis and into the juvenile phase, providing a potential record of larval exposures in post-recruitment stages. However, abrasion or overgrowth of the larval shell might complicate use of the larval carbonates as proxies, and the calcified structures of echinoderm larvae usually are not retained in post-larval stages. Many mollusks (e.g., loliginid squid and gastropods) deposit embryos within jelly capsules on the seafloor. This isolates statoliths or shells from direct contact with the seawater environment during early development, also complicating proxy development.

GEOCHEMICAL PROXIES: FROM PALEOCEANOGRAPHIC TO MODERN APPLICATIONS

Boron Isotopes
Of the boron species in seawater, borate is the dominant form incorporated in marine carbonates, and theoretically δ¹¹B (see footnote¹) and the boron concentration in carbonates should increase as seawater pH increases (Hemming and Hanson, 1992; Box 1). This occurs because the equilibrium reaction between the two dominant aqueous boron species shifts their relative abundance from boric acid (B(OH)₃) at low pH to borate (B(OH)₄⁻) at high pH (Box 1). Among foraminifers, the hypothesis above has been verified in laboratory experiments with planktonic species (Sanyal et al., 1996, 2001; Henehan et al., 2013), one shallow-water benthic foraminiferal species (Rollion-Bard and Erez, 2010), and inorganic carbonates (Sanyal et al., 2000; Figure 1). The deep-sea benthic foraminifers typically used for paleoceanographic reconstructions are difficult to culture in the laboratory, and existing boron isotope calibrations are therefore restricted to core-top sediment observations from depth transects with limited pH gradients (Hönisch et al., 2008; Rae et al., 2011).

Deviations from predicted δ¹¹B can be caused by photosynthesis in species with photosynthetic symbionts, by respiration, and by calcification, all of which modify pH in the foraminiferal micro-environment (Hönisch et al., 2003; Zeebe et al., 2003; Henehan et al., 2013), and by partial test dissolution, which lowers the δ¹¹B originally recorded at the sea surface (Hönisch and Hemming, 2004). Fortuitously, physiological effects appear to be constant in the largest calcareous foraminiferal species (Hönisch and Hemming, 2004; Henehan et al., 2013); their δ¹¹B agrees with laboratory calibrations, and the largest tests are also more resistant to partial dissolution (Hönisch and Hemming, 2004).

Metazoans
Culture experiments with several species of living corals have confirmed that δ¹¹B in coral skeletons systematically increases with pH (Figure 1 and references therein). Application of these relationships to a live massive Porites from the western Coral Sea revealed a clear interdecadal pH oscillation equating to a 0.3 unit range of exposure (Pelejero et al., 2005). Similar to symbiont-bearing foraminifers, the coral’s calcifying environment can also be modified by photosynthesis and respiration where pH at the site of calcification is elevated in the light and depleted in the dark (Al-Horani et al., 2003; Venn et al., 2011). Venn et al. (2013) furthermore observed that pH elevation at the site of calcification in Stylophora is greater under lower ambient pH conditions, which they interpreted as a means to mitigate the negative effects of acidification on coral calcification. Similar arguments have been raised by Trotter et al. (2011) and

¹ Boron has two stable isotopes ¹⁰B and ¹¹B, with 20% and 80% occurrence, respectively. The variation in isotopic composition is defined as:

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\delta^{11}B = \left( \frac{^{11}B/^{10}B}_{\text{sample}} / \frac{^{11}B/^{10}B}_{\text{standard}} - 1 \right) \times 1000,
$$

where $^{11}B/^{10}B_{\text{sample}}$ denotes the ¹¹B-to-¹⁰B boron isotopic ratio of the sample and $^{11}B/^{10}B_{\text{standard}}$ that of a standard (usually NBS SRM951 boric acid).
McCulloch et al. (2012a,b), who interpreted the lower $\delta^{11}B$ sensitivity to seawater pH in corals compared to the aqueous prediction (Klochko et al., 2006) as a pH upregulation mechanism under low ambient pH conditions. However, similarly reduced pH sensitivity has been observed in synthetic calcite (Sanyal et al., 2000), which cannot be ascribed to a vital effect. The universally lower-than-predicted $\delta^{11}B$ vs. pH sensitivity observed in marine biogenic and synthetic carbonates (Figure 1) may instead reflect on a not-yet-identified aqueous or adsorption process. This conundrum highlights the danger of inferring physiological processes (i.e., internal pH upregulation) from incompletely understood proxy systematics, and more instrumental observations are needed to confirm the pH regulation in marine calcifiers. Boron isotopes in brachiopod shells are also sensitive to pH (Penman et al., 2013), whereas $\delta^{11}B$ does not vary with seawater pH in shells of the mussel *Mytilus edulis*, but rather reflects variability induced by physiological modification of the calcification fluid (Heinemann et al., 2012).

### B/Ca Ratios

The prediction that boron incorporation into marine carbonates should also increase at higher pH with the aqueous abundance of borate in seawater has been tested in core-top (Yu et al., 2007; Foster, 2008) and laboratory (Allen et al., 2011, 2012) settings. Neither of these approaches found B/Ca to be very sensitive in planktonic foraminifers, although culture experiments verified pH to be the dominant control, with minor influences of temperature, salinity, and a potential competition with $\text{CO}_3^{2-}$ (Figure 2). An

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**Box 1. Overview of Mechanisms Underlying Geochemical Proxies Proposed as Indicators of Ocean Acidification**

**$\delta^{11}B$ and B/Ca** – The sensitivity of boron isotopes ($\delta^{11}B$) and B/Ca ratios in marine carbonates to seawater pH is rooted in the same basic principle. The equilibrium reaction between the two dominant aqueous boron species shifts their relative abundance from boric acid ($\text{B(OH)}_3$) at low pH to borate ($\text{B(OH)}_4^-$) at high pH (Figure A). Because of differences in vibrational energy and molecular geometry, $^{10}B$ is preferentially fractionated into borate and $^{11}B$ into boric acid, such that the isotopic composition ($\delta^{11}B$) of borate increases with its relative abundance at higher pH (Figure B). Marine carbonates record $\delta^{11}B$ similar to aqueous borate, suggesting that borate is the species incorporated and $\delta^{11}B$ of marine carbonates should consequently increase with higher pH (Figure B). Similarly, boron incorporation into marine carbonates should also increase with the aqueous abundance of borate in seawater (i.e., at higher pH; Figure A).

**U/Ca** – The theoretical basis for an ocean acidification effect on U incorporation into biogenically precipitated carbonate is rooted in the changing abundance of the triscarbonato complex ($\text{UO}_2(\text{CO}_3)_3^{4-}$) with pH (Figure C; redrawn from Djogic et al., 1986), and/or the observation that uranium adsorption onto shell surfaces is inhibited by $\text{CO}_3^{2-}$. Consequently, U/Ca should decrease at higher $\text{CO}_3^{2-}$.

**Other trace elements** – Trace element to calcium ratios such as Mg/Ca, Sr/Ca, Cd/Ca, and Zn/Ca recorded in marine carbonates respond to a number of primary environmental controls. However, many elements share a collective sensitivity to CaCO$_3$ saturation, which is likely related to a precipitation rate control.

**Carbonate polymorphs** are different forms of CaCO$_3$ (amorphous carbonate, high-Mg calcite, aragonite, calcite, vaterite) with differing solubilities. These may change in response to saturation state and altered Mg content of seawater.
evaluation of previous paleoceanographic reconstructions found that application of partition coefficients for estimating past ocean changes obfuscates true proxy controls and should be avoided (Allen and Hönsisch, 2012).

In contrast to planktonic foraminifers, benthic foraminiferal B/Ca does not directly respond to seawater pH, but core-top observations reveal a convincing dependency on $\Delta$CO$_3^-$ (i.e., the carbonate ion saturation; Yu and Elderfield, 2007; Brown et al., 2011; Rae et al., 2011; Raitzsch et al., 2011a). Although a mechanistic understanding for this $\Delta$CO$_3^-$ control is currently missing, core-top observations from various ocean basins are mutually consistent and do not suggest any additional environmental controls other than species-specific sensitivities. Importantly, B/Ca sensitivity to $\Delta$CO$_3^-$ is greatest in epifaunal species, which record bottom-water conditions and are thus most useful for paleoceanographic reconstructions. In addition, the B/Ca to $\Delta$CO$_3^-$ response is linear and does not lose sensitivity at low $\Delta$CO$_3^-$, which is a complication of the boron isotope proxy. In contrast to calcitic foraminifers, B/Ca ratios in corals appear more sensitive to temperature and less sensitive to carbonate chemistry (Trotter et al., 2011).

Calcium Isotopes
Calcium isotope ratios in biogenic carbonates have been related to temperature variations (Nägler et al., 2000), but additional effects seem to be exerted by [CO$_3^-$] and carbonate saturation state ($\Omega$) (Gussone et al., 2005). While Lemarchand et al. (2004) showed that inorganic calcite exhibits a strong increase in $^{44}$Ca uptake as pH, $\Omega$$_{calc}$ and the carbonate ion concentration decrease, the relative contributions of [CO$_3^-$] and temperature remain convoluted (Gussone et al., 2003, 2006; Gussone and Filipsson, 2010). Additional research is required to determine whether the [CO$_3^-$] signal can be distinguished from temperature.

U/Ca Ratios
U/Ca is predicted to decrease at higher [CO$_3^-$] because abundance of the triscarbonate complex (UO$_2$(CO$_3$)$_2$) declines with increasing pH, and uranium adsorption onto test surfaces is inhibited by CO$_3^-$ (Box 1). Russell et al. (1996) found U/Ca ratios in glacial planktonic foraminiferal tests to be ~25% lower compared to their Holocene relatives and suggested the observed glacial-interglacial U/Ca difference may be controlled by seawater [CO$_3^-$] or temperature. This hypothesis has been confirmed in culture experiments with the living planktonic foraminifers Orbulina universa and Globigerina bulloides (Russell et al., 2004) and a benthic foraminiferal species (Ammonia sp., Keul et al., 2013). All calibrations display inverse correlations between foraminiferal U/Ca and [CO$_3^-$] (Figure 3). Raitzsch et al. (2011b) made similar observations with core-top samples of the benthic foraminifers Planulina wuellerstorfi and Cibicidoides mundulus, although they found a stronger relationship with carbonate saturation, $\Delta$CO$_3^-$, than with [CO$_3^-$].

Although first applications demonstrated that last glacial maximum surface ocean [CO$_3^-$] estimates are comparable to reconstructions using $\delta^{11}$B and size-normalized foraminiferal weights (Russell et al., 2004), the U/Ca proxy has found little downcore application to date. This is partly due to authigenic overgrowths complicating the [CO$_3^-$] signal in reducing sediments (Boiteau et al., 2012), but U/Ca is also susceptible to partial dissolution in corrosive bottom water, which decreases the original ratio (Russell et al., 1994; Yu et al., 2008). U/Ca is elevated in larger planktonic foraminiferal

**FIGURE 1.** Laboratory (open symbols) and field calibrations (closed symbols) of boron isotopes ($\delta^{11}$B) in marine carbonates versus seawater pH. Green symbols = deep-sea corals (Anagnostou et al., 2012), red symbols = shallow water corals (Hönisch et al., 2004; Reynaud et al., 2004; Krief et al., 2010; Trotter et al., 2011), light blue symbols = planktonic foraminifers (Henehan et al., 2013; Hönisch et al., 2009; Sanyal et al., 2001), black symbols = inorganic calcite (Sanyal et al., 2000), orange symbols = brachiopods (Penman et al., 2013), and dark blue symbols = benthic foraminifers (Rollion-Bard and Erez, 2010; Rae et al., 2011). $\delta^{11}$B$_{borate}$ is calculated for K$_{br}$ = 8.60 at 25°C and S = 35, using the aqueous fractionation factor of Klochko et al. (2006). Note that variability in the field calibrations is partially due to their range of temperature, pressure, and salinity conditions, which affect boron dissociation (Dickson, 1990). Field calibrations typically cover a much smaller pH range than is achievable in the laboratory.
tests compared to smaller conspecifics, and it may be related to ontogenetic differences in calcification rate and/or habitat depth in the water column (Ni et al., 2007). Finally, a temperature influence on U incorporation remains controversial (Russell et al., 2004; Yu et al., 2008).

Similar to foraminifers, the aragonitic skeletal U/Ca ratio of the coral Acropora digitifera increases with decreasing pH (Inoue et al., 2011); the same is true for the larval prodissocochn shells of Mytilus californianus and M. galloprovincialis (Frieder et al., 2014). The U/Ca sensitivity to \([\text{CO}_3^{2–}]\) or pH differs greatly between different calcifiers (Figure 3), making species-specific empirical calibrations a necessity. Evidence from Crassostrea gigas indicates that initial shell formation is influenced by seawater carbonate saturation state (Waldbusser et al., 2013), favoring the potential utility of U/Ca as a pH proxy in larval bivalve stages. In habitats where pH and \([\text{CO}_3^{2–}]\) do not covary, such as in estuaries (because of salinity and alkalinity gradients), the U/Ca proxy should be tested for dependence on pH or \([\text{CO}_3^{2–}]\) (Keul et al., 2013).

The use of U/Ca as an OA proxy may be limited for encapsulated larvae, later ontogenetic stages, or fossilized material. In a laboratory-controlled study, statoliths of Doryteuthis opalescens (squid) became enriched in U/Ca when embryos were exposed to low pH and low oxygen in combination (characteristic of upwelling), but not under low pH alone, suggesting an oxygen-induced loss of acid-base regulation in the endolymph of the squid embryo statocyst (Navarro et al., 2014). This finding could reflect an indirect physiological response to pH stress rather than an environmental pH signal and may apply more broadly to encapsulated embryos developing in isolation from seawater.

Evidence from the butter clam, Saxidomus gigantea, indicates that U/Ca is under ontogenetic and diagenetic, not environmental, forcing in post-larval stages; U/Ca becomes undetectable in older specimens, and it decreases from the outside of the shell toward the inside (Gillikian and Dehairs, 2013). U/Ca also changes over time and has been found to be much higher in fossils on land (Broecker, 1963; Kaufmann et al., 1971); thus, its application to shell material from archaeological middens may be limited. U/Ca has also been considered as an environmental indicator for temperature in corals (e.g., Min et al., 1995; Shen and Dunbar, 1995; Felis et al., 2009; but see Wei et al., 2000), indicating a need to isolate the influence of temperature and \([\text{CO}_3^{2–}]\) on uranium incorporation into the carbonate complex before the proxy can be reliably utilized in the context of OA.

### Trace Element Sensitivity to the Carbonate System

#### Foraminifera

Mg/Ca, Sr/Ca, Cd/Ca, and Zn/Ca in foraminiferal tests all decrease with decreasing CaCO₃ saturation at the seafloor. This phenomenon has been observed in core-top foraminifers (Marchitto et al., 2000, 2002, 2005; Rosenthal et al., 2006; Elderfield et al., 2010) and in a laboratory culture experiment (Raitzsch et al., 2010), although the culture experiment modified CaCO₃ saturation by varying \([\text{Ca}^{2+}]\) instead of \([\text{CO}_3^{2–}]\). Although the primary sensitivities of the trace element ratios to temperature or their respective concentrations in seawater complicate their application for reconstructing \(\Delta\text{CO}_3^{2–}\), their common sensitivity to this parameter suggests it may be caused by biological elevation of the calcifying fluid’s saturation state to allow for CaCO₃ precipitation under challenging calcification conditions (de Nooijer et al., 2014). These observations and a growing number of laboratory experiments (e.g., Dissard et al., 2010; Haynert et al., 2014) provide an opportunity to gain greater insight into how foraminifera have evolved to cope with low saturation conditions in their seafloor environment.

#### Metazoans

Despite a broad literature on trace element concentrations in metazoan carbonate structures (e.g., Thorrold et al., 2007), little research has focused directly on these elements as proxies for OA. Mg is the element most often cited in the context of environmental reconstruction, and it has been widely studied in echinoderms. Echinoderm skeletons are precipitated intracellularly and 3–18% wt of Ca²⁺ is replaced by Mg²⁺ (high-Mg CaCO₃). This percentage varies by taxon, by type of plate or structure, and with environmental temperature or seawater chemistry. Analysis of Mg/Ca in Antarctic echinoderms yields decreasing Mg % wt in asteroids > ophiuroids > holothurians > echinoids.
consistent with fossil and tropical studies (McClintock et al., 2011). High-Mg calcite is precipitated within vesicles that regulate pH, $pCO_2$, and trace elemental composition of the calcification compartment (Weiner and Dove, 2003). It is also recognized that sea urchins employ several calcification pathways that involve varying proportions of metabolic vs. inorganic carbon (e.g., Ebert, 2007; Ries, 2011). Taxon variation in Mg/Ca is interpreted to reflect relative dissolution vulnerabilities to OA because CaCO$_3$ solubility increases with increasing Mg content (McClintock et al., 2011; Ries, 2011), and it changes with the [Mg] and [Ca] composition of seawater over geologic time (Dickson, 2002, 2004). Taxa with high-Mg calcite or aragonite can evolve or switch morphs, or construct thicker skeletons when Mg/Ca is higher in seawater (Stanley, 2006; Gorzalek et al., 2013).

Adult purple sea urchins (Strongylocentrotus purpuratus) do not vary their skeletal Mg/Ca or Sr/Ca ratios under different upwelling (lower pH) regimes in their natural environment (La Vigne et al., 2013). In contrast, S. purpuratus larvae exposed to experimentally elevated $pCO_2$ exhibited increased Sr/Ca (by 8%) in skeletal structures of newly settled juveniles, but only for settlers spawned from adults originating in more pH benign conditions in southern California, not for those from further north (where upwelling events are more intense and pH is lower; La Vigne et al., 2013). Geochemical response by larval or juvenile urchins to elevated $pCO_2$ could offer adaptive potential, but the functional consequences remain unknown. In crinoids, Mg is positively correlated with water temperature, but significant Mg/Ca variation within specific structures, such as stereom tribecula or ossicles, and between individuals makes use as a proxy difficult (Gorzalek et al., 2013). While Mg/Ca and Sr/Ca ratios may vary with $pCO_2$ conditions for some echinoid stages, the effect is likely to be indirect (e.g., via calcification rate) and not directly mediated by carbonate chemistry. Given the diversity of influences on calcification rates (e.g., temperature, food availability, age), Mg/Ca and Sr/Ca are not likely to serve as robust OA proxies in metazoans. However, they may be useful indicators of calcification rate.

Mg content of biogenic carbonate, while responsive to $CO_2$ in some taxa, does not exhibit consistent trends. Mediterranean mussels (M. galloprovincialis) transplanted into low-pH ($CO_2$ vent) environments without a change in temperature revealed increasing Mg content in calcite layers but no other elemental response (e.g., Sr, Ba, Mn, Fe; Hahn et al., 2012). Bryozoans (Myriapora truncata) transplanted to sites of $CO_2$ venting exhibited reduced Mg incorporation at acidic sites (mean pH 7.43) relative to controls (mean pH 8.07) (Lombardi et al., 2010). However, recent analysis of 53 species of Antarctic bryozoans showed no relationship of Mg/Ca to water depth, despite a 0.4 unit decline in pH with depth (Borszcz et al., 2013). With decreasing $\Omega_{arag}$, the Mg/Ca ratio in the tubes of serpulid polychaetes increases, whereas it declines in red coralline algae; notably, Mg/Ca in these taxa exhibits a much larger response to seawater temperature and salinity than to $\Omega_{arag}$ (Ries, 2004, 2006, 2011).

Frieder et al. (2014) reported a negative relationship between Sr/Ca and pH in aragonitic larval shells of two Mytilus species exposed experimentally to varying pH conditions. However, because Sr/Ca was correlated with larval size in M. californianus and because it is known to vary with temperature and salinity in aragonite (Campana, 1999), we do not consider Sr/Ca a viable acidification proxy for mollusks. Munday et al. (2011b) raised the anemone fish Amphiprion percula at pH$_{NRS}$ 7.16–8.15 and found no influence on Ba, Sr, Mn, Mg, and Li to Ca ratios. Studies have shown trace element concentrations in fish otoliths to be linked to salinity (e.g., Martin et al., 2004)—the extent that this is controlled.
by alkalinity, reflects pH, or is due to some other aspect of the carbonate system has not yet been addressed.

In radiotracer experiments Lacoue-Labarthe et al. (2011) demonstrated increasing $^{65}$Zn incorporation into squid statoliths with decreasing pH, whereas $^{110}$Ag, $^{109}$Cd, $^{57}$Co, $^{203}$Hg, and $^{54}$Mn did not vary with pH. These were correlated with statolith enlargement (but not embryo enlargement), so it is unclear whether there is proxy potential here. On the other hand, scleractinian corals (Stylophora pistillata) exhibit greater incorporation of $^{65}$Zn in coral tissue and skeleton with increasing pH, independent of photosynthesis, photosynthetic efficiency, and gross calcification (Houlbrèque et al., 2012).

Efforts to use multi-elemental fingerprints as proxies for OA exposure might offer some success. For example, a principal components analysis with U, Sr, Cu, and Zn was a viable predictor of pH, explaining >97% of variance in larval shell elemental composition for M. californianus and M. galloprovincialis (Frieder et al., 2014). However, a similar quest for a multi-elemental proxy in market squid (D. opalescens) statoliths was not successful (Navarro et al., 2014). A suite of elements with strong mechanistic rationale (e.g., B, U, and Mg) might offer the best outcomes.

**Carbonate Polymorphs**

Among invertebrates, many taxa precipitate different forms of CaCO$_3$ (polymorphs) with differing solubilities and potentially varying sensitivities to OA (Andersson et al., 2008). Variations in polymorph mineralogy among taxa largely reflect the seawater Mg/Ca ratios at the time when each taxon evolved (Lowenstein et al., 2014). CaCO$_3$ solubility increases with increasing Mg content and may make organisms that secrete high-Mg calcite (defined as > 4 mol% MgCO$_3$; Boggild, 1930), such as echinoderms, bryozoans, calcareous red algae, and some foraminifers, more vulnerable to OA. Aragonite, with intermediate solubility, is precipitated by corals and mollusks, and most extant fish species produce otoliths made of aragonite or vaterite (Gauldie, 1993). Most calcareous foraminifers and articulate brachiopods precipitate calcite, although Hoeglundina (Epistomina) elegans secretes aragonite tests. Some invertebrate species shift their carbonate morph ontogenetically or upon environmental forcing (Ries, 2011). Mussel and oyster larvae (for example, precipitate aragonitic shells as larvae in the water column, but secrete calcite and aragonite as benthic adults; this may increase larval vulnerability to OA relative to adults (Byrne, 2011). Fitzier et al. (2014) found juvenile mussels can precipitate aragonite under normal pCO$_2$, but only calcite at 1,000 µatm pCO$_2$. It is possible that these variations in carbonate polymorph could be considered as possible proxies for pCO$_2$ ([CO$_3^-$]$_2$, or pH, as discussed above for Mg content. Why do some taxa exhibit variability in their carbonate polymorph but most do not? We are just discovering the reasons why animals might have evolved mechanisms to alter trace elemental chemistry or carbonate morph. High magnesium increases hardness in echinoids (Ma et al., 2009) and biminarlic layering reduces crack propagation in bivalves (Ries, 2011). More work is required to determine whether these patterns reflect adaptation or resilience to OA.

**DISCUSSION**

Although proxy development is fairly well advanced for paleoceanographic applications, the use of geochemical proxies to understand exposures of modern or living species is in its infancy. Boron-based ($^{88}$B, B/Ca) and U/Ca proxies exhibit the greatest promise as OA proxies because they directly reflect chemical mechanisms for carbonate system influence on incorporation from seawater (Box 1). It may be that U/Ca, which achieves higher concentrations in aragonite (Figure 3), is a more suitable proxy for carbonates of bivalves, corals, and fish, whereas B/Ca is a more faithful reflector of seawater conditions in calcitic taxa like foraminifers and brachiopods. The other trace element proxies discussed here most likely reflect biologically mediated responses to changes in carbonate chemistry, and many also vary with temperature, alkalinity, nutrients, or other aspects of hydrography.

The feasibility of using geochemical proxies may differ in different environments, depending on the nature of pH or pCO$_2$ variability. Many intertidal or shallow ecosystems (coral reefs, rock pools, seagrass beds, and kelp forests) exhibit high, high-frequency pH variability (Hofmann et al., 2011; Frieder et al., 2012). In southern California, this variation can be semiannual and diurnal, associated with respiration cycles or internal tides, wind-driven (one- to two-week events), seasonal, interannual (El Niño-Southern Oscillation-driven), or associated with regime changes (Levin et al., 2015). Also, daily vertical migrations of larvae or holoplankton can expose organisms to considerable high-frequency variability in pH, pCO$_2$, and saturation state. Understanding how high-frequency environmental variability is integrated into a geochemical signal will be particularly relevant when applying OA proxies that infer exposure history in highly variable settings such as estuaries, continental shelves, and coral reefs. One high-resolution (ICPMS-based) laboratory study demonstrated that mussel larval shell geochemistry (U/Ca) remains the same whether precipitated under semidiurnal pH variations ($±0.15$ pH units) or constant pH conditions (Frieder et al., 2014). However, daily variations in trace elements such as Mg/Ca have been detected in planktonic foraminifera (Spero et al., 2015) and might offer potential to evaluate high-frequency variation in physiology. Ability to resolve high-frequency patterns will depend not only on the integration of the signal in the calcified structure but also on the resolution of the method used.

Deep-sea ecosystems exhibit much lower carbonate precipitation rates, and they potentially offer records (such as
in cold-water corals) that integrate historical time scales. Unfortunately, there is greater unexplained proxy variability, for example, in δ¹³B in the deep-sea coral *Lophelia*, than might be expected from a stable environment (Blamart et al., 2007). This variability is often attributed to vital effects; Adkins et al. (2003) propose a mechanism to explain stable isotope variability in deep-sea corals that is based on a thermodynamic response to a biologically induced pH gradient in the calcifying region. More work is required to determine whether such information could prove useful for understanding biological response to environmental change in the deep sea.

There is growing recognition that different sources of OA often involve additional stressors, especially in coastal waters (Duarte et al., 2013; Breitburg et al., 2015, in this issue). Using additional trace elements, it may be possible to reconstruct the sources of exposure to low pH by resolving additional features such as warming, hypoxia (Limburg et al., 2011, 2015), coastal upwelling in the Northeast Pacific (Lares and Orians, 1997; Segovia-Zavala et al., 1998), or freshwater and alkalinity (Hönisch et al., 2011).

**CONCLUSIONS AND PROSPECTS**

Many challenges remain in developing and calibrating geochemical proxies for paleoceanographic or modern carbonate system reconstruction and exposure studies. In metazoans, which have complex tissues that interact with carbonates, understanding species-specific controls on carbonate chemistry at the site of calcification poses the greatest challenge. Rate of precipitation and crystallization can influence elemental partitioning in carbonates. The influence of growth and calcification on element incorporation is often explained as a Rayleigh fractionation effect (e.g., Elderfield et al., 1996; Bockmon et al., 2013), but there are still other factors.

In OA proxy development could be facilitated by (1) availability of experimental facilities that allow control of multiple factors and can mimic realistic conditions (e.g., Bockmon et al., 2013), (2) use of field calibrations that allow outplanting or transplanting of organisms under known (measured) conditions, and (3) collaboration of chemists and biologists to investigate novel proxies and their mechanistic basis. Finally, we suggest that simultaneous application of geochemical proxies to infer exposure to multiple stressors will advance mechanistic, context-dependent understanding of OA.

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