

1 **Environmental Controls on the Boron and Strontium**  
2 **Isotopic Composition of Aragonite Shell Material of**  
3 **Cultured *Arctica islandica***

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11  
12 **Abstract**

13 Ocean acidification, the decrease in ocean pH associated with increasing atmospheric CO<sub>2</sub>, is  
14 likely to impact marine organisms, particularly those that produce carbonate skeletons or  
15 shells. Therefore it is important to investigate how environmental factors (seawater pH,  
16 temperature and salinity) influence the chemical compositions in biogenic carbonates. In this  
17 study we report the first high-resolution strontium (<sup>87</sup>Sr/<sup>86</sup>Sr and δ<sup>88/86</sup>Sr) and boron (δ<sup>11</sup>B)  
18 isotopic values in the aragonite shell of cultured *Arctica islandica* (*A. islandica*). The <sup>87</sup>Sr/<sup>86</sup>Sr  
19 ratios from both tank water and shell samples show ratios nearly identical to the open ocean,  
20 which suggests that the shell material reflects ambient ocean chemistry without terrestrial  
21 influence. The <sup>84</sup>Sr-<sup>87</sup>Sr double spike resolved shell δ<sup>88/86</sup>Sr and Sr concentration data show no  
22 resolvable change throughout the culture period and reflect no theoretical kinetic mass  
23 fractionation throughout the experiment despite a temperature change of more than 15 °C.  
24 The δ<sup>11</sup>B records from the experiment show at least a 5 ‰ increase through the 29-week the  
25 culture season (January, 2010 – August, 2010), with low values from the beginning to week  
26 19 and higher values hereafter. The larger range in δ<sup>11</sup>B in this experiment compared to  
27 predictions based on other carbonate organisms (2 to 3 ‰) suggests that a species-specific  
28 fractionation factor may be required. A significant correlation between the ΔpH (pH<sub>shell</sub>-pH<sub>sw</sub>)  
29 and seawater pH (pH<sub>sw</sub>) was observed (R<sup>2</sup> = 0.35), where the pH<sub>shell</sub> is the calcification pH of

1 the shell calculated from boron isotopic composition. This negative correlation suggests that  
2 *A. islandica* partly regulates the pH of the extrapallial fluid. However, this proposed  
3 mechanism only explains approximately 35 % of the variance in the  $\delta^{11}\text{B}$  data. Instead, a  
4 rapid rise in  $\delta^{11}\text{B}$  after week 19 suggests that the boron uptake of the shell changes when a  
5 temperature threshold of 13 °C is reached.

6

## 7 **1 Introduction**

8 Since the onset of the Industrial Revolution (ca. 1750 AD) the global ocean has absorbed  
9 approximately 30 % of the emitted anthropogenic carbon dioxide ( $\text{CO}_2$ ) causing ocean  
10 acidification (IPCC, 2013). The ecological effects from lowering the pH of the surface ocean  
11 on marine organisms, especially those which calcify, will likely be substantial (Doney et al.,  
12 2009; Hofmann et al., 2010; Orr et al., 2005), highly variable among taxa (Kroeker et al.,  
13 2010; Riebesell et al., 2013), and extend throughout the global ocean (Feely et al., 2004; Orr  
14 et al., 2005). Furthermore, anthropogenic  $\text{CO}_2$  is not evenly distributed among the ocean  
15 basins. In particular, the North Atlantic Ocean stores about 23 % of anthropogenic  $\text{CO}_2$   
16 despite representing only 15 % of the global ocean area (Sabine et al., 2004). Consequently,  
17 the North Atlantic Basin, compared to other regions, might be more susceptible to ocean  
18 acidification. In situ measurements of surface ocean pH are rare prior to about 1990 (Byrne et  
19 al., 2010; Dore et al., 2009), however, instrumental data show that the surface global ocean  
20 pH has decreased by approximately 0.05 pH units in the last 20 to 25 years (IPCC, 2013).  
21 Time-series data from the North Pacific and North Atlantic Oceans indicate that the surface  
22 ocean pH has been changing between -0.0015 to -0.0024 pH units per year (IPCC, 2013;  
23 Vázquez-Rodríguez et al., 2012).

24 This global rise in atmospheric  $\text{CO}_2$  has resulted in changes in surface ocean pH and shows a  
25 projected persistence in the near future. Therefore, proxy records from the geologic record  
26 sensitive to oceanic carbon dynamics are highly desired to place modern pH trends into  
27 context (e.g., Hönisch et al., 2012). Biogenic proxy archives calcifying within the surface  
28 waters of the global oceans have the unique potential to reveal spatial and temporal patterns  
29 and trends in pH using boron isotopes (e.g., Anagnostou et al., 2012; Shinjo et al., 2013).  
30 However, in the dynamic coastal regions of the global ocean, local and regional processes  
31 have the potential to complicate the boron-pH proxy (described in detail below). Freshwater  
32 mixing has the potential to change (1) temperature, salinity, and pH, (2) nutrient availability

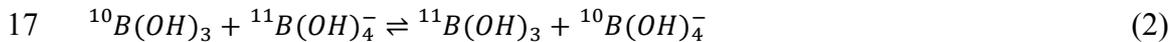
1 and productivity leading to changes in pH, and (3) local seawater boron isotopic composition.  
2 Additionally, potential species-specific biological effects that occur during calcification need  
3 to be evaluated. Here we apply multiple isotope systems to evaluate the boron-pH proxy in  
4 the northern North Atlantic coastal and shelf-dwelling marine bivalve *Arctica islandica* (*A.*  
5 *islandica*) exposed to ambient conditions. We use radiogenic strontium isotopes to assess  
6 terrestrial river water influence on seawater and shell geochemistry for elements with long  
7 residence times in coastal water, such as boron. We utilize stable strontium isotopes from  
8 shell material to evaluate the potential impacts of growth rates during biomineralization.

### 9 **1.1 Boron isotopes as pH indicators in biogenic carbonates**

10 | Boron has two natural stable isotopes,  $^{10}\text{B}$  and  $^{11}\text{B}$ , which comprise 19.9(7)% and 80.1(7)%  
11 of total boron, respectively (Berglund and Wieser, 2011). The dominant aqueous species of  
12 boron in seawater are  $\text{B}(\text{OH})_3$  and  $\text{B}(\text{OH})_4^-$ . The relative proportion of these two species in an  
13 aqueous environment is a function of pH with the following relationship:



15 At low pH, boron exists as  $\text{B}(\text{OH})_3$  in solution, conversely at high pH, boron exists as  
16  $\text{B}(\text{OH})_4^-$ . The governing reaction for isotope exchange between these two species is:



18 The stable isotope  $^{11}\text{B}$  is enriched in  $\text{B}(\text{OH})_3$  compared to  $\text{B}(\text{OH})_4^-$ , and the combination of  
19 Eq. (1) and (2) can be used to determine the distribution of the two boron species and the  
20 isotopic composition of each for a given pH. The isotopic composition of boron is generally  
21 reported as:

$$22 \quad \delta^{11}\text{B} = \left[ \frac{({}^{11}\text{B}/{}^{10}\text{B})_{\text{sample}}}{({}^{11}\text{B}/{}^{10}\text{B})_{\text{SRM 951a}}} - 1 \right] \times 1000 \text{ (‰)} \quad (3)$$

23 where SRM 951 is the internationally recognized boron isotope standard. Because the  
24 residence time of seawater boron is approximately 14 million years (Lemarchand et al., 2000),  
25 boron isotopic composition in the open ocean is considered constant over this time period,  
26 with an average seawater  $\delta^{11}\text{B}$  value of 39.61 ‰ (Foster et al., 2010). Therefore,  $\delta^{11}\text{B}$  has the  
27 following relationship: at low pH, the isotopic composition of  $\text{B}(\text{OH})_3$  is equal to the isotopic  
28 composition of the total dissolved boron (39.61 ‰). In contrast, at high pH, the isotopic  
29 composition of  $\text{B}(\text{OH})_4^-$  is equal to the isotopic composition of the total dissolved boron.

1 Therefore the  $\delta^{11}\text{B}$  is enriched in  $\text{B}(\text{OH})_3$  by about 20 ‰ with respect to  $\text{B}(\text{OH})_4^-$  at any  
2 equilibrium pH based on a constant fractionation factor.

3 During growth, it is assumed that marine carbonates primarily incorporate  $\text{B}(\text{OH})_4^-$  into the  
4 carbonate structure. Building on these relationships, seawater pH dictates the amount of  
5  $\text{B}(\text{OH})_4^-$  in seawater and thus the isotopic composition of boron in marine carbonates. The  
6 possibility of trigonal  $\text{B}(\text{OH})_3$  incorporation in carbonates, especially in calcite, is still under  
7 debate (Klochko et al., 2009; Rollion-Bard et al., 2011a); but after thorough calibration in  
8 targeted marine carbonate species, the  $\delta^{11}\text{B}$  to pH transfer function can be applied. Changes in  
9 the  $\delta^{11}\text{B}$  composition of shell carbonates are based on the equation:

$$10 \quad pH = pK_b - \log \left( \frac{\delta^{11}\text{B}_{\text{sw}} - \delta^{11}\text{B}_{\text{carbonate}}}{\alpha \delta^{11}\text{B}_{\text{carbonate}} - \delta^{11}\text{B}_{\text{sw}} + 1000(\alpha - 1)} \right) \quad (4)$$

11 where  $pK_b$  is the pK value for boric acid at a given temperature and salinity, and is 8.5975 at  
12 25 °C and 35 PSU salinity (DOE, 1994),  $\delta^{11}\text{B}_{\text{sw}}$  is the isotopic composition of seawater, and  $\alpha$   
13 is the equilibrium isotopic fractionation factor between boric acid and borate ion  $\left( \alpha \equiv \right.$   
14  $\left. \frac{(^{11}\text{B}/^{10}\text{B})_{\text{B}(\text{OH})_3}}{(^{11}\text{B}/^{10}\text{B})_{\text{B}(\text{OH})_4^-}} \right)$ . Of these variables, only the seawater composition can be considered known

15 and constant for all geographic locations and carbonate-precipitating species. Temperature,  
16 salinity and the fractionation factor must be estimated. Two empirical and analytical values of  
17  $\alpha$  are suggested for seawater: (1)  $\alpha = 1.0194$ , a theoretical result of Kakihana et al. (1977),  
18 which has been applied widely on paleo-reconstructions (Hönisch et al., 2004; Sanyal et al.,  
19 1995); and (2)  $\alpha = 1.0272$ , which was empirically obtained from Klochko et al. (2006) and is  
20 considered to better describe the distribution of the two boron species in nature today (Foster,  
21 2008; Rollion-Bard et al., 2011b; Rollion-Bard and Erez, 2010). Recent work of Nir et al.  
22 (2015), using reverse osmosis membrane under controlled pH, also suggests a higher  
23 fractionation factor than the theoretical result from Kakihana et al. (1977). However, due to  
24 the ability of calcifying organisms to buffer their own local environments, species specific  
25 fractionation factors and transfer functions are likely more appropriate than theoretical  $\alpha$   
26 values (Anagnostou et al., 2012; Hönisch et al., 2004; Krief et al., 2010; Rae et al., 2011;  
27 Reynaud et al., 2004; Reynaud et al., 2008; Trotter et al., 2011). Thus far, the pH- $\delta^{11}\text{B}$   
28 relationship has been tested extensively on some biogenic marine carbonates (foraminifera  
29 and coral) with broad success (Anagnostou et al., 2012; Henehan et al., 2013; Hönisch et al.,  
30 2004; Ni et al., 2007; Rae et al., 2011; Reynaud et al., 2004), a few measurements have been

1 made on calcite shells (Heinemann et al., 2012; Penman et al., 2012), but no published data  
2 exists for aragonite bivalves.

3 As indicated earlier, one of the primary assumptions in applying the boron isotope/pH proxy  
4 technique is that the boron isotopic composition of the seawater from which the biogenic  
5 carbonates form remains constant. Therefore, in order to use such a proxy to understand pH  
6 changes through the geological past, a technique is required to evaluate variability in local  
7 seawater geochemistry.

## 8 **1.2 Radiogenic strontium isotopes as a water mass tracer**

9 To evaluate the potential impacts of freshwater on local or regional ocean chemistry, it is  
10 necessary to differentiate sources that influence the chemical composition of the target water  
11 mass. This is especially important in coastal regions where fresh water input from river,  
12 surface runoff and/or groundwater mix with seawater often modifying both physical (e.g.  
13 temperature, salinity or turbidity) and chemical (e.g. nutrients, trace metal and/or isotopic  
14 compositions) conditions. Even for isotopic systems with long residence times in seawater  
15 (including boron), observations indicate that large fluxes of freshwater can cause substantial  
16 variations in coastal environments where conservative mixing behavior is generally assumed  
17 but not always achieved (Chung et al., 2009; D'Olivo et al., 2014; Huang and You, 2007;  
18 Huang et al., 2011; Widerlund and Andersson, 2006).

19 Radiogenic strontium has successfully been used to trace unique water masses. There are four  
20 naturally occurring isotopes of strontium:  $^{84}\text{Sr}$ ,  $^{86}\text{Sr}$ ,  $^{87}\text{Sr}$  and  $^{88}\text{Sr}$ , with the only radiogenic  
21 isotope being  $^{87}\text{Sr}$ , which decays from  $^{87}\text{Rb}$ . Therefore, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is widely used to  
22 trace provenance in geological studies (Aarons et al., 2013; Bataille and Bowen, 2012; Huang  
23 and You, 2007; Jahn et al., 2001). Because the residence time of Sr in seawater is more than  
24 4 My, similar to the residence time of boron (Broecker, 1963; Goldberg, 1963),  $^{87}\text{Sr}/^{86}\text{Sr}$  is  
25 considered spatially homogeneous in seawater at any instant of geological time. However, in  
26 coastal areas, radiogenic Sr isotopes vary as inputs from continental sources are released from  
27 terrestrial sediments to fresh water and then exported to the open ocean (Huang et al., 2011).  
28 The variability in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in natural archives on seasonal and annual timescales has  
29 been used as a proxy for quantitatively evaluating proportions of different water mass sources  
30 in coastal regions (Huang et al., 2011). The similarity of residence times of Sr and B in the  
31 ocean, and potential large differences between ocean and terrestrial isotopic compositions,

1 suggests that the radiogenic strontium composition of shell material can be used to determine  
2 if there is a potential offset between local seawater and open-ocean B isotopic composition.

3 Prior studies have shown that bivalve shells record both ambient seawater composition and  
4 mixing of water sources in the ambient seawater. Widerlund and Anderson (Widerlund and  
5 Andersson, 2006) developed a two-endmember mixing model of terrestrial fluvial water and  
6 seawater radiogenic Sr in the Baltic Sea and compared the modern bivalve  $^{87}\text{Sr}/^{86}\text{Sr}$  inferred  
7 salinity across the Baltic Sea from inland to the Atlantic coast to the *in-situ* measurements of  
8 water salinity. Their results indicate conservative characteristics of  $^{87}\text{Sr}/^{86}\text{Sr}$  vs. salinity in the  
9 Baltic Sea. Maurer et al. (2012) also measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in a freshwater bivalve species:  
10 the Sr isotopic ratios recorded in the shells, in both early and late ontogeny ages, agreed with  
11 local water samples, indicating their usefulness in investigating the effects of anthropogenic  
12 contamination in rivers.

### 13 **1.3 Stable strontium isotopes in biogenic carbonates**

14 In addition to  $^{87}\text{Sr}/^{86}\text{Sr}$ , the stable isotopes of Sr ( $\delta^{88/86}\text{Sr}$ , the deviation in  $^{88}\text{Sr}/^{86}\text{Sr}$  of a sample  
15 relative to a standard given in parts per mil) in natural archives are potential indicators of  
16 environmental controls on vital effects, although the applicability is less well established and  
17 still controversial. Previously reported  $\delta^{88/86}\text{Sr}$  values of coral, foraminifera and  
18 coccolithophores from the literature show  $^{88}\text{Sr}$  depleted results compared to the  $\delta^{88/86}\text{Sr}$  value  
19 in seawater, which suggest the lighter  $^{86}\text{Sr}$  is preferentially incorporated into biogenic  
20 carbonates (Böhm et al., 2012; Fietzke and Eisenhauer, 2006; Krabbenhöft et al., 2010;  
21 Rüggeberg et al., 2008; Stevenson et al., 2014). If the fractionation of stable Sr isotopes in  
22 precipitated biogenic carbonates is dominated by kinetic isotope effects and not under  
23 equilibrium conditions, the  $\delta^{88/86}\text{Sr}$  ratio would likely have a strong correlation with  
24 precipitation temperature and/or precipitation rate. As an environmental proxy,  $^{88}\text{Sr}/^{86}\text{Sr}$  in  
25 both deep sea and tropical corals has been reported as a temperature proxy (Fietzke and  
26 Eisenhauer, 2006; Rüggeberg et al., 2008). However, more recent, higher precision work, has  
27 indicated either a null relationship (foraminifera) or negative relationship (coccolithophores)  
28 between  $\delta^{88/86}\text{Sr}$  and ambient seawater temperature, suggesting growth rate controls the  
29 uptake of Sr isotopes into biogenic carbonates (Böhm et al., 2012; Stevenson et al., 2014).  
30 Although stable strontium applications are in their infancy, the potential for vital effects  
31 (growth rate) fractionating  $\delta^{88/86}\text{Sr}$  indicates it could be useful for assessing vital effects  
32 during calcification in bivalve archives.

#### 1 **1.4 The Biogenic Archive *Arctica islandica***

2 The long-lived bivalve mollusk *A. islandica*, common in the shelf seas of the temperate to  
3 sub-polar North Atlantic Ocean, is an excellent high-resolution marine archive with a huge  
4 potential for monitoring pH as well as other seawater properties (for a recent review, see  
5 Schöne, 2013). This stationary benthic clam lives in water depths ranging from ~10 m to as  
6 deep as 500 m and thrives in full marine conditions, yet can also tolerate salinities as low as  
7 28 PSU for short time intervals (Merrill and Ropes, 1968; Nicol, 1951). *Arctica islandica*  
8 lives within the sediment and extends its relatively short siphons into the main water column,  
9 exchanging water to feed and remove waste. Weidman (1995) demonstrated that the  
10 geochemical signature in the shell material reflects that of the ambient water conditions and  
11 not pore water. *Arctica islandica* is highly suitable for environmental and ocean studies  
12 because: (1) *A. islandica* is extremely long-lived - up to 5 centuries (Butler et al., 2013;  
13 Schöne et al., 2005; Wanamaker Jr. et al., 2008a), (2) it produces annual growth increments in  
14 its shell (Jones, 1980), (3) regional increment series can be crossdated, demonstrating a  
15 common response to environmental forcing(s) (Schöne et al., 2003), (4) fossil shells can be  
16 crossdated and floating shell chronologies can be constructed after radiocarbon dating  
17 (Scourse et al., 2006), (5) live-caught shells can be crossdated with fossil shells to assemble  
18 very long, absolutely dated growth records (Butler et al., 2009; Butler et al., 2011; Marchitto  
19 et al., 2000), (6) master shell chronologies can be created that are as statistically robust as tree  
20 ring chronologies (Butler et al., 2010), (7) it precipitates its aragonitic shell in oxygen isotope  
21 equilibrium with ambient seawater (Weidman et al., 1994), and (8) the geochemical signature  
22 (e.g.,  $^{14}\text{C}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ ) from shell material has been used to reconstruct ocean circulation,  
23 hydrographic changes, seasonal changes in ocean conditions, and ecosystem dynamics (Butler  
24 et al., 2009; Schöne et al., 2005; Schöne et al., 2011a; Scourse et al., 2012; Wanamaker Jr. et  
25 al., 2012; Wanamaker Jr. et al., 2008a; Wanamaker Jr. et al., 2011; Wanamaker Jr. et al.,  
26 2009; Wanamaker Jr. et al., 2008b; Weidman and Jones, 1993; Weidman et al., 1994;  
27 Witbaard and Bergman, 2003). Despite their effectiveness as environmental proxies,  
28 applications of non-traditional isotopic systems ( $\delta^{11}\text{B}$  and  $\delta^{88/86}\text{Sr}$ ) that have the potential to  
29 reveal additional environmental factors have not been explored.

30

## 1 **2 Methods**

### 2 **2.1 Sample preparation**

3 Living shells from the Gulf of Maine were collected from Jonesport, Maine, USA on 21  
4 November 2009 with a commercial quahog-fishing vessel, F. V. Three of A Kind. The live-  
5 caught animals were then transported to the Darling Marine Center (University of Maine) in  
6 Walpole, Maine, USA for the culture experiment. Seawater was pumped from the  
7 Damariscotta River estuary from ~10 m and delivered to the flowing seawater laboratories.  
8 The shells were reared in flow-through seawater tanks without filtration, in which the  
9 temperature, salinity and seawater pH were monitored *in-situ* concurrently and continuously  
10 (Fig. 1 (d)). The pH of the culture seawater was also measured a total of seven times with a  
11 highly accurate Metrohm handheld pH meter ( $\pm 0.003$  units). Additional details of the culture  
12 conditions are given in Beirne et al. (2012). Tank seawater was sampled biweekly throughout  
13 the culture period and filtered through a 0.45  $\mu\text{m}$  filter. Two additional samples, one from the  
14 Gulf of Maine surface seawater and one from auxiliary water flow pumped into the culture  
15 tanks, were also sampled to evaluate if the culture experiment was representative of the  
16 natural marine environment. Boron isotopic data from seawater samples during the  
17 experiment were previously measured and published by Liu et al. (2013).

18 Shell subsamples were collected at Iowa State University via a Dremel hand drill, with 10  
19 intervals throughout the 8-month culturing (Fig. 1 (c)). Based on the calcein staining (see  
20 Beirne et al., 2012 for details) and natural marking on the external shell, the timing of the  
21 winter (January to March), spring (March to May), and summer (May to August) growing  
22 seasons were evident (Fig. 1 (a) – (c)). These markings were used to establish growth rates  
23 during each season as well as to provide temporal controls on the sampled shell material. The  
24 instrumental data and shell growth-rates have been published by Beirne et al. (2012) and the  
25 average seawater salinity, temperature, and pH for shell record comparison are summarized in  
26 Table 1.

27 The boron and strontium concentrations in *A. islandica* shells are about 10 ppm and 1000  
28 ppm, respectively (Zhang, 2009); 1 mg of shell material, after cleaning (details noted below),  
29 is required for B (Liu et al., 2013) and Sr (Stevenson et al., 2014) isotopic measurements.  
30 Because shell growth rates differ throughout the year, and throughout ontogeny (Beirne et al.,

1 2012), we have one subsample from January to March, but we have four subsamples from  
2 March to May and five subsamples from May to August.

3 The subsample cleaning procedure was modified from Barker et al. (2003). In summary: coral  
4 and shell powders were first cleaned with Super-Q (SQ) water (Millipore, > 18.2 MΩ) in an  
5 ultrasonic bath three times and the suspension solution was extracted after centrifuging.  
6 Samples were then treated with 10 % H<sub>2</sub>O<sub>2</sub> at room temperature overnight to remove organic  
7 particles embedded in skeletons. The samples were rinsed with SQ water, 0.001 N HNO<sub>3</sub> and  
8 SQ water again. After drying at 60 °C, samples were weighed and then dissolved in ~1.7 N  
9 HCl with a boron concentration equal to about 750 ppb.

10 Seawater samples were diluted to [B] = ~750 ppb in 1.7 N HCl for boron measurements.  
11 Because the strontium concentration in seawater is about 9 ppm, the residual after sublimation  
12 (see below) is not enough for strontium isotopes measurements. Therefore for strontium  
13 isotope analysis of seawater, an additional 100 μL of seawater sample was dried and  
14 redissolved in concentrated HNO<sub>3</sub> three times and brought into solution in 7 N HNO<sub>3</sub> for  
15 column chemistry. Spiked samples were mixed to have a sample to spike ratio of 1:1, with at  
16 least 600 ng of sample Sr in solution prior to preconditioning steps.

17 Separation of the boron and strontium elemental fractions was achieved using a combination  
18 of micro-sublimation (Liu et al., 2013) and elemental specific ion exchange resin. Briefly, <  
19 50 μL of sample solution was loaded in the cap of a conic-bottom vials in an up-side-down  
20 position and put into the high-throughput system. After 12 hours of sublimation at 70-74 °C,  
21 the purified boron sample solution is condensed and collected on the conic tip. To further  
22 improve the reproducibility for carbonate samples, an extra 2 μL of 30 % H<sub>2</sub>O<sub>2</sub> was added to  
23 the purified solutions (~ 90 % of the volume before sublimation) for all the runs after 23 April  
24 2014. Sample solutions were then set stagnant in the vial, and the cap of the conic vial was  
25 then loosened for two hours to reduce the organic levels and liberate the product oxygen gas.  
26 The micro-sublimation method only extracts boron from the sample solution, therefore the  
27 residual on the cap of the vials is reserved for Sr separation and analysis.

28 All measurements were conducted on a Thermo Fisher Triton PLUS multicollector thermal  
29 ionization mass spectrometer operating in positive ion mode for strontium isotope analysis  
30 and negative ion mode for boron isotope analysis at the Glaciochemistry and Isotope  
31 Geochemistry Lab (GIGL) at the Department of Earth and Environmental Sciences,  
32 University of Michigan.

## 1 **2.2 Radiogenic and Stable Strontium Isotope analysis**

### 2 **2.2.1 General**

3 The residuals of carbonate samples after sublimation were redissolved in concentrated HNO<sub>3</sub>  
4 to [Sr] = ~10 ppm, which is approximately the same Sr concentration in seawater. A small  
5 aliquot of sample was spiked with our <sup>84</sup>Sr-<sup>87</sup>Sr double spike solution. Both unspiked normal  
6 sample and spiked mixture sample solutions were then dried down three times, then dissolved  
7 in 7 N HNO<sub>3</sub> for Sr column chemistry. In order to separate strontium element from matrix,  
8 samples were passed through a 50-100 μm Sr-spec resin (Eichrom), and 0.035 N HNO<sub>3</sub> was  
9 used to elute Sr after using 7 N HNO<sub>3</sub> to elute the others. The eluted Sr aliquots were refluxed  
10 with 30 % H<sub>2</sub>O<sub>2</sub> overnight, dried, and finally dissolved in concentrated HNO<sub>3</sub> for loading  
11 (Liu, 2010).

12 For radiogenic isotopic measurements, 100-200 ng of Sr sample was loaded onto outgassed  
13 Re filaments with TaF<sub>5</sub> activator solution. Each sample was heated to an intensity of ~8 V  
14 <sup>88</sup>Sr. Four hundred cycles of data were collected for each measurement to determine the Sr  
15 isotopic ratios with within run precision better than 10 ppm (2 SE). Mass 84 to 86 were  
16 detected with five Faraday cups position from L1 to H3, respectively, with <sup>85</sup>Rb measured in  
17 the center cup. The long-term reproducibility of <sup>87</sup>Sr/<sup>86</sup>Sr for SRM987 Sr standard was  
18 0.710268 ± 21 (2σ, n = 140) from the time of Triton installation in January 2012 to April  
19 2014. In May 2014, problems associated with the H3 Faraday cup resulted in change to a <sup>86</sup>Sr-  
20 centered cup configuration (June to October 2014). After H3 cup replacement, the <sup>85</sup>Rb-  
21 centered cup configuration was established again. The new SRM987 <sup>87</sup>Sr/<sup>86</sup>Sr average value  
22 is 0.719246 ± 13 (2σ, n = 42) for data collected after June 2014 (for both cup configurations)  
23 and sample data were normalized based on this new SRM987 standard ratio. The reported  
24 <sup>87</sup>Sr/<sup>86</sup>Sr data in this study were all normalized to SRM987 = 0.710250 for inter-laboratory  
25 comparisons.

### 26 **2.2.2 Double spike (84-87) Sr**

27 High precision <sup>88</sup>Sr/<sup>86</sup>Sr isotopic compositions are measured by double spike (DS);  
28 measurements by DS removes instrumental mass bias associated with thermal ionization  
29 during sample runs. The double spike method was first developed in the 1960s: Dodson  
30 (1963) outlined a methodology for determining the unknown mass discrimination factor  
31 directly if the sample is mixed with a double spike, consisting of an enriched mixture of two

1 stable isotopes. Later, Krogh (1964) worked out a graphical method for a spike enriched in  
 2 both  $^{84}\text{Sr}$  and  $^{86}\text{Sr}$ . Finally, Long (1966) showed that the correction factor for mass  
 3 discrimination can be calculated by using three elements, and it depends on the fractional  
 4 abundances in the normal and spike elements. A simpler expression was then published by  
 5 Boelrijk (1968). These pioneering studies founded the basis of the Sr double spike method,  
 6 and Sr double spikes have already been successfully used to determine the Sr isotopic  
 7 composition of the early solar system (Patchett, 1980b; 1980a). Optimal spike compositions  
 8 are determined using a 3D data reduction method (Galer, 1999). The choice of isotope ratios  
 9 used in the equations, the mathematical formulation to solve for the mass discrimination  
 10 factor  $\epsilon$  and the influence of the spike-to-sample ratio in the mixture should be taken into  
 11 consideration. With the addition of “tuning” with IAPSO seawater standards (Krabbenhöft et  
 12 al., 2009), this double spike method could produce more precise true isotopic compositions in  
 13 an unknown sample solution.

14 A  $^{84}\text{Sr}$ - $^{87}\text{Sr}$  double spike solution was prepared at GIGL at the University of Michigan  
 15 followed the method from Liu (2010). The optimal value of the spike depends on the angle of  
 16 two planes, defined by normal sample (N) fractionation line and spike point and mixture (M)  
 17 fractionation line and spike point, respectively (Fig. 2), expressed as  $\theta$  here. Because all the  
 18 measured points have their own errors, when the angle between these two planes approximate  
 19 a right angle, the intersected area reaches a minimum and thus that the N-M-S line will be  
 20 defined precisely (Fig. 2 (b)). Therefore, by checking different portions of spike mixing with  
 21 normal (Qs) mathematically, the distribution of  $\theta$  to spike composition can be derived (Fig. 2  
 22 (c)). For our  $^{84}\text{Sr}$ - $^{87}\text{Sr}$  double spike with an  $^{84}\text{Sr}/^{87}\text{Sr}$  ratio of 0.8679, the optimal Qs is about  
 23 0.5, which makes the two planes perpendicular to each other. Within a range of  $Q_s = 0.45$ -  
 24 0.55, the  $\theta$  is still in a range of  $\pm 2$  degree, which supports a tolerance of spiking samples with  
 25 a slight deviation from a 1:1 sample to spike ratio. The stable Sr data were reported as  $\delta^{88/86}\text{Sr}$ ,  
 26 which was defined as:

$$27 \quad \delta^{88/86}\text{Sr} = \left[ \frac{(^{88}\text{Sr}/^{86}\text{Sr})_{\text{sample}}}{(^{88}\text{Sr}/^{86}\text{Sr})_{\text{SRM 987}}} - 1 \right] \times 1000 (\text{‰}) \quad (5)$$

28 In this study, a Python script was applied to evaluate true normal values of the shells. We  
 29 assumed the measured isotopic ratios of normal sample and spiked mixture would follow  
 30 exponential law, and the equations of each  $^{87}\text{Sr}$ -based isotopic ratio for both normal and

1 mixture sample could be stated. After inputting the known isotopic composition of the spike,  
2 the true value of each isotope concentration can be solved using a least squares minimization  
3 of the residual of the non-linear equations.

4 For the spiked sample solution, 200-250 ng of Sr samples was loaded onto outgassed Re  
5 filaments with TaF activator solution and run the same as the unspiked samples described in  
6 the previous section. The deconvolved  $\delta^{88/86}\text{Sr}$  values for seawater standard IAPSO 141 and  
7 an inter-laboratory coral standard JCp-1 are  $0.390 \pm 16 \text{ ‰}$  ( $2\sigma$ ,  $n = 4$ ) and  $0.144 \pm 26 \text{ ‰}$  ( $2\sigma$ ,  
8  $n = 3$ ), respectively. According to Krabbenhöft et al. (2009), using the IAPSO seawater  
9 standard to fine-tune the Sr double spike composition provides an optimal  $\delta^{88/86}\text{Sr}$  result. Here  
10 we used the same technique and obtained a compatible  $\delta^{88/86}\text{Sr}$  value for the IAPSO seawater  
11 standard to the reported values from Krabbenhöft et al. (2009). However, due to the H3 cup  
12 deficiency, the deconvolved  $\delta^{88/86}\text{Sr}$  value of inter-laboratory carbonate standard JCp-1 is  
13 about 0.05 ‰ lighter than the reported values between April 2014 and June 2014. This offset  
14 was fixed, with the alternate cup configuration and replacement of H3 cup, after June 2014  
15 and new values of IAPSO =  $0.365 \pm 73 \text{ ‰}$  ( $2\sigma$ ,  $n = 4$ ) and JCp-1 =  $0.195 \pm 21 \text{ ‰}$  ( $2\sigma$ ,  $n = 4$ )  
16 (Fig. 3) were obtained.

### 17 **2.3 Boron Isotope Analysis**

18 The procedure used for obtaining B isotopic compositions by total evaporation (TE) is  
19 described in Liu et al. (2013). In summary: one  $\mu\text{L}$  of boron free synthetic seawater matrix  
20 was loaded onto outgassed single Re filament at 0.8 A current, followed by 1  $\mu\text{L}$  of sample  
21 solution with 30 seconds of waiting between the two steps. Samples were then dried down at  
22 2 A current for 10 seconds and then the filaments were flashed to a dull red color in the center  
23 of the filament (about 2.5 A) and ready for analysis. Data collection was initiated when the  
24 intensity of mass 42 reached 20 mV, and terminated after the signal dropped lower than the  
25 initial 20 mV.

26 The long-term reproducibility (18 months) of  $^{11}\text{B}/^{10}\text{B}$  for boric acid standard SRM 951a is  
27  $4.0332 \pm 0.0064$  ( $2\sigma$ ,  $n = 97$ ) before treated with 30 %  $\text{H}_2\text{O}_2$  and is  $4.0316 \pm 0.0084$  ( $2\sigma$ ,  $n =$   
28 19) after the extra treatment. The precisions of  $\delta^{11}\text{B}$  for seawater and biogenic carbonate  
29 standards without addition of peroxide are  $40.46 \pm 1.29 \text{ ‰}$  ( $2\sigma$ ,  $n = 54$ ) and  $24.94 \pm 2.35 \text{ ‰}$   
30 ( $2\sigma$ ,  $n = 39$ ) for IAEA B-1 and JCp-1, respectively;  $41.70 \pm 1.13 \text{ ‰}$  ( $2\sigma$ ,  $n = 8$ ) and  $24.93 \pm$   
31  $1.83 \text{ ‰}$  ( $2\sigma$ ,  $n = 18$ ) for IAEA B-1 and JCp-1, respectively, with  $\text{H}_2\text{O}_2$  treatment (Fig. 4).

1

## 2 **3 Results**

3 The *in-situ* seawater salinity, temperature and pH results are summarized in Table 1. To  
4 compare the instrumental data to the shell records, the instrumental results were averaged  
5 with respect to the subsampling intervals. The seawater and shell results are summarized in  
6 Table 2 and Table 3, respectively

### 7 **3.1 $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{88/86}\text{Sr}$**

#### 8 **3.1.1 $^{87}\text{Sr}/^{86}\text{Sr}$**

9 The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of seawater range from 0.709177 to 0.709192, with an average of  
10  $0.709185 \pm 8$  ( $2\sigma$ ,  $n = 13$ ). There is no distinguishable difference between samples from  
11 offshore Gulf of Maine seawater, auxiliary flow and tank waters (Fig. 5 (a)). For shell  
12 carbonate, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios range from 0.709163 to 0.709210, with an average of  $0.709183$   
13  $\pm 23$  ( $2\sigma$ ,  $n = 27$ ). Both of the seawater and shell  $^{87}\text{Sr}/^{86}\text{Sr}$  are identical to the mean seawater  
14 values (Fig. 5 (a) and (b)). Because all the radiogenic Sr results are identical within error,  
15 there is no relationship with either seawater salinity, temperature or pH.

#### 16 **3.1.2 $\delta^{88/86}\text{Sr}$**

17 Two sets of shell samples, A103JV and A105JV, were spiked for stable Sr measurements.  
18 However, due to the defect of H3 cup, the A103JV double spike results are underestimated  
19 and are not listed. The deconvolved  $\delta^{88/86}\text{Sr}$  for A105JV and Sr concentrations values for both  
20 of A103JV and A105JV from high-resolution shell records are shown in Fig. 5 (c) and (d),  
21 respectively. The  $\delta^{88/86}\text{Sr}$  values ranges from 0.215 to 0.296 ‰ with an average of  $0.248 \pm 48$   
22 ‰ ( $2\sigma$ ,  $n = 10$ ) and the concentration ranges from 1200-1800 ppm. Considering analytical  
23 uncertainty, no distinctive differences or trend for either Sr concentration or stable Sr isotopic  
24 compositions in the shells are observed throughout the culture season. Similarly, no  
25 correlation can be found between stable Sr or Sr concentration to any measured ambient  
26 seawater conditions.

## 3.2 Boron isotopic composition ( $\delta^{11}\text{B}$ ) in ambient seawater the shell and aragonite shell

Boron isotopic compositions of 11 tank water samples are from 37.51 ‰ to 47.83 ‰, and the average for 36 sample runs is  $39.20 \pm 1.73$  ‰. The  $\delta^{11}\text{B}$  values for additional seawater samples from the offshore Gulf of Maine and the auxiliary flow to the culture tanks are 36.93 ‰ and 40.38 ‰, respectively (Fig. 6 (a)) (Liu et al., 2013). Similar to what has been observed from radiogenic Sr data in seawater, the boron isotopic composition of our culture seawater is invariant and identical to the open ocean composition reported by Foster et al. (2010).

High-resolution boron isotopic composition records from 4 juvenile shells (A101JV, A102JV, A103JV and A105JV) show nearly identical patterns and trends throughout the experiment. The shell  $\delta^{11}\text{B}$  values range from 11.09 to 18.81 ‰ before week 19 and from 17.41 to 25.12 ‰ after week 19 (Fig. 6 (b)). Compared to seawater temperature and average shell growth rates in three growth seasons, we found a distinct rise in  $\delta^{11}\text{B}$  for temperatures over  $\sim 13$  °C. However, this rapid change in  $\delta^{11}\text{B}$  did not correlate to the rapid change in shell growth during the culture period.

In order to evaluate the pH dependency of the  $\delta^{11}\text{B}$ , Eq. (4) is used. Based on *in-situ* temperature, salinity and pH measurements throughout the culture experiment, we calculated the predicted range in  $\delta^{11}\text{B}$  of the *A. islandica* shell with  $\alpha = 1.0272$ , which was empirically obtained from Klochko et al. (2006) and is considered to better describe the distribution of the two boron species in natural system (Foster, 2008; Rollion-Bard et al., 2011b; Rollion-Bard and Erez, 2010) (Fig. 6 (b)). Another prediction line calculated based on independently derived fractionation factor from (Nir et al., 2015) is also shown for reference (Fig. 6 (b)). The predictions suggest a slight increase in  $\delta^{11}\text{B}$  throughout the culture season, primarily due to more than a 15 °C temperature increase. A 0.2 pH unit drop, observed between week 24 and 26, should have decreased the  $\delta^{11}\text{B}$  value by about 2 ‰ even with the large temperature change. Most of the shell boron isotopic composition data followed the two prediction lines, before week 19, with some of the data points lower than the predictions. After week 19, the  $\delta^{11}\text{B}$  deviate significantly and trend toward higher and higher  $\delta^{11}\text{B}$  compositions. Duplications on different individual shells all suggest the same trend.

We also calculated shell pH ( $\text{pH}_{\text{shell}}$ ) with  $\alpha = 1.0272$  based on average tank water  $\delta^{11}\text{B}$ , shell  $\delta^{11}\text{B}$  and the corresponding average seawater temperature and salinity values. The results

1 show a significant negative relationship between  $\Delta\text{pH}$  and  $\text{pH}_{\text{sw}}$  ( $R^2 = 0.35$ ;  $p\text{-value} \leq 0.001$ )  
2 (Fig. 7).

3

## 4 **4 Discussion**

### 5 **4.1 Radiogenic Sr isotope incorporation into *A. islandica***

6 In this study, we measured the radiogenic Sr isotope ratios in seawater to estimate the source  
7 water contributions to the culture site, which is situated within the Damariscotta River  
8 estuary. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in cultured seawater showed identical values to the open surface  
9 seawater ratio. Bedrock types in the Gulf of Maine coastal region are dominated by late  
10 Proterozoic and lower Paleozoic sedimentary rocks (Osberg et al., 1985), which would  
11 provide a terrestrial source with high  $^{87}\text{Sr}/^{86}\text{Sr}$  values. Considering rock type, age and  
12 freshwater flux, a recent model of  $^{87}\text{Sr}/^{86}\text{Sr}$  for flux-weighted catchment water suggests the  
13  $^{87}\text{Sr}/^{86}\text{Sr}$  value to be in a range of 0.7099 to 0.7145 (Bataille and Bowen, 2012). If river fluxes  
14 influence the Sr isotopic composition of coastal seawater, the value should be enriched in  
15  $^{87}\text{Sr}$ , driving the  $^{87}\text{Sr}/^{86}\text{Sr}$  higher than the current seawater ratio. Therefore the Sr isotopic  
16 results suggest a negligible amount of fresh water input into the culture water at the Darling  
17 Marine Center.

18 The mean  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in the shell is consistent with the isotopic composition in the culture  
19 seawater, but with a relatively larger variation between individual shell samples. Therefore,  
20 incorporation of radiogenic Sr ratios into the shells occurs without measurable fractionation  
21 and reflects the composition of ambient seawater. Although the shell  $^{87}\text{Sr}/^{86}\text{Sr}$  values have a  
22 larger standard deviation compared to the seawater values, they are within the range of the  
23 long-term precision of Triton plus at the Department of Earth and Environmental Sciences,  
24 University of Michigan (see Sect. 2.2.1). The high content of calcium in carbonate samples,  
25 which cannot be fully separated using Sr-specific ion exchange column chemistry, may  
26 contribute to the larger variation of shell  $^{87}\text{Sr}/^{86}\text{Sr}$  compared to seawater.

### 27 **4.2 Stable Sr isotope incorporation into *A. islandica*, and Sr concentrations**

28 In this study we observed no statistically significant correlation of  $\delta^{88/86}\text{Sr}$  or Sr  
29 concentrations with respect to seawater temperature. The stable Sr isotopic composition of

1 some biogenic carbonates has been suggested to reflect ambient seawater temperature due to  
2 mass dependent kinetic fractionation, in which the relative mass difference of the isotopes  
3 involved accounts for the inverse correlation to the ion mass in a kinetic fractionation process  
4 (Fietzke and Eisenhauer, 2006; Rüggeberg et al., 2008). However, more recent work has  
5 shown no relationship between seawater temperature and  $\delta^{88/86}\text{Sr}$  values from various  
6 biogenic archives (Böhm et al., 2012; Raddatz et al., 2013; Stevenson et al., 2014; Vollstaedt  
7 et al., 2014). Our results support the argument that a simple temperature dependent kinetic  
8 effect is not the primary control on  $\delta^{88/86}\text{Sr}$  in the aragonitic shell of *A. islandica*. The  
9 temperature range in the experiment is over 15 °C (2.4 – 17.6 °C) and growth rate more than  
10 doubles (0.24 – 0.68 mm/week) during the experiment, which could result in over 1.5 ‰  
11 change in  $\delta^{88/86}\text{Sr}$  if *A. islandica* behaved similarly to coccolithophores (Stevenson et al.,  
12 2014). The lack of a consistent relationship between  $\delta^{88/86}\text{Sr}$  with temperature or shell growth  
13 rate during the experiment indicates that  $\delta^{88/86}\text{Sr}$  is not controlled by temperature or growth  
14 rates. Thus it is likely that  $\delta^{88/86}\text{Sr}$  records derived from *A. islandica* shells reflect ambient  
15 seawater conditions, and could be a potential archive for studying the global Sr cycle in the  
16 context of chemical weathering (Krabbenhöft et al., 2010; Raddatz et al., 2013; Vollstaedt et  
17 al., 2014). More work is needed to fully evaluate this potential proxy.

18 Our deconvolved shell Sr concentrations show no resolvable relationship with the seawater  
19 temperature, despite a possible physiological control on Sr uptake into bivalve shell material.  
20 In general, co-precipitation of Sr to Ca in aragonite decreases with temperature increases due  
21 to a declining distribution coefficient, which has been both measured and derived  
22 theoretically (Dietzel et al., 2004). The negative correlation between skeletal Sr/Ca ratios  
23 observed in some massive corals with ambient seawater temperature has been widely  
24 established and applied to reconstruct paleo seawater temperature (Beck et al., 1992; Corrège,  
25 2006; de Villiers, 1999; McCulloch et al., 1994; McCulloch et al., 1996; Shen et al., 1996;  
26 Weber, 1973; Yan et al., 2013; Yu et al., 2005). However, these relationships may be biased  
27 by the influence from symbionts causing a vital effect (Böhm et al., 2012; Cohen et al., 2006;  
28 Cohen et al., 2002; Purton et al., 1999; Stevenson et al., 2014). Schöne et al. (2011b) observed  
29 a strong physiological regulation to Sr/Ca and Mg/Ca on ontogenically old adult *A. islandica*  
30 records. The metal to calcium ratio (Me/Ca) increased with shell age when the annual  
31 increment widths were below 30 to 200  $\mu\text{m}$ . However, Schöne et al. (2013) concluded that the  
32 faster-growing juvenile portion of the shells showed a weak relationship between Me/Ca and  
33 ambient temperature and results from different specimens were variable. Schöne et al. (2013)

1 proposed that pronounced vital effects may control the trace metal uptake in juvenile shells.  
2 Our results are also consistent with the study of Schöne et al. (2011b), where the juvenile  
3 portion of the two *A. islandica* shells with annual growth increments larger than 750  $\mu\text{m}$ ,  
4 showed no clear relationship between Me/Ca ratios and growth rates.

### 5 **4.3 Controls on $\delta^{11}\text{B}$ in *A. islandica* and an evaluation of the proxy archive as** 6 **a seawater pH indicator**

7 The range in measured shell  $\delta^{11}\text{B}$  values lies between the prediction lines (Fig. 6 (b)), which  
8 suggest that the shell boron content generally reflects the ambient seawater conditions.  
9 However, our data do not consistently follow either prediction line. Previous studies on  
10 foraminifera have shown offsets between different genera and the empirical  $\delta^{11}\text{B}$ -pH  
11 relationship of  $\alpha = 1.0194$  (Hönisch and Hemming, 2004). The inconsistency between shell  
12  $\delta^{11}\text{B}$  and either prediction is therefore strong evidence that a species-specific fractionation  
13 factor is required for bivalves.

14 The offsets between our shell data with the predicted trends (Fig. 6 (b)) are likely from vital  
15 effects during biomineralization. Previous studies suggested a range of fractionation factors  
16 might be applied, and an additional constant offset might better describe the empirical  $\delta^{11}\text{B}$ -  
17 pH relationship (Anagnostou et al., 2012; Hönisch et al., 2004; Rae et al., 2011). Therefore a  
18 species-specific offset may account for the smaller variations before week 19, where many of  
19 the results lie under the prediction lines and the negative  $\Delta\text{pH}$ . In this study, because the  
20 temperature and salinity are variable, we cannot determine a precise transfer function for *A.*  
21 *islandica*. However, the total variation throughout the experiment is about 10 ‰, and has an  
22 obvious trend after week 19, suggesting other controls on boron incorporation in the shell.

23 Rollion-Bard and Erez (2010) and Trotter et al. (2011) evaluated vital effects in corals and  
24 foraminifera, and the potential use of the  $\delta^{11}\text{B}$ -pH relationship in such biogenic carbonates.  
25 They observed a pH offset between calcifying fluid and ambient seawater, and this pH  
26 discrepancy ( $\Delta\text{pH}$ ) increases with decreasing ambient seawater pH ( $\text{pH}_{\text{sw}}$ ). As the  
27 environment becomes more acidic, marine calcifiers likely adjust their internal  
28 microenvironment during calcification, resulting in larger  $\Delta\text{pH}$  values than expected. Under  
29 careful culture conditions, species-specific  $\Delta\text{pH}$ - $\text{pH}_{\text{sw}}$  relationships can be developed, and  
30 after calibration, the corresponding ambient seawater pH can be determined.

1 It has been argued that bivalves have the ability to regulate their inner shell fluid chemistry,  
2 more specifically the extrapallial fluid (EPF), to achieve a carbonate saturation state in order  
3 to facilitate biomineralization (Crenshaw, 1980). Stemmer (2013) observed pH gradients  
4 between inner shell surface and outer mantle epithelium (OME) of *A. islandica* via *in-situ* pH  
5 microscopy method. During a short-term monitoring, Stemmer (2013) also observed the  
6 measured pH rose rapidly as the probe approached the OME. They concluded this elevation  
7 was due to active proton uptake by the epithelium. This result suggests a pH self-regulation  
8 occurs for *A. islandica* shell precipitation, and the shell will record the regulated calcification  
9 pH in the carbonate shell.

10 The calculated  $\Delta\text{pH}$  shows a statistically significant negative relationship to  $\text{pH}_{\text{sw}}$  ( $R^2 = 0.35$ ;  
11  $\text{p-value} \leq 0.001$ ) (Fig. 7). The negative correlation supports the argument that *A. islandica*  
12 regulate their EPF pH for calcification. However, only 35 % of the variability can be  
13 explained by  $\Delta\text{pH}$ , which indicates that the pH regulation in the EPF is likely not the  
14 prevailing factor. Instead, we found a rapid increase of the shell  $\delta^{11}\text{B}$  when temperature  
15 increased over 13 °C (Fig. 8 (a)). This rapid change in boron isotopic composition can be  
16 explained with respect to two factors: (1) a growth rate controlled vital effect, or (2) a  
17 temperature controlled vital effect.

18 Herfort et al. (2008) suggested that carbonate species are the limiting factor in coral calcium  
19 precipitation rather than calcium: when ambient seawater temperature increases,  $[\text{CO}_2]_{\text{aq}}$   
20 decreases, and leads to rising  $[\text{CO}_3^{2-}]$  as well as calcification rate. The rapid growth rate  
21 change is likely related to spring bloom. Our data shows no correlation between boron  
22 isotopic composition in the shell and the shell growth, indicating shell growth is not the  
23 primary control to the boron deviation in the culture experiment (Fig. 8b). Therefore,  
24 combined correlation with temperature and lack of correlation with growth to the shell  $\delta^{11}\text{B}$   
25 cannot be explained by a temperature controlled growth/precipitation effect.

26 Alternatively, a proton removal mechanism via  $\text{Ca}^{+2}$ -ATpase from the site of calcification has  
27 been proposed. This mechanism raises the pH of the calcification solution (Dissard et al.,  
28 2012; Rollion-Bard et al., 2011b). In this scenario, the activity of the enzyme is enhanced  
29 when a certain temperature has been reached, accelerating the proton removal process and  
30 resulting in a higher boron isotopic composition in the calcification solution with respect to  
31 the elevated pH. We suggest that there may be a temperature threshold of the boron  
32 incorporation into the aragonite *A. islandica*. This proposed threshold may be related to the

1 upper-end thermal tolerance of *A. islandica*. For example, below 13 °C, the  $\delta^{11}\text{B}$  values  
2 closely matched the predicted model of Klochko et al. (2006), supporting the assumption that  
3 borate is the dominate species incorporated into the shell and so can reflect the ambient  
4 seawater pH. At temperatures above 13 °C the utility of the  $\delta^{11}\text{B}$  values as a pH indicator is  
5 questionable and likely unreliable. We suggest that the thermal tolerance of *A. islandica* was  
6 exceeded in the summer growing season in the culture conditions, causing biological stress on  
7 the animals.

8

## 9 **5 Conclusion**

10 Here we examined boron and strontium isotopic compositions recorded in the shell material  
11 of cultured aragonitic *A. islandica* with *in-situ* seawater temperature, salinity and pH  
12 measurements. Both seawater and shell  $^{87}\text{Sr}/^{86}\text{Sr}$  show identical values to the mean global  
13 seawater composition, suggesting there is trivial influence from local continental runoff. Shell  
14  $\delta^{88/86}\text{Sr}$  and Sr concentration values during the culture season were not influenced by seawater  
15 temperature or calcification rates. These results suggest that well-preserved sub-fossil  
16 specimens may be use to determine the past isotopic composition ( $^{87}\text{Sr}/^{86}\text{Sr}$  and  $\delta^{88/86}\text{Sr}$ ) of  
17 seawater.

18 The boron isotope results from the cultured aragonite *A. islandica* shells generally are within  
19 the range of two prediction lines utilizing previously published fractionation factors. Although  
20 to first order, these results indicate that the shell  $\delta^{11}\text{B}$  values reflect ambient conditions,  
21 substantial variability not related to pH changes was noted. The 5 to 8 ‰ increase in shell  
22  $\delta^{11}\text{B}$  values is larger than theoretical predictions based on *in-situ* seawater temperature,  
23 salinity, pH and conventional boron fractionation factors for corals and foraminifera. A  
24 species-specific  $\delta^{11}\text{B}$ -pH transfer function is recommended for bivalve species because of  
25 their inherent ability to self regulate calcifying fluids. The fairly strong relationship ( $r^2 = 0.35$ )  
26 between  $\Delta\text{pH}$  and  $\text{pH}_{\text{sw}}$  indicates that *A. islandica* does regulate the EPF pH during  
27 calcification, but self-regulation is not the primary control on shell  $\delta^{11}\text{B}$ . The largest increase  
28 in shell  $\delta^{11}\text{B}$  values was observed after crossing an apparent temperature threshold at 13 °C,  
29 suggesting a possible influence from biological processes. To better evaluate the potential of  
30  $\delta^{11}\text{B}$  as a seawater pH indicator, a pH controlled culture experiment with limited seawater  
31 temperature and salinity variation is needed.

32

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6

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- 17
- 18

1 Table 1. Summary of *in-situ* instrumental data of tank seawater salinity, temperature, pH  
 2 during culture season

Week number (dates)	Salinity (PSU) <sup>1</sup>	Average to subsampling interval <sup>3</sup>	Temperature (°C) <sup>1</sup>	Average to subsampling interval <sup>3</sup>	pH <sup>1</sup>	Average to subsampling interval <sup>3</sup>	Predicted $\delta^{11}\text{B}$ with $\alpha =$ 1.0260 <sup>2</sup>	Predicted $\delta^{11}\text{B}$ with $\alpha =$ 1.0272 <sup>2</sup>
<b>1</b> (1/17 - 1/23)	31.036		3.029		--		--	--
<b>2</b> (1/24 - 1/30)	30.508		3.030		--		--	--
<b>3</b> (1/31 - 2/6)	30.520		2.375		--		--	--
<b>4</b> (2/7 - 2/13)	31.066	30.608 ± 0.683	2.369	2.960 ± 0.511	8.018	8.033 ±0.019	15.84	14.79
<b>5</b> (2/14 - 2/20)	31.186		2.776		8.037		16.00	14.95
<b>6</b> (2/21 - 2/27)	30.755		3.367		8.059		16.18	15.14
<b>7</b> (2/28 - 3/6)	29.183		3.774		8.019		15.90	14.85
<b>8</b> (3/7 - 3/13)	29.288		4.596		8.029		16.03	14.99
<b>9</b> (3/14 - 3/19 (3/20))	29.743	29.516 ± 0.322	4.800	4.698 ± 0.145	8.049	8.039 ± 0.014	16.20	15.16
<b>10</b> (3/21 - 3/27)	29.751	29.149 ± 0.851	5.646	5.735 ± 0.127	8.094	8.089 ± 0.006	16.59	15.57
<b>11</b> (3/28 - 4/3)	28.547		5.825		8.085		16.49	15.47
<b>12</b> (4/4 - 4/10)	28.011		7.252		8.085		16.60	15.58
<b>13</b> (4/11 (4/14) - 4/17)	29.478	28.745 ± 1.037	7.158	7.205 ± 0.066	8.078	8.082 ± 0.005	16.61	15.59
<b>14</b> (4/18 - 4/24)	29.751	29.827 ± 0.107	7.594	8.246 ±0.922	8.102	8.102 ± 0.000	16.85	15.84

<b>15</b> (4/25 - 5/1)	29.902		8.898		8.102		16.99	15.99
<b>16</b> (5/2 - 5/8)	30.335		9.662		8.095		17.03	16.03
<b>17</b> (5/9 - 5/15)	30.762	30.702 ± 0.341	9.364	9.784 ± 0.494	8.150	8.128 ± 0.029	17.48	16.50
<b>18</b> (5/16 - 5/22)	31.008		10.328		8.139		17.51	16.53
<b>19</b> (5/23-5/29)	31.102		12.653		8.118		17.59	16.61
<b>20</b> (5/30-6/5)	31.227	31.099 ± 0.129	13.385	13.170 ± 0.450	8.120	8.105 ± 0.025	17.69	16.72
<b>21</b> (6/6-6/12)	30.969		13.472		8.076		17.30	16.31
<b>22</b> (6/13-6/19)	31.067		13.427		8.078		17.32	16.33
<b>23</b> (6/20-6/26)	31.115	30.829 ± 0.454	14.676	14.494 ± 0.989	8.125	8.121 ± 0.041	17.88	16.91
<b>24</b> (6/27-7/3)	30.306		15.380		8.159		18.24	17.30
<b>25</b> (7/4-7/10)	30.508		16.445		8.015		17.07	16.08
<b>26</b> (7/11-7/17)	30.555	30.521 ± 0.030	17.209	17.076 ± 0.577	7.960	7.985 ± 0.028	16.71	15.70
<b>27</b> (7/18-7/24)	30.500		17.576		7.981		16.91	15.90
<b>28</b> (7/25-7/31)	30.305		17.188		7.966		16.75	15.73
<b>29</b> (8/1-8/5)	30.626	30.466 ± 0.227	17.026	17.107 ± 0.115	7.985	7.976 ± 0.013	16.89	15.89

1 <sup>1</sup>. Data from Beirne et al. (2012)

1 <sup>2</sup> Calculation based on Eq. (4), where  $\alpha \equiv \frac{({}^{11}\text{B}/{}^{10}\text{B})_{\text{B(OH)}_3}}{({}^{11}\text{B}/{}^{10}\text{B})_{\text{B(OH)}_4^-}}$  and

2 
$$pK_b = -\log \left( \exp \left( \frac{-8966.90 - 2890.53S^{\frac{1}{2}} - 77.942S + 1.728S^{\frac{3}{2}} - 0.0996S^2}{T} + 148.0248 + 137.1942S^{\frac{1}{2}} + \right. \right.$$

3 
$$\left. \left. 1.62142S - (24.4344 + 25.085S^{\frac{1}{2}} + 0.2474S) \ln T + 0.053105S^{\frac{1}{2}}T \right) \right) \text{ (DOE, 1994).}$$

4 <sup>3</sup> Standard deviation of the data

5

6

1 Table 2. Summary of seawater data

Sample ID	Week	$^{87}\text{Sr}/^{86}\text{Sr}$ (2 SE)	$\delta^{11}\text{B}$ (‰)
Tank A 031710	9	0.709182(4)	38.48
Tank A 041810	14	0.709192(5)	39.41
Tank A 050810	16	0.709183(4)	39.47
Tank A 052210	18	0.709186(4)	38.04
Tank A 081610	31	0.709188(4)	38.10
Tank B 031910	9	0.709185(4)	39.31
			39.97
			38.50
			40.59
			38.51
			40.10
			39.45
			40.33
			39.81
			37.91
Flow A 021410	5	0.709184(4)	39.00
Flow A 070510	25	0.709188(4)	38.65
Flow A 072210	27	0.709187(4)	38.33
			39.36
			38.57
			38.48
			38.40
			39.02

			40.77
			40.78
Flow #2 013010	2	0.709180(4)	37.51
			38.53
			39.01
			39.91
			40.83
			40.01
			39.08
			38.98
			39.78
			39.14
Flow #2 022710	6	0.709189(4)	39.16
Aux.	33	0.709182(5)	36.93
GoM 112309	33	0.709177(4)	40.38

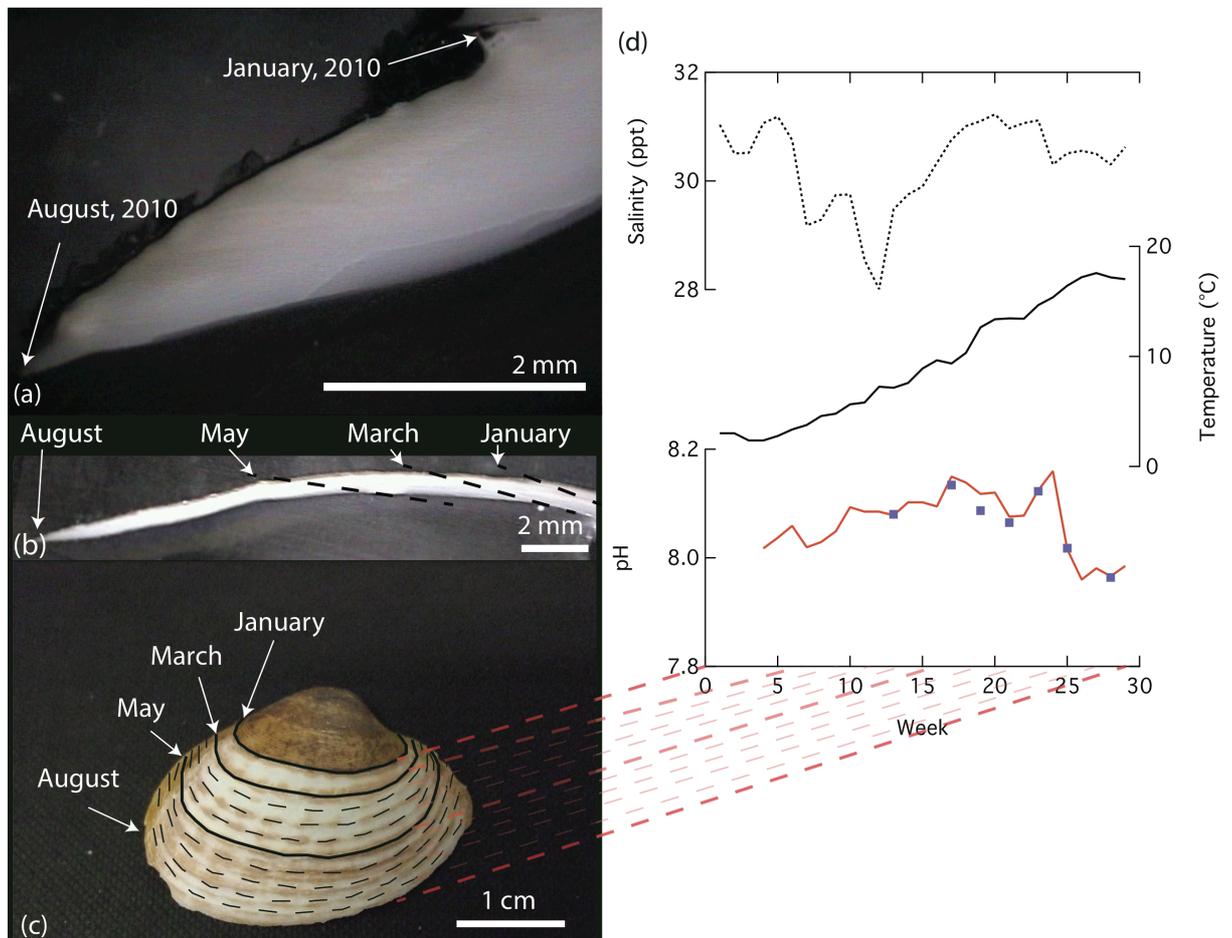
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1 Table 3. Summary of shell data

Sample ID	Week number	$^{87}\text{Sr}/^{86}\text{Sr}$ (2 SE)	$\delta^{88/86}\text{Sr}$ (‰)	[Sr] (ppm)	$\delta^{11}\text{B}$ (‰)	Average growth rate (mm/week) <sup>1</sup>	$\text{pH}_{\text{shell}}^2$	$\Delta\text{pH}^3$	2 STD for calculated $\text{pH}^4$
A101JV-8	22	--	--	--	20.32	--	8.419	0.298	0.22
A101JV-9	24.8	--	--	--	23.34	--	8.595	0.610	0.10
A102JV-1	4	0.709181 (7)	0.132	1403	15.68	0.27	8.139	0.105	0.11
A102JV-2	8.5	0.709210 (10)	0.151	1282	14.94		8.020	-0.019	0.08
A102JV-3	10.5	0.709181 (10)	0.158	1422	14.28		7.898	-0.191	0.42
A102JV-4	12.5	0.709157 (15)	0.112	1452	--	0.53			
A102JV-5	14.5	0.709201 (7)	0.118	1435	13.77		7.756	-0.346	0.15
A102JV-6	16.4	0.709194 (12)	0.124	1319	13.65		7.705	-0.423	0.14
A102JV-7	19.2	0.709169 (13)	0.154	1474	16.80		8.132	0.028	0.10
A102JV-8	22	0.709170 (5)	0.099	1558	18.04	0.65	8.236	0.115	0.13
A102JV-9	24.8	0.709178 (6)	0.112	1491	20.23		8.382	0.397	0.02
A102JV-10	27.6	0.709184 (6)	0.097	--	21.93		8.502	0.527	0.02
A103JV-1	4	--	--	--	16.57	0.24	8.242	0.209	0.25
A103JV-2	8.5	--	--	--	16.57		8.225	0.186	0.25
A103JV-3	10.5	0.709191 (7)	--	--	18.41		8.389	0.300	0.03
A103JV-4	12.5	0.709187 (5)	--	--	16.04	0.51	8.138	0.056	0.17
A103JV-5	14.5	0.709195 (9)	--	--	16.28		8.145	0.043	0.11
A103JV-6	16.4	0.709204 (15)	--	--	17.71		8.265	0.138	0.13
A103JV-7	19.2	--	--	--	16.57		8.108	0.003	0.06
A103JV-8	22	0.709188 (6)	--	--	17.45		8.182	0.061	0.24
A103JV-9	24.8	0.709182(5)	--	--	19.21	0.61	8.305	0.319	0.07
A103JV-10	27.6	0.709182 (5)	--	--	25.12		8.708	0.733	0.22
A105JV-1	4	0.709178 (6)	0.227	1414	14.53	0.24	7.970	-0.064	0.53
A105JV-2	8.5	0.709191 (7)	0.255	1534	13.75		7.802	-0.237	0.39
A105JV-3	10.5	0.709183 (6)	0.251	1482	12.22	0.60	7.175	-0.914	0.76
A105JV-4	12.5	0.709184 (5)	0.263	1453	17.15		8.257	0.176	0.21

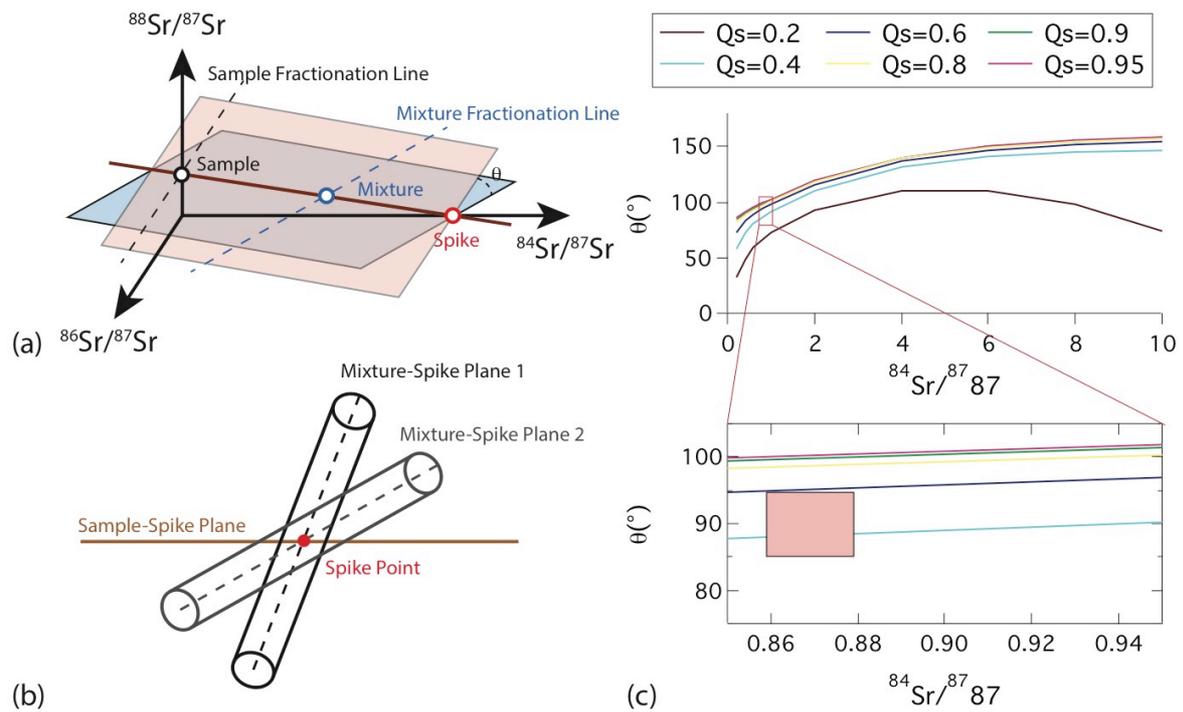
A105JV-5	14.5	0.709175 (7)	0.216	1521	14.59		7.916	-0.186	0.25
A105JV-6	16.4	0.709179 (6)	0.273	2559	11.09		--	--	0.30
A105JV-7	19.2	0.709183 (5)	0.296	2048	12.52		7.272	-0.833	0.33
A105JV-8	22	0.709163 (5)	0.230	2204	17.41	0.68	8.178	0.057	0.17
A105JV-9	24.8	0.709186 (6)	0.234	1850	19.43		8.322	0.337	0.09
A105JV-10	27.6	0.709177 (6)	0.238	1795	22.11		8.514	0.539	0.23

- 1        1. Data from Beirne (2011)
- 2        2.  $\text{pH}_{\text{shell}}$  was calculated with Eq. 4, in-situ temperature and salinity data was used to
- 3        determine  $\text{pK}_b$  and  $\alpha = 1.0272$  was used.
- 4        3.  $\Delta\text{pH}$  ( $\text{pH}_{\text{shell}} - \text{pH}_{\text{sw}}$ )
- 5        4. Propagation error determined from within run standard deviation of shell  $\delta^{11}\text{B}$
- 6        (duplication or triplication of the same sample solution).
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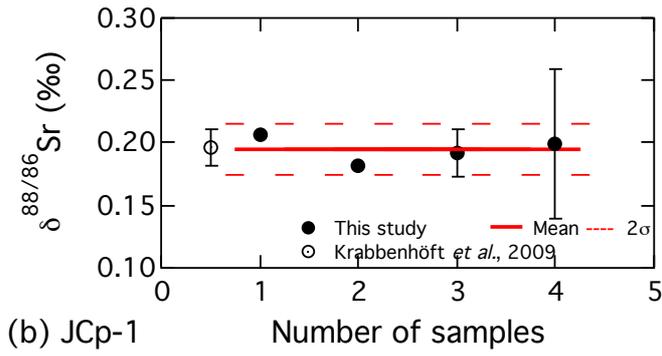
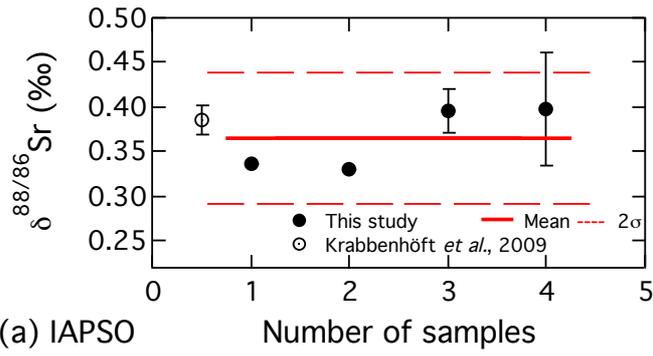
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Figure 1. Photos of (a) adult and (b) (c) juvenile *A. islandica* from the culture experiment. One can see nature marking on the external shell, or calcein mark in the cross-section under microscope to constrain the shell growth. (d) shows the corresponding *in-situ* measurements of tank water salinity, temperature and pH during the 31-week culture experiment. The juvenile shells were sampled in 10 intervals for this study (c). Note that because the growth rates differ during the season, each internal represents different durations (c) and (d).



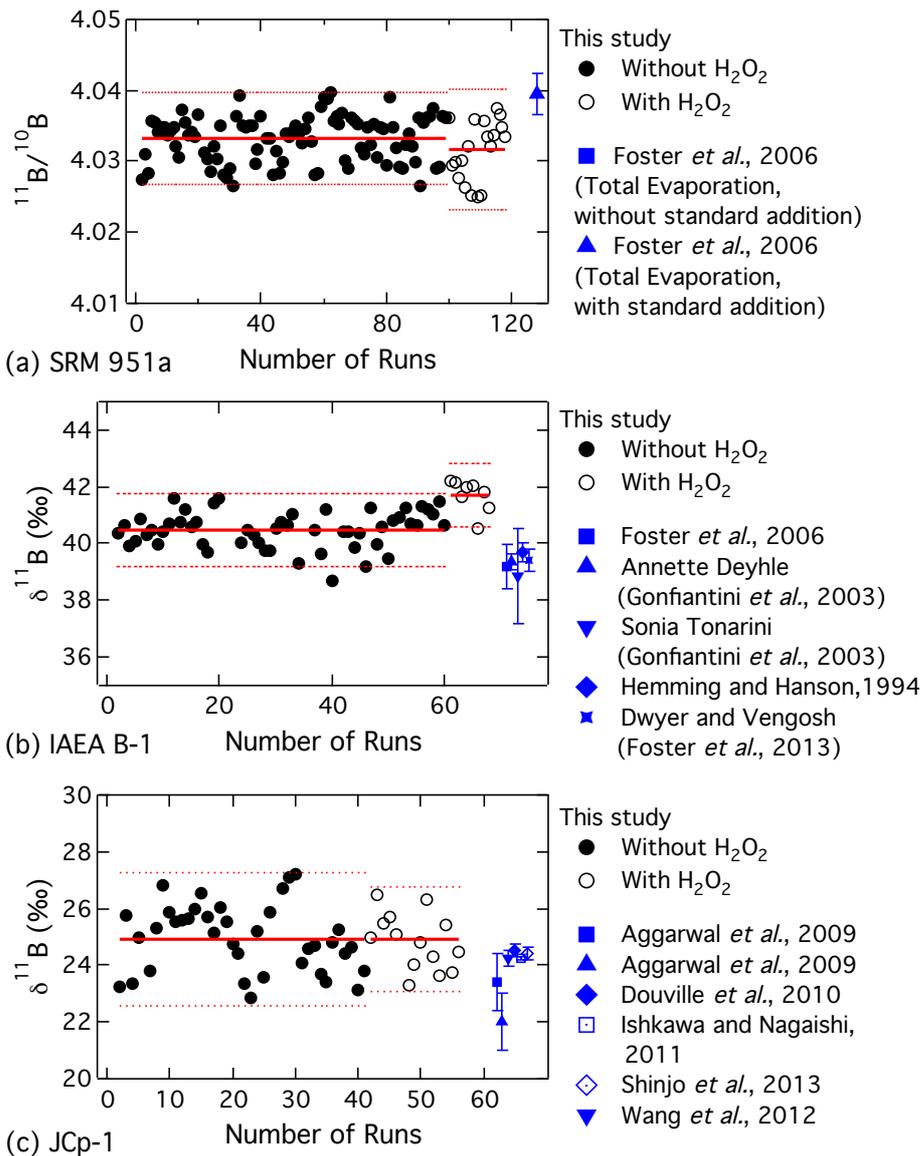
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Figure 2. The illustrations of (a) the 84-87 Sr double spike method, (b) how the angle between Mixture-Spike plane and Sample-Spike plane can influence the precision of the deconvolved result, and (c) the optimal sample-spike ratio in our study.



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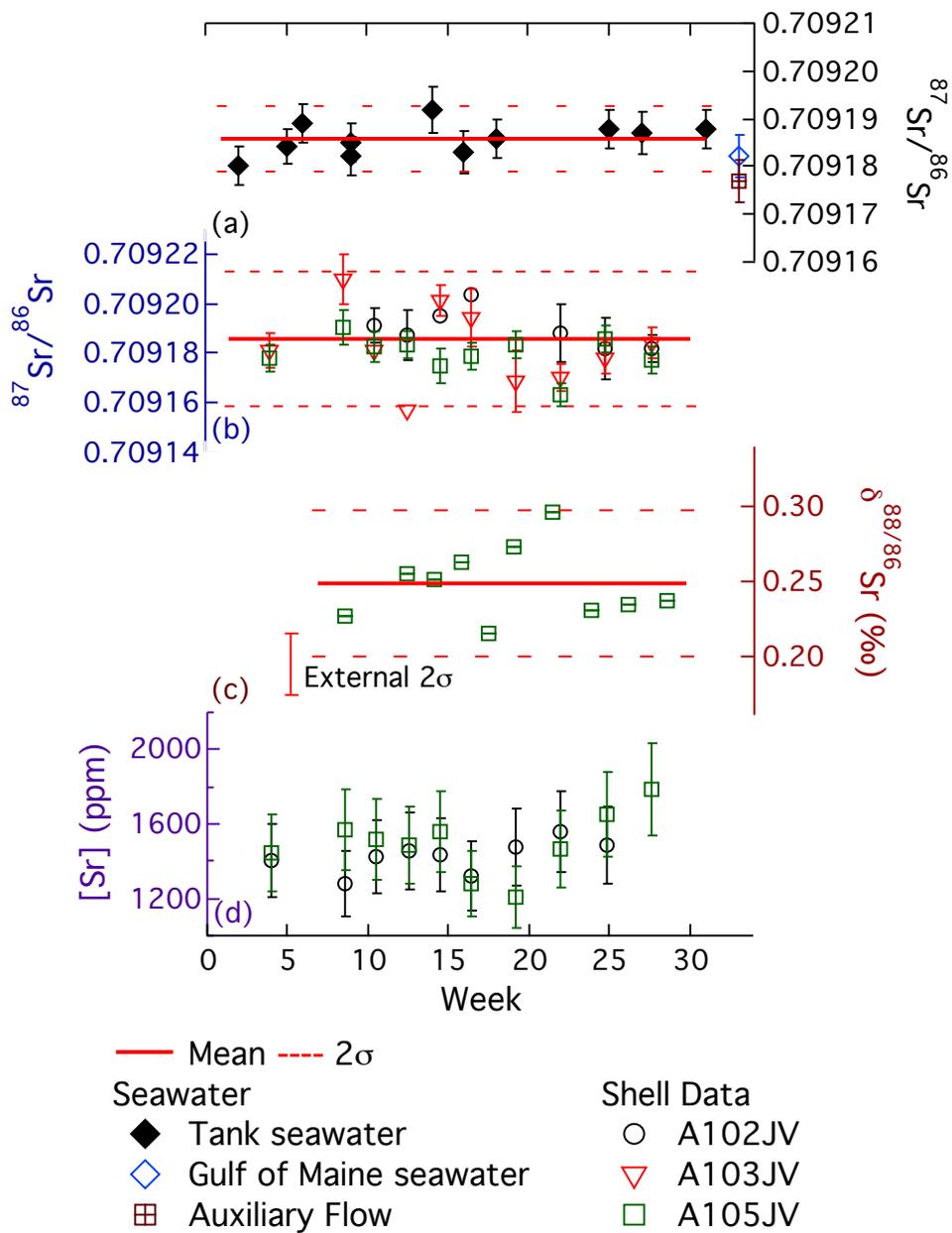
Figure 3. Stable Sr results for (a) seawater standard IAPSO and (b) inter-laboratory biogenic carbonate standards JCp-1.



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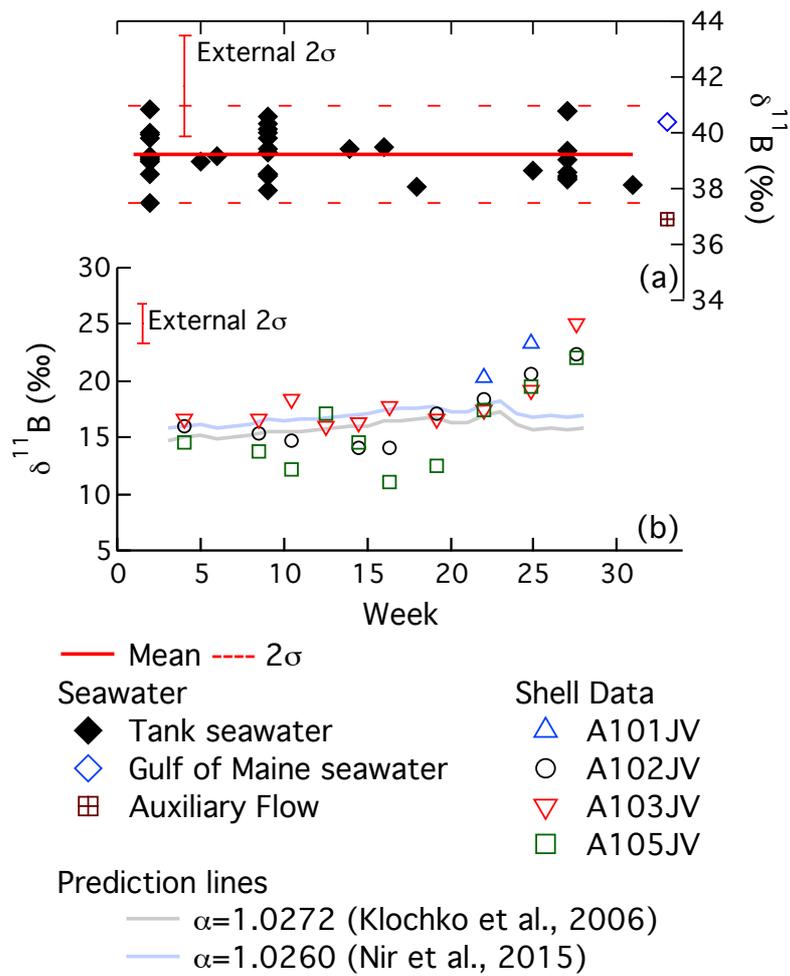
3 Figure 4. Long-term precision of (A) boric acid standard SRM 951a, (B) seawater standard  
 4 IAEA B-1, and (C) inter-laboratory carbonate standard JCP-1.



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3 Figure 5. GoM  $^{87}\text{Sr}/^{86}\text{Sr}$  data for (a) seawater samples and (b) shell samples and the double  
 4 spike deconvoluted (c)  $\delta^{88/86}\text{Sr}$  values and (d) Sr concentrations for the juvenile shell.



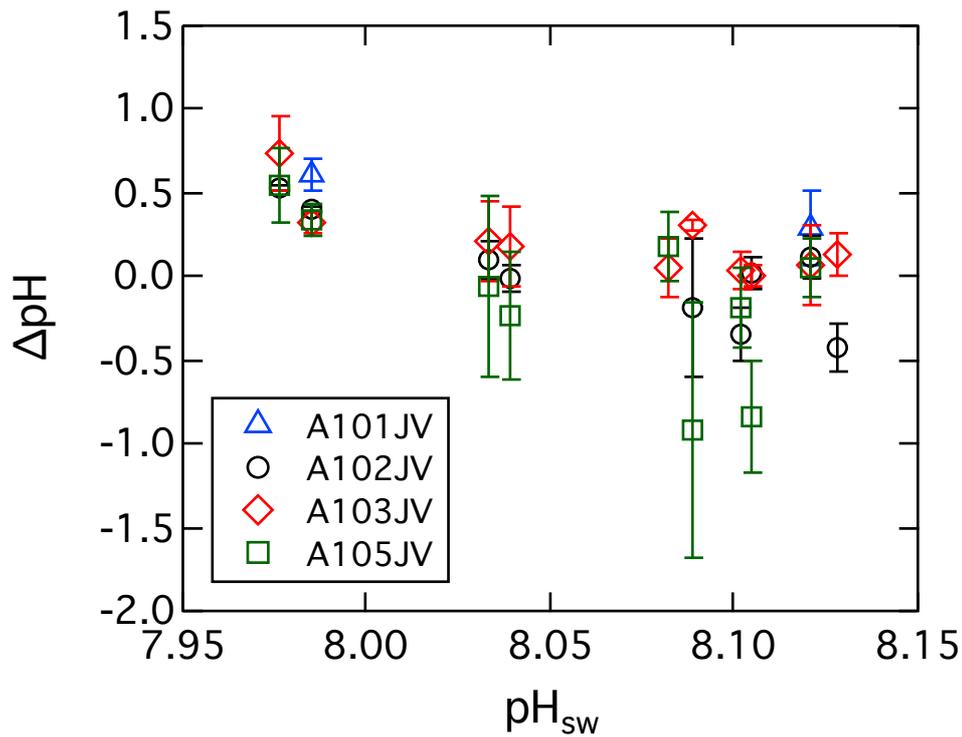
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3 Figure 6. GoM boron data for (a) seawater samples and (b) shell samples. Two prediction  
 4 lines listed were calculated based on our instrumental culture seawater pH, temperature, and  
 5 salinity data, and two boron fractionation factors:  $\alpha = 1.0272$  (Klochko et al., 2006) and  $\alpha =$

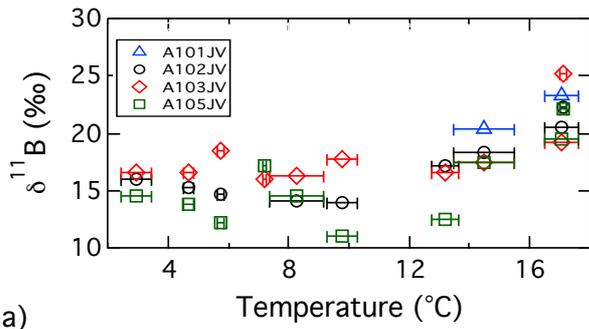
6  $1.0260$  (Nir et al., 2015), where  $\alpha \equiv \frac{(^{11}\text{B}/^{10}\text{B})_{\text{Boric acid}}}{(^{11}\text{B}/^{10}\text{B})_{\text{Borate}}}$ . Equation 4 was used for the

7 calculation.

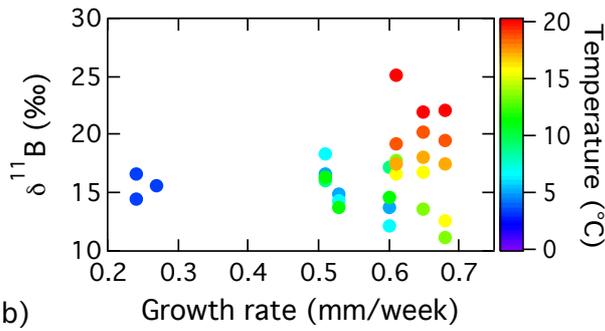


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Figure 7 The calculated pH discrepancy ( $\Delta\text{pH} = \text{pH}_{\text{shell}} - \text{pH}_{\text{sw}}$ ) shows a statistically significant negative relationship to  $\text{pH}_{\text{sw}}$  ( $R^2 = 0.35$ ;  $p\text{-value} \leq 0.001$ ). The negative correlation supports the argument that *A. islandica* regulate their EPF pH for calcification. The shell calcification pH ( $\text{pH}_{\text{shell}}$ ) were calculated based on in-situ water temperature  $\alpha = 1.0272$  (Klochko et al., 2006).



(a)



(b)

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3 Figure 8 The comparisons between the shell  $\delta^{11}\text{B}$  to (a) the corresponding culture water  
 4 temperature and (b) the growth rates for individual shells. Colors shown in (b) represent the  
 5 temperature corresponded to each data point, with low temperature in blue to high  
 6 temperature in red.