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Intratest Variations in Trace Element Composition of Amphistegina lessonii Using Femtosecond-Laser Ablation-ICP-Mass Spectrometry: A Field Study From Akajima, Okinawa Prefecture, Japan

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Abstract Amphistegina are common larger benthic foraminifer in coral reefs, with a nearly circumtropical distribution, and are major contributors to the CaCO3 budget of shallow environments. The family Amphisteginidae is dominant in Cenozoic carbonates. However, its potential as a proxy for paleoclimate reconstruction has not been completely explored. The intratest variability in trace elements of Amphistegina lessonii has been investigated using femtosecond-laser ablation-inductively coupled plasma-mass spectrometry (fs-LA-ICP-MS). We collected and analyzed adult specimens of A. lessonii in September 2003, November 2003, January 2004, and March 2004, from ~2 m water depth in the coral reefs of Akajima, Okinawa, Japan. Tests of A. lessonii from these four collections were analyzed for Mg/Ca of the septa to observe Test Size-Lifespan relationships. The lifespan of a specimen of A. lessonii of 1.200 µm in diameter is estimated at ~3 and ~2 months for specimens 900 µm in size. Over the estimated lifespans, Mg/Ca of individual specimens of A. lessonii is highly variable and displays co-variation with temperature and tidal heights. Future projects may apply and further test this approach for the reconstruction of the tropical shallow marine paleoenvironments.

Plain Language Summary Larger Benthic Foraminifera (LBF) have slow growth rates and can be used as a proxy to reconstruct seasonal variations in temperature. Amphistegina, a common genus of LBF found within the Cenozoic era including the past 55 million years, was sampled across four seasons from a modern coral reef of Okinawa, Japan. The trace element concentrations in nine specimens of A. lessonii were measured using femtosecond LA-ICP-MS. The intratest variation in Mg/Ca co-varied with tidal heights and seawater temperature, assuming the ages of the specimens to be 2–3 months. We provide a temperature calibration equation for A. lessonii for reconstructing paleotemperatures of shallow marine environments of the Cenozoic.

1. Introduction

Foraminiferal calcite is the most widely used biogenic carbonate to generate proxy geochemical data for paleoclimate reconstruction (Fischer & Wefer, 1999; Kucera, 2007). Trace element ratios in foraminifera are commonly used as proxies to reconstruct temperature, salinity, pH and redox potential, over seasonal or longer-term time intervals (Evans et al., 2015; Geerken et al., 2018; Lea et al., 1999; Raja et al., 2005; Wefer & Berger, 1980).

The Larger Benthic Foraminifera (LBF) are K-strategists. Their size is proportional to their lifespan (Wefer & Berger, 1980). To understand the growth rates of the various LBF species, different techniques have been explored like stable isotope analyses, micro-CT Scan, and culture studies (Hallock, 1981; Hohenegger et al., 2019; Wefer & Berger, 1980). The oxygen and carbon isotope ratios of the LBF Marginopora vertebralis (test diameter: 6.8 mm) and Cyclorubiculina compressa (test diameter: 5.2 mm) suggest lifespans of ~2 and ~1 year, respectively (Wefer & Berger, 1980). Purton and Brasier (1999) assume a lifespan of at least 6 years for Eocene Nummulites using stable isotopes of carbon and oxygen. Recently, Hohenegger et al. (2019) used the micro-CT Scan technique to estimate the growth rates of Nummulites venosus and estimated the life
expectancy of the schizont and gammont generations to be 18 months. The culture experiments by Hallock (1981) and verified by Hallock et al. (1986) suggest the life-expectancy of Amphistegina lessonii to vary between 3–4 months. To conclude, the longevity of the LBF spans from a few months to more than a year and makes them suitable to establish past seasonality.

The growth of various marine invertebrates is thought to be influenced by tidal and synodic lunar cycles, sea-level changes, and temperature (e.g., Carre et al., 2005; Goodwin et al., 2001; Schiebel & Hemleben, 2017; Siccia et al., 2012). Briguglio and Hohenegger (2014) and Hohenegger et al. (2019) suspect that growth in LBF is influenced by seasonality and various cyclic events such as lunar cycles, including tidal currents affecting sediment suspension and light attenuation, and increasing nutrient availability of the foraminifer’s photosymbionts. Warter and Müller (2017) observed that Element/Ca in giant clams of the Miocene and present-day was related to growth and tidal fluctuations. Peaks of light intensity in controlled growth experiments in mollusks are correlated to a minimum and maximum Element/Ca values. However, relating ontogenetic variations in Mg/Ca of LBF to seasonality, tidal fluctuation, and temperature is yet to be studied.

Amphistegina are photosymbiont-bearing hyaline-walled foraminifera, which are dominant in oligotrophic environments of clear and quiet marine lagoons, in sandy reefal and phytal substrates of the upper fore-reef, and even invades coastal ecosystems (Hohenegger, 1994; Triantaphyllou et al., 2012). Amphistegina lessonii occurs most frequently down to 30 m water depths but can be found down to 100 m depth in clear waters (Hallock, 1999). Amphistegina are composed of intermediate-Mg calcite \( \sim 25–70 \text{ mmol/mol} \), that is, <4% MgCO3 (Chave, 1962; Engel et al., 2015; Raja et al., 2007; Segev & Erez, 2006; Toler et al., 2001). The growth period of Amphistegina lobifera typically varies between 6 and 12 months while that of A. lessonii is considered to vary between 3 and 4 months as confirmed by culture studies (Hallock, 1981; Hallock et al., 1986). The adult test size of A. lessonii usually varies between \( \sim 800 \) and 1,300 µm (Prazeres et al., 2017).

In this study, we have measured the trace elements along the septa of adult specimens of A. lessonii of varying sizes, aiming to understand variation in Mg/Ca across the septa during ontogeny of this species and how that variation relates to environmental parameters like temperature, lifespan, tidal cycles, and salinity. The field-grown specimens from Akajima analyzed here facilitate analyses of naturally interacting environmental variables including seawater temperature, salinity, pH, and tidal oscillations, and comparison with results from culture experiments (e.g., Hallock, 1981).

Temperature calibration equations based on the lifespan of larger benthic foraminifera were proposed for Operculina ammonoides, Marginopora kudakajimensis, and Calcarina gaudichaudi (Evans et al., 2015, 2013 and Maeda et al., 2017). A field-based Mg/Ca temperature calibration study on Marginopora kudakajimensis was carried out by Raja et al. (2005), analyzing specimens collected live from Akajima, Japan. We present a temperature calibration using Mg/Ca in A. lessonii tests to be applied to fossil Amphistegina for the interpretation of paleotemperatures in shallow marine. As the first occurrence of Amphistegina is in the Paleogene, our results on the modern trace-element chemistry may help investigate climate change over the past 55 Myrs from shallow marine platform carbonates (Khanolkar & Saraswati, 2015; Schiebner & Speijer, 2008).

2. Materials and Methods

2.1. Study Area and Sample Collection

The investigated area lies within the subtropical Aka Island (Akajima), Okinawa Prefecture, in southwestern Japan (Figure 1; 26° 3' 52" N, 127° 5' 30" E). Akajima is part of the Ryukyu Island arc, which spans between 31°N and 24°N and extends for about 1,200 km. Toward the northwestern part of the islands lies the Okinawa trough with about 2000 m water depth (Kimura et al., 1991), along which flows the warm Kuroshio currents facilitating the growth of coral reefs in the shallow waters of the island. The seasonal sea surface temperature variation in this region is \( \sim 7.3 \text{ K} \), ranging between about 29°C in summer and 21°C in winter, which makes this site suitable to study the impact of seasonal temperature on LBF test formation and chemistry. The LBF assemblage from the Akajima comprises dominantly of Marginopora, Sorites, Calcarina, Baculogypsina, Peneroplis, Neorotalia, and subordinately of Amphistegina and Heterostegina (Saraswati...
The samples for this study were collected from the Akajima during September 2003, November 2003, January 2004, and March 2004. *Amphistegina* specimens were collected from seagrass leaves and hard substrate of the reef-flat at ∼2 m water depth. The golden-brown color of the diatom symbionts of *Amphistegina* facilitates picking live specimens.

### 2.2. Data Collection

Sea-surface temperature (SST) and salinity (SSS) over 12 months from April 2003 to March 2004 were logged at 15-min intervals by the Akajima Marine Science Laboratory and made available for this study (Data set files uploaded in Zenodo Repository). The average daily variations in tidal heights from April 2003 to March 2004 (See Figure S1 in Supporting Information file) were obtained and processed using the software Tide Predictor (http://tbone.biol.sc.edu/). The average tidal height and average SST per week were calculated from April 2003 to March 2004 (Figure 2).

![Figure 1.](image1.png)  
*Figure 1.* (a) Location map of the investigated area. (b) Samples were collected from Hizushi Bay, along the coral reefs of Okinawa, Japan (Satellite image is taken from Google Earth, January 27, 2017. Width of photo is 4,150 m).

![Figure 2.](image2.png)  
*Figure 2.* Average weekly SST (°C) versus Average tidal height (meters) in Okinawa, Japan from April 2003 to March 2004. SST, Sea-surface temperature.
2.3. Geochemical Analysis

The field-collected live specimens of microspheric *Amphistegina lessonii* were separated under a stereo-zoom binocular microscope and air-dried for later analyses. To understand the variations in trace elements across the septa of the specimens, we analyzed the samples using fs-LA-ICP-MS at Max Planck Institute for Chemistry, Mainz, Germany. Equatorial sections the tests of *A. lessonii* were prepared by polishing on a glass plate using 1,000 and 1,200 µm carborundum powder (Figure 3). The sections were fine polished with alumina, ultrasonicated in distilled water to remove alumina powder, and cleaned before analysis as outlined by Barker et al. (2003). As our goal was to understand the variations of trace elements along with the growth profile of the specimen, we analyzed the septa of the specimens, starting from the outermost chamber toward the proloculus (Figure 3). We chose to analyze the specimens along the septa, as septa represent instantaneous biomineralization and are not covered by additional layers of calcite at later growth stages. However, we did not analyze the first few chambers of the specimens because the individual septa could not be well distinguished and were too narrow and closely spaced for laser ablation. In the following, we discuss the variation of the elements Mg, Ca, Na, and Sr. In total, 271 sites from sections of nine specimens were analyzed for trace element composition, to develop a temperature calibration, and an equation for temperature calculation and reconstruction of past seasonal seawater-temperature variability from single adult tests of *A. lessonii*.

The 200-nm wavelength–NWR fs-laser ablation system NWR Femto from ESI combined with the Thermo Element 2 ICP mass spectrometer was used for the fs-LA-ICP-MS analyses (Jochum et al., 2014). The fs-laser is particularly well suited for this type of analyses because its short wavelength of 200 nm minimizes the elemental and isotopic fractionation effects when analyzing calcium carbonate (Jochum et al., 2019), by using the certified silicate reference material NIST SRM610 for calibration. To test the accuracy of the measurements, we also analyzed the USGS MACS-3 carbonate reference material. The data agree with the reference values (Jochum et al., 2019) within the uncertainty limits between 7% and 12%. For maximum spatial resolution, a small spot size of 30 µm was chosen, at a low fluence of about 0.2 J cm⁻², and a pulse repetition rate (PRR) of 15 Hz. Uncertainties (RSD in percentage) mainly depend on the trace element abundances and range from 5% to 15%.

3. Results

3.1. Intratest Heterogeneity in Trace Elements

Test sizes for nine *A. lessonii* specimens that were analyzed for trace elements vary between ~900 and 1,200 µm (Table 1). The lifespan of *A. lessonii* is assumed at ~3–4 months according to the growth curve.
produced from culture experiments by Hallock (1981) for the specimens within the size range corresponding to 900–1,200 µm (Figure 4 and Table 1). Growth rates in LBF decrease during the ontogeny from an initial growth rate of about one chamber per day over the first four chambers (and septa), to one chamber per 2 days for the following 4–6 weeks, and subsequently about one chamber per 3 days for the largest sized specimens of ~1,200 µm in diameter (specimens 1-1-F, 1-2-F, and 1-3-F), resulting in an assumed lifespan of ~90 days (Figure 4, Table 1). The lifespan of specimens with diameters varying ~900 µm (specimens 2-1-F, 2-2-F, 3-1-F, 3-2-F, 4-1-F, and 4-2-F) are estimated at ~60 days and may not include the final stage of slow growth, that is, the formation of one chamber at a 3 day-long time interval. The relatively cool winter temperatures could have slightly slowed growth and delayed maturation and reproduction in the largest size specimens (Specimens: 1-1-F, 1-2-F, and 1-3-F; Size: ~1,200 µm diameter), which were collected in January 2004.

Nine specimens of different sizes and corresponding growth rates collected from different sampling months were analyzed along the septa to assess the intratest heterogeneity in Mg, Ca, Na, and Sr using fs-LA-ICP-MS. Consequently, we compared the average SST and tidal heights corresponding to the approximated growth rates of each specimen to Element/Ca data (Figures 5–8). The intratest heterogeneity in Mg/Ca (9.2–23.3 mmol/mol) and Na/Ca (9.2–23.3 mmol/mol) is quite high, while being lower in Sr/Ca (1.5–2.5 mmol/mol). The specimens 1-1-F, 1-2-F, and 1-3-F (test diameter ~1,200 µm) show covariation between Mg/Ca and the average temperature along the estimated growth rate, over their entire lifespans assumed to range ~3 months (Figure 5). The intratest heterogeneity between Mg/Ca of other specimens (2-1-F, 2-2-F, 3-1-F, 3-2-F, 4-1-F, and 4-2-F) with test diameters ~900 µm, covary with SST changes over a total lifespan of ~2 months (Figures 5–8). In contrast, we do not observe any obvious pattern in covariation between Na/Ca and Sr/Ca versus SST among any of the analyzed specimens of A. lessonii (Figures 5–8).

A strong covariation ($R^2 = 0.61$) occurred between the average week-long tidal height and SST at Akajima from April 2003 to March 2004 (Figure 9). We also observed a covariation between the tidal heights, temperature, and Mg/Ca values over the assumed growth rates of A. lessonii for the nine specimens (Figures 5–8).

### Table 1

| Specimen | Month and year of sampling | Test diameter (µm) | Number of spots analyzed using fs-LA-ICP-MS | Estimated lifespan (days) |
|----------|---------------------------|-------------------|--------------------------------------------|--------------------------|
| 1-1-F    | Jan-2004                  | 1,200             | 33                                         | 90                       |
| 1-2-F    | Jan-2004                  | 1,200             | 34                                         | 90                       |
| 1-3-F    | Jan-2004                  | 1,200             | 33                                         | 90                       |
| 2-1-F    | Mar-2004                  | 900               | 26                                         | 60                       |
| 2-2-F    | Mar-2004                  | 900               | 31                                         | 60                       |
| 3-1-F    | Nov-2003                  | 900               | 26                                         | 60                       |
| 3-2-F    | Nov-2003                  | 900               | 27                                         | 60                       |
| 4-1-F    | Sep-2003                  | 900               | 26                                         | 60                       |
| 4-2-F    | Sep-2003                  | 900               | 18                                         | 60                       |

Abbreviation: fs-LA-ICP-MS, femtosecond-laser ablation-inductively coupled plasma-mass spectrometry.
Figure 5. Element/Ca (mmol/mol) that is measured along the septa of *A. lessonii*, average weekly tidal heights (m) and average of SST depending on the growth rate in specimens (a)1-1-F, (b) 1-2-F, and (c)1-3-F collected in January 2004. SST, Sea-surface temperature.
The tidal heights in Okinawa, Japan, may also be affected by tropical storms/typhoons. The Japan Meteorological Survey recorded a typhoon during the end of August 2003 in Okinawa, which may have increased the tidal heights and thus also increased the Mg/Ca values of the specimens (4-1-F and 4-2-F) during this time-period (Figure 8).

### 3.2. Mg/Ca-Temperature Relationship in *A. lessonii*

For temperature calibration, we consider the average Mg/Ca values of *A. lessonii* test calcite and correlate it with the average temperature considering the lifespan of the specimen of ∼2–3 months, as displayed by test size variations and growth rate from culture studies by Hallock (1981).

A significant statistical correlation is observed between the average Mg/Ca values of each individual specimen versus average temperature over ∼2–3 months growth period (Refer Figure S2 in Supporting Information file), described by the exponential equation

\[ \text{Mg/Ca (mmol/mol)} = 26.8 e^{0.015SST} \pm 0.94 \left( R^2 = 0.45, p < 0.05 \right) \]  

and the following linear equation
Average Mg/Ca of all specimens for each sampling month correlate with the average temperature of the growth period of the specimens to an even higher degree (Figure 10a), given the exponential equation

$$\text{Mg/Ca (mmol/mol)} = 0.607T + 24.5 \pm 0.94 \left( R^2 = 0.46, p < 0.05 \right)$$

and the following linear equation

$$\text{Mg/Ca (mmol/mol)} = 25.904e^{0.0172T} \pm 1.31 \left( R^2 = 0.78, p < 0.05 \right)$$

Intratest variation in Mg/Ca is high, ranging between 21.6 and 77.6 mmol/mol. Error bars are denoted by calculating the standard error (SE) of the mean ($\pm 2\text{SE}$) and represent the biogeochemical heterogeneity within the tests of *A. lessonii* (Figure 10a).

Little variation of Sr/Ca results in a low correlation of Sr/Ca versus average temperature of lifespans for single *A. lessonii* specimens, and the ontogenetic profiles of all specimens, with $R^2 = 0.10$ (linear and exponential equations; Figure S2 in Supporting Information) and $R^2 = 0.38$, respectively (linear and exponential equations).
equations; Figure 10b). Similarly, Na/Ca is not correlated with the average temperature over the lifespan of single specimens ($R^2 = 0.10$, for linear and exponential equations; Figure S2 in Supporting Information), and the combined data of all specimens correlate at $R^2 = 0.54$ (linear equation) and $R^2 = 0.61$ (exponential equation, Figure 10c). Sea surface salinity at Okinawa varies at 34–35 PSU. Sea-surface salinity during the growth period of each specimen of *A. lessonii* exhibits no statistically significant correlation with Mg/Ca ($R^2 = 0.15$), Na/Ca ($R^2 = 0.03$), and Sr/Ca ($R^2 = 0.20$) (Figure 11 and Figure S3 in Supporting Information).

4. Discussion

4.1. Possible Causes for Intratest Variations in Trace Elements of *A. lessonii*: Tidal Cycles, SST, and Salinity

The Element/Ca of individual foraminifera may vary due to (a) seasonal changes in temperature and salinity of ambient seawater, (b) differences in ecology and micro-habitat of individuals including (c) varying light intensity with water depth and micro-habitat (Evans et al., 2015; Raja et al., 2005). Other factors may affect the chemical composition of tests in sediment samples, such as differential diagenesis and preservation, but
are not discussed here because our analyses are based on live individuals of the same species. Also, the effects of differential ecologies and microhabitats may be limited because all our specimens were picked from the same phytal habitat. Therefore, the observed intratesser variation in Element/Ca of the symbiont-bearing *A. lessonii* is assumed to mainly result from seasonal changes in seawater temperature, salinity, and changes in light intensity. Piniak et al. (2008) showcased by a field study in coral reefs (2–2.5 m average water depth) at Hawaii that high tides increased the turbidity of seawater, which attenuated light intensity as an important limiting factor for the distribution and growth of the symbiont-bearing coral *Montipora capitata*. Light intensity is also assumed to be one of the factors, which may cause fluctuations in trace elements in marine invertebrates including mollusks and foraminifera (Dämmer et al., 2019; Fehrenbacher et al., 2017; Warter & Müller, 2017). The micro-analyses of symbiont-bearing planktic foraminifera have shown that there are bands of high Mg/Ca values in the tests that cannot be explained by temperature alone (Spero et al., 2015). The intensity of light affecting symbiosis seems to control Mg/Ca variation within the foraminifer test wall (Eggins et al., 2004; Fehrenbacher et al., 2017; Spero et al., 2015). Previous culture studies on *A. lessonii* documented an interspecimen variation in Mg/Ca but do not provide a further explanation (e.g., de Nooijer et al., 2014; Geerken et al., 2018). Geerken et al. (2018) suggest that Mg/Ca (and Na/Ca) variability is associated to organic linings within *Amphistegina lessonii*. Dämmer et al. (2019) show that average Mg/Ca values per chamber in *A. lessonii* are also strongly affected by light exposure. The Mg/Ca of a chamber completely calcified in the dark is 23 mmol/mol higher compared with one completely formed under light conditions. Levi et al. (2019) showed by culture studies that variation in dissolved inorganic carbon (DIC) did not have any significant effect on high and low Mg bands in the central knob area of *A. lessonii*. The culture studies performed by Mewes et al. (2015) indicate that Mg/Ca of the test calcite in *A. lessonii* is not controlled by absolute seawater [Ca$^{2+}$] and [Mg$^{2+}$] but by their ratios in seawater. Since ambient Mg/Ca in seawater is $\sim 5.12$ mol/mol (Nozaki, 2001), the intratesser variations in *A. lessonii* analyzed in this study cannot be related to changes in seawater Mg/Ca.

Recent studies on Mg-banding in the planktic foraminifer *Orbulina universa* suggest that Mg gets preferably incorporated during the night and the resulting Mg banding is due to diurnal cyclicity of dark-and-light conditions (Fehrenbacher et al., 2017; Spero et al., 2015). Thus, the Mg/Ca in the test calcite of a foraminifer that hosts algal symbionts is higher in low-light than high-light conditions. Also, covariation exists between Mg/Ca, average tidal heights, and ambient seawater temperature at the estimated growth stage of the specimen (Figures 5–8). Therefore, we assume that the changes in the Mg/Ca of *A. lessonii* from Akajima are affected by tidal cyclic changes in tidal height, that is, lowering of light intensity through increased turbidity during times of high tidal range.

Apart from tidal height, there can be an additional influence of typhoons, which regularly occur in the Okinawa region and may cause an increase in turbidity/resuspension of fine sediments. The one typhoon recorded by the Japan Meteorological Survey in the Okinawa region during the growth intervals of the
sampled A. lessonii (Figure 8) may have affected Mg/Ca in addition to changes in tidal range and sediment resuspension. However, since the exact growth rate of an individual specimen in natural conditions cannot be determined for each point of time, correlation to singular events such as a typhoon is difficult. Tidal currents occur in conjunction with strong tidal range, and hence their effect on resuspension of sediments can be estimated along with variation in tidal fluctuations. Whereas the Mg/Ca composition of the foraminiferal shell, including modern A. lessonii from Okinawa may be related to various environmental parameters such as ambient SST and tidal range (Figure 5678 and 10a), we observe little correlation with ambient seawater salinity (Figure 11a). So far, there is an inadequate quantitative understanding of the effect of salinity on the incorporation of trace elements into the shell calcite of LBF, though studies on planktic foraminifers indicate that salinity may affect the incorporation of Mg (Friedrich et al., 2012; Groeneveld et al., 2008; Lea et al., 1999; Nürnberg et al., 1996). However, the Mg/Ca data on A. lessonii from Aka-jima show no significant statistical relationship (R² = 0.1) with salinity (Figure 11a). The same is true for sodium (Na/Ca) and strontium (Sr/Ca) (Figures 11b and 11c). However, interstitial incorporation of sodium had been assumed by Ishikawa and Ichikuni (1984) and may be different for strontium and magnesium, which would explain differences in the systematic distribution of these elements in foraminiferal shell calcite. Alternative explanations for concentrations of alkaline elements like Sr may be provided by crystal lattice strain in A. lessonii (Geerken et al., 2019), and element banding because of laminar calcification (Erez, 2003; A. Sadekov et al., 2005).

4.2. Calibration Equation for Sea Surface Temperature Using Mg/Ca in A. lessonii

Several studies on planktic and smaller (i.e., not LBF) benthic foraminifera have concluded that temperature is the decisive mechanism controlling Mg/Ca of the test carbonate (e.g., Anand et al., 2003; Elderfield et al., 2006; Lea et al., 1999; Nürnberg et al., 1996; A. Y. Sadekov et al., 2014). So far, only few calibrations have been provided for any species of larger benthic foraminifera, LBF (Evans et al., 2013, 2015; Maeda et al., 2017; Raja et al., 2005). Through field studies and whole shell analysis of Amphistegina lessonii, Marginopora kudakajimensis, Calcarina gaudichaudii, Amphisorus hemprichii, Neorotalia calcar, Raja et al. (2007) have shown that the Mg/Ca varies within a range of ~50–350 mmol/mol among different LBF species and showcased poor correlation with temperature during the individual lifespan. However, Maeda et al. (2017) later reported through culture studies that the Mg/Ca in Calcarina gaudichaudii and Marginopora kudakajimensis varies within the range of 136–158 mmol/mol and has a strong correlation with temperature as observed through whole-test analysis. The LA-ICP-MS analysis of specimens of Operculina ammonoides indicates Mg/Ca within the range of 120–160 mmol/mol and exhibits strong linearity with temperature (Evans et al., 2013, 2015).

From the above discussion it is evident that there is a necessity to develop Mg/Ca-temperature calibration for different species of LBF, which are dominant components of carbonate platforms during times of global climate warming through the Cenozoic, and hence are useful paleotemperature archives. Some of the concerns of proxy development in LBF were raised by Raja et al. (2005), including (i) a large variation in Mg/Ca of different species calcified under the same temperature and salinity conditions, and (ii) sig-
significant heterogeneity in Mg/Ca within the tests of some species; these issues imply a more scrupulous selection of species for paleoclimate reconstruction.

The following relationship between test Mg/Ca of *A. lessonii* and ambient seawater temperature is observed in this study:

(i) The intratest variation in Mg/Ca within the same species, *A. lessonii*, is large across the four sampling months
(ii) Despite intratest variability in Mg/Ca in specimens of *A. lessonii*, a significant correlation is observed between Mg/Ca of the test and average ambient seawater temperature assuming an individual lifespan of ~2–3 months 

The Mg/Ca to temperature relationship is represented by the exponential equation

$$\text{Mg/Ca (mmol/mol)} = 25.904 e^{0.0172T} \pm 1.31 \quad (R^2 = 0.78, p < 0.05)$$  \hspace{1cm} (5)

and the linear equation

$$\text{Mg/Ca (mmol/mol)} = 0.67T + 23.08 \pm 1.31 \quad (R^2 = 0.80, p < 0.05) \hspace{1cm} (6)$$

We observe a 1.72% increase in Mg/Ca per 1 K temperature increase using *A. lessonii*, which is comparable to the previous results of Evans et al. (2013) for *Operculina ammonoides* (1.9% increase in Mg/Ca per 1 K) and by Raja et al. (2005) for *Marginopora kudakajimaensis* (3.1% increase in Mg/Ca per 1 K). In inorganically precipitated calcite, an exponential relationship exists between Mg/Ca and ambient seawater temperature (e.g., Katz, 1973; Mucci, 1987; Oomori et al., 1987). The exponential component for the calibration equations in our study is also comparable to the study of Evans et al. (2013) for recent *O. ammonoides*, as well as inorganic calcite (Burton & Walter, 1991).

Our results suggest that the intratest variations of Mg/Ca in *A. lessonii* are related to both tidal fluctuations and seasonal SST variations. However, *A. lessonii* may exhibit additional metabolic intratest variability in Mg/Ca. Therefore, additional field and culture studies would be required for better paleoclimate reconstruction. It is also necessary to examine the tests of fossil *A. lessonii* and other LBF for diagentic alteration before geochemical analysis, as diagensis may affect the Mg/Ca values in foraminiferal shell calcite (Evans et al., 2018), and the equation presented here may not be applicable to different fossil species.

### 5. Conclusions

Intratest heterogeneity in Element/Ca of the larger benthic foraminifer *A. lessonii* was analyzed by fs-LA-ICP-MS. In adult specimens with an assumed lifespan of 2–3 months, a significant correlation exists between Mg/Ca and ambient seasonal seawater temperature, best described by the equation: Mg/Ca (mmol/mol) = 25.904 e^{0.0172T} ± 1.31 (R^2 = 0.78, p < 0.05), for live specimens collected from Akajima, Okinawa, Japan. In addition to ambient seawater temperature, the Mg/Ca of *A. lessonii* seems to be affected by the tidal range. High tides cause sediment resuspension and light attenuation, which may affect the photosymbionts hosted by the foraminifer, and explain fluctuations in the Mg/Ca incorporation, analogous to similar mechanisms described for the symbiont-bearing planktic foraminifera (Spero et al., 2015). Our findings are
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Data Availability Statement
All the data used in this study could be accessed from Zenodo Repository (http://doi.org/10.5281/zenodo.4305748).

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