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Magnesium (Mg/Ca, $\delta^{26}Mg$), boron (B/Ca, $\delta^{11}B$), and calcium ($[Ca^{2+}]$) geochemistry of *Arctica islandica* and *Crassostrea virginica* extrapallial fluid and shell under ocean acidification

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Abstract. The geochemistry of biogenic carbonates has long been used as proxies to record changing seawater parameters. However, the effect of ocean acidification on seawater chemistry and organism physiology could impact isotopic signatures and how elements are incorporated into the shell. In this study, we investigated the geochemistry of three reservoirs important for biomineralization - seawater, the extrapallial fluid (EPF), and the shell - in two bivalve species, *Crassostrea virginica* and *Arctica islandica*. Additionally, we examined the effects of three ocean acidification conditions (ambient: 500 ppm CO_2, moderate: 900 ppm CO_2, and high: 2800 ppm CO_2) on the geochemistry of the same three reservoirs for *C. virginica*. We present data on calcification rates, EPF pH, measured elemental ratios (Mg/Ca, B/Ca), and isotopic signatures ($\delta^{26}Mg$, $\delta^{11}B$). In both species, comparisons of seawater and EPF Mg/Ca and B/Ca, $[Ca^{2+}]$, and $\delta^{26}Mg$ indicate that the EPF has a distinct composition that differs from seawater. Shell $\delta^{11}B$ did not faithfully record seawater pH and $\delta^{11}B$-calculated pH values were consistently higher than pH measurements of the EPF with microelectrodes, indicating that the shell $\delta^{11}B$ may reflect a localized environment within the entire EPF reservoir. In *C. virginica*, EPF Mg/Ca and B/Ca, as well as absolute concentrations of Mg, B, and $[Ca^{2+}]$, were all significantly affected by ocean acidification, indicating that OA affects the physiological pathways regulating or storing these ions, an observation that complicates their use as proxies. Reduction in EPF $[Ca^{2+}]$ may represent an additional mechanism underlying reduction in calcification in *C. virginica* in response to seawater acidification. The complexity of dynamics of EPF chemistry suggest boron proxies in these two mollusc species are

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

not straightforwardly related to seawater pH, but ocean acidification does lead to both a decrease in microelectrode pH and boron-isotope-based pH, potentially showing applicability of boron isotopes in recording physiological changes. Collectively, our findings show that bivalves have high physiological control over the internal calcifying fluid, which presents a challenge to using boron isotopes for reconstructing seawater pH.

1 Introduction

The elemental geochemistry of marine biogenic carbonate shells is widely used to track and reconstruct environmental change (Broeker and Peng, 1982; Elderfield, 2006). The incorporation of elements within the skeleton of marine calcifiers has been shown to be correlated with different environmental parameters, such as temperature (Dunbar et al., 1994, Alibert and McCulloch 1997) and pH (e.g. Hemming and Hanson, 1992; Hönisch et al., 2004; McCulloch et al., 2018). However, it has long been recognised that elemental and isotopic signatures of biogenic carbonate deviate from inorganic carbonate grown under the same conditions, complicating the use and interpretation of these theoretical models for paleo-reconstructions (e.g.. Urey, 1951; Craig, 1953; reviewed by Weiner and Dove, 2003). The physiological processes alter the geochemistry of biominerals and consequently offset the environmental signal incorporated in biogenic carbonates, termed "vital effects" (Urey, 1951) which includes the different biomineralization strategies that can modify the chemistry of the calcification fluid (Weiner and Dove, 2003). For organisms to calcify, a semi-isolated calcification space will be, to varying degrees, separated from seawater for supersaturation to be achieved in support of calcification (Weiner and Dove, 2003). In intracellular calcification, biominerals can be formed within cells using specialized vesicles or vacuoles, whereas in extracellular cases, calcification may occur on an organic matrix template, with ions transported as necessary for crystal nucleation to occur (Weiner and Dove, 2003; Addadi et al., 2006; reviewed by Gilbert et al., 2022). Additionally, the geochemistry of the calcification fluid can be altered due to differing degrees of isolation from the parent fluid, seawater, as well as the modulation of the calcification fluid chemistry via different methods of passive or active ion transport to the site of calcification (Weiner and Dove 2003; McCulloch et al., 2017; Sutton et al., 2018; Liu et al., 2020). A mechanistic understanding of such vital effects is desirable for the accurate interpretation of geochemical proxies preserved in the shells of these organisms.

Molluscs have long been recognized as valuable archives for climate reconstructions, given the annual resolution growth bands, long lifespans, and wide geographic distributions (Gibson et al., 2001; Peharda et al., 2021). However, it is also well established that mollusc shell carbonates can express significant vital effects in many geochemical parameters (Schöne, 2008). For example, the $\delta^{11}B$ proxy for seawater pH in foraminifera and corals seems relatively insensitive in many molluscs examined, including *Mytilus edulis, Mercenaria mercenaria*, and *Crassostrea virginica* (Heinemann et al., 2012; Foster and Rae, 2016; McCulloch et al., 2017; Liu et al., 2020; Eagle et al., 2022). Shell B/Ca has been shown to be correlated to internal fluid pH in *Mytilus edulis* (Heinemann, 2012) and *Mercenaria mercenaria* (Ulrich et al., 2021), but relationships to seawater pH were less clear. Reported Mg/Ca are widely used as temperature proxies in many marine calcifiers

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

(Wannamaker 2008), however it is also long established that molluscs can regulate and actively exclude [Mg^{2+}] from their shells (Lorens and Bender, 1977; Planchon et al., 2013), showing that biological regulation of biocalcification and the parent fluids for shell formation can have a strong influence on Mg-based geochemical proxies. Mg isotope analyses can potentially inform the [Mg^{2+}] transport process in molluscs. Although few Mg isotope studies of molluscs have been done, a study by Planchon et al. (2013) investigated $\delta^{26}Mg$ across *Ruditapes philippinarum* tissues, shell, and fluid reservoirs and found that seawater and extrapallial fluid magnesium signatures similar, suggesting that seawater is the source of [Mg^{2+}] ions within the extrapallial fluid. Additionally, Planchon et al. (2013) found that Mg signatures within the shell varied between specimens and were either in line with or deviated from inorganically precipitated aragonite, suggesting an ability for some clams to physiologically alter or regulate [Mg^{2+}] within the extrapallial fluid.

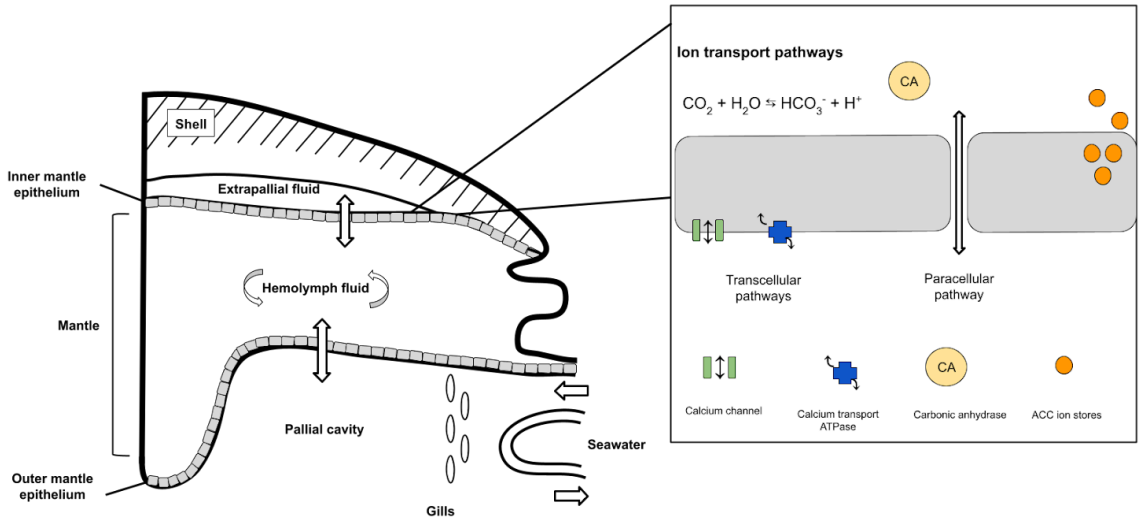

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Figure 1. Schematic of a bivalve cross section showing the flow of between biomineralization ion reservoirs. The box on the right shows a zoomed in schematic across the inner mantle epithelium cells that show transcellular and paracellular ion transport pathways in and between epithelial cells. Figure adapted from Planchon et al. (2013) and Zhao et al. (2016).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

Understanding the structure of mollusc tissues, internal fluid reservoirs, mechanisms of calcification and ion transport to the site of calcification is critical to understanding these vital effects (Fig 1). It may also give insight into the sensitivity of bivalves to CO_2-induced ocean acidification, a major environmental challenge to ocean ecosystems and commercial shellfish fisheries (Gazeau et al., 2013; Stewart-Sinclair et al., 2020). Typically, bivalves are amongst the more sensitive group of marine calcifier species to acidification (Ries et al., 2009; Kroecker et al., 2011).

	Control *A. islandica*	Control *C. virginica*	Moderate OA *C. virginica*	High OA *C. virginica*
Measured seawater parameters				
pH (total scale)	7.93 ± 0.09	8.01 ± 0.08	7.75 ± 0.07	7.29 ± 0.11
DIC (µmol/kg)	n/d	1966 ± 44	1998 ± 212	2177 ± 160
TA (µmol/kg)	n/d	2120 ± 46	2120 ± 42	1511 ± 40
Mg/Ca (mol/mol)	5.13 ± 0.07	5.15 ± 0.07	5.23 ± 0.06	5.12 ± 0.03
δ^{26}Mg (‰)	-0.82 0.06 ‰	-0.77 ± 0.01	-0.82 ±0.03	-0.76 ± 0.09
B/Ca (mol/mol)	41.75 ± 1.52	41.66 ± 1.07	43.08 ± 2.9	42.11 ± 1.8
δ^{11}B (‰)	39.88 ± 0.13	40.29 ± 0.33	39.39 ± 0.33	39.82 ± 0.33
Calculated seawater parameters				
pCO_2 (ppm)	n/d	570 ± 90	990 ± 173	2912 ± 373
$[CO_3^{2-}]$ (µM)	n/d	120 ± 12	79 ± 13	31 ± 4
$\Omega_{Calcite}$	n/d	2.95 ± 0.30	1.93 ± 0.32	0.75 ± 0.09
$\Omega_{Aragonite}$	n/d	1.89 ± 0.19	1.24 ± 0.21	0.48 ± 0.06
δ^{11}B-calculated EPF pH (total scale)	7.76 ± 0.07	8.12 ± 0.09	8.06 ± 0.10	8.01 ± 0.08

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

$\triangle pH_{SW\text{-}\delta 11B.pH}$	0.17	0.64	0.77	0.8
EPF geochemistry				
microelectrode EPF pH (total scale)	7.41 ± 0.14	7.48 ± 0.15	7.29 ± 0.10	7.21 ± 0.10
$\triangle pH_{SW\text{-}EPF}$	0.52	0.53	0.46	0.08
Mg/Ca (mol/mol)	4.25 ± 0.67	4.55 ± 0.50	5.73 ± 0.34	5.58 ± 0.46
$\delta^{26}Mg$ (‰)	-0.69 0.01 ‰	-0.88 ± 0.06	-0.87 ± 0.07	-0.9 ± 0.1
B/Ca (mol/mol)	31.17 ± 4.87	33.66 ± 2.81	42.22 ± 3.33	43.26 ± 2.82
$\delta^{11}B_{EPF}$ (‰)	39.5 ± 0.4	39.3 ± 1.0	38.9 ± 0.47	n/d
Shell geochemistry				
Mg/Ca (mmol/mol)	0.8 ± 0.2	13.8 ± 1.7	13.4 ± 2.3	12.3 ± 1.5
$\delta^{26}Mg$ (‰)	n/d	-3.2 ± 0.1	-3.1 ± 0.1	-3.0 ± 0.2
B/Ca (µmol/mol)	57 ± 17	114 ± 22	125 ± 11	124 ± 9
$\delta^{11}B_{Shell}$ (‰)	15.26 ± 0.41	18.34 ± 0.59	16.91 ± 0.56	16.84 ± 0.35

Table 1. Seawater and extrapallial fluid carbonate chemistry parameters (pH, DIC, TA, Ω, δ11B-calculated EPF pH, and △pH) for both C. virginica and A. islandica under control conditions and C. virginica for OA conditions.. Seawater, extrapallial fluid, and shell geochemical parameters (Mg/Ca, δ26Mg, B/Ca, δ11B) for both C. virginica and A. islandica under control conditions and C. virginica for OA conditions. Parameters that were not measured or calculated are marked with 'n/d.'

The bivalve mollusc extrapallial fluid (EPF) is an internal fluid reservoir physically semi-separated from seawater that circulates in the pallial cavity, between the outer mantle epithelium (OME) and shell. Seawater enters the pallial cavity when

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

valves are open, then the internal hemolymph fluid circulates within the organs of the mollusc and finally can also be transported across the mantle to the EPF (Table 1; Zhao et al., 2018). Bivalve mollusc shell calcification is thought to occur at the interface of the EPF and growing shell where the ions for calcification interact with organic matrices, such as polypeptide molecules (Crenshaw, 1972; Wheeler and Sikes, 1984; Wilbur and Bernhardt, 1984; Addadi, 2006) and proteins within the EPF that act as a scaffolding template for nucleation and are important in the calcification process (Crenshaw, 1972; Wilber and Bernhardt, 1984). Additionally, molluscs can calcify though a transient amorphous calcium carbonate precursor phase in which disordered calcium carbonate crystals can be stored and then transported to the calcification front (Addadi, 2003; Immenhauser et al., 2016), which can act as another source of potential geochemical vital effects. Therefore, it is expected that EPF chemistry will differ from seawater and that knowledge of EPF geochemistry may inform our knowledge of vital effects in bivalve molluscs.

Unlike the calcifying fluid reservoirs in most organisms, bivalve EPF has a large enough volume that it can be directly sampled, allowing for direct measurements of the reservoir to compare with seawater geochemistry and elucidate in situ changes in EPF chemistry. A foundational study by Crenshaw (1972) found that, in three mollusc species, the EPF calcification fluid had a different chemical composition and pH from seawater and from the mollusc hemolymph fluid (Crenshaw et al., 1972). Crenshaw, (1972) reported that EPF pH was significantly lower than seawater pH, that cationic compositions of the EPF could also differ from seawater, and that the total C (including all species of dissolved inorganic carbon) of the EPF was higher than that of seawater. Additionally, Crenshaw also showed that EPF calcium concentration and pH co-varied significantly over time during the opening and closing of valves, or the ventilation cycle. When valves are closed pH is lower and calcium concentration higher, resulting from dissolution of shell material and return of calcium to the EPF (Crenshaw, 1972). A previous study on the king scallop, *Pecten maximus*, by Cameron et al. (2019) showed that EPF pH was lower than seawater and also depended on pCO_2 and temperature. Ramesh et al., (2017) reported, using a microelectrode approach, that pH and $[CO_3^{2-}]$ were elevated proximal to the growing shell in larval *Mytilus edulis* shells. In the quahog *Arctica islandica*, Stemmer et al. (2019) reported synchronous short-term fluctuations in $[Ca^{2+}]$ and pH at the outer mantle epithelium surface. They attributed this to active ion pumping across mantle epithelial cells, which created significant differences between carbonate saturation and pH of the bulk EPF and the EPF close to the outer mantle epithelium.

Boron proxies utilise boron speciation and isotope fractionation in seawater to reconstruct pH and $[CO_3^{2-}]$ of seawater from the chemistry of calcium carbonate shells (Hemming and Hanson, 1992; Hönisch et al., 2004). In seawater, the speciation of boric acid $[B(OH)_3]$ and borate ion $[B(OH)_4^-]$ varies as a function of pH (Hemming and Hanson 1992). In addition to the pH dependence of their relative abundances, the boron proxy also makes use of a large isotopic fractionation between the two boron species (Klochko et al., 2006, Nir et al., 2015). A key assumption of the proxy is that boron, in the form of borate ion, is the predominant form incorporated into the crystal lattice of calcite via carbonate ion substitution during the precipitation of calcium carbonate (Hemming and Hanson 1992). The $\delta^{11}B$ of the carbonate ($\delta^{11}B_{CaCO3}$) should then, in theory, reflect the boron isotopic composition of the borate ion in seawater ($\delta^{11}B_{CaCO3}$). Accurate reconstruction of seawater pH can then be

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

achieved using specific empirical relationships between the $\delta^{11}B_{CaCO3}$ and $\delta^{11}B_{CaCO3}$, which can in turn be used to determine pH. The marine boron system is also utilized in the development of B/Ca proxies, which utilize the substitution of boron for $[CO_3^{2-}]$ in the crystal lattice and the relationship between the partition coefficient (K_D), B/Ca, and $[CO_3^{2-}]$ to create a proxy for $[CO_3^{2-}]$ of seawater or calcifying fluid (reviewed by DeCarlo et al., 2018). Using the exchange reactions for the substitution of boron during aragonite or calcite precipitation, the founding assumption of the proxy is that B/Ca of the shell can be used to calculate the $[CO_3^{2-}]$ of the solution from which the aragonite or calcite precipitated. Inorganic aragonite precipitation experiments have validated the B/Ca proxy by allowing for the calculation of the partition coefficient (K_D) between aragonite and seawater and fitting of experimental B/Ca data (Mavromatis et al., 2015; Holcomb et al., 2016; Allison 2017; reviewed by DeCarlo et al., 2018). However the B/Ca proxy also has limitations, as it has only been developed for aragonite samples and because of remaining unresolved differences in the formulation of the K_D, exchange reactions, and fitting of B/Ca experimental data between studies (Allison et al., 2017; McCulloch et al., 2017; DeCarlo et al., 2018; Holcomb et al., 2016). Together, both $\delta^{11}B$ (pH_{CF}) and B/Ca ($[CO_3^{2-}]$) proxies can be used to constrain the full carbonate system of the calcifying medium (DeCarlo et al., 2018).

Vital effects of the $\delta^{11}B$ can be species-specific. In the case of foraminifera, vital effects are relatively minor (Hönisch et al., 2004; Foster and Rae, 2016). However, other calcifying organisms, such as corals, coralline red algae, and molluscs, show significant $\delta^{11}B$ deviations from relationships predicted from theoretical calculations (e.g.. Donald et al., 2017; Schoepf et al., 2017; McCulloch et al., 2018; Sutton et al. 2018, Anagnostou et al., 2019; Liu et al., 2020). There are different theories to explain the divergence of $\delta^{11}B$ from the seawater theoretical model. It is hypothesized for some taxa that $\delta^{11}B$ may not faithfully record seawater pH, but rather the pH of the discrete fluid from which ions are sourced for calcification that may be isolated or semi-isolated from seawater (Gilbert et al., 2022). Previous work on corals has used the boron proxy analyses, along with other approaches, to probe internal carbonate chemistry of the calcification fluid (Ries, 2011; Holcomb et al., 2014; Guillermic et al., 2021; Cameron et al., 2022; Eagle et al., 2022; Allison et al., 2023). All approaches, both geochemical and physiological, indicate that corals elevate the pH and $[CO_3^{2-}]$ of their calcifying fluid to induce calcification, but this mechanism is sensitive to ocean acidification and has yet to be fully understood (Liu et al., 2020; Guillermic et al., 2021; Cameron et al., 2022; Eagle et al., 2022).

Beyond corals, few taxa have been studied using combined geochemical tracer work to determine the chemistry of calcification fluid pools and sources of ions to the calcification front. Work by Sutton et al. (2018) noted that $\delta^{11}B$ values in urchin spines were lower than seawater borate $\delta^{11}B$. Stumpp et al. (2013) showed that the internal pH of sea urchin larvae was typically lower than seawater pH. Short et al. (2015), Donald et al. (2017), Anagnostou et al. (2019), and Liu et al (2020) found high $\delta^{11}B$ in calcite produced by coralline algae, which is potentially consistent with elevation of calcifying fluid pH in support of calcification either through enzymatic proton removal and/or photosynthetically driven removal of dissolved inorganic carbon from the calcifying fluid. To date, one study has investigated the B/Ca and $\delta^{11}B$ of shell and EPF of the bivalve *Mytilus edulis* (Heinemann et al., 2012).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

The mollusc extrapallial fluid is an attractive target to investigate geochemical vital effects because not only can it be probed with electrodes, like for corals, but it can also be extracted and analyzed. In this study, we investigate the $\delta^{11}B$, B/Ca, $\delta^{26}Mg$, and Mg/Ca in extracted extrapallial fluid and aragonite shell of the quahog, *Arctica islandica*, and the calcite shell of the eastern oyster, *Crassostrea virginica*. This allows for the investigation of the tripartite fractionation between seawater, extrapallial fluid, and shell. Individuals were grown in controlled laboratory experiments, with extrapallial fluid pH determined with microelectrodes, and other physiological parameters, such as calcification rate and tissue production, determined by conventional methods (Downey-Wall et al., 2020). Specimens of *C. virginica* were also cultured in three different treatments of pCO_2: ambient, moderate and high ocean acidification conditions. Geochemical analysis of the seawater, shell, and extrapallial fluid thereby allow novel insights into the transport of ions from seawater to the extrapallial fluid, and the fractionation of isotopes and elements between the extrapallial fluid and shell under both control and acidified conditions.

2 Materials and Methods

2.1 Experimental Conditions

A detailed explanation of the collection and culturing of *C. virginica* and *A. islandica* is outlined in Downey-Wall et al. (2020). Seawater salinity, temperature, and pH (total scale) were monitored and maintained throughout the experiment. Seawater was maintained at a pH of 8.01 ± 0.08, temperature of 18.2 ± 1 °C, and salinity of 31 psu for the calcitic oyster *C. virginica.* Seawater was maintained at a pH of 7.93 ± 0.09, temperature of 18.2 ± 1 ° C, and salinity of 35 psu for the aragonitic clam *A. islandica* in the control conditions (Downey-Wall et al., 2020).

Adult *C. virginica* specimens were collected from three intertidal sites on Plum Island Sound, Massachusetts, USA (Site 1, 42.75 N, -70.84 E; Site 2,: 42.73 N, -70.86 E; Site 3, 42.68, -70.81) and transferred to Northeastern University's Marine Science Center. Following a 33-day period of acclimation to laboratory conditions, oysters from each collection site were exposed to control (mean pCO_2 ± SE = 570 ± 14 ppm; $\Omega_{calcite}$ = 2.95 ± 0.30), moderate OA (990 ± 29 ppm, $\Omega_{calcite}$ = 1.93 ± 0.32), or high OA (2912 ± 59 ppm, $\Omega_{calcite}$ = 0.75 ± 0.09) treatments. Target pCO_2 treatment was achieved by mixing compressed CO_2 and compressed ambient air using solenoid-valve-controlled mass flow controllers at flow rates that target pCO_2 conditions. The treated seawater was introduced to the flow-through aquaria at a rate of 150 mL min^{-1}. Tank salinity, temperature, and DIC and TA were measured for the duration of the experiment and used to calculate pH (total scale), $\Omega_{calcite}$, $[CO_3^{2-}]$, $[HCO_3^-]$, $[CO_2]$, and pCO_2 of each tank using CO2SYS version 2.1 (Pierrot et al. 2011; see Downey-Wall et al. 2020). Measured and calculated seawater parameters are reported in Table 1. Oysters were fed 1% Shellfish Diet 1800® twice daily following best practices outlined in Helm and Bourne (2004).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

2.2 Calcification rate measurements

Net calcification rate was calculated using the dry weight at the start and end of the experiment. Initial dry weight was measured at the start of exposure, on day 33 or 34, after the acclimation period (Downy-Wall et al., 2020). The buoyant weight was measured on either day 50 or 80 and the final dry weight was derived using a linear relationship between oyster dry weight and oyster buoyant weight (Ries et al., 2009).

2.3 Extrapallial fluid sampling

Sampling of the extrapallial fluid (EPF) was previously described in Downey-Wall et al. (2020). Briefly, a hole was drilled onto the shell to expose the EPF cavity, a port was inserted and sealed with epoxy to directly sample the EPF with a syringe and prevent seawater intrusion. Oysters recovered for 4 days before being transferred to experimental tanks for acclimation before the experiment. To sample the EPF, oysters were removed from the tanks and EPF was extracted by inserting a sterile 5 mL syringe with a flexible 18-gauge polypropylene tip through the port. EPF samples were stored in 2 mL microcentrifuge tubes and refrigerated at 6˚C for further analysis. pH (Total scale) of the EPF was measured directly after extraction using a micro-pH probe. EPF measurements were collected at the end of the experiment, on day 71, for *C. virginica* and day 14 for *A. islandica*. EPF pH diel variability was also explored by measuring EPF pH at 6 timepoints to produce time series for both species in a 24-hour period.

2.4 Shell sampling

Following EPF extraction, oysters were shucked and cleaned in 90% ethanol. The cleaned shells were dried at room temperature for 48 hours and sealed in plastic bags for analysis. For skeletal geochemical and elemental ratio analysis, the inner (lamellar) layer of the oyster shell was gently shaved with a diamond-tipped Dremel tool and about 5 mg of ground powder was stored in sealed microcentrifuge tubes.

2.4 Elemental ratio analysis

For the shells, about 2.5 mg of powder was sub-sampled from each specimen shell and cleaned with a 0.3 % hydrogen peroxide in 0.1 N sodium hydroxide solution to remove organic matter as described in Barker et al. (2003). Carbonate samples were dissolved in 1 N double-distilled HCl (see Guillermic et al., 2021, for details). Elemental ratios were measured on a Thermo Fisher Scientific Element XR HR-ICP-MS at the PSO (Plouzané, France) after Ca analyses on an Agilent ICP-AES Varian 710 at the University of California, Los Angeles (UCLA, Los Angeles, USA). Data quality and external reproducibility were maintained and quantified via repeated measurements of international standard JC_P-1 during a

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

particular session (Gutjahr et al., 2021). Typical measured concentrations of procedural blanks for the trace element analyses for sessions in which samples are diluted to 30 ppm Ca are ^{7}Li < 3%, ^{11}B < 4%, ^{25}Mg < 0.1%, ^{87}Sr < 0.1%, and ^{43}Ca < 0.1%. Typical analytical uncertainties on the X/Ca elemental ratios are 0.3 µmol/mol for Li/Ca, 21 µmol/mol for B/Ca, 0.09 mmol/mol for Mg/Ca, and 0.01 mmol/mol for Sr/Ca (2 SD, n = 28).

For EPF and seawater samples, 10 µL of sample was added to 490 µL of a solution of 0.1 N HNO_3/0.3 M HF. Mono-elemental solution of indium was added to reach a concentration of 1 ppb to monitor any matrix effect or drift of the instrument during a particular session. Standards were prepared by diluting an in-house seawater standard spiked with indium. International standards NRC-NASS-6 was used to ensure quality of the data.

2.5 Boron isotope analyses

Boron purification for the different samples was achieved via microdistillation following the method described in Guillermic et al. (2021) and originally developed by Gaillardet et al. (2001) and modified for Ca-rich matrix by Wang et al. (2010). 2.5-3.0 mg of oxidatively cleaned shell powders were dissolved in 1N HCl. For the EPF, 25 µL of EPF was added to 40 µL of 1N HCl. For the seawater, 50 µL of concentrated HCl was added to 450 µL of seawater. 60µL of each of the solutions was loaded for microdistillation. Boron isotopes were analyzed at the Pôle Spectrométrie Océan (PSO), Plouzané, on a Thermo Neptune inductively coupled plasma mass spectrometry (MC-ICP-MS) equipped with 10^{11} Ohm Faraday cup.

The certified boron isotope liquid standard ERM© AE120 (δ^{11}B = -20.2 ± 0.6 ‰, Vogl and Rosner, 2011) was used to monitor reproducibility and drift during each session. Samples measured for boron isotopes in carbonates were typically run at 80 ppb B (~30 ng B per <0.5 mL), whereas samples of EPF and seawater were typically run at 150-200 ppb B (~150 ng B per mL). Sensitivity on ^{11}B was 10 mV/ppb B (e.g., 10 mV for 1 ppb B) in wet plasma at 50 µL/min sample aspiration rate. Procedural boron blanks ranged from 0.3 to 0.4 ng B and the acid blank during analyses was measured at 3 mV on the ^{11}B, indicating a total blank contribution of <2% of the sample signal with no memory effect within and across sessions. External reproducibility was ensured by the measurements of carbonate standard microdistilled at the same time as the samples. Results for the isotopic composition of the JC_P-1 is δ^{11}B =24.67 ± 0.28 ‰ (2 SE, n=41), within error of published values (24.36 ± 0.45 ‰, 2SD, Gutjahr et al., 2021).

2.6 Magnesium isotope analyses

Carbonate samples were dissolved in 0.1 N buffered acetic acid ammonium hydroxide solution over four hours in a sonicator. Samples were then centrifuged and aliquots of the supernatant were transferred into cleaned 15 mL centrifuge tubes. Aliquots of the bulk supernatants were then diluted ~30-fold and calcium and magnesium were separated and purified in different runs via a Thermo-Dionex ICS-5000+ ion chromatograph equipped with a fraction collector according to established methods outlined by Husson et al. (2015). EPF samples contained organics that obscured elution profiles, thus limiting the elemental yield and purification. Therefore, samples were digested on a hot plate in hydrogen peroxide and nitric

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

acid to remove organics prior purification. Seawater and EPF samples were purified through the Thermo-Dionex ICS-5000+ ion chromatograph using another elution method than for carbonate samples. Seawater and carbonate standards were also purified at the same time to ensure quality of the method.

Samples were then dried and then rehydrated in a solution of 2% nitric acid. Magnesium isotopic ratios were measured at Princeton University using a Thermo Neptune+ (MC-ICP-MS) spectrometer according to methods outlined in Higgins et al. (2018) and Ahm et al. (2021). Samples were introduced via an ESI Apex-IR sample introduction system. Magnesium isotope ratios ($^{26}Mg/^{24}Mg$) were measured in low resolution mode, with every sample bracketed by the analysis of standards. Results are reported relative to the Dead Sea Magnesium-3 standard (DSM-3). Long term external precision on magnesium isotope results at the Higgins Lab (Princeton) was determined through repeated measurements of the Cambridge-1 standard (-2.59 ± 0.07‰, 2 SD, n = 19) and modern seawater (-0.82 ± 0.14 ‰, 2 SD, n = 21) and is reported in Ahm et al. (2021). Measured standards during the analytical session are given for the Cambridge-1 standard (-2.60 ± 0.20 ‰, 2 SD, n = 2) and for modern seawater (-0.82 ± 0.06 ‰, 2 SD, n=2).

2.7 Calculation of boron proxies and EPF carbonate chemistry

The use of boron proxies to reconstruct pH and [CO_3^{2-}] of the precipitating solution (i.e., the organism's calcifying fluid) is based upon boron speciation and fractionation in seawater (Hemming and Hanson, 1992; Hönisch et al., 2004). In seawater-type solutions, the speciation of boric acid [$B(OH)_3$] and borate ion [$B(OH)_4^-$] varies as a function of pH (Hemming and Hanson 1992). In addition to the pH dependence of their relative abundances, the boron proxy also relies upon the large isotopic fractionation between the two boron species (Klochko et al., 2006, Nir et al., 2015). A key assumption of the proxy is that boron, in the form of borate ion, is the predominant form incorporated into the crystal lattice of calcite via carbonate ion substitution during the precipitation of calcium carbonate (Hemming and Hanson 1992). The $\delta^{11}B$ of the carbonate ($\delta^{11}B_{CaCO3}$) should then, in theory, reflect the boron isotopic composition of the borate ion ($\delta^{11}B_{B(OH)4-}$) in the bivalve calcifying fluid (extrapallial fluid), which in turn reflects pH of the calcifying (extrapallial) fluid.

The boron isotopic signature of the shell ($\delta^{11}B_{carb}$) was used to calculate pH of the calcifying fluid (pH_{CF}) using the following equation (Hemming and Hanson, 1992; Zeebe and Wolf-Gladrow, 2001):

$$pH_{cf} = pK_B - \log\left(\frac{\delta^{11}B_{SW} - \delta^{11}B_{carb}}{\delta^{11}B_{SW} - \alpha * \delta^{11}B_{carb} - \varepsilon}\right) \quad \text{eq. 1}$$

In equation 1, pK_B is the dissociation constant, $\delta^{11}B_{sw}$ represents the measured boron isotopic composition of seawater, $\delta^{11}B_{carb}$ represents the boron isotopic composition of the shell, and α/ε represents the boron isotopic fractionation factor/ fractionation between boric acid and borate ion (Klochko et al. 2006).

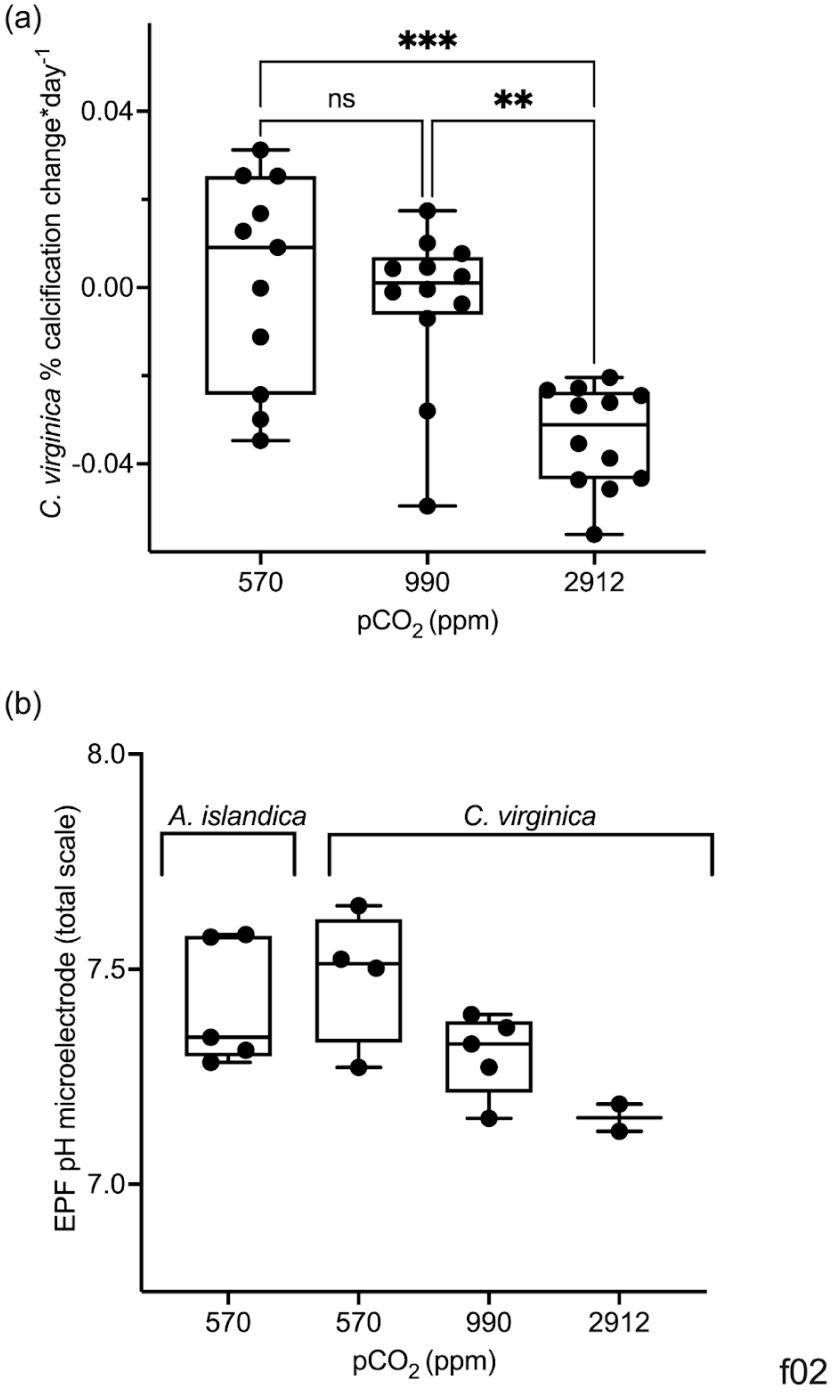
https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

The saturation state of calcite (Ω_{cacite}) and aragonite ($\Omega_{aragonite}$) of the EPF for each species were calculated using temperature, salinity, pressure, measured EPF Ca, measured EPF Mg, pH either from microelectrode pH or δ^{11}B-calculated pH, and literature values of DIC (3000 for *A. islandica* from Stemmer et al. 2013, and 4200 for *C. virginica* from McNally et al., 2022). The saturation states were calculated using Seacarbx with maximum input of [Mg^{2+}] allowed by the code for samples presenting higher EPF [Mg^{2+}] than the limit allowed by the code (Raitzsch et al., 2021). Those saturation state values are limited by the fact that no direct measurements of EPF DIC was performed during this study, and a range of [Ca^{2+}] and [Mg^{2+}] values were measured in the EPF, resulting in a range of calculated saturation states as presented in Table 3.

3 Results

3.1 Previous Culturing experiment, calcification rates, seawater chemistry, and EPF chemistry

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024



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Preprint. Discussion started: 12 August 2024

Figure 2. (a) Box plots showing percent calcification change over the experiment for C. virginica for each treatment. Stars denote statistically different means and 'ns' signify non significant mean differences in a pairwise t-test (at significance $p < 0.05$). (b) Averaged microelectrode EPF pH for A. islandica under control conditions and C. virginica for OA conditions.

Crassostrea virginica specimens were previously cultured in experimental tanks with seawater that was continuously bubbled with gas mixtures comprising three pCO_2 levels (400 ppm, 900 ppm, 2800 ppm; see Downey-Wall *et al*., 2020). The highest pCO_2 treatment produced seawater values with a $\Omega_{calcite} < 1$, which does not favor calcification (Table 1). In this study, we present unpublished EPF pH microelectrode data for *A, islandica* cultured at a single control condition (400 ppm pCO_2) and we present published EPF microelectrode data for the *C. virginica* acidification experiment of Downey-Wall et al. (2020). Measured and calculated seawater parameters from the culture experiments are presented in Table 1. Percent change in calcification per day (Fig 2a), as well as EPF pH as measured by microelectrode (Fig 2B), decreased in *C. virginica* with increasing pCO_2 Both species had similar EPF pH (Fig 2b). Downey-Wall et al 2020 reported that *C. virginica* calcification decreased as pCO_2 increased and that, for each acidification treatment, the mean EPF pH during the experiment was lower than the corresponding seawater pH. Additionally, they report that using a linear model, pCO_2 treatment had a significant effect on EPF pH (linear model, $p<0.05$) and that at the highest pCO_2 treatment, EPF pH was significantly lower than seawater pH (Table 1; Fig 2; post hoc p-value<0.05 see Downey-Wall *et al*., 2020). We note that the *C. virginica* average ΔpH (seawater pH - EPF pH) decreased with decreasing seawater pH. The ΔpH for the control treatment was 0.53, the moderate OA treatment was 0.46, and the high OA treatment was 0.08. Here we report that at the control pCO_2 level, the EPF pH of *A. islandica* was 7.41, compared to 7.48 for *C. virginica* and the ΔpH for *A. islandica* was 0.52 (Table 1).

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Preprint. Discussion started: 12 August 2024

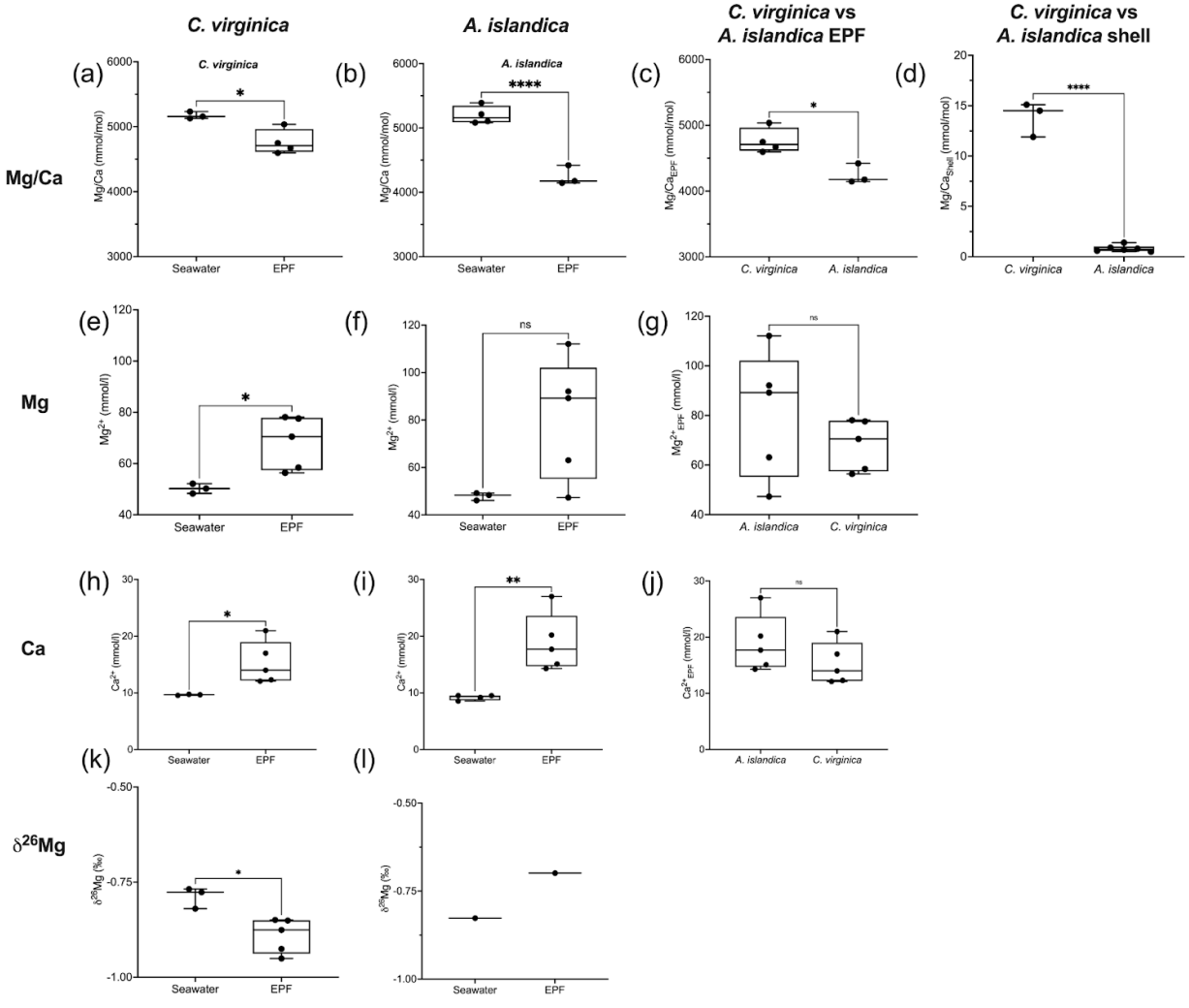

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Figure 3. Box plots of Mg/Ca comparing seawater and extrapallial fluid for (a) C. virginica and (b) A. islandica, (c) comparing EPF Mg/Ca between species, and (d) shell Mg/Ca between species. Box plots of [Mg] comparing seawater and extrapallial fluid for (e) C. virginica and (f) A. islandica, (g) comparing EPF [Mg] between species. Box plots of [Ca] comparing seawater and extrapallial fluid for (h) C. virginica and (i) A. islandica, (j) comparing EPF [Ca] between species. Box plots of 26Mg comparing seawater and extrapallial fluid for (k) C. virginica and (l) A. islandica. Stars denote statistically different means and 'ns' signify non significant mean differences in a pairwise t-test (at significance $p < 0.05$). No comparison was tested on (l) due to limited sample size.

3.2 Mg/Ca of seawater, EPF, and bivalve shell

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

There was a significant decrease in EPF Mg/Ca compared to seawater Mg/Ca for both *A. islandica* and *C. virginica* (t-test, n=2, p-value<0.05; Fig 3a-b). The Mg/Ca of *C. virginica* EPF was 4.55± 0.50 mol/mol and significantly higher than *A. islandica* EPF which was 4.25±0.67 mol/mol (Fig 3d; Table 1). For both species, the low EPF Mg/Ca versus seawater Mg/Ca was driven by higher [Ca^{2+}] concentrations in the EPF relative to seawater (Fig 3h-i). Considering the elemental concentrations alone, instead of as a ratio, there was no significant difference in EPF [Mg^{2+}] or [Ca^{2+}] concentrations between species (Fig 3g and 3j). Shell Mg/Ca for the calcitic *C. virginica* was 13.8±1.7 mmol/mol and significantly higher than the aragonitic *A. islandica* shell which was 0.8±0.02 mmol/mol, in line with shell polymorph mineralogy. The apparent partition coefficient (K_{Mg}) between the seawater and the shell was 0.003 in *C. virginica* and 0.002 in *A. islandica* (Table 2). K_{Mg} between EPF and shell was 0.003 in *C. virginica* and 0.002 in *A. islandica*. K_{Mg} between seawater and the EPF is 0.9 for *C. virginica* and 0.8 for *A. islandica* (Table 2).

		EPF/SW		Shell/SW		Shell/EPF	
		A. islandica	*C. virginica*	*A. islandica*	*C. virginica*	*A. islandica*	*C. virginica*
$K_{Mg/Ca}$	400	0.8	0.9	0.0002	0.003	0.0002	0.003
	900		1.1		0.002		0.002
	2000		1.2		0.002		0.002
$K_{B/Ca}$	400	0.7	0.8	0.001	0.003	0.002	0.003
	900		0.9		0.003		0.003
	2000		1.1		0.003		0.003

Table 2. Partition coefficients between EPF and seawater, seawater and the mineral, and EPF and the mineral for Mg/Ca and B/Ca.

C. virginica seawater and EPF δ^{26}Mg were -0.77 ± 0.01 ‰ and -0.88 ± 0.06 ‰, respectively and displayed a significant decrease in EPF δ^{26}Mg compared to seawater for *C. virginica* (t-test, n1=3 n2=5, p-value< 0.05; Table 1, Fig 3k-l). For *A. islandica*, seawater and EPF δ^{26}Mg were -0.82 ± 0.06 ‰ and -0.69 ± 0.01 ‰, respectively, but no statistical analysis could be done between the two reservoirs owing to the small sample size (Table 1). The average shell δ^{26}Mg for *C. virginica* was

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

-3.2 ± 0.1‰, but *A. islandica* shell $\delta^{26}Mg$ could not be analyzed because of low shell [Mg^{2+}] content and limited sample material.

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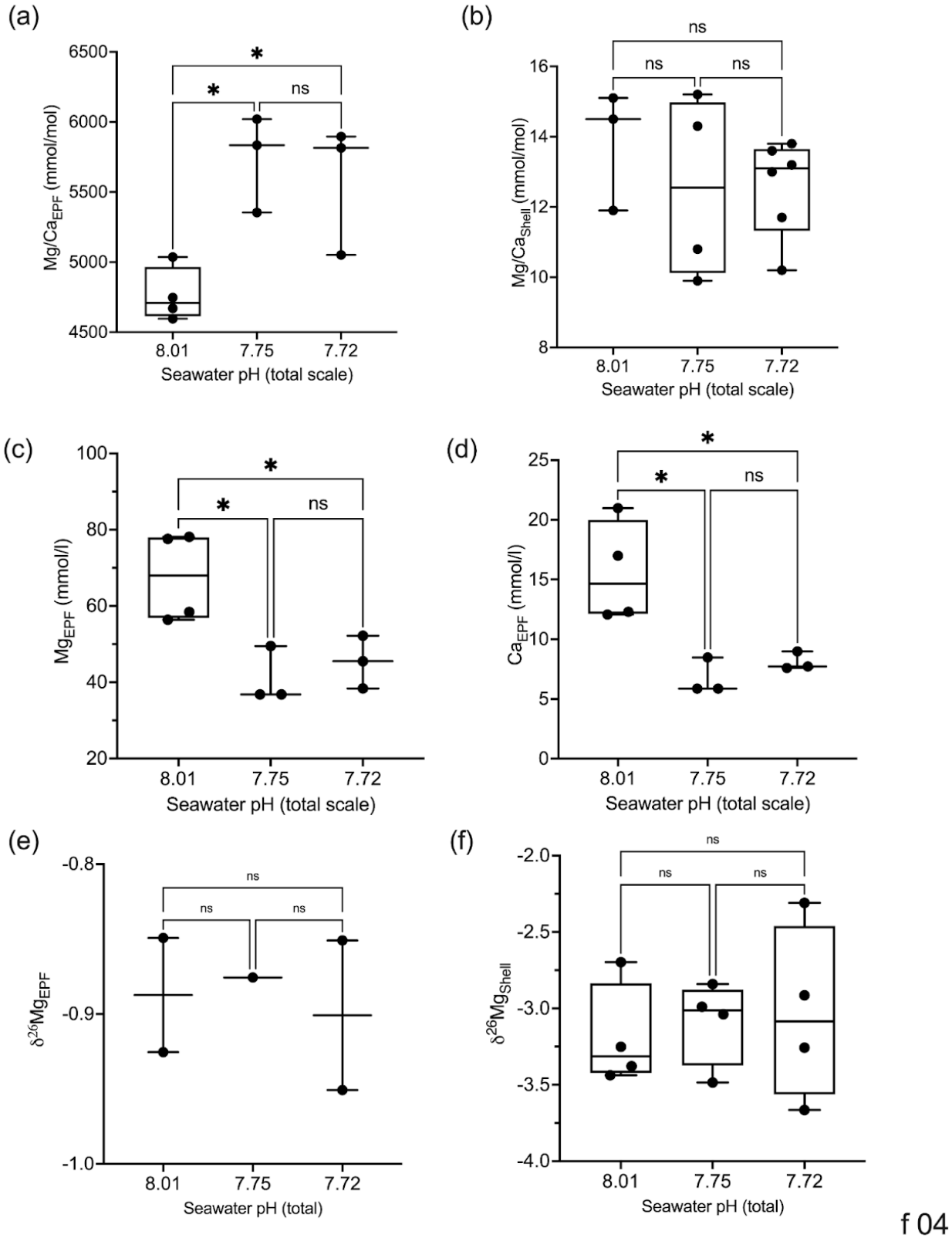
Preprint. Discussion started: 12 August 2024



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Preprint. Discussion started: 12 August 2024

Figure 4. Box plots showing C. virginica (a) EPF Mg/Ca and (b) shell Mg/Ca across seawater pH treatments. Additionally, box plots show (c) EPF [Mg], (d) EPF [Ca], (e) EPF 26Mg, and (f) shell 26Mg. Stars denote statistically different means and 'ns' signify non significant mean differences in a pairwise t-test (at significance $p < 0.05$).

In the *C. virginica* acidification experiment, EPF but not shell Mg/Ca was found to increase as EPF pH decreased (regression, n=10, p-value<0.05; Fig 5a-b). OA treatment had a significant effect on shell Mg/Ca (ANOVA, n=10, p-value<0.05, Fig 4a-b). The concentration of both $[Ca^{2+}]$ and $[Mg^{2+}]$ in the EPF decreased with decreasing EPF pH (regression, n=10, p-value< 0.05; Fig 5c-d). However, when binning by seawater pH treatments, only the $[Ca^{2+}]$ and $[Mg^{2+}]$ of the ambient condition was significantly elevated compared to the moderate and high ocean acidification treatments (Tukey HSD, n1=4 n2=3, p<0.05, Fig 4c-d). The EPF and shell $\delta^{26}Mg$ did not change as a function of EPF or seawater pH (Fig 4e-f and 5e-f).

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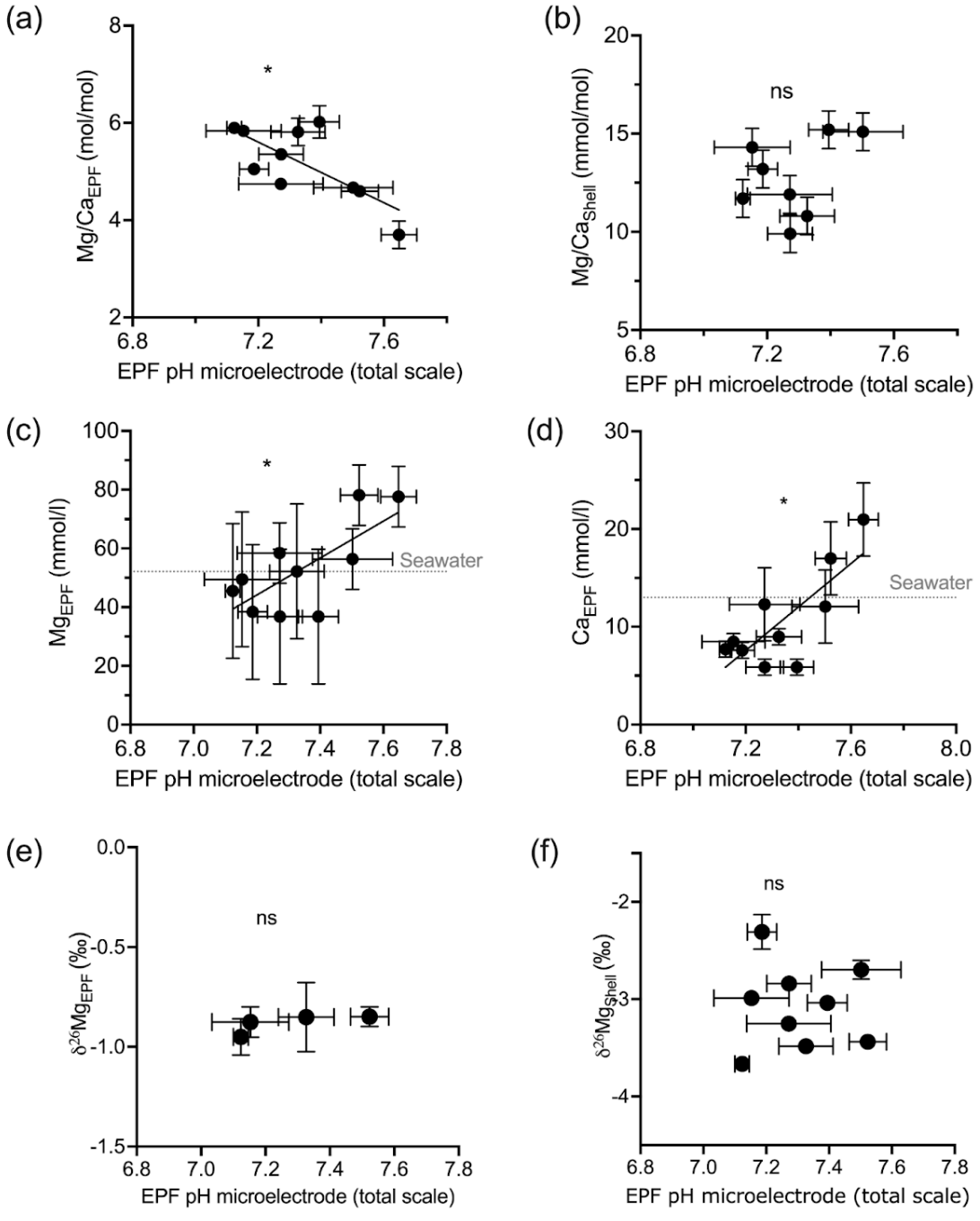
Preprint. Discussion started: 12 August 2024



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Preprint. Discussion started: 12 August 2024

Figure 5. Scatter plots showing C. virginica individual specimen (a) EPF Mg/Ca and (b) shell Mg/Ca across corresponding microelectrode pH. Additionally, scatter plots (c) EPF [Mg], (d) EPF [Ca], (e) EPF 26Mg, and (f) shell 26Mg across microelectrode EPF pH. Stars denote statistically significantly nonzero regression slopes and 'ns' signify non significant regressions (at significance $p < 0.05$). Dotted gray lines on (c) and (d) show the average [Mg] and [Ca] seawater concentration, respectively.

3.3 Boron geochemistry of seawater, EPF, and shell

A. islandica EPF B/Ca was 27.91 ± 4.87 mmol/mol and was significantly lower than seawater B/Ca which was 41.75 ± 1.52 mmol/mol (t-test, n1=7 n2=5, p-value<0.05, Fig 6a). *C. virginica* EPF B/Ca was 41.66 ± 1.07 mmol/mol and was significantly lower than seawater B/Ca which was 33.66 ± 2.81 mmol/mol (t-test, n1=6 n2=5, p-value<0.05 Fig 6b) The boron concentration was not significantly different between seawater and EPF for both *C. virginica* and *A. islandica* (Fig 6e-f). There was no significant difference in shell or EPF B/Ca between *C. virginica* and *A. islandica* (Fig 6c-d). The apparent partition coefficient (K_B) between the seawater and the shell was 0.003 in *C. virginica* and 0.001 in *A. islandica*. K_B between EPF and shell was 0.003 in *C. virginica* and 0.002 in *A. islandica*. K_B between seawater and the EPF is 0.8 in *C. virginica* and 0.7 for *A. islandica* (Table 3).

	Control *A. islandica* ($\Omega_{aragonite}$)	Control *C. virginica* ($\Omega_{calcite}$)	Moderate OA *C. virginica* ($\Omega_{calcite}$)	High OA *C. virginica* ($\Omega_{calcite}$)
Ω using EPF pH (range)	1.7 (1.0-3.8)	3.7 (1.3-11.4)	1.1 (0.5-2)	0.9 (0.5-1.2)
Ω using δ^{11}B-calculated pH (range)	3.8 (2.9-6.7)	15.4 (6.7-37)	6.1 (3-11.7)	6.5 (3.4-9.7)

Table 3. Table of calculated saturation state (Ω) with respect to calcite (C. virginica) or aragonite (A. islandica) for the average EPF pH value based on microelectrode measurements or δ11B-calculated EPF pH.

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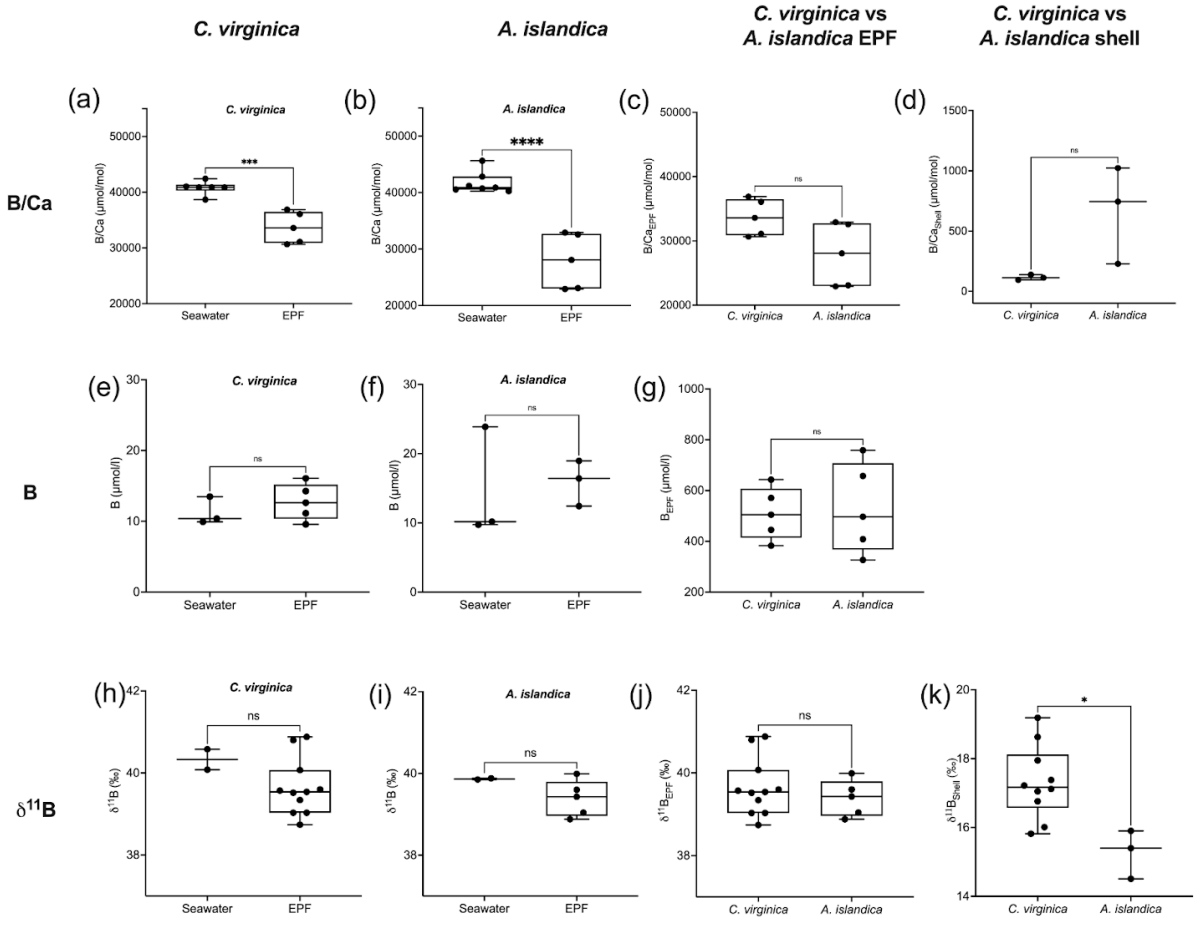
Preprint. Discussion started: 12 August 2024



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377 Figure 6. Box plots of B/Ca comparing seawater and extrapallial fluid for (a) C. virginica and (b) A. islandica, (c)
378 comparing EPF B/Ca between species, and (d) shell B/Ca between species. Box plots of [B] comparing seawater and
379 extrapallial fluid for (e) C. virginica and (f) A. islandica, (g) comparing EPF [B] between species. Box plots of 11B
380 comparing seawater and extrapallial fluid for (h) C. virginica and (i) A. islandica, comparing EPF 11B between species, and
381 (d) shell 11B between species. Stars denote statistically different means and 'ns' signify non significant mean differences in a
382 pairwise t-test (at significance $p < 0.05$).

383 There was no significant difference in $\delta^{11}B$ between seawater and EPF for both species in the control condition (Fig 6h-l).
384 There was also no significant difference in EPF $\delta^{11}B$ between species(Fig 6j); however, there was a significant difference in
385 shell $\delta^{11}B$ between *C. virginica* and *A. islandica* (t-test, n1=10 n2=3, p-value<0.05, Fig 6k). Under control conditions, shell
386 $\delta^{11}B$ was measured to be 15.26 ± 0.41‰ (2 SD, n=3) for *C. virginica* and 18.34 ± 0.59 ‰ (2 SD, n = 3) for *A. islandica*.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

3.4 *Crassostrea virginica* ocean acidification experiment geochemistry

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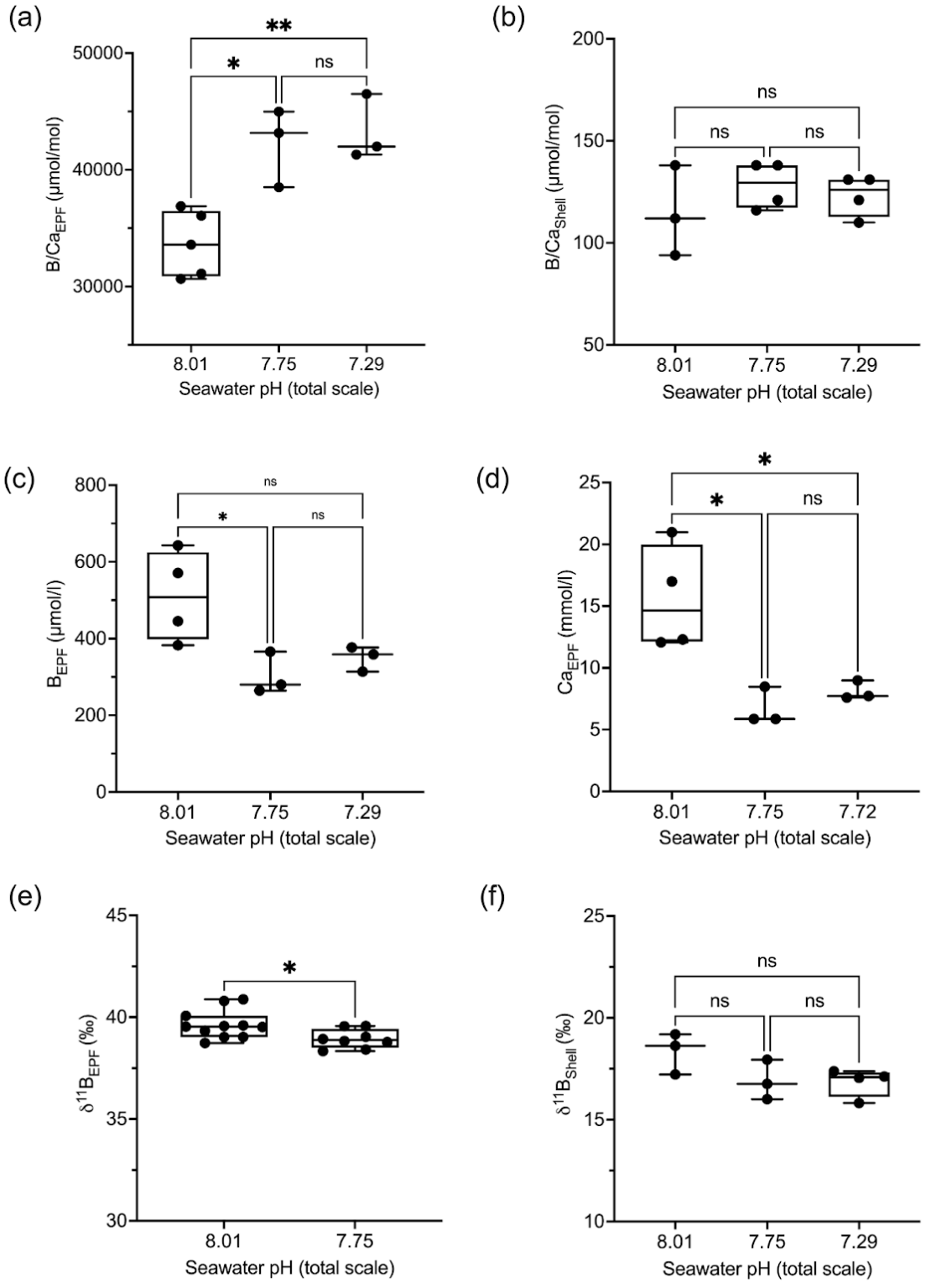

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Preprint. Discussion started: 12 August 2024

Figure 7. Box plots showing C. virginica (a) EPF B/Ca and (b) shell B/Ca across seawater pH treatments. Additionally, box plots show (c) EPF [B], (d) EPF [Ca], (e) EPF 11B, and (f) shell 11B. Stars denote statistically different means and 'ns' signify non significant mean differences in a pairwise t-test (at significance $p < 0.05$). The sample set for (e) was limited and we were unable to analyze the lowest pH treatment.

In the *C. virginica* acidification experiment, EPF B/Ca but not shell B/Ca was found to increase as seawater pH decreased (ANOVA p-value<0.05, compare Fig 7a-b). The EPF but not shell B/Ca was found to increase as EPF pH decreased (regression p-value< 0.05, Fig 8a-b). The boron concentration of the EPF, but not the shell, significantly decreased with decreasing EPF pH (regression p-value< 0.05, Fig 8c). The EPF B concentration increased with increasing seawater pH (ANOVA p-value< 0.05, Fig 8c); however, shell boron concentrations did not significantly change with seawater pH. Due to small EPF sample volume, EPF for the oysters in the lowest seawater pH treatment was not measured for $\delta^{11}B$. There was a significant difference in mean EPF $\delta^{11}B$ between the control pH treatment which was 39.39 ‰ and moderate pH treatment which was 38.92 ‰ (t-test, n1=11 n2=7, p-value<0.05, Fig 7e-f). The difference between seawater $\delta^{11}B$ and EPF $\delta^{11}B$ was 0.91 ‰ for the control treatment and decreased to 0.47 ‰ for the moderate pH treatment. Shell $\delta^{11}B$, but not EPF $\delta^{11}B$, significantly decreased with decreasing EPF pH (regression p-value<0.05, Fig 8e-f).

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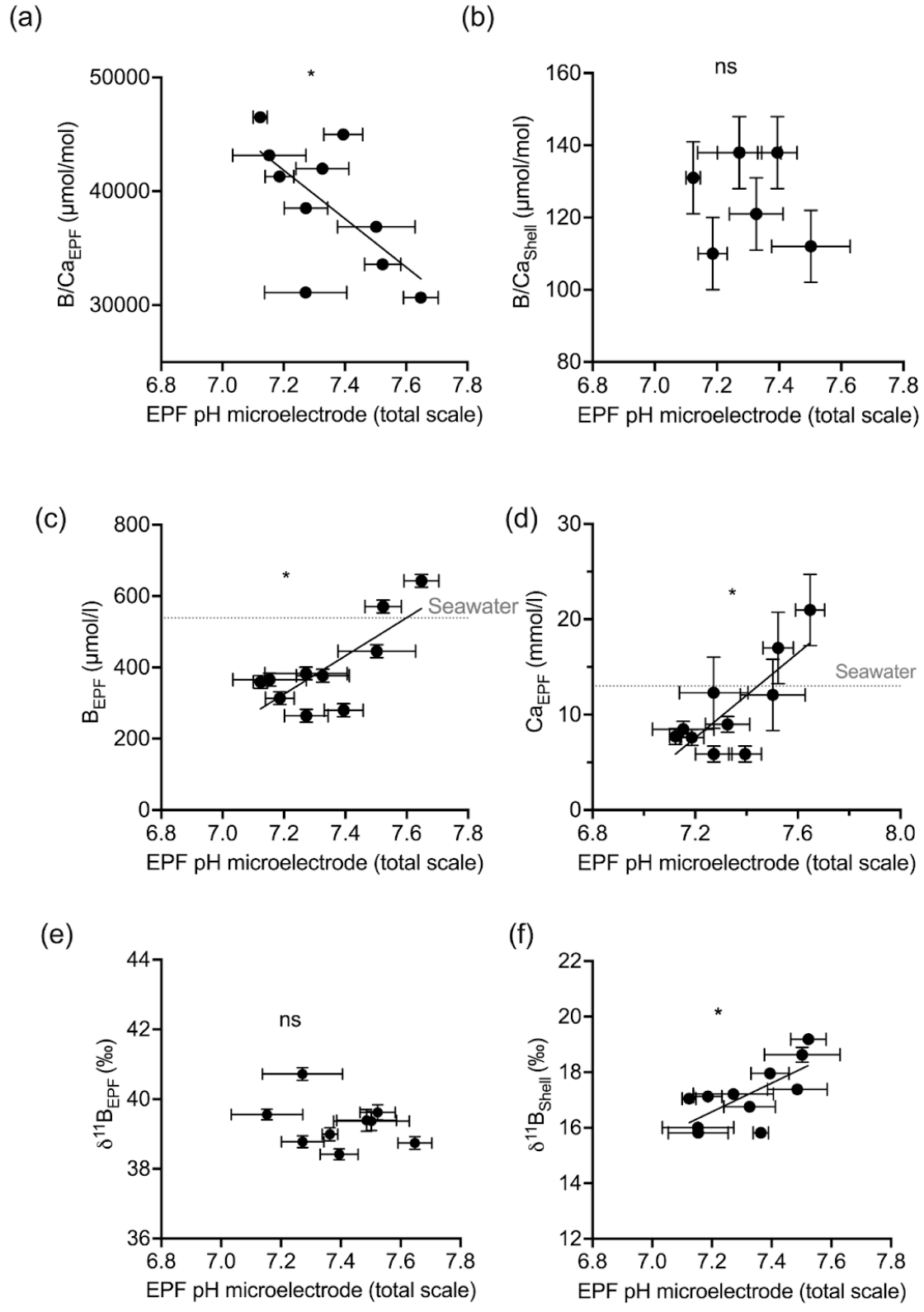
Preprint. Discussion started: 12 August 2024



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404 Figure 8. Scatter plots showing C. virginica individual specimen (a) EPF B/Ca and (b) shell B/Ca across corresponding
405 microelectrode EPF pH. Additionally, scatter plots of (c) EPF [B], (d) EPF [Ca], (e) EPF 11B, and (f) shell 11B across
406 microelectrode EPF pH. Stars denote statistically significantly nonzero regression slopes and 'ns' signify non significant
407 regressions (at significance $p < 0.05$). Dotted gray lines on (c) and (d) show the average [B] and [Ca] seawater
408 concentration, respectively.

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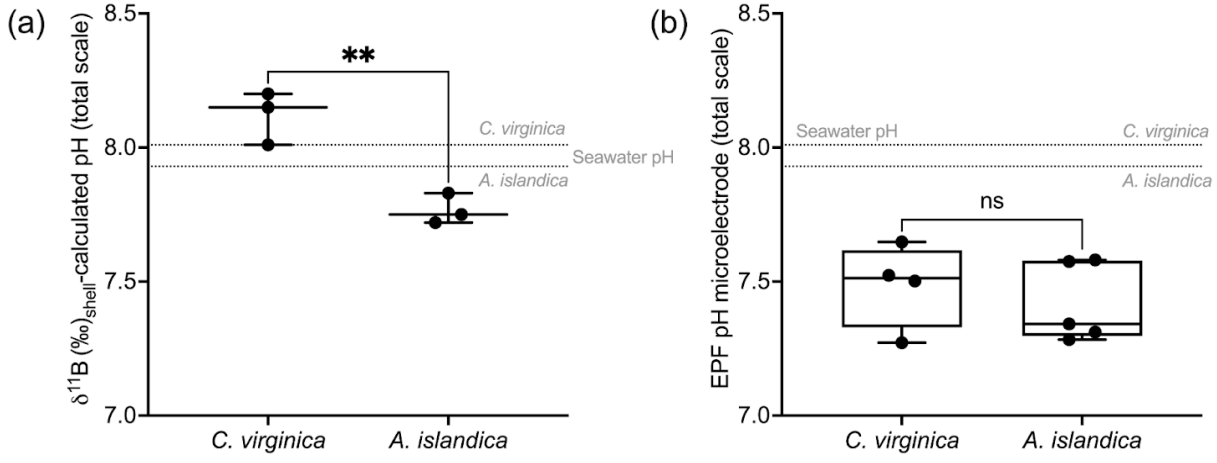

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411 Figure 9. (a) Box plot of 11B-calculated pH for C. virginica and A. islandica. (b) Box plot of measured microelectrode pH
412 for C. virginica and A. islandica. The grey line shows seawater pH for C. virginica and A. islandica. Stars denote statistically
413 different means and 'ns' signify non significant mean differences in a pairwise t-test (at significance $p < 0.05$).

414 The control condition δ^{11}B-calculated EPF pH for *C. virginica* was 8.12 ± 0.08 ‰ (2 SD, n=3) and for *A. islandica* was 7.93
415 ± 0.09 ‰ (2 SD, n=3), which yielded a statistically significant difference between the two species (t-test, n1=3 n2=3,
416 p-value<0.05, Fig 9a). For *C. virginica*, the δ^{11}B-calculated EPF was 0.1 pH units higher than the seawater pH and 0.6 lower
417 than measured EPF pH. Conversely, the *A. islandica* δ^{11}B-calculated EPF was 0.1 pH units lower than the seawater pH and
418 0.3 higher than the measured EPF pH (Fig 9). Fig 10a shows the measured EPF pH, the δ^{11}B-calculated EPF, and seawater to
419 EPF 1:1 pH line graphed across the *C. virginica* acidification experiment. The slope of the measured microelectrode EPF pH
420 versus seawater pH linear regression was 0.3, and lies below the seawater to EPF 1:1 pH line, but intersects the seawater to
421 EPF 1:1 pH line at lowest pH/highest pCO_2 culture conditions (Fig 10). Conversely, the slope of the δ^{11}B-calculated EPF pH

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

versus seawater pH linear regression was 0.1, lies above the seawater to EPF 1:1 pH line, but intersected the seawater to EPF 1:1 pH line at higher culture pH conditions (Fig 10).

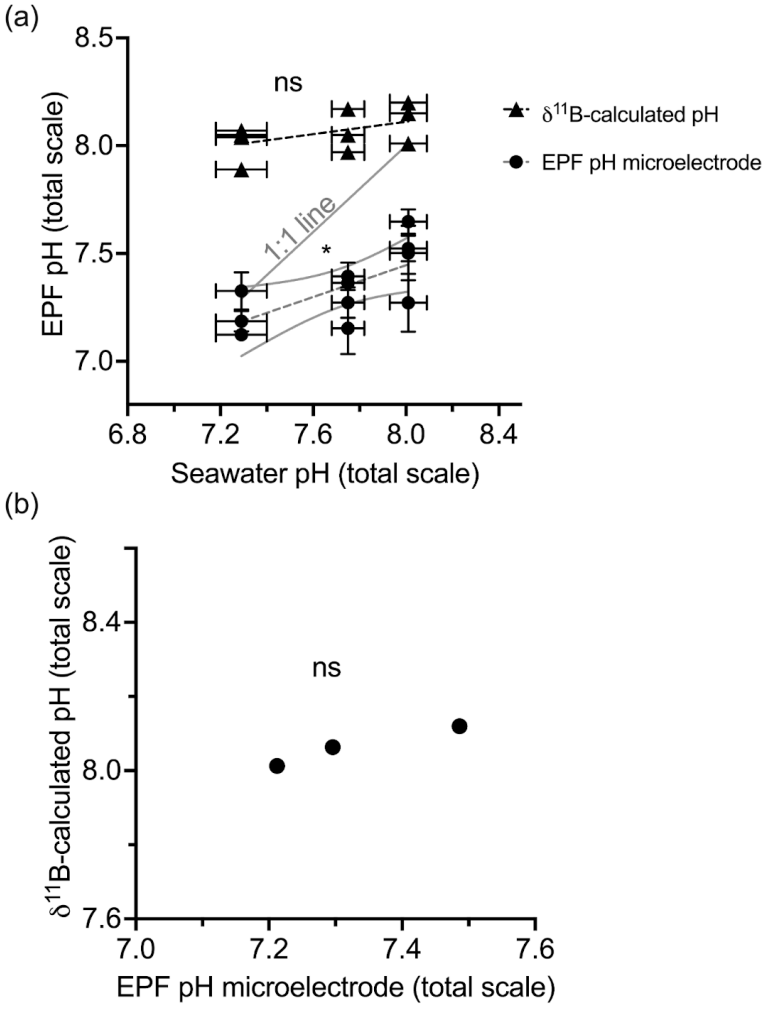

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Figure 10. (a) Scatter plot of 11B-calculated pH and microelectrode EPF pH across seawater pH treatments. The gray line shows the 1:1 seawater to EPF pH line. In the seawater pH: EPF pH space, the 11B-calculated pH regression line is statistically nonzero (at significance $p < 0.05$), with a slope of 0.368. The microelectrode EPF pH line was not significantly nonzero and had a slope of 0.143. (b) shows the averaged 11B-calculated pH versus microelectrode EPF pH. Stars denote statistically significantly nonzero regression slopes and 'ns' signify non significant regressions (at significance $p < 0.05$).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

For the *C. virginica* acidification experiment, Downey-Wall et al., (2020) measured the EPF pH of individual specimens in each acidification treatment over a 24-hour period (n_{total}=108 and n=6 per time point per treatment). Fig 11 shows how the EPF pH for each individual fluctuated over 24 hours. The control treatment EPF pH of individuals did intersect the averaged seawater pH for the treatment tanks, however, the EPF pH in the moderate and high pH treatments fell below the corresponding average treatment seawater pH lines. For all treatments, the time series EPF pH lines fell below the corresponding treatment averaged δ^{11}B-calculated EPF pH line.

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Preprint. Discussion started: 12 August 2024

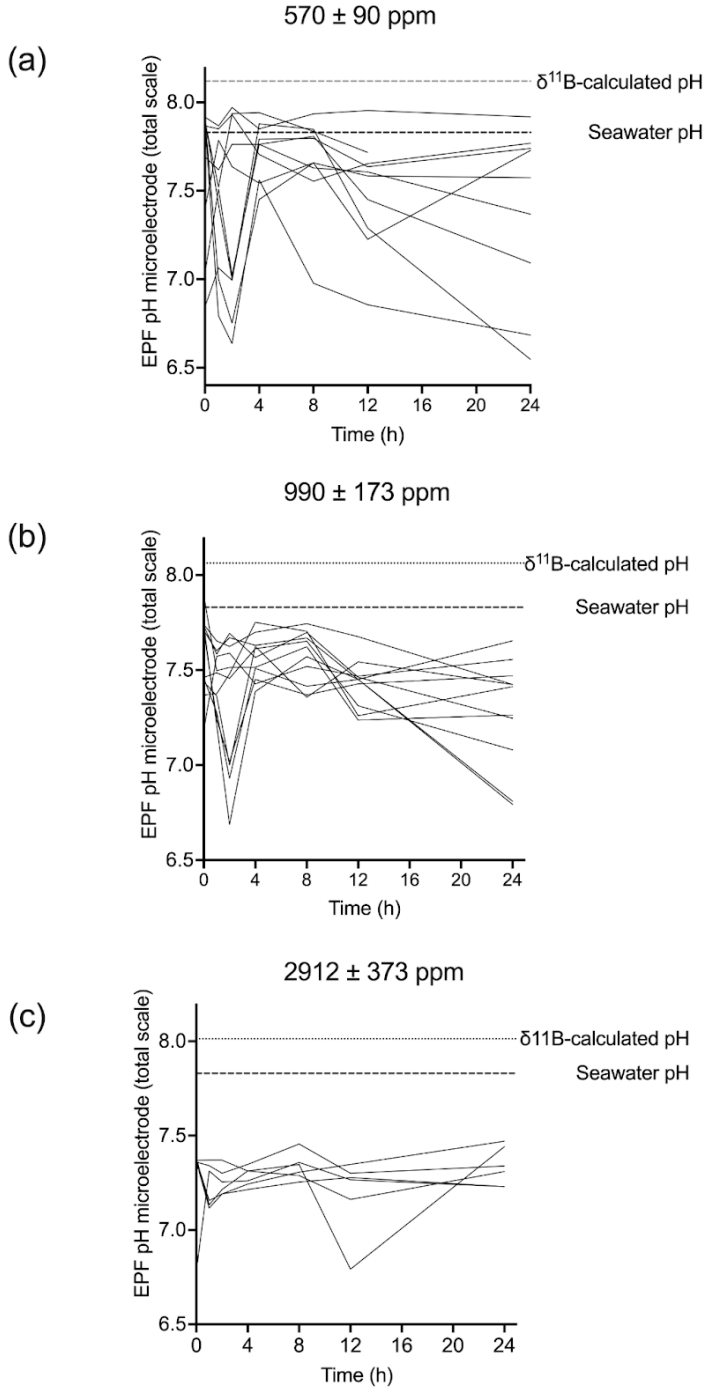

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Figure 11. Time series (in hours) of microelectrode EPF pH over a 24 hour period for (a) control (b) moderate and (c) high pCO2 treatments. Each line represents the microelectrode EPF pH for each individual specimen measured in that treatment.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

The small dotted line shows the corresponding average 11B-calculated pH for the treatment and the larger dotted line shows the average seawater pH for the treatment.

In Table 3, the EPF aragonite saturation state ($\Omega_{aragonite}$) for *A. islandica* and EPF calcite saturation state ($\Omega_{calcite}$) for *C. virginica* were calculated using the averaged measured EPF pH and averaged δ^{11}B-calculated EPF pH, averaged measured [Mg^{2+}] and [Ca^{2+}], and literature values of DIC (3000 µmol/L for *A. islandica* taken from Stemmer et al. (2013) and 4200 µmol/L for *C. virginica* from McNally et al. (2022). Under control conditions, the *A. islandica* $\Omega_{aragonite}$ and *C. virginica* $\Omega_{calcite}$ that was calculated using δ^{11}B-calculated EPF pH and measured EPF pH (Table 3). Under the ocean acidification experiment, EPF $\Omega_{calcite}$ decreased with decreasing seawater pH when using either EPF pH or δ^{11}B-calculated EPF pH to calculate EPF $\Omega_{calcite}$. There were large differences in *A. islandica* $\Omega_{aragonite}$ and *C. virginica* $\Omega_{calcite}$ when using either EPF pH ($\Omega_{aragonite}$=1.7 and $\Omega_{calcite}$=3.7) or the δ^{11}B-calculated pH ($\Omega_{aragonite}$=3.8 and $\Omega_{calcite}$=15.4).

4. Discussion

4.1 [Mg^{2+}] and [Ca^{2+}] concentrations in the EPF and shell

This study examined tripartite element and isotope fractionation between different reservoirs involved in the biomineralization of two bivalves species, aragonitic *A. islandica* and calcitic *C. virginica*. Marine bivalves source ions for internal fluids from seawater and previous studies by Crenshaw (1972) have highlighted that the extrapallial fluid, the internal ion reservoir pool for calcification, is chemically different from seawater. Seawater enters the hemolymph fluid within the bivalve tissues through the gills, filter feeding, and passive diffusion. Thereafter, the ions sourced from seawater are modulated either passively or actively across the outer mantle epithelium (OME) cells into the extrapallial cavity, a semi-isolated space that separates the outer mantle epithelium tissue from the shell. Here, ions are sourced to the site of calcification where biomineralization occurs. The exact mechanisms behind bivalve biomineralization is still a topic of active research and evidence has been put forth for several distinct pathways, primarily regulation of calcification constituents across the OME and transport of a precursor phase of $CaCO_3$ to promote calcification (Addadi 2003; Checa 2020).

In the complementary study by Downey-Wall et al. (2020), it was found that the *C. virginica* calcification rates decreased with seawater pH (Downey-Wall et al., 2020; Fig 2). The reduction of calcification under ocean acidification conditions is well documented in other seawater pH experiments on different bivalve species (e.g., Ries et al., 2009; Beniash et al., 2010; Waldbusser et al., 2011; Downey-Wall et al., 2020). This result is consequential as the shell is important in protecting the animal from predation, desiccation, and the effects of transient changes in seawater chemistry (Gosling 2008). Under ambient control conditions, *C. virginica* and *A. islandica* microelectrode EPF pH was lower than seawater pH. Additionally, under both the moderate and high experimental ocean acidification treatments, the average microelectrode EPF pH of *C. virginica* was lower than seawater pH. These findings are in line with previous work on bivalves, which show that

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

the EPF pH is regularly lower than seawater pH (Crenshaw 1972, Heinemann et al., 2012, Stemmer et al., 2013, Sutton et al., 2018; Cameron et al. 2019, Liu et al., 2020) and that simulated ocean acidification results in a decreased EPF pH (Michaelidis et al., 2005; Thomsen et al., 2013, Zittier et al., 2015, Cameron et al., 2019; Downey-Wall et al., 2020). However, the change in pH between EPF and seawater pH (△pH) decreased with decreasing pH, resulting in an EPF pH that was closer to seawater pH under acidified conditions (Table 1).

Here we show that, under ambient conditions, both the EPF Mg/Ca and B/Ca of both *C. virginica* and *A. islandica* were lower than that of seawater, indicating that the EPF has a distinct geochemical make up different from seawater (Fig 3; Downey-Wall et. al., 2022). This is consistent with the anatomical understanding in bivalves that EPF is semi-isolated from seawater and its geochemistry can be influenced by ion fluxes across the OME as well as other ion pathways (Crenshaw 1972; Stemmer et al., 2013; Sillanpaa et al., 2018). However, we also find that for both Mg/Ca and B/Ca, this result is driven by an increase in absolute [Ca^{2+}] in EPF, so we do not find evidence for dilution or concentration of the absolute [Mg^{2+}] or Bin the EPF (Fig 3). Previous work on bivalves has shown that magnesium can inhibit calcite crystal nucleation and there is evidence for exclusion of [Mg^{2+}] from the EPF (Lorens and Bender, 1977). In line with other studies, we show that *C. virginica* and *A. islandica* have lower Mg/Ca in EPF than seawater (Lorens and Bender, 1977; Planchon et al., 2013); however, we note that the EPF Mg/Ca trend is driven by changes in EPF Ca. *C. virginica* and *A. islandica* EPF Mg/Ca were significantly different, with lower EPF Mg/Ca for *A. islandica*, possibly due to different controls over EPF [Ca^{2+}] between both species. The partition coefficient between EPF and the shell was calculated to be 0.003 for *C. virginica* 0.0002 for *A. islandica*, which is consistent with previous studies on bivalves and with the Mg/Ca mineralogical difference between the calcite produced by *C. virginica* and the aragonite produced by *A. islandica* (Ulrich et al. 2021).

We found that the EPF $\delta^{26}Mg$ of *C. virginica* was depleted compared to seawater $\delta^{26}Mg$ (Fig 3). Our $\delta^{26}Mg$ values for the EPF and shell were in line with previous work on bivalves (Planchon et al., 2013). Planchon et al. (2013) found a -0.23 ± 0.25 ‰ (2 SD, n=5) difference between EPF and seawater in the aragonitic manila clam, *Ruditapes philippinarum*. Similarly, in the present study, a difference of -0.11 ± 0.06 ‰ was observed for the calcitic *C. virginica*, but no $\delta^{26}Mg$ data were collected for *A. islandica* due to sample limitation. Both Planchon et al. (2013) and the present study show depleted EPF $\delta^{26}Mg$ relative to seawater $\delta^{26}Mg$, indicating a potential biological modulation of EPF [Mg^{2+}] which has been previously attributed to heavier isotopes being incorporated into soft tissues or magnesium fixation within organic molecules (Planchon et al., 2013). However, it is important to note that the difference between EPF and seawater $\delta^{26}Mg$ is low and the $\delta^{26}Mg$ fractionation between the shell and seawater (2.43‰) was slightly larger than but still in line with inorganic calcite precipitation studies (Mavromatis et al., 2013; Saulnier et al., 2012).

Only *C. virginica* was cultured under ocean acidification (OA) treatments representing control, moderate, and high OA treatments. As mentioned above, the control experiment showed elevation of EPF [Ca^{2+}] and EPF [Mg^{2+}] relative to seawater. However, as EPF pH decreased, the EPF [Ca^{2+}] and [Mg^{2+}] significantly decreased as well (Fig 3 & 5). Ion transporters such as voltage gated Ca-channels tend to also affect chemically similar ions like [Mg^{2+}] and a reduction of such a transporter could possibly explain the similar trends in [Ca^{2+}] and [Mg^{2+}] concentrations under OA (Hess et al., 1986).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

Under OA conditions, EPF [Ca^{2+}] decreased to concentrations that were similar to or below seawater Ca, indicating a reduced ability of the organism to upregulate these ions under OA conditions. Previous studies have found a similar tight coupling between pH and Ca. For example, Stemmer et al. (2013) found synchronous patterns between pH and [Ca^{2+}] dynamics in *A. islandica* that they explained to be the result of calcium-transporting ATPase, which exchanges protons and calcium ions across the OME and has proven to be important for acid-base regulation and calcium transport in bivalves (Stemmer et al., 2013; Sillanpaa et al., 2018, 2020). Although calcium transporting ATPase could explain this increase in [Ca^{2+}] under ambient conditions, this transport mechanism may be reduced under acidified conditions, thereby impairing the bivalve's ability to regulate protons and calcium ions in the extrapallial fluid, rendering EPF [Ca^{2+}] and pH more similar to that of seawater.

Alternatively, the simultaneous reduction in [Ca^{2+}] and [Mg^{2+}] under OA conditions could point to an ion storage mechanism. The reduction of both calcium and magnesium within the EPF under moderate and high OA treatments could possibly be linked to changes of storage and budgets of ions under stressful conditions (Mount 2004; Johnstone et al., 2015; Wang et al. 2017). Further, several studies have highlighted significant changes in bivalve [Ca^{2+}] ion transport and storage in different extracellular and subcellular compartments associated with shell damage and repair under acidified conditions (Sillanpaa et al., 2016; Mount et al., 2004; Fitzer et al., 2016). Lastly, the EPF [Ca^{2+}] could simply reflect the balance between calcification and dissolution of the shell, despite the decrease in calcification rate over the experimental period, as exemplified by a study on *C. virginica* conducted by Ries et al. (2016) that found that under similarly low saturation states, localized shell calcification was maintained despite net dissolution of the shell. Regardless of the exact mechanism, the reduction in extrapallial fluid [Ca^{2+}] under ocean acidification is a significant result that could impact the ability of bivalves to calcify by decreasing the $CaCO_3$ saturation state of the EPF.

4.2 Boron geochemistry

The boron isotopes and B/Ca proxies have been used as paleo-pH and CO_3^{2-} proxies, respectively, recording changes in seawater carbonate chemistry in the shells of foraminifera (Hemming and Hanson 1992; Sanyal et al., 2001; Foster and Rae 2016). In corals, however, there is evidence that these proxies monitor changes in the carbonate chemistry of the internal calcifying fluid, which may be different from seawater geochemistry (Allison and Finch 2010; Sutton et al., 2018; Guillermic et al., 2021). The boron isotopes proxy has also been applied to other marine species (Sutton et al., 2018, Liu et al., 2020, Cornwall et al., 2017), but independent measurements are needed to fully understand the systematics of this proxy in other organisms. In the present study, we constrained the B/Ca and $\delta^{11}B$ of the main reservoirs involved in the biomineralization (seawater, extrapallial fluid, and shell) of two species of bivalves, the oyster *C. virginica* and the clam *A. islandica.*

For both *A. islandica* and *C. virginica*, there were no significant changes nor correlation observed between $\delta^{11}B$ of the EPF and seawater (Fig 6). Shell $\delta^{11}B$ was significantly different between species, with *A. islandica* recording lower shell $\delta^{11}B$ (15.26 ± 0.41 ‰) than *C. virginica* (18.34 ± 0.59 ‰). Using boron isotope systematics, the $\delta^{11}B$-based EPF pH was

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

determined to be 7.76 ± 0.07 for *A. islandica* and 8.12 ± 0.09 for *C. virginica*. The δ^{11}B-based pH was significantly different between the two species (t-test p value <0.05) and also significantly different from the direct EPF microelectrode pH measurements of 7.41 ± 0.14 and 7.48 ± 0.15 for *A. islandica* and *C. virginica*, respectively (t-test p value < 0.05). In other words, the use of canonical δ^{11}B proxy systematics to calculate δ^{11}B based pH does not match direct measurements of EPF pH. Microelectrode EPF pH was consistently lower than seawater for both species. δ^{11}B-based pH also revealed EPF pH lower than seawater pH for *A. islandica* (but to a lesser extent than direct microelectrode measurement), but an EPF pH greater than seawater for *C. virginica*. This observation in the control experiments holds true under ocean acidification, where the δ^{11}B-based pH is systematically higher than microelectrode EPF pH (Fig 10). Both δ^{11}B-based pH and measured EPF pH record a decrease in pH under acidified conditions (regression p<0.05 for microelectrode pH). However, the offset between microelectrode EPF pH and the δ^{11}B-calculated pH was 0.3 pH units and increased to 0.6 and 0.8 pH units for the moderate and high OA treatments, respectively (Table 1). This demonstrates that, under OA conditions, the incongruence between δ^{11}B based pH and measured EPF pH increases and potentially renders the seawater pH proxy impractical, even after species-specific empirical calibration. Shell δ^{11}B was not correlated with seawater pH, but was significantly correlated to microelectrode pH. These data indicate that microelectrode EPF pH does not fully resolve δ^{11}B vital effects. However it is important to note the differences in timescales associated with δ^{11}B-calculated EPF pH and microelectrode pH. Our microelectrode pH measurements, although averaged across several time points, show snapshots in time and is variable due different behavioral scenarios such asn open (feeding, high pH) and closed (respiring into a closed system, low pH) cycles. Conversely, the δ^{11}B approach represents EPF pH integrated average EPF pH over the interval that the sampled shell was formed, which could range from days to weeks. Furthermore, the δ^{11}B method will only record EPF pH when the shell is forming, which can skew the archiving of the δ^{11}B (pH) signal in the shell to higher values because the crystal only forms when saturation states and calcification rates are higher. This potential bias is also consistent with our δ^{11}B-calculated EPF pH data being higher than the microelectrode pH data, and similar to trends seen in the corals (Cameron et al, 2022).

A possible explanation for the incongruence between δ^{11}B-based pH and measured EPF pH arises from boron isotope systematics. The boron isotope proxy assumes that only the charged borate ion is incorporated as BO_4 into the mineral but has been shown that boric acid can also be incorporated as BO_3, and NMR studies have shown the presence of BO_3 in the shells of different marine organisms (Rollion Bard et al., 2011; Cusack et al., 2015). However, the presence of BO_3 does not obviously translate to a strong bias in the δ^{11}B signature of the mineral due to the potential re-coordination of BO_4 to BO_3 within the crystal lattice (Klochko et al., 2009). A simple calculation shows that 14-17% boric acid incorporation could explain the observed difference between EPF pH and δ^{11}B-calculated pH for *C. virginica*, with only 6% boric acid incorporation needed for *A. islandica*, which could very well explain the discrepancy. Alternatively, shell δ^{11}B could also be affected by seawater or extrapallial fluid DIC, which bivalves are known to modulate under ambient and OA conditions (Crenshaw 1972, Stemmer et al., 2012). Gagnon et al. (2021) found that the shell δ^{11}B of deep-water coral is independently sensitive to changes in seawater DIC as a result of diffusion of boric acid (Gagnon et al., 2021), though no similar studies have looked at the same effect in bivalves this mechanism is still possible. Taken together, these findings could explain the

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

offset between δ^{11}B-based pH and seawater or EPF pH. Nevertheless, this remains speculative as there is no further evidence of boric acid incorporation in these species.

Furthermore, boron isotope derived pH can be influenced by diffusion of boric acid across cell membranes (Stoll et al., 2012; Liu et al., 2018; Liu et al., 2021; Gagnon et al., 2021). At two extremes, diffusion between seawater and the calcifying fluid pool can be fast, resulting in chemically and isotopic equilibrium between both pools, or diffusion can be slow, resulting in calcifying fluid being isolated from seawater such that the boron isotopes would record the chemistry of the calcifying fluid under physiological control. If diffusion is fast compared to other processes, then seawater and the calcifying fluid would be in equilibrium and the δ^{11}B would not differ between the two pools. Our data show no difference between seawater and EPF δ^{11}B. However, differences in Ca, Mg, and δ^{26}Mg between seawater and EPF does provide evidence for physiological modulation of the EPF, despite similar δ^{11}B signatures.

In the case where there is not a strong diffusion of boric acid, then the pH calculated from boron isotopes should reflect the pH at the site of calcification and physiological control over the calcifying fluid. The difference between microelectrode EPF pH and δ^{11}B-based EPF pH implies that pH measured with boron isotopes probes a localized site of calcification rather than the entire EPF pool measured with microelectrode. A spatial and temporal study conducted by Stemmer et al. (2019) measured the EPF of *Arctica islandica* and showed highly dynamic changes in pH, [Ca^{2+}] and DIC from the surface of the shell to the outer mantle epithelium (OME), with localized environment at the OME reaching pH values up to 9.5. Due to this high variability, it is possible that the EPF microelectrode measurements in this study did not capture the full variability of the EPF. Stemmer et al. (2019) presented EPF pH values measured at the shell surface ranging [7.1-7.6] for *A. islandica*, comparable to the values measured from microelectrode in this study. Additionally, Stemmer et al. (2019) found large influxes of DIC which could not have been explained just from metabolic activity, but instead indicated intense DIC pumping and bursts of calcification. These findings are in line with the holistic view of biomineralization outlined in Checa (2018) and Johnstone (2015) that argue that crystal deposition is a series of periodic events under biological regulation. In our study, a time-series of microelectrode EPF pH shows that at no point, during ventilation and closed cycles, does the EPF pH reach the δ^{11}B-calculated pH (Fig 11). The fact that microelectrode EPF pH is systematically lower than seawater pH for both of our bivalve species may reflect localized differences in pH associated with zones of calcification. The two environments (site of calcification and bulk EPF) can act distinctly, with low pH and high DIC EPF being a source of carbon for the site of calcification, and with the elevated pH of the site of calcification supporting the conversion of the DIC species to [CO_3^{2-}] in support of mineral precipitation. Further work would be needed to assess this highly dynamic and localized environment, however our study shows that boron isotopes may reflect the pH of the microenvironment where calcification occurs within the EPF, which has previously been inferred by prior studies using non-geochemical approaches (Ramesh et al., 2017; Stemmer et al., 2019).

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

Conclusion

In this study, we used numerous approaches constraining the geochemical composition of and partitioning between the tripartite reservoirs of bivalve mineralization system--seawater, the EPF and the shell. Our study presents Mg/Ca and B/Ca, and absolute [Ca^{2+}] data of the seawater, EPF and shell. Comparisons of seawater and extrapallial fluid Mg/Ca and B/Ca, Ca, and $\delta^{26}Mg$ indicate that the EPF has a distinct composition that differs from seawater. Additionally, our OA experiments show that the EPF Mg/Ca and B/Ca, as well as absolute Mg, B, and Ca, all were significantly affected by CO_2-induced ocean acidification, demonstrating that the biological pathways regulating or storing these ions involved in calcification are impacted by ocean acidification. Decreased calcium ion concentration within the extrapallial fluid due to OA could impair calcification by lowering the saturation state of the EPF with respect to $CaCO_3$. Additionally, our results show that shell $\delta^{11}B$ does not faithfully record seawater pH. However, shell $\delta^{11}B$ is correlated with EPF pH, despite an offset from *in situ* microelectrode pH measurements. Both microelectrode pH and $\delta^{11}B$-calculated pH decreased with decreasing pH. However, the $\delta^{11}B$-calculated pH values were consistently higher than microelectrode pH measurements, indicating that the shell $\delta^{11}B$ may reflect pH at a more localized site of calcification, rather than pH of the bulk EPF. Furthermore, the offset between the $\delta^{11}B$-calculated pH and microelectrode pH increased with decreasing pH under ocean acidification, indicating OA has a larger effect on bulk pH of the EPF measured via microelectrode than on site of calcification pH—the latter of which the bivalve may have more physiological control over to ensure continued calcification, even under chemically unfavorable conditions. These complex dynamics of EPF chemistry suggest that boron proxies in these two bivalve species are not straightforwardly related to seawater pH, precluding utilization of those species for reconstructing the carbonate chemistry of seawater. Moreover, the $\delta^{11}B$ proxy may not be suitable for reconstructing seawater pH for bivalves with high physiological control over their internal calcifying fluid and is further complicated under conditions of moderate and extreme ocean acidification, where $\delta^{11}B$ EPF pH deviates further from bulk microelectrode pH, possibly due to the effect of DIC on shell $\delta^{11}B$ or the tendancy for shell $\delta^{11}B$ to reflect EPF pH at the more localized site of calcification, rather than pH of the bulk EPF.

Author contribution

LPC, AD, JBR, and KL designed the experiments and carried them out. BAC, MG, and RAE developed the geochemical study. BAC and Mg performed geochemical analysis with the help of JNS and JAH. BAC, MG, and RAE prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

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References

1. Addadi, L., Raz, S., and Weiner, S.: Taking Advantage of Disorder: Amorphous Calcium Carbonate and Its Roles in Biomineralization, Advanced Materials, 15, 959–970, https://doi.org/10.1002/adma.200300381, 2003.
2. Addadi, L., Joester, D., Nudelman, F., and Weiner, S.: mollusc Shell Formation: A Source of New Concepts for Understanding Biomineralization Processes, Chemistry A European J, 12, 980–987, https://doi.org/10.1002/chem.200500980, 2006.
3. Ahm, A.-S. C., Bjerrum, C. J., Hoffman, P. F., Macdonald, F. A., Maloof, A. C., Rose, C. V., Strauss, J. V., and Higgins, J. A.: The Ca and Mg isotope record of the Cryogenian Trezona carbon isotope excursion, Earth and Planetary Science Letters, 568, 117002, https://doi.org/10.1016/j.epsl.2021.117002, 2021.
4. Alibert, C. and McCulloch, M. T.: Strontium/calcium ratios in modern *porites* corals From the Great Barrier Reef as a proxy for sea surface temperature: Calibration of the thermometer and monitoring of ENSO, Paleoceanography, 12, 345–363, https://doi.org/10.1029/97PA00318, 1997.
5. Allison, N.: Reconstructing coral calcification fluid dissolved inorganic carbon chemistry from skeletal boron: An exploration of potential controls on coral aragonite B/Ca, Heliyon, 3, 2017.
6. Allison, N. and Finch, A. A.: δ11B, Sr, Mg and B in a modern Porites coral: the relationship between calcification site pH and skeletal chemistry, Geochimica et Cosmochimica Acta, 74, 1790–1800, 2010.
7. Anagnostou, E., Williams, B., Westfield, I., Foster, G. L., and Ries, J. B.: Calibration of the pH-δ11B and temperature-Mg/Li proxies in the long-lived high-latitude crustose coralline red alga Clathromorphum compactum via controlled laboratory experiments, Geochimica et Cosmochimica Acta, 254, 142–155, 2019.
8. Barker, S., Greaves, M., and Elderfield, H.: A study of cleaning procedures used for foraminiferal Mg/Ca paleothermometry, Geochem Geophys Geosyst, 4, 2003GC000559, https://doi.org/10.1029/2003GC000559, 2003.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

9. Beniash, E., Ivanina, A., Lieb, N. S., Kurochkin, I., and Sokolova, I. M.: Elevated level of carbon dioxide affects metabolism and shell formation in oysters Crassostrea virginica, Marine Ecology Progress Series, 419, 95–108, 2010.
10. Broecker, W. S. and Peng, T.-H.: Tracers in the Sea, Lamont-Doherty Geological Observatory, Columbia University Palisades, New York, 1982.
11. Cameron, L.P., Reymond, C.E., Bijma, J., Büscher, J.V., de Beer, D., Guillermic, M., Eagle, R.A., Gunnell, J., Müller-Lundin, F., Schmidt-Grieb, G.M., *Westfield, I., Westphal, H., Ries, J.B., 2022, Impacts of warming and acidification on coral calcification linked to photosymbiont loss and deregulation of calcifying fluid pH, Journal of Marine Science and Engineering, 10, 1106. https://doi.org/10.3390/jmse10081106.
12. Cameron, L. P., Grabowski, J. H., Ries, J. B., 2022, Impact of ocean acidification and warming on calcification rate, survival, extrapallial fluid chemistry, and respiration of the Atlantic sea scallop Placopecten magellanicus, Limnology & Oceanography, 1-17. https://doi: 10.1002/lno.12153
13. Checa, A. G.: Physical and Biological Determinants of the Fabrication of Molluscan Shell Microstructures, Frontiers in Marine Science, 5, 2018.
14. Cornwall, C. E., Comeau, S., and McCulloch, M. T.: Coralline algae elevate pH at the site of calcification under ocean acidification, Global Change Biology, 23, 4245–4256, https://doi.org/10.1111/gcb.13673, 2017.
15. Craig, H.: The geochemistry of the stable carbon isotopes, Geochimica et cosmochimica acta, 3, 53–92, 1953.
16. Crenshaw, M. A.: THE INORGANIC COMPOSITION OF MOLLUSCAN EXTRAPALLIAL FLUID, The Biological Bulletin, 143, 506–512, https://doi.org/10.2307/1540180, 1972.
17. Cusack, M., Kamenos, N. A., Rollion-Bard, C., and Tricot, G.: Red coralline algae assessed as marine pH proxies using 11B MAS NMR, Sci Rep, 5, 8175, https://doi.org/10.1038/srep08175, 2015.
18. DeCarlo, T. M., Holcomb, M., and McCulloch, M. T.: Reviews and syntheses: Revisiting the boron systematics of aragonite and their application to coral calcification, Biogeosciences, 15, 2819–2834, 2018.
19. Donald, H. K., Ries, J. B., Stewart, J. A., Fowell, S. E., and Foster, G. L.: Boron isotope sensitivity to seawater pH change in a species of Neogoniolithon coralline red alga, Geochimica et Cosmochimica Acta, 217, 240–253, 2017.
20. Downey-Wall, A. M., Cameron, L. P., Ford, B. M., McNally, E. M., Venkataraman, Y. R., Roberts, S. B., Ries, J. B., and Lotterhos, K. E.: Ocean acidification induces subtle shifts in gene expression and DNA methylation in mantle tissue of the Eastern oyster (Crassostrea virginica), Frontiers in Marine Science, 7, 566419, 2020.
21. Dunbar, R. B., Wellington, G. M., Colgan, M. W., and Glynn, P. W.: Eastern Pacific sea surface temperature since 1600 A.D.: The δ^{18}O record of climate variability in Galápagos Corals, Paleoceanography, 9, 291–315, https://doi.org/10.1029/93PA03501, 1994.
22. Eagle, R. A., Guillermic, M., De Corte, I., Alvarez Caraveo, B., Bove, C. B., Misra, S., Cameron, L. P., Castillo, K. D., and Ries, J. B.: Physicochemical Control of Caribbean Coral Calcification Linked to Host and Symbiont Responses to Varying p CO2 and Temperature, Journal of Marine Science and Engineering, 10, 1075, 2022.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

23. Elderfield, H., Yu, J., Anand, P., Kiefer, T., and Nyland, B.: Calibrations for benthic foraminiferal Mg/Ca paleothermometry and the carbonate ion hypothesis, Earth and Planetary Science Letters, 250, 633–649, 2006.
24. Fitzer, S. C., Chung, P., Maccherozzi, F., Dhesi, S. S., Kamenos, N. A., Phoenix, V. R., and Cusack, M.: Biomineral shell formation under ocean acidification: a shift from order to chaos, Sci Rep, 6, 21076, https://doi.org/10.1038/srep21076, 2016.
25. Foster, G. L. and Rae, J. W. B.: Reconstructing Ocean pH with Boron Isotopes in Foraminifera, Annual Review of Earth and Planetary Sciences, 44, 207–237, https://doi.org/10.1146/annurev-earth-060115-012226, 2016.
26. Gagnon, A. C., Gothmann, A. M., Branson, O., Rae, J. W. B., and Stewart, J. A.: Controls on boron isotopes in a cold-water coral and the cost of resilience to ocean acidification, Earth and Planetary Science Letters, 554, 116662, https://doi.org/10.1016/j.epsl.2020.116662, 2021.
27. Gaillardet, J., Lemarchand, D., Göpel, C., and Manhès, G.: Evaporation and Sublimation of Boric Acid: Application for Boron Purification from Organic Rich Solutions, Geostandards Newsletter, 25, 67–75, https://doi.org/10.1111/j.1751-908X.2001.tb00788.x, 2001.
28. Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., Pörtner, H.-O., and Ross, P. M.: Impacts of ocean acidification on marine shelled molluscs, Mar Biol, 160, 2207–2245, https://doi.org/10.1007/s00227-013-2219-3, 2013.
29. Gibson, R., Barnes, M., and Atkinson, R.: Molluscs as archives of environmental change, Oceanogr. Mar. Biol. Annu. Rev, 39, 103–164, 2001.
30. Gilbert, P. U. P. A., Bergmann, K. D., Boekelheide, N., Tambutté, S., Mass, T., Marin, F., Adkins, J. F., Erez, J., Gilbert, B., Knutson, V., Cantine, M., Hernández, J. O., and Knoll, A. H.: Biomineralization: Integrating mechanism and evolutionary history, Science Advances, 8, eabl9653, https://doi.org/10.1126/sciadv.abl9653, 2022.
31. Gosling, E.: Bivalve molluscs: biology, ecology and culture, John Wiley & Sons, 2008.
32. Guillermic, M., Cameron, L. P., De Corte, I., Misra, S., Bijma, J., De Beer, D., Reymond, C. E., Westphal, H., Ries, J. B., and Eagle, R. A.: Thermal stress reduces pocilloporid coral resilience to ocean acidification by impairing control over calcifying fluid chemistry, Sci. Adv., 7, eaba9958, https://doi.org/10.1126/sciadv.aba9958, 2021.
33. Gutjahr, M., Bordier, L., Douville, E., Farmer, J., Foster, G. L., Hathorne, E. C., Hönisch, B., Lemarchand, D., Louvat, P., McCulloch, M., Noireaux, J., Pallavicini, N., Rae, J. W. B., Rodushkin, I., Roux, P., Stewart, J. A., Thil, F., and You, C.: Sub-Permil Interlaboratory Consistency for Solution-Based Boron Isotope Analyses on Marine Carbonates, Geostandard Geoanalytic Res, 45, 59–75, https://doi.org/10.1111/ggr.12364, 2021.
34. Heinemann, A., Fietzke, J., Melzner, F., Böhm, F., Thomsen, J., Garbe-Schönberg, D., and Eisenhauer, A.: Conditions of Mytilus edulis extracellular body fluids and shell composition in a pH-treatment experiment: Acid-base status, trace elements and δ 11 B, Geochem Geophys Geosyst, 13, 2011GC003790, https://doi.org/10.1029/2011GC003790, 2012.
35. Helm, M. M., Bourne, N., and Lovatelli, A.: Hatchery culture of bivalves: a practical manual, 2004.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

36. Hemming, N. G. and Hanson, G. N.: Boron isotopic composition and concentration in modern marine carbonates, Geochimica et Cosmochimica Acta, 56, 537–543, 1992.
37. Higgins, J. A., Blättler, C. L., Lundstrom, E. A., Santiago-Ramos, D. P., Akhtar, A. A., Crüger Ahm, A.-S., Bialik, O., Holmden, C., Bradbury, H., Murray, S. T., and Swart, P. K.: Mineralogy, early marine diagenesis, and the chemistry of shallow-water carbonate sediments, Geochimica et Cosmochimica Acta, 220, 512–534, https://doi.org/10.1016/j.gca.2017.09.046, 2018.
38. Holcomb, M., DeCarlo, T. M., Gaetani, G. A., and McCulloch, M.: Factors affecting B/Ca ratios in synthetic aragonite, Chemical Geology, 437, 67–76, 2016.
39. Hönisch, B., Hemming, Ng., Grottoli, A. G., Amat, A., Hanson, G. N., and Bijma, J.: Assessing scleractinian corals as recorders for paleo-pH: Empirical calibration and vital effects, Geochimica et Cosmochimica Acta, 68, 3675–3685, 2004.
40. Husson, J. M., Higgins, J. A., Maloof, A. C., and Schoene, B.: Ca and Mg isotope constraints on the origin of Earth's deepest δ13C excursion, Geochimica et Cosmochimica Acta, 160, 243–266, 2015.
41. Immenhauser, A., Schöne, B. R., Hoffmann, R., and Niedermayr, A.: Mollusc and brachiopod skeletal hard parts: Intricate archives of their marine environment, Sedimentology, 63, 1–59, https://doi.org/10.1111/sed.12231, 2016.
42. Johnstone, M. B., Gohad, N. V., Falwell, E. P., Hansen, D. C., Hansen, K. M., and Mount, A. S.: Cellular orchestrated biomineralization of crystalline composites on implant surfaces by the eastern oyster, Crassostrea virginica (Gmelin, 1791), Journal of Experimental Marine Biology and Ecology, 463, 8–16, https://doi.org/10.1016/j.jembe.2014.10.014, 2015.
43. Klochko, K., Kaufman, A. J., Yao, W., Byrne, R. H., and Tossell, J. A.: Experimental measurement of boron isotope fractionation in seawater, Earth and Planetary Science Letters, 248, 276–285, 2006.
44. Kroeker, K. J., Micheli, F., Gambi, M. C., and Martz, T. R.: Divergent ecosystem responses within a benthic marine community to ocean acidification, Proc. Natl. Acad. Sci. U.S.A., 108, 14515–14520, https://doi.org/10.1073/pnas.1107789108, 2011.
45. Liu, Y.-W., Sutton, J. N., Ries, J. B., and Eagle, R. A.: Regulation of calcification site pH is a polyphyletic but not always governing response to ocean acidification, Sci. Adv., 6, eaax1314, https://doi.org/10.1126/sciadv.aax1314, 2020.
46. Liu, Y.-W., Wanamaker Jr, A. D., Aciego, S. M., Searles, I., Hangstad, T. A., Chierici, M., and Carroll, M. L.: Resistant calcification responses of Arctica islandica clams under ocean acidification conditions, Journal of Experimental Marine Biology and Ecology, 560, 151855, 2023.
47. Lorens, R. B. and Bender, M. L.: Physiological exclusion of magnesium from Mytilus edulis calcite, Nature, 269, 793–794, 1977.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

48. Mavromatis, V., Montouillout, V., Noireaux, J., Gaillardet, J., and Schott, J.: Characterization of boron incorporation and speciation in calcite and aragonite from co-precipitation experiments under controlled pH, temperature and precipitation rate, Geochimica et Cosmochimica Acta, 150, 299–313, 2015.
49. McCulloch, M. T., D'Olivo, J. P., Falter, J., Holcomb, M., and Trotter, J. A.: Coral calcification in a changing world and the interactive dynamics of pH and DIC upregulation, Nature Communications, 8, 15686, 2017.
50. McCulloch, M. T., D'Olivo, J. P., Falter, J., Georgiou, L., Holcomb, M., Montagna, P., and Trotter, J. A.: Boron Isotopic Systematics in Scleractinian Corals and the Role of pH Up-regulation, in: Boron Isotopes, edited by: Marschall, H. and Foster, G., Springer International Publishing, Cham, 145–162, https://doi.org/10.1007/978-3-319-64666-4_6, 2018.
51. McNally, E. M., Downey-Wall, A. M., Titmuss, F. D., Cortina, C., Lotterhos, K., and Ries, J. B.: Parental exposure of Eastern oysters (Crassostrea virginica) to elevated p CO 2 mitigates its negative effects on early larval shell growth and morphology, Limnology & Oceanography, 67, 1732–1745, https://doi.org/10.1002/lno.12162, 2022.
52. Michaelidis, B., Ouzounis, C., Paleras, A., and Pörtner, H. O.: Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels Mytilus galloprovincialis, Marine Ecology Progress Series, 293, 109–118, 2005.
53. Mount, A. S., Wheeler, A. P., Paradkar, R. P., and Snider, D.: Hemocyte-Mediated Shell Mineralization in the Eastern Oyster, Science, 304, 297–300, https://doi.org/10.1126/science.1090506, 2004.
54. Nir, O., Vengosh, A., Harkness, J. S., Dwyer, G. S., and Lahav, O.: Direct measurement of the boron isotope fractionation factor: Reducing the uncertainty in reconstructing ocean paleo-pH, Earth and Planetary Science Letters, 414, 1–5, 2015.
55. Peharda, M., Schöne, B. R., Black, B. A., and Correge, T.: Advances of sclerochronology research in the last decade, Palaeogeography, Palaeoclimatology, Palaeoecology, 570, 110371, 2021.
56. Pierrot, D. E., Wallace, D. W. R., and Lewis, E.: MS Excel program developed for CO2 system calculations, Carbon dioxide information analysis center, 2011.
57. Planchon, F., Poulain, C., Langlet, D., Paulet, Y.-M., and André, L.: Mg-isotopic fractionation in the manila clam (Ruditapes philippinarum): New insights into Mg incorporation pathway and calcification process of bivalves, Geochimica et cosmochimica acta, 121, 374–397, 2013.
58. Ramesh, K., Melzner, F., Griffith, A. W., Gobler, C. J., Rouger, C., Tasdemir, D., and Nehrke, G.: In vivo characterization of bivalve larval shells: a confocal Raman microscopy study, J. R. Soc. Interface., 15, 20170723, https://doi.org/10.1098/rsif.2017.0723, 2018.
59. Ries, J. B., Cohen, A. L., and McCorkle, D. C.: Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification, Geology, 37, 1131–1134, 2009.
60. Ries, J.B. A physicochemical framework for interpreting the biological calcification response to CO2-induced ocean acidification. Geochimica et Cosmochimica Acta 75: 4053-4064, 2011.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

61. Ries, J. B., Ghazaleh, M. N., Connolly, B., Westfield, I., and Castillo, K. D.: Impacts of seawater saturation state (ØmegaA= 0.4–4.6) and temperature (10, 25 C) on the dissolution kinetics of whole-shell biogenic carbonates, Geochimica et Cosmochimica Acta, 192, 318–337, 2016.
62. Rollion-Bard, C., Blamart, D., Trebosc, J., Tricot, G., Mussi, A., and Cuif, J.-P.: Boron isotopes as pH proxy: A new look at boron speciation in deep-sea corals using 11B MAS NMR and EELS, Geochimica et cosmochimica acta, 75, 1003–1012, 2011.
63. Sanyal, A., Bijma, J., Spero, H., and Lea, D. W.: Empirical relationship between pH and the boron isotopic composition of *Globigerinoides sacculifer* : Implications for the boron isotope paleo-pH proxy, Paleoceanography, 16, 515–519, https://doi.org/10.1029/2000PA000547, 2001.
64. Saulnier, S., Rollion-Bard, C., Vigier, N., and Chaussidon, M.: Mg isotope fractionation during calcite precipitation: An experimental study, Geochimica et Cosmochimica Acta, 91, 75–91, https://doi.org/10.1016/j.gca.2012.05.024, 2012.
65. Schoepf, V., Jury, C. P., Toonen, R. J., and McCulloch, M. T.: Coral calcification mechanisms facilitate adaptive responses to ocean acidification, Proc. R. Soc. B., 284, 20172117, https://doi.org/10.1098/rspb.2017.2117, 2017.
66. Schöne, B. R.: The curse of physiology—challenges and opportunities in the interpretation of geochemical data from mollusc shells, Geo-Marine Letters, 28, 269–285, 2008.
67. Short, J. A., Pedersen, O., and Kendrick, G. A.: Turf algal epiphytes metabolically induce local pH increase, with implications for underlying coralline algae under ocean acidification, Estuarine, Coastal and Shelf Science, 164, 463–470, 2015.
68. Sillanpää, J. K., Ramesh, K., Melzner, F., Sundh, H., and Sundell, K.: Calcium mobilisation following shell damage in the Pacific oyster, Crassostrea gigas, Marine Genomics, 27, 75–83, https://doi.org/10.1016/j.margen.2016.03.001, 2016.
69. Sillanpää, J. K., Sundh, H., and Sundell, K. S.: Calcium transfer across the outer mantle epithelium in the Pacific oyster, Crassostrea gigas, Proc. R. Soc. B., 285, 20181676, https://doi.org/10.1098/rspb.2018.1676, 2018.
70. Sillanpää, J. K., Cardoso, J. C. dos R., Félix, R. C., Anjos, L., Power, D. M., and Sundell, K.: Dilution of seawater affects the Ca2+ transport in the outer mantle epithelium of Crassostrea gigas, Frontiers in Physiology, 11, 496427, 2020.
71. Stemmer, K., Brey, T., Gutbrod, M. S., Beutler, M., Schalkhausser, B., and De Beer, D.: In situ measurements of pH, Ca2+, and DIC dynamics within the extrapallial fluid of the ocean quahog Arctica islandica, Journal of Shellfish Research, 38, 71–78, 2019.
72. Stewart-Sinclair, P. J., Last, K. S., Payne, B. L., and Wilding, T. A.: A global assessment of the vulnerability of shellfish aquaculture to climate change and ocean acidification, Ecology and Evolution, 10, 3518–3534, https://doi.org/10.1002/ece3.6149, 2020.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

73. Stoll, H., Langer, G., Shimizu, N., and Kanamaru, K.: B/Ca in coccoliths and relationship to calcification vesicle pH and dissolved inorganic carbon concentrations, Geochimica et cosmochimica acta, 80, 143–157, 2012.
74. Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F., and Dupont, S.: Digestion in sea urchin larvae impaired under ocean acidification, Nature climate change, 3, 1044–1049, 2013.
75. Sutton, J. N., Liu, Y.-W., Ries, J. B., Guillermic, M., Ponzevera, E., and Eagle, R. A.: δ^{11}B as monitor of calcification site pH in divergent marine calcifying organisms, Biogeosciences, 15, 1447–1467, https://doi.org/10.5194/bg-15-1447-2018, 2018.
76. Thomsen, J., Casties, I., Pansch, C., Körtzinger, A., and Melzner, F.: Food availability outweighs ocean acidification effects in juvenile *M ytilus edulis* : laboratory and field experiments, Global Change Biology, 19, 1017–1027, https://doi.org/10.1111/gcb.12109, 2013.
77. Ulrich, R. N., Guillermic, M., Campbell, J., Hakim, A., Han, R., Singh, S., Stewart, J. D., Román-Palacios, C., Carroll, H. M., and De Corte, I.: Patterns of element incorporation in calcium carbonate biominerals recapitulate phylogeny for a diverse range of marine calcifiers, Frontiers in earth science, 9, 641760, 2021.
78. Urey, H. C., Lowenstam, H. A., Epstein, S., and McKinney, C. R.: Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States, Geological Society of America Bulletin, 62, 399–416, 1951.
79. Vogl, J., Rosner, M., and Pritzkow, W.: Development and validation of a single collector SF-ICPMS procedure for the determination of boron isotope ratios in water and food samples, Journal of analytical atomic spectrometry, 26, 861–869, 2011.
80. Waldbusser, G. G., Voigt, E. P., Bergschneider, H., Green, M. A., and Newell, R. I. E.: Biocalcification in the Eastern Oyster (Crassostrea virginica) in Relation to Long-term Trends in Chesapeake Bay pH, Estuaries and Coasts, 34, 221–231, https://doi.org/10.1007/s12237-010-9307-0, 2011.
81. Wanamaker Jr, A. D., Kreutz, K. J., Wilson, T., Borns Jr, H. W., Introne, D. S., and Feindel, S.: Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve calcite for Mytilus edulis: implications for paleotemperature reconstructions, Geo-Marine Letters, 28, 359–368, 2008.
82. Wang, B.-S., You, C.-F., Huang, K.-F., Wu, S.-F., Aggarwal, S. K., Chung, C.-H., and Lin, P.-Y.: Direct separation of boron from Na-and Ca-rich matrices by sublimation for stable isotope measurement by MC-ICP-MS, Talanta, 82, 1378–1384, 2010.
83. Wang, X., Wang, M., Jia, Z., Qiu, L., Wang, L., Zhang, A., and Song, L.: A Carbonic Anhydrase Serves as an Important Acid-Base Regulator in Pacific Oyster Crassostrea gigas Exposed to Elevated CO2: Implication for Physiological Responses of mollusc to Ocean Acidification, Mar Biotechnol, 19, 22–35, https://doi.org/10.1007/s10126-017-9734-z, 2017.
84. Weiner, S., Levi-Kalisman, Y., Raz, S., and Addadi, L.: Biologically Formed Amorphous Calcium Carbonate, Connective Tissue Research, 44, 214–218, https://doi.org/10.1080/03008200390181681, 2003.

https://doi.org/10.5194/egusphere-2024-1957
Preprint. Discussion started: 12 August 2024

85. Wheeler, A. P., Rusenko, K. W., Swift, D. M., and Sikes, C. S.: Regulation of in vitro and in vivo CaCO 3 crystallization by fractions of oyster shell organic matrix, Marine Biology, 98, 71–80, 1988.
86. Wilbur, K. M. and Bernhardt, A. M.: EFFECTS OF AMINO ACIDS, MAGNESIUM, AND MOLLUSCAN EXTRAPALLIAL FLUID ON CRYSTALLIZATION OF CALCIUM CARBONATE: IN VITRO EXPERIMENTS, The Biological Bulletin, 166, 251–259, https://doi.org/10.2307/1541446, 1984.
87. Zeebe, R. E. and Wolf-Gladrow, D.: CO2 in Seawater: Equilibrium, Kinetics, Isotopes, Gulf Professional Publishing, 382 pp., 2001.
88. Zhao, L., Milano, S., Walliser, E. O., and Schöne, B. R.: Bivalve shell formation in a naturally CO2-enriched habitat: Unraveling the resilience mechanisms from elemental signatures, Chemosphere, 203, 132–138, https://doi.org/10.1016/j.chemosphere.2018.03.180, 2018a.
89. Zhao, L., Yang, F., Milano, S., Han, T., Walliser, E. O., and Schöne, B. R.: Transgenerational acclimation to seawater acidification in the Manila clam Ruditapes philippinarum: Preferential uptake of metabolic carbon, Science of the Total Environment, 627, 95–103, 2018b.
90. Zittier, Z. M., Bock, C., Lannig, G., and Pörtner, H. O.: Impact of ocean acidification on thermal tolerance and acid–base regulation of Mytilus edulis (L.) from the North Sea, Journal of experimental marine biology and ecology, 473, 16–25, 2015.