Environmental and biological factors influencing trace elemental and microstructural properties of *Arctica islandica* shells

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Long-term and high-resolution environmental proxy data are crucial to contextualize current climate change. The extremely long-lived bivalve, *Arctica islandica*, is one of the most widely used paleoclimate archives of the northern Atlantic because of its fine temporal resolution. However, the interpretation of environmental histories from microstructures and elemental impurities of *A. islandica* shells is still a challenge. Vital effects (metabolic rate, ontogenetic age, and growth rate) can modify the way in which physiochemical changes of the ambient environment are recorded by the shells. To quantify the degree to which microstructural properties and element incorporation into *A. islandica* shells is vitally or/and environmentally affected, *A. islandica* specimens were

reared for three months under different water temperatures (3, 8 and 13 °C) and food concentrations (low, medium and high). Concentrations of Mg, Sr, Na, and Ba were measured in the newly formed shell portions by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The microstructures of the shells were analyzed by Scanning Electron Microscopy (SEM). Shell growth and condition index of each specimen were calculated at the end of the experimental period.

Findings indicate that no significant variation in the morphometric characteristics of the microstructures were formed at different water temperatures or different food concentrations. Shell carbonate that formed at lowest food concentration usually incorporated the highest amounts of Mg, Sr and Ba relative to Ca^{+2} (except for Na) and was consistent with the slowest shell growth and lowest condition index at the end of the experiment. These results seem to indicate that, under food limitation, the ability of *A. islandica* to discriminate element impurities during shell formation decreases. Moreover, all trace element-to-calcium ratios were significantly affected by shell growth rate. Therefore, physiological processes seem to dominate the control on element incorporation into *A. islandica* shells.

Keywords:

Bivalve, environmental proxy, vital effects, temperature, phytoplankton concentration, sclerochronology

1. Introduction

Proxy records are crucial to study climate change in areas where instrumental records are absent (Freitas et al., 2006). In the last two decades, bivalve shells have become an important bioarchive tool and the number of respective studies has greatly increased (Gillikin et al., 2005; Freitas et al., 2006; Wanamaker et al., 2008; Schöne et al., 2011; Milano et al., 2017a). Microstructural properties and trace element-to-calcium ratios can reflect the environment in which the bivalves lived (Schöne et al., 2013; Milano et al., 2017a). The long-living species *Arctica islandica* (up to 507 years old; Wanamaker et al., 2008, Butler et al., 2013), also known as ocean quahog, has been widely used for multicentennial paleoclimatic reconstruction (Butler et al., 2013), but the study of its microstructural and geochemical shell properties as an environmental proxy is still under development. Therefore, knowledge of the interacting effects of extrinsic (environmental) and intrinsic (physiological) factors on *A. islandica* shells are essential to interpret this long-living bioarchive (Abele et al., 2009).

Levels of strontium (Sr) and magnesium (Mg) in bivalve shells have been proposed as proxies for water temperature (Klein et al., 1996; Schöne et al., 2013). However, Sr/Ca and Mg/Ca ratios are still difficult to interpret because the incorporation of trace and minor impurities in the shell carbonate is partly physiologically controlled (e.g., Urey et al., 1951; Purton et al., 1999; Lorrain et al., 2005; Freitas et al., 2005, 2006; Schöne et al., 2010, 2013; Marali et al., 2017a; Geeza et al., 2018). Published results on the relationship between the Sr/Ca and Mg/Ca ratios of bivalve shells and temperature are highly ambiguous; previous studies have reported a positive relationship (Stecher et al., 1996; Hart and Blusztajn, 1998; Toland et al., 2000), negative relationship (Dodd, 1965; Stecher et al., 1996; Surge and Walker, 2006; Schöne et al., 2011), and no relationship (Gillikin et al., 2005; Strasser et al., 2008; Izumida et al., 2011; Wanamaker and

Gillikin, 2018). The variation on the type of relationship (positive, negative, or neutral) have been identified as species-specific and can even change depending on the season of the year (e.g., Gillikin et al., 2005; Freitas et al., 2006). In addition, Sr/Ca and Mg/Ca ratios can even differ among specimens of the same population, but the causes are not yet clear (Vander Putten et al., 2000; Lorrain et al., 2005; Freitas et al., 2006; Foster et al., 2008, 2009).

The potential use of other element-to-calcium ratios of bivalve shells as environmental proxies has also been explored. For example, Na/Ca is strongly correlated to salinity (Rucker and Valentine, 1961; O'Neil and Gillikin, 2014) and water pH (Zhao et al., 2017a). Furthermore, it has been suggested that Ba/Ca and Na/Ca ratios are linked to primary production (e.g., Stecher et al., 1996; Gillikin et al., 2006; Poulain et al., 2015; Klünder et al., 2008). Ba/Ca profiles are typically characterized by a relatively flat background interrupted by episodic sharp peaks (e.g., Stecher et al., 1996; Vander Putten et al., 2000; Gillikin et al., 2006, 2008; Thébault et al., 2009; Elliot et al., 2009; Hatch et al., 2013), which are usually highly reproducible among specimens (e.g., Gillikin et al., 2008; Elliot et al., 2009; Marali et al., 2017a, b). Although the factors controlling the formation of Ba/Ca peaks are still controversially debated, the Ba/Ca ratio of bivalve shells is potentially strongly influenced by an environmental forcing (Gillikin et al., 2006, 2008; Poulain et al., 2015).

Previous studies of mollusks show that environmental parameters can also influence the microstructure of the shell (Lutz, 1984; Tan Tiu and Prezant, 1987; Tan Tiu, 1988; Nishida et al., 2012) and therefore, can serve as potential proxy for environmental conditions (Tan Tiu, 1988; Tan Tiu and Prezant, 1989; Schöne et al., 2010; Milano et al., 2017b). For example, the size and elongation of individual biominerals in *Cerastoderma edule* (Milano et al., 2017b) and the cyclical changes in thickness of the outer layer of *Scapharca broughtonii* (Nishida et al., 2012)

shells are related to temperature changes. Moreover, the relationship between food conditions and microstructure have lately been explored; some studies reported an accumulation of pigments in mollusk shells due to the ingestion of pigment-enriched microalgae (polyenes; Hedegaard et al. 2006; Soldatov et al.., 2013), while others argued that diets do not influence shell pigment composition, and that polyenes are likely species-specific and habitat independent (Nehrke and Nouet, 2011; Stemmer and Nehrke, 2014; Milano et al., 2017a). The study of shell microstructure can therefore help to develop alternative techniques to reconstruct environmental variables from bivalve shells (Milano et al., 2017a).

The objective of the present study is to clarify the effect of external (environmental) and internal (physiological) factors on microstructural properties and element incorporation into *A. islandica* shells. Under laboratory conditions, *A. islandica* individuals from the same population were reared at different temperatures and food concentrations. Several studies have used controlled laboratory experiments to determine the relationship between the elemental composition of bivalve shells and variable environmental parameters (Lorens and Bender, 1980; Strasser et al., 2008; Wanamaker et al., 2008; Poulain et al., 2015; Wanamaker and Gillikin, 2018). However, fewer studies have tested the effect of the environment on the shell microstructure. Furthermore, most previous studies only focused on single parameter validation and did not take into account the possible interaction among multiple parameters (e.g., Lorens and Bender, 1980; Poulain et al., 2015). Here, we present for the first time a controlled laboratory experiment that aim to provide a better understanding of the interplay between environmental (food and temperature) and physiological influence (shell growth and condition index) on the geochemical properties and shell microstructure of *A. islandica* shells.

2. Materials and methods

2.1. Sample collection

In July 2014, live juvenile specimens of *A. islandica* were collected from the Kiel Bay, Baltic Sea (54° 32′ N, 10° 42′ E) and used in a laboratory growth experiment conducted at Royal Netherlands Institute for Sea Research (NIOZ) between 22 March and 23 June 2016 (14 weeks; Ballesta-Artero et al., 2018). The specimens were divided among 12 different treatments, i.e., combinations of four food concentrations (no, low, medium, and high food) and three different temperatures (3 °C, 8 °C, and 13 °C; Table 1). There were 3 replicates per treatment (3 aquaria), which meant a total of 36 aquaria (4 food levels x 3 temperatures x 3 replicates). Five *A. islandica* juveniles were randomly assigned to each aquarium, amounting to a total 180 *A. islandica* specimens. Specimens reared without food were not analyzed in this study because there was insufficient (or none) newly formed shell material for chemical analyses (Ballesta-Artero et al., 2018). Therefore, we only analyzed 9 treatments (27 trials) and a total of 73 specimens for the present study. Bivalves were fed 8 times per day with a commercial mix of marine microalgae containing *Isochrysis* sp., *Tetraselmis* sp., *Pavlova* sp., *Thalassiosira* sp. and *Nannochloropsis* spp (Mixalgae; Acuinuga, Spain). Numbers of cells in each aquarium were checked once per week with a flow cytometer (BD Accuri C6), while temperature and salinity were monitored on a daily basis with a portable multiparameter probe (HI98192; Hanna instruments, USA).

The starting shell height of the experimental animals ranged between 8.31 and 14.34 mm (± 0.01 mm). Prior to the start of the experiment, the specimens were soaked in a calcein solution of 125mg/l for 24 hours (Linard et al., 2011; Ambrose et al., 2012). This solution allowed us to accurately identify the newly formed shell portion that grew under experimental conditions.

	3 °C	8 °C	13 °C
Low			
Na/Ca	23.94 ± 0.70	23.27 ± 0.75	23.58 ± 1.39
Mg/Ca	0.44 ± 0.05	0.40 ± 0.02	0.47 ± 0.08
Sr/Ca	1.58 ± 0.17	1.49 ± 0.06	1.68 ± 0.17
Ba/Ca	8.48 ± 2.78	8.27 ± 1.26	8.32 ± 3.75
Condition Index	5.27 ± 0.21	3.72 ± 0.57	4.33 ± 0.37
Shell growth	0.40 ± 0.28	0.28 ± 0.22	0.39 ± 0.44
[cells/ L] x 10^6	0.85 ± 0.43	0.22 ± 0.02	0.53 ± 0.18
mg DW /ind· /d	0.62 ± 0.01	0.62 ± 0.01	0.62 ± 0.01
Medium			
Na/Ca	25.42 ± 1.54	24.99 ± 0.40	24.25 ± 1.45
Mg/Ca	0.38 ± 0.14	0.32 ± 0.06	0.36 ± 0.02
Sr/Ca	1.55 ± 0.21	1.43 ± 0.06	1.50 ± 0.04
Ba/Ca	9.56 ± 7.18	11.84 ± 6.84	5.26 ± 1.69
Condition Index	7.83 ± 0.22	5.59 ± 0.57	6.92 ± 0.96
Shell growth	0.90 ± 0.24	1.65 ± 0.35	1.82 ± 0.52
[cells/ L] x 10^6	6.19 ± 0.64	2.34 ± 0.17	1.53 ± 0.05
mg DW /ind· /d	5.33 ± 0.21	5.33 ± 0.21	5.33 ± 0.21
High			
Na/Ca	25.98 ± 2.08	24.79 ± 0.23	23.95 ± 0.76
Mg/Ca	0.43 ± 0.17	0.31 ± 0.04	0.35 ± 0.11
Sr/Ca	1.41 ± 0.17	1.42 ± 0.09	1.75 ± 0.10
Ba/Ca	6.29 ± 3.56	7.17 ± 0.48	5.86 ± 1.91
Condition Index	7.62 ± 2.64	9.01 ± 2.13	9.24 ± 1.02
Shell growth	0.72 ± 0.39	1.34 ± 0.40	1.48 ± 0.05
[cells/ L] x 10^6	24.56 ± 2.85	12.99 ± 0.82	6.45 ± 2.08
mg DW /ind· /d	13.69 ± 0.03	13.69 ± 0.03	13.69 ± 0.03
T real (°C)	2.49 ± 0.02	7.94 ± 0.07	13.11 ± 0.05
Salinity(PPT)	30.26 ± 0.10	30.38 ± 0.08	29.39 ± 0.24

Table 1: Summary of treatments (mean ± SD). Results are given in µm/mol for Ba/Ca and mmol/mol for the other elements. Shell growth was measured in height (mm). DW= Dry weight and ind= individual.

2.2. Shell preparation

The right valve of each specimen was glued to a plexiglass cube, covered with a layer of JB KWIK epoxy resin and dried overnight. A low speed saw (Buehler IsoMet 1000; 250 rpm) was used to cut 3-mm-thick section from each specimen along the axis of maximum growth. Subsequently, the slabs were embedded in Struers EpoFix resin and air-dried overnight. The blocks were then ground using Buehler silicon carbide papers of different grit sizes (P320, P600, P1200, P2500) mounted on a Buehler Metaserv 2000 grinder-polisher machine. After each grinding step the blocks were rinsed in an ultrasonic bath for ca. 2 min. The samples were polished with a Buehler diamond polycrystalline suspension (3 µm) and rinsed once more. Prior to the analyses, shell sections were examined under a fluorescence light stereomicroscope (Zeiss AxioImager A1m fluorescent microscope equipped with a Zeiss HBO 100 mercury lamp for UV light, and Zeiss filter set 18 with an excitation wavelength of ~450-500 nm and an emission wavelength of ~500-550 nm), which clearly highlighted the calcein marks, indicating the newly formed shell portion. Photographs were taken using a Canon EOS 600D digital camera connected to the microscope. Newly formed increment widths (total growth over 14 weeks) were measured using the free image processing software, ImageJ (National Institutes of Health, USA).

2. 3. Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS)

Element concentrations of sodium (measured as ^{23}Na), magnesium (^{25}Mg), strontium (^{86}Sr) and barium (^{137}Ba) were determined in 73 *A. islandica* shells (9 ± 2 specimens per treatment) at the Institute of Geosciences, University of Mainz. We used an Agilent 7500ce inductively coupled plasma-mass spectrometer (ICP-MS) coupled to an ESI NWR193 ArF excimer laser ablation (LA) system which was equipped with a TwoVol^2 ablation cell.

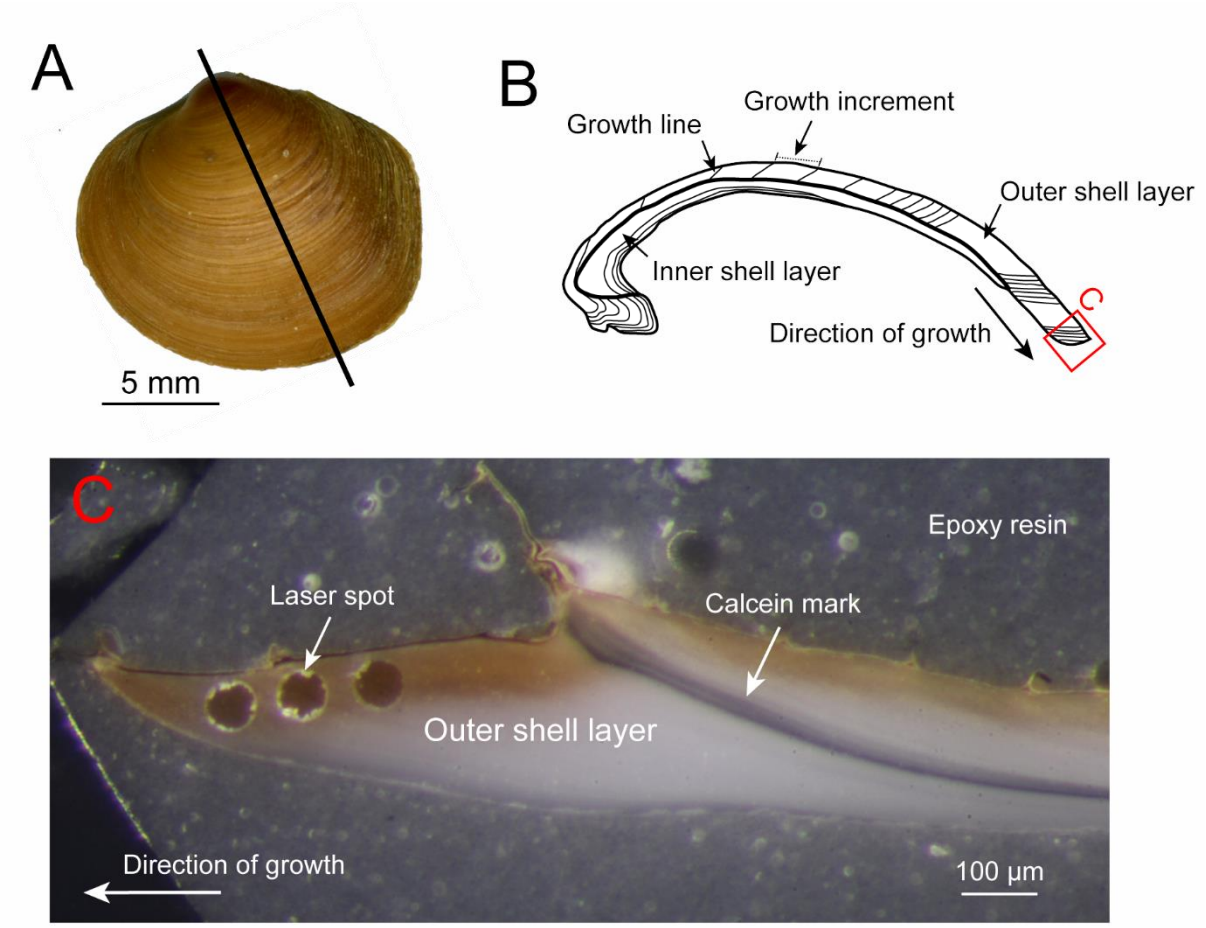


Fig. 1: Shell of juvenile *Arctica islandica* A) outer shell surface with line of maximum growth (black line) B) cross-sectioned shell with major structures C) magnification of the outer shell margin revealing calcein mark and laser spots.

The ArF LA system was operated at a pulse repetition rate of 10 Hz, an energy density of ~3 J/cm², and an ablation spot diameter of 55µm. Background measurement were performed for 20 s, followed by ablation times of 40 s, and wash out times of 20 s. Ablation was carried out under a He atmosphere and the sample gas was mixed with Ar before entering the plasma. For each shell, measurements were performed at three equidistant spots in the middle of the aragonite layer of the newly formed shell portion (Fig. 1). The multi-element synthetic glass NIST SRM 610 was used as calibration material, applying as the "true" concentrations the preferred values

reported in the GeoReM database (http://georem.mpch-mainz.gwdg.de/; Jochum et al., 2005, 2011). For all materials, ^{43}Ca was used as internal standard. For the reference materials, we applied the Ca concentrations reported in the GeoReM database, and for the samples 56.03 wt.%, the stoichiometric CaO content of $CaCO_3$. During each analytical session, we analyzed homogeneous basaltic USGS BCR-2G and synthetic carbonate USGS MACS-3 as quality control materials (QCMs). Reproducibility, which was expressed as the relative standard deviation based on repeated measurements of the QCM, was always better than 1.6 % for USGS BCR-2G (n = 9) and 7.5 % for MACS-3 (n = 9). The measured Na, Mg, Sr, and Ba concentrations of USGS BCR-2G agree within 1.4 %, 12.5 %, 2.1 %, and 0.5 % with the preferred values of the GeoReM database and within 1.1 %, 5.0 %, 0.6 %, and 0.7 % with the preliminary reference values for USGS MACS-3 (personal communication S. Wilson, USGS; Jochum et al., 2012). In the following, the average of the element concentrations (of the three spots measurements) are reported relative to Ca in mmol/mol for Sr, Mg and Na, and µmol/mol for Ba.

2.4. Shell microstructure

A. islandica produces a shell composed of a single calcium carbonate polymorph (aragonite) organized in layers characterized by different microstructures (Milano et al., 2017a). The outer portion of the outer shell layer (oOSL) consists of homogenous microstructure, whereas the inner portion of the outer shell layer (iOSL) and the inner shell layer (ISL) are dominated by crossed-acicular microstructures (Dunca et al., 2009; Schöne et al., 2013; Milano et al., 2017a). The homogenous microstructure is characterized by granular biomineral units distributed without a specific structural arrangement (Carter et al., 2012). The crossed-acicular microstructure contains elongated biomineral units obliquely aligned. The present study focuses on the ventral margin of

the shells outside the pallial line. In this area, the ISL is missing. SEM analyses were carried out in the iOSL which has been previously identified as being potentially sensitive to environmental changes in *Cerastoderma edule* (Milano et al., 2017b).

The microstructure of 32 *A. islandica* shells was analyzed using a Scanning Electron Microscope (SEM). On average, four specimens were investigated per treatment (4 ± 2). The selection of specimens was based on the amount of aragonite deposited during the experimental phase. Shells with limited or no growth were omitted from the analysis. To study the microstructures, samples were etched in 1 vol% HCl for 10 s and bleached in 6 vol% NaClO for 30 min. After being dried from air, the samples were coated with a 2 nm-thick platinum layer by using a sputter coater (Leica EM ACE200). The microstructures were qualitatively analyzed with a scanning electron microscope (LOT Quantum Design 2nd generation Phenom Pro desktop SEM) with backscattered electron detector and 10kV accelerating voltage. SEM images were taken over 100 µm away from the calcein line to avoid bias associated with the marking stress. In each specimen, the SEM images represent an area of ca. 2 mm^2 located in the middle of the shell portion formed during the experiment. In specimens with a large amount of aragonite deposited during the experiment, a second area was selected for SEM analysis ca. 300 µm away from the first area.

2.5. Shell growth

Shell growth was measured as the width between the ventral (outer) edge and the calcein mark that had formed at the start of the experimental period (mm; 93 days). In this study, shell growth is analogous to growth rate as all shells were grown over the same length of period.

223 *2.6. Condition Index*

224 Condition index (CI = dry soft tissue mass/ dry shell mass) of each specimen was calculated at
225 the end of the experimental period as a measure of their physiological state at the end of the
226 experiment. Dry weight was determined after drying the soft tissue at 60 ºC for 3 days.

227

228 *2.7. Statistical analysis*

229 Experimental data were analyzed with R version 3.2.2. Since individual specimens within one
230 experimental unit (aquarium) were interdependent pseudo-replicates, we calculated single
231 average values for trace elements, condition index, and shell growth for each aquarium. Then,
232 data were checked for normality (Shapiro–Wilk's test; $p < 0.05$) and homogeneity of variance
233 (Levene's *F*–test ; $p < 0.05$) before applying a two-way analysis of variance (ANOVA). ANOVA
234 was used to test for significant effects among the different food-temperature treatments. Ba/Ca
235 and Mg/Ca ratios were log-transformed to follow ANOVA assumptions.

236 Univariate relationships between the average Na/Ca, Mg/Ca, Sr/Ca and Ba/Ca ratios of *A.*
237 *islandica* shells per aquarium, environmental parameters (temperature and food), shell growth,
238 and condition index were estimated by means of Pearson correlation analysis. Statistically
239 significant differences were set at a $p < 0.05$.

240

241

Variable response	Effect	df	Sum Sq	Mean Sq	F-value	Pr (> F)
Na/Ca	Food (F)	2	8.6698	4.3344	3.0953	0.0699
	Temperature (T)	2	7.3328	3.6664	2.6182	0.1004
	Interaction F*T	4	2.2196	0.5549	0.3963	0.8087
	Residuals	18	25.2060	1.4003		
log(Mg/Ca)	Food	2	0.0558	0.0279	2.9061	0.0806
	Temperature	2	0.0242	0.0121	1.2607	0.3073
	Interaction F*T	4	0.0115	0.0029	0.2996	0.8743
	Residuals	18	0.1728	0.0096		
Sr/Ca	Food	2	0.0367	0.0183	1.0657	0.3652
	Temperature	2	0.1862	0.0931	5.4131	0.0144
	Interaction F*T	4	0.1174	0.0294	1.7066	0.1924
	Residuals	18	0.3096	0.0172		
log(Ba/Ca)	Food	2	0.0656	0.03281	0.9155	0.4182
	Temperature	2	0.1060	0.05298	1.4781	0.2545
	Interaction F*T	4	0.0769	0.01923	0.5366	0.7107
	Residuals	18	0.6451	0.03584		
CI	*Food*	2	79.0870	39.5430	24.8314	6.67E-06
	Temperature	2	3.5250	1.7630	1.1069	0.3521
	Interaction F*T	4	12.3600	3.0900	1.9403	0.1473
	Residuals	18	28.6650	1.5920		
GH	*Food*	2	5.4311	2.7155	22.0637	2.52E-05
	Temperature	2	1.4654	0.7327	5.9530	0.0117
	Interaction F*T	4	0.8693	0.2173	1.7658	0.1851
	Residuals	16	1.9692	0.1231		

242

243 **Table 2**: Two-way ANOVA test on the effects of temperature and food level over the different
244 response variables (n=27, 27 trials). Significant factors per model are highlighted in italic. CI =
245 condition index, GH = shell growth in height.

246 Finally, a multi-regression analysis was performed with all the individual data to identify
247 which factor/s (temperature, food, shell growth, and electric conductivity: EC) were
248 mathematically linked to the trace element content of the shells using the following equation:

249 $$y_i = \beta_0 + \beta_1 \times Temperature_i + \beta_2 \times Food_i + \beta_3 \times Shell\ growth_i + \beta_4 \times EC_i + \varepsilon_i$$

250 Where y_i was the trace element-to-calcium ratio of specimen i (i range 1–73), β_0 was the
251 intercept, β_{1-4} were the estimated coefficients of the different explanatory variables
252 (temperature, food concentration, shell growth, and EC, respectively), and ε_i was the model error
253 for each specimen (ε_i ~ N(0,1)). To avoid collinearity, explanatory variables were included in the
254 analyses only when they had a (Pearson) correlation coefficient ≤±0.5 (Graham 2003; Duncan
255 2011; Ieno and Zuur 2015).

3. Results

3.1. Experimental conditions

During the experiment, water temperature and salinity were constant in the different treatments of the experiment (Table 1). The concentration of phytoplankton cells, however, decreased with temperature (at the same food level). For instance, despite equal supply to the different temperature treatments, the average phytoplankton cells concentration at medium food level, varied from 6.19 (cells/L x 10^6) at 3 °C to 1.53 at 13 °C (Table 1).

The average shell growth increased with increasing food supply, from 0.28 to 1.82 mm in 14 weeks (3µm/day - 19µm/day; Table 1, Fig. 2A). At the lowest food level, shell growth was not affected by temperature, but temperature had a positive effect at medium and high food levels (Fig. 2A; Table 2). The condition index was not affected by temperature, but showed an increase with increasing food level (Fig. 2B; Table 2).

3.2. Sodium

In the different treatments, the average Na/Ca ranged between 23.27 and 25.98 mmol/mol (Table 1; Fig. 3A, B, C). The correlation matrix in Table 3 suggests that the average Na/Ca in the newly deposited carbonate is inversely related to temperature (r= -0.38) and positively related to food level (r =0.42). Shell growth at 3 and 8 °C showed higher Na/Ca values ($p = 0.07$; Table 2; Fig. 3A, B, C) with higher amounts of supplied food. Only at 13° C, the trend was not clear (Fig. 3C). When the Pearson coefficients were analyzed both food level and temperature showed a significant correlation with Na/Ca values (r =0.42 and r= -0.38 respectively, $p < 0.05$; Table 3). At increasing food levels, *A. islandica* shells showed higher Na/Ca values ($p = 0.07$; Table 2; Fig. 3A, B, C). Only at 13° C, the trend was not clear (Fig. 3C).

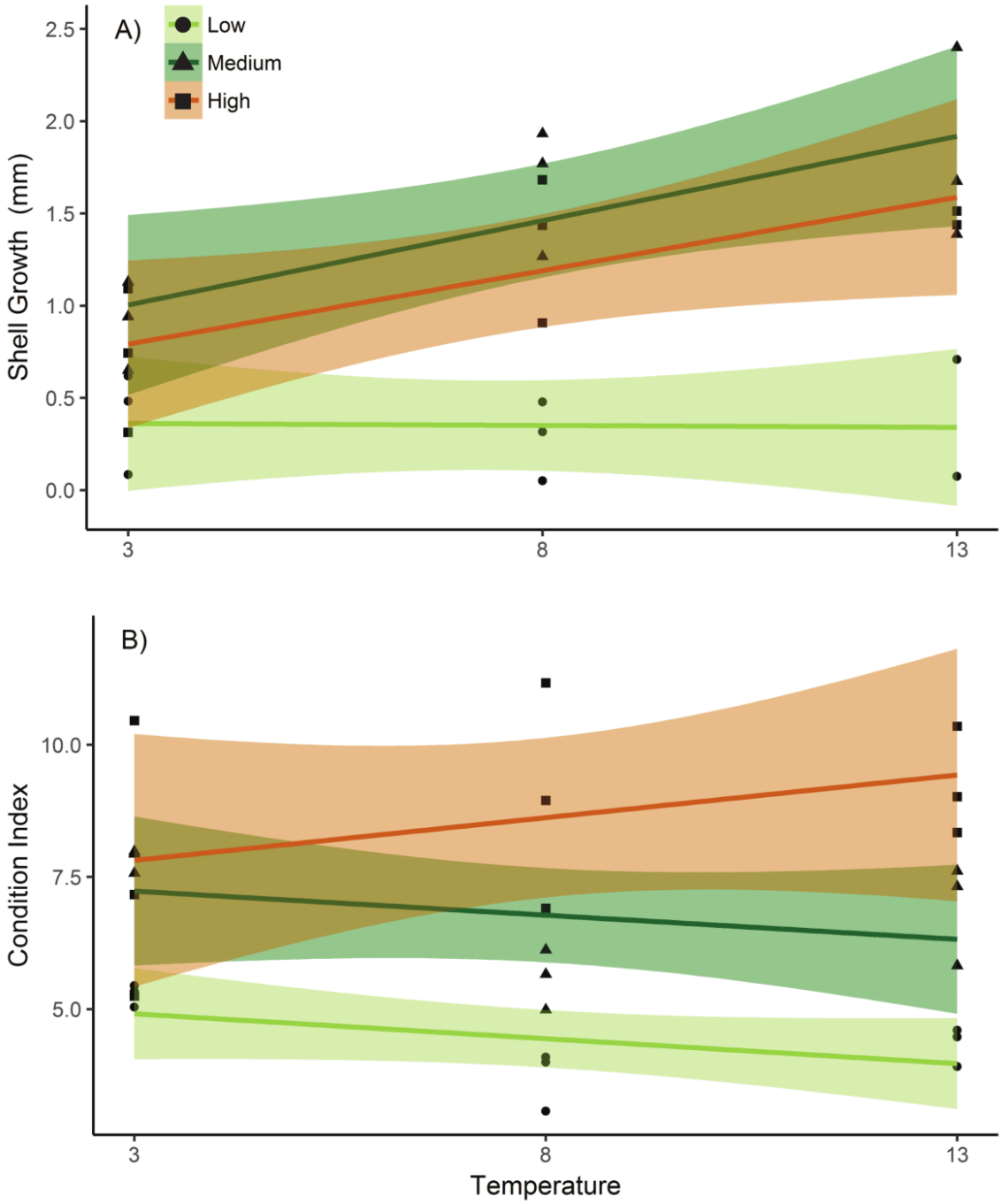


Fig. 2: Linear regression of the A) average shell growth and B) average condition index of *Arctica islandica* by treatment (3 temperature x 3 food level x 3 replicates = 27 observations). Shadow colors show confident intervals. Circle = low food, triangle = medium food, square = high food.

We calculated a linear multiple regression model (n = 73 observations) for Na/Ca ratios as a function of the supplied food, temperature, electrical conductivity (EC), and shell growth to identify whether external and internal factor(s) are linked to the incorporation of sodium into the shell carbonate. Na/Ca values are best explained by a combination of shell growth rate and temperature (Table 4, $p < 0.05$; Fig. 4A, B, C, D). A 32% of the variability in the Na/Ca (adjusted-R^2= 0.316) was explained by these two factors (correlation between these factors was 0.3). Although a univariate regression model suggested that Na and Condition Index (CI) were significantly correlated (r = 0.41; Table 3), CI was not included in the multiple regression model because this variable is directly related to shell growth.

	Temp	**Food**	**Na/Ca**	**log(Mg/Ca)**	**Sr/Ca**	**log(Ba/Ca)**	**GH**	**CI**
Temp		1	0.0487	0.6237	0.0727	0.3799	0.0392	0.9392
Food	0.00		0.0289	0.0384	0.4662	0.1989	0.0100	0.0000
Na/Ca	-0.38	0.42		0.0079	0.0013	0.0017	0.0982	0.0343
log(Mg/Ca)	-0.10	-0.40	-0.50		0.0043	0.0099	0.0011	0.0138
Sr/Ca	0.35	-0.15	-0.59	0.53		0.0694	0.1701	0.9619
log(Ba/Ca)	-0.18	-0.26	-0.58	0.49	0.35		0.1469	0.2598
GH	0.41	0.51	0.34	-0.62	-0.28	-0.30		0.0105
CI	-0.02	0.8	0.41	-0.47	-0.01	-0.22	0.50	

Table 3: Correlation per treatment (27 observations) among the average element-to-Ca ratios in the shell and food, temperature (Temp), and physiological factors (shell growth in height (GH) and condition index (CI) at the end of the experiment). The top right part shows the P-values of the corresponding correlations.

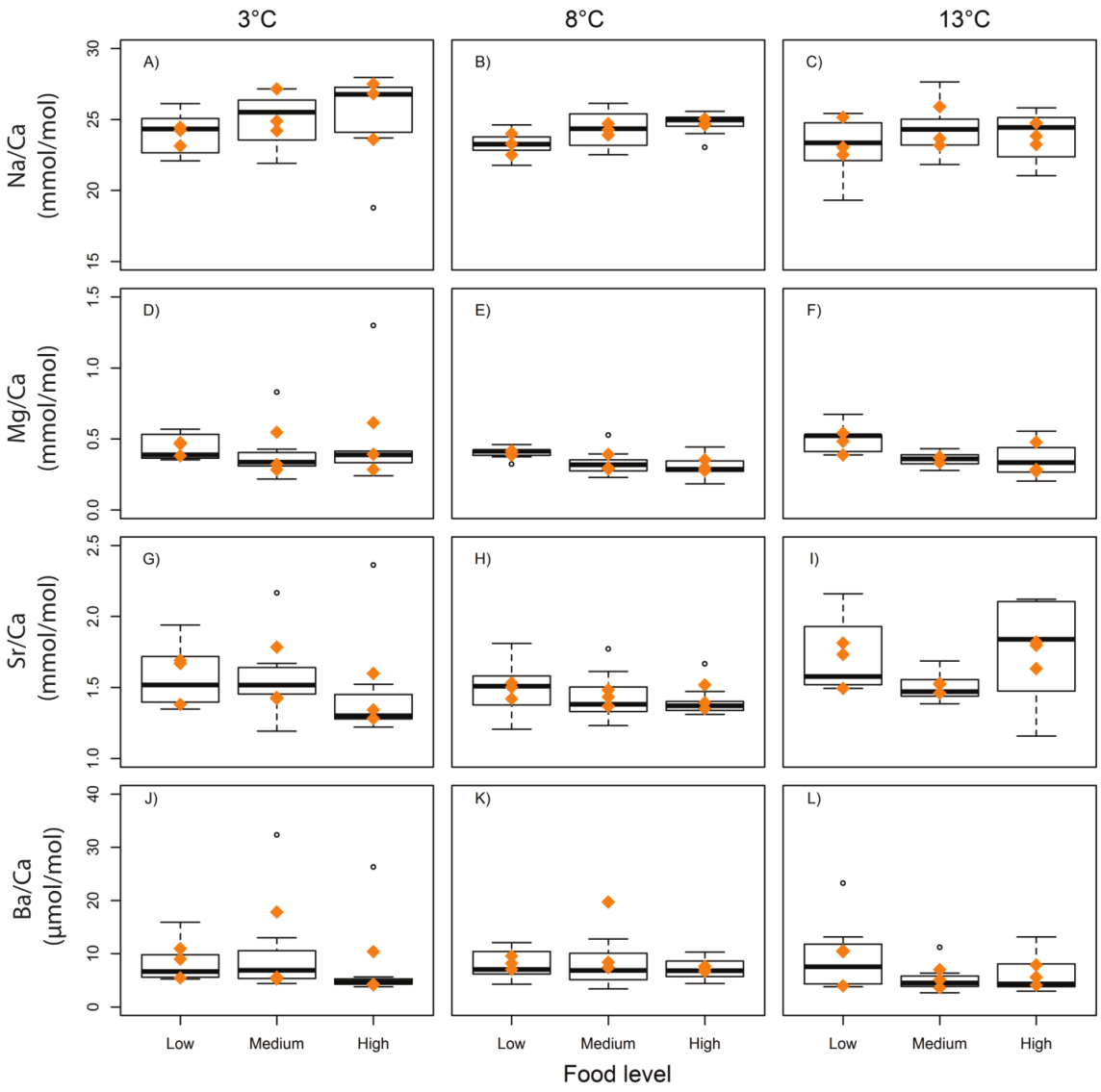


Fig. 3: Shell trace-element values per treatment. Orange diamonds indicate average per aquarium (data used for ANOVA analysis) and boxplot showed the inter-specimens variation (n = 73).

3.3. Magnesium

The average Mg/Ca of *A. islandica* varied between 0.31 and 0.47 mmol/mol (Table 1; Fig. 3D, E, F). The Mg/Ca correlated inverse but significantly with food level ($r = -0.40$ $p < 0.05$) and CI ($r = -0.47$, $p < 0.05$; Table 3) but in a two way ANOVA neither the temperature, food level nor the

interaction between these two factors showed statistically significant effects on the average Mg/Ca (Two-way ANOVA, $p > 0.05$; Table 2). The linear multiple regression model however identified shell growth rate as the most likely factor to explain the variation in Mg/Ca ratios between shells (adjusted-R^2= 0.234 , Table 4; Fig. 4E, F, G, H).

3.4. Strontium

Sr/Ca varied between 1.41 and 1.75 mmol/mol in the freshly grown shell material among the different treatments (Table 1; Fig. 3G, H, I). The Sr/Ca ratio only showed a weak correlation with temperature and had a Pearson correlation coefficient of r = 0.35 **(**p = 0.07; Table 3). The correlation with other factors (food level, condition index and shell growth) were insignificant. In the two way ANOVA (to test the effect of temperature, food and their interaction) only temperature showed a statistically significant effect on Sr/Ca (two-way ANOVA, $p \leq 0.05$; Table 2). The Sr-trend was negative between 3 and 8 °C but positive if we considered the entire temperature range (between 3-13° C), i.e., levels of Sr were lowest in shells grown at 8 °C.

When the role of external and internal factors were considered in a multiple regression model, the stepwise variable selection procedure included the variables growth rate and temperature (adjusted-R^2=15%, Table 4; Fig. 4I, J, K, L). Therefore, not only temperature, but also shell growth rate were statistically linked to Sr/Ca.

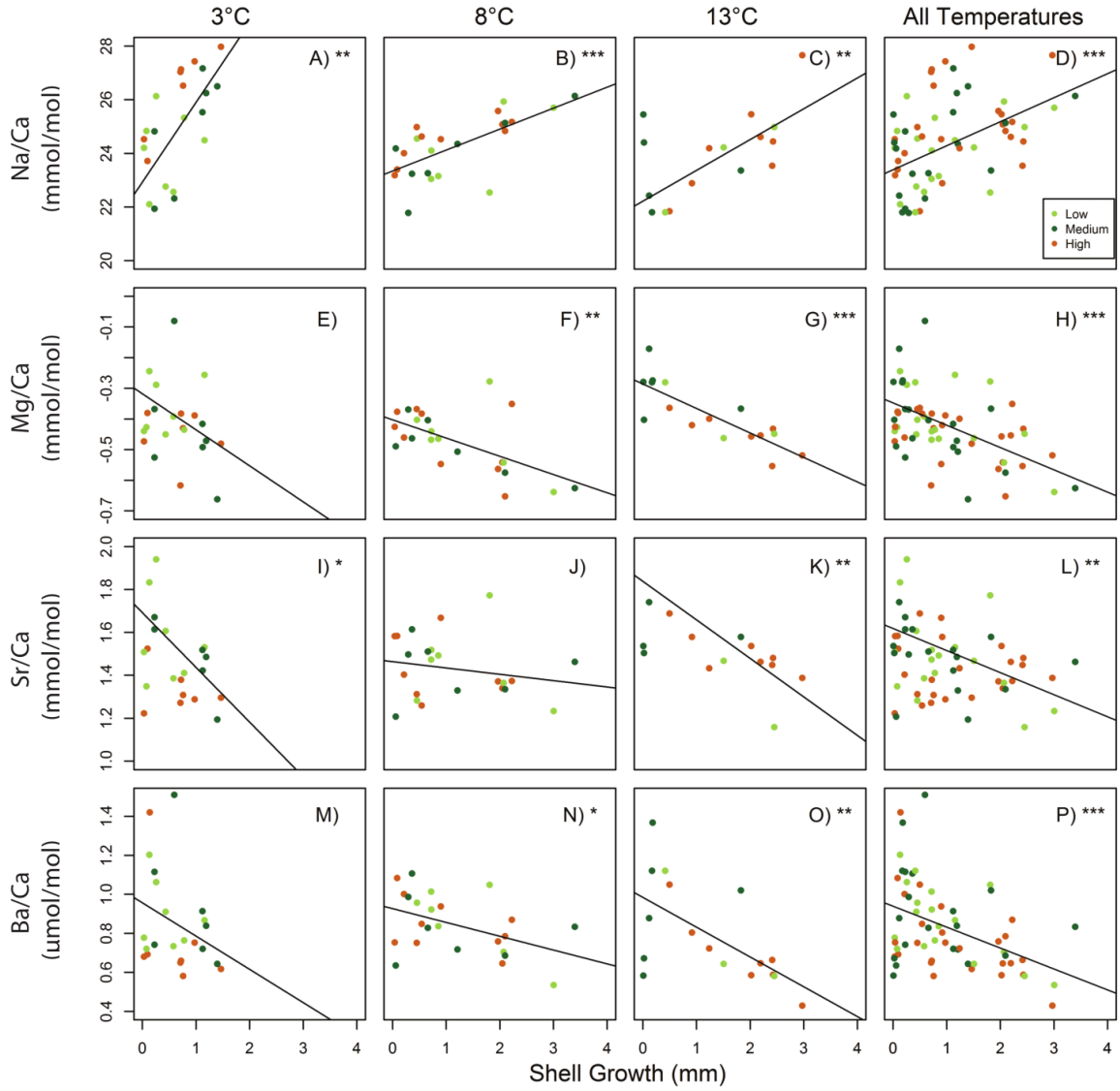
3.5. Barium

Ba/Ca ratios varied greatly among specimens of the same and different treatments. Mean values ranged from 5.26 to 11.84 µmol/mol (Table 1; Fig. 3J, K, L). However, some specimens had

peaks higher than 20 µmol/mol (Fig. 3J, K, L). Temperature, food level and the interaction between these factors did not have a statistically significant effect on Ba/Ca (Two-way ANOVA, $p > 0.05$; Table 2). When external and internal factors were considered in a multiple regression model, only shell growth rate was significantly linked to Ba/Ca (adjusted-R^2= 0.229, Table 4; Fig. 4M, N, O, P).

Model	Variables	R^2-adjusted	F-statistic
M_Na	Na ~ *Temperature* + Food + *GH* + EC	0.303	7.85
	Na ~ *Temperature* + *GH*	**0.316**	15.57
	Na ~ *GH*	0.173	14.19
	Na ~ *Temperature*	0.035	3.58
M_Mg	Log(Mg) ~ Temperature +Food + *GH* + EC	0.217	5.36
	Log(Mg) ~ Temperature + *GH*	0.235	10.71
	Log(Mg) ~ *GH*	**0.228**	19.70
	Log(Mg) ~ Temperature	0.000	0.46
M_Sr	Sr ~ Temperature + Food + *GH* + EC	0.125	3.25
	Sr ~ Temperature + *GH*	**0.149**	6.52
	Sr ~ *GH*	0.116	9.24
	Sr ~ Temperature	0.026	2.88
M_Ba	Log(Ba) ~Temperature + Food + *GH* + EC	0.144	3.64
	Log(Ba) ~Temperature + *GH*	0.236	10.71
	Log(Ba) ~ *GH*	**0.229**	19.70
	Log(Ba) ~Temperature	0.000	0.46

Table 4: Regression models for each trace element ratio with the significant effect variable highlighted in italic ($p < 0.05$). Stepwise selection (both directions) selected the model highlighted in bold for each element (n=73, 73 specimens). EC =electric conductivity (µS/cm) and GH = shell growth in height.



337

338 **Fig. 4:** Linear relationships between trace element ratios and shell growth in height (73

339 observations). Dot colors indicate the different food levels: low (light green), medium (dark

340 green) or high (orange). Statistically significant relationship are expresses as: '***' ($p < 0.001$)

341 '**'($p < 0.01$) '*' ($p < 0.05$).

342

343

In summary, shell carbonates that had been formed at lowest food concentration, leading to the lowest shell growth rates, usually incorporated the highest amounts of Mg, Sr and Ba in the shell. Only for Na the opposite was found (Fig. 3, Table 1). All trace element-to-calcium ratios were statistically significantly linked to shell growth rate (Fig. 4; Table 4). Moreover, the Na levels in the shell were also linked to food level and temperature, but Mg only to food, and Sr to temperature. None of the external factors studied (temperature, food, or EC) showed a significant influence on the incorporation of Ba into *A. islandica* shell material grown during this experiment.

3.6. Microstructure

The morphology of the shell microstructures did not significantly vary in relation to the different temperatures and food levels (Fig. 5). In the shells grown at 3 °C, the structural units were characterized by a bulky appearance with no considerable difference between specimens grown at food levels "low" and "medium" (Fig. 5B). Shells growth at the highest food level showed a slight increase in the size of individual biomineral units (Fig. 5B). A similar increase in biomineral unit size was observed in shells reared at 13 °C and medium food level. A minor shift toward more elongated microstructural units was visible in shells exposed to a water temperature of 8 °C and medium food level (Fig. 5B). The most significant morphological difference in shell microstructure was detected in shells grown at 13 °C and low food and in shells reared at 8 °C and high food (Fig. 5B). In these cases, the microstructures resembled a well-pronounced crossed-acicular appearance commonly found in the ISL. However, this microstructural variation was not shared by all shells grown at the same treatment. Therefore, on basis of SEM we could not detect a significant variation of *A. islandica* shell microstructures reared at different temperatures and food levels.

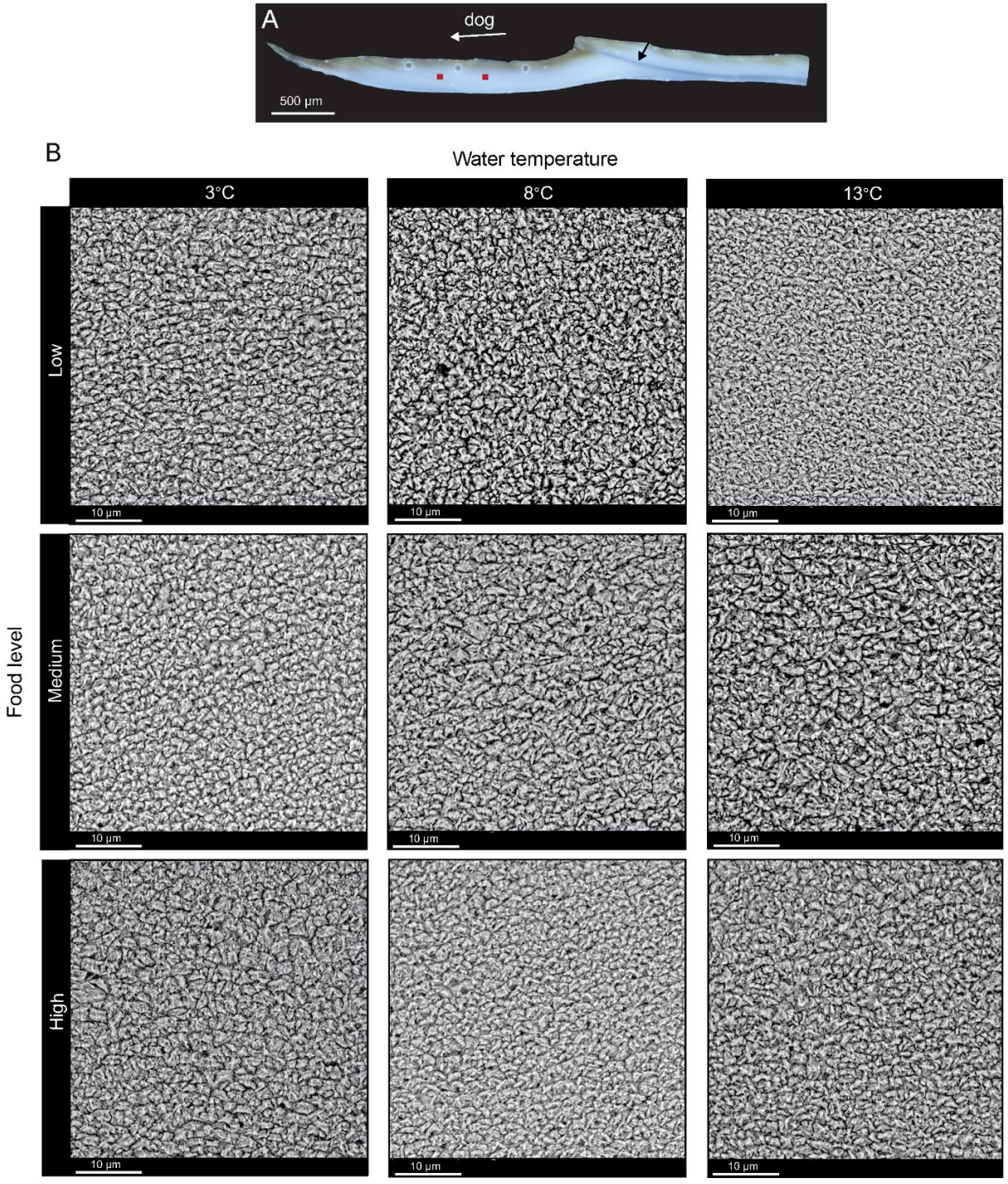


Fig. 5: Microstructural organization of *A. islandica* reared in different environmental conditions. (A) Shell slab with well visible calcein line marking the start of the experiment (black arrow). The red squares indicate the approximate location of the SEM images. The three circular spots on the shell surface are the marks left behind by the LA-ICP-MS measurements. dog = direction of growth (B) Microstructures formed during the exposure at different water temperatures (3 °C, 8 °C, 13 °C) and food levels (low, medium, and high).

4. Discussion

This study demonstrates that shell growth rate exerts a large control on incorporation of Sr, Mg, Ba and Na into shells (Table 4). The effect of temperature and food on shell chemistry seems overarched by shell growth and varied among the studied elements. Na seems to be affected by a combination of food and temperature, Mg by food, Sr by temperature, and for Ba, we could not demonstrate any effect of food or temperature (Table 3). Therefore, the findings of our study suggest that trace element incorporation into the shell of *A. islandica* is controlled by a complex interplay between environmental and physiological processes. Moreover, the comparison of the microstructural properties of shell growth by SEM could not find external or internal factors affecting *A.islandica* shell microstructural properties.

4.1. Controls on trace elemental incorporation into Arctica islandica shells

In many organisms, Sr/Ca and Mg/Ca ratios serve as useful paleo-temperature proxies (e.g., foraminifera, Nürnberg et al., 1996; corals, Goodkin et al., 2007; sclerosponges, Rosenheim et al., 2004; bivalves, Zhao et al., 2017b). As shown by several studies, in the bivalve *A. islandica* vital effects have an important effect on the incorporation of these two trace elements into the shell (Toland et al., 2000; Foster et al., 2008, 2009; Schöne et al., 2011, 2013; Marali et al., 2017a; Wanamaker and Gillikin, 2018). Most of these studies, which are based on small samples sizes (3-8 specimens vs. 73 this study), concluded that it is unclear whether these metal-to-calcium ratios in the shells of *A. islandica* can be used as temperature proxies. After mathematically eliminating effects of ontogenetic age and growth rate, Schöne et al., (2011) found a significant negative correlation between temperature and Sr/Ca and Mg/Ca ratios (explaining 41 and 27 % of the variability, respectively). In our controlled laboratory study, the

amount of Sr incorporated into *A. islandica* was, however, positively and significantly ($r = 0.35$; $p < 0.05$; Table 3) correlated to seawater temperature (Table 2, 3). The difference between both studies could be due to the fact that we used juveniles and not adults, that the specimens came from different populations (Baltic Sea vs. Iceland), or to the small sample size analyzed by Schöne et al., (2011). Hart and Bluzstajn (1998) found, as in this study, a positive relationship between Sr/Ca ratios and temperature in *A. islandica.* Similar observations were previously reported for other bivalve species by Lorrain et al., (2005), Gillikin et al., (2005) and Izumida et al., (2011). Because thermodynamics predict a negative correlation between Sr/Ca and temperature in aragonite, our results corroborate the idea that biological processes play a dominant role in the incorporation of Sr in *A. islandica* shells (Gillikin et al, 2005; Izumida et al., 2011), which is supported by the strong negative relation between shell growth and Sr concentration (Fig. 4).

In contrast to Sr/Ca, and despite the broad range of temperatures tested in the present study (3 to 13 °C), Mg/Ca was significantly correlated to food level ($r=-0.40$), shell growth ($r=-0.62$) and CI ($r=-0.67$) rather than temperature (Table 3; Fig. 4E, F, G, H). Moreover, multiple regression analysis showed that shell growth rate explained 23 % of the Mg/Ca variability, and identified the effect of temperature as being negligible in our data set (Table 4). Thus, it appears that the Sr/Ca and Mg /Ca ratios of *A. islandica* shells to a large proportion reflect physiological processes and do not exclusively reflect water temperature (Gillikin et al., 2005; Freitas et al., 2006; Elliot et al., 2009; Foster et al., 2008, 2009; Wanamaker et al., 2008; Geeza et al., 2018; Wanamaker and Gillikin, 2018).

Although Ba/Ca ratios in some marine bivalves are used as proxy for paleo-productivity (e.g., Stecher et al., 1996; Vander Putten et al., 2000; Gillikin et al., 2008; Thébault et al., 2009; Hatch

et al., 2013), we could not find a clear relationship between Ba/Ca ratios of our experimental *A. islandica* shells and food availability. The results however again suggest that Ba levels were related to shell growth rate, which could explain 23% of the variability in the shell Ba concentration, comparable to what has been found for the freshwater pearl mussel *Hyriopsis* sp. (Izumida et al., 2011). The large Ba peaks detected in some of our specimens did not show any pattern in relationship to the food concentration where those bivalves grew, instead we observed strong Ba/Ca peaks even at the lowest food level (Fig. 3J, K, L). Moreover, not all specimens from the same aquarium (or same treatment) showed large Ba peaks, as it was previously reported about wild populations of *A. islandica* (Gillikin et al., 2008; Marali et al., 2017a, b). Although we kept the food concentration constant (per treatment) over the entire experimental period, and the food used was chemically homogenous, slight variations in the food supply could be the reason for the asynchrony of Ba peaks between individuals. Additionally, the time lag between feeding and incorporation of Ba into the shell, i.e., the moment that the animals really deposited their shell, can differ between specimens and these differences can be more evident in studies of short-period of time such as ours (experimental period =14 weeks). Moreover, since different specimens had different shell growth rates the laser spots made to sublimate the carbonate might have reflected different periods or period lengths and thus have led to different sampled time frames or time frames with different time resolution (this could apply to all trace-elements analyzed). To get better insight about the timing of Ba peaks, constant series of laser spots should have been done over all the newly formed shell. Therefore, even though we did not find synchrony in the Ba peaks among specimens, we cannot exclude it either.

Interestingly, Na/Ca of *A. islandica* exhibited a significant correlation with food ($r = 0.42$) and temperature ($r = -0.38$; Table 3). When several different external and internal factors were

simultaneously considered in a multiple regression model, shell growth and temperature explained about 1/3 of the variability of Na/Ca (adjusted-R^2 = 32 %, Table 4). Since salinity was kept stable during the course of the experiment (indicating that variation of Na/Ca ratio in the water was small), water chemistry interference on Na/Ca variations are unlikely (Table 2, 4). A possible explanation for the observed correlation between food and Na/Ca or temperature and Na/Ca (Table 3) could be that Na/Ca reflects the metabolic rate of *A. islandica*, with the latter being strongly affected by temperature and food supply (Winter 1978). Support for such an interpretation comes from the finding that the condition index was also significantly correlated with Na/Ca ($r= 0.41$; $p <0.05$). Zhao et al., (2017a) demonstrated that Na/Ca is related to the acid-base and ionic regulation in the calcifying fluid of *Mytilus edulis* shells, a process strongly linked to biomineralisation and highly dependent on the metabolic activity. Hence, when the change of Na/Ca_{water} is small, physiological influences may exert a major control on the incorporation of Na into bivalve shells.

Our findings seem to indicate that, under food limitation, *A. islandica* is less efficient in discriminating against trace and minor element impurities of the carbonate matrix and that physiological processes play an important role in the control on elemental incorporation into *A. islandica* shells.

4.2. Controls on microstructural characteristics of A. islandica shells

According to the results of the present study, the shell microstructure of *Arctica islandica* was not significantly affected by water temperature or dietary regimes. Although some slight differences were visible among the treatments (i.e., biomineral size increase and shape variation), the lack of consistency of these specific alterations suggest that the observed differences may not be related to

464 the two studied environmental variables. The small morphological variability may be explained by
465 differences among specimens. Our observations are in good agreement with Milano et al., (2017a)
466 which results also indicated that the morphology of biomineral units in *A. islandica* shells is not
467 influenced by water temperature or diet. Note that in their study, the role of these two
468 environmental factors on crystal morphology were investigated in separate experiments whereas
469 we went further in the current research by studying the effect of both environmental variables in
470 one experiment. We did not find, however, an interaction effect on crystal morphology, size, or
471 orientation at the temperature and food conditions considered.

472 Previously, microstructures of other mollusk species were shown to be influenced by the
473 environment (Hedegaard et al., 2006; Nehrke and Nouet, 2011; Soldatov et al.., 2013; Stemmer
474 and Nehrke, 2014; Milano et al., 2017a). For instance, water temperature was identified as the
475 major factor controlling size and shape of individual biomineral units in the non-denticular
476 composite prismatic microstructure of *Cerastoderma edule* (Milano et al., 2017b). Similarly, the
477 relative thickness of the composite prismatic layer of *Scapharca broughtonii* was shown to be
478 negatively correlated with water temperature in naturally and laboratory-based grown specimens
479 (Nishida et al., 2012, 2015). In these species, the sensitivity of the shell microstructure to
480 environmental fluctuations, especially temperature, offers the potential to use microstructural
481 properties as environmental proxies. However, the possibility of using shell architecture for
482 paleoenvironmental reconstructions largely depends on the type of microstructure considered, the
483 species under study, and the methodology applied (Nishida et al., 2012; Milano et al., 2017 b;
484 Purroy et al., 2018). Among the different mollusk species, microstructures are highly diversified,
485 coming with different morphometric and mineralogical properties (Nishida et al., 2012; Milano et
486 al., 2017 b). In the case of homogenous microstructures as in *A. islandica,* the lack of a specific
487 alignment among the biomineral units together with their irregular shape, challenges the

identification of potential structural changes. Unlike prismatic and crossed-lamellar structures, where variations in morphometric and alignment parameters can be easily detected using SEM, *A. islandica* microstructures may require assessments on the crystallophic properties, more than from the morphometric ones (Milano et al., 2017a).

5. Conclusions

Factors influencing the incorporation of trace elements into biogenetic carbonates are complex (Stecher et al., 1996) and species-specific (Gillikin et al., 2005; Freitas et al., 2006; Zhao et al., 2017b). Although element-to-calcium ratios in *A. islandica* shells contain environmental information, this information cannot be easily distinguished from physiological controls (mainly shell growth rate). Specifically, the pathways of elements from the water and food into the shell needs further study. Our study, however, supports the conclusion of Wanamaker and Gillikin (2018) that at present, there is not yet enough and consistent information that would allow to use trace element-to-calcium ratios of *A. islandica* shells as reliable environmental proxies.

With the SEM technique used in this study, we could not find a significant variation in the morphometric characteristics of *A. islandica* microstructures relating on the studied environmental variables (i.e. temperature and food). We think, however, that subsequent studies with different and more advanced methods (for instance: Confocal Raman Microscopy and Electron Backscatter Diffraction; Milano et al., 2017a) can identify physical properties of microstructures as proxies for paleonvironmental reconstructions.

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References

Abele et al., 2009. Bivalve models of aging and the determination of molluscan lifespans. Exp. gerontol. 44: 307–315.doi: 10.1016/j.exger.2009.02.012

Ambrose et al., 2012. Growth line deposition and variability in growth of two circumpolar bivalves (*Serripes groenlandicus*, and *Clinocardium ciliatum*). Polar Biol. 35: 345–354. doi: 10.1007/s00300-011-1080-4

Ballesta-Artero et al., 2018. Interactive effects of temperature and food availability on the growth of *Arctica islandica* (Bivalvia) juveniles. Mar. Environ. Res. 133:67–77. doi: 10.1016/j.marenvres.2017.12.004

Butler et al., 2013. Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve *Arctica islandica*. Palaeogeogr Palaeoclimatol Palaeoecol 373:141–151. doi:10.1016/j.palaeo.2012.01.016

Carter et al., 2012. Illustrated glossary of the Bivalvia. Treatise Online: 1-209.

Dodd, 1965. Environmental control of strontium and magnesium in *Mytilus*. Geochim. Gosmochim. Acta. 29: 385–398. doi: 10.1016/0016-7037(65)90035-9

Dunca et al., 2009. Using ocean quahog (*Arctica islandica*) shells to reconstruct palaeoenvironment in Öresund, Kattegat and Skagerrak, Sweden. Int J Earth Sci 98:3–17. doi:10.1007/s00531-008-0348-6

Duncan, 2011. Healthcare risk adjustment and predictive modeling. Actex Publications.

Elliot et al., 2009. Profiles of trace elements and stable isotopes derived from giant long-lived *Tridacna gigas* bivalves: potential applications in paleoclimate studies. Palaeogeogr. Palaeoclimatol. Palaeoecol. 280: 132–142. doi: 10.1016/j.palaeo.2009.06.007

Foster et al., 2009. Strontium distribution in the shell of the aragonite bivalve *Arctica islandica*. Geochem. Geophys. Geosyst. 10(3). doi: 10.1029/2007GC001915

Foster et al., 2008. Mg in aragonitic bivalve shells: Seasonal variations and mode of incorporation in *Arctica islandica*. Chem. Geol. 254: 113–119. doi: /10.1016/j.chemgeo.2008.06.007

Freitas et al., 2006. Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. Geochim. Gosmochim. Acta. 70: 5119–5133. doi: 10.1016/j.gca.2006.07.029

Freitas et al., 2005. Mg/Ca, Sr/Ca, and stable-isotope (δ18O and δ13C) ratio profiles from the fan mussel *Pinna nobilis*: Seasonal records and temperature relationships. Geochem. Geophys. Geosyst. 6(4). doi: 10.1029/2004GC000872

Geeza, et al., 2018. Controls on magnesium, manganese, strontium, and barium concentrations recorded in freshwater mussel shells from Ohio. Chem. Geol. doi: 10.1016/j.chemgeo.2018.01.001

Gillikin et al., 2008. Synchronous barium peaks in high-resolution profiles of calcite and aragonite marine bivalve shells. Geo-Mar. Lett. 28: 351–358. doi: 10.1007/s00367-008-0111-9

555 Gillikin et al., 2006. Barium uptake into the shells of the common mussel (*Mytilus edulis*) and the
556 potential for estuarine paleo-chemistry reconstruction. Geochim. Gosmochim. Acta. 70: 395–
557 407. doi: 10.1016/j.gca.2005.09.015

558 Gillikin et al., 2005. Strong biological controls on Sr/Ca ratios in aragonitic marine bivalve shells.
559 Geochem. Gosmochim. Acta. 6 (5). doi: 10.1029/2004GC000874

560 Goodkin et al., 2007. A multicoral calibration method to approximate a universal equation relating
561 Sr/Ca and growth rate to sea surface temperature. Paleoceanography 22. doi:
562 10.1029/2006PA001312

563 Graham, 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84:2809–
564 2815. doi:10.1890/02-3114

565 Hart and Blusztajn, 1998. Clams as recorders of ocean ridge volcanism and hydrothermal vent field
566 activity. Science 280: 883–886. doi: 10.1126/science.280.5365.883

567 Hatch et al., 2013. Ba/Ca variations in the modern intertidal bean clam *Donax gouldii*: An
568 upwelling proxy. Palaeogeogr. Palaeoclimatol. Palaeoecol. 373: 98–107. doi:
569 10.1016/j.palaeo.2012.03.006

570 Hedegaard et al., 2006.Molluscan shell pigments: An in situ resonance Raman study, J. Mollus.
571 Stud. 72: 157–162. doi:10.1093/mollus/eyi062

572 Ieno and Zuur, 2015. A beginner's guide to data exploration and visualization with R. Highland
573 Statistics Ltd., Newburgh, United Kingdom

574 Izumida et al., 2011. Biological and water chemistry controls on Sr/Ca, Ba/Ca, Mg/Ca and $\delta^{18}O$
575 profiles in freshwater pearl mussel *Hyriopsis* sp. Palaeogeogr. Palaeoclimatol. Palaeoecol.309:
576 298–308. doi: 10.1016/j.palaeo.2011.06.014

577 Jochum et al., 2012. Accurate trace element analysis of speleothems and biogenic calcium
578 carbonates by LA-ICP-MS. Chem. Geol. 318-319, 31-44. doi:
579 10.1016/j.chemgeo.2012.05.009

580 Jochum et al., 2011. Determination of reference values for NIST SRM 610-617 glasses following
581 ISO guidelines. Geostand. Geoanal. Res. 35: 397-429. doi: 10.1111/j.1751-
582 908X.2011.00120.x

583 Jochum, et al., 2005. GeoReM: a new geochemical database for reference materials and isotopic
584 standards. Geostand. Geoanal. Res. 29: 87-133. doi: 10.1111/j.1751-908X.2005.tb00904.x

585 Klein et al., 1996. Sr/Ca and $^{13}C/^{12}C$ ratios in skeletal calcite of *Mytilus trossulus*: Covariation with
586 metabolic rate, salinity, and carbon isotopic composition of seawater. Geochim. Gosmochim.
587 Acta. 60: 4207–4221. doi: 10.1016/S0016-7037(96)00232-3

588 Klünder et al., 2008. Laser ablation analysis of bivalve shells – archives of environmental
589 information. Geol. Surv. Denm. Greenl. Bull. 15: 89–92.

590 Linard et al., 2011. Calcein staining of calcified structures in pearl oyster *Pinctada margaritifera*
591 and the effect of food resource level on shell growth. Aquaculture 313: 149–155.
592 doi:10.1016/j.aquaculture.2011.01.008

593 Lorens and Bender, 1980. The impact of solution chemistry on *Mytilus edulis* calcite and aragonite.
594 Geochim. Gosmochim. Acta. 44: 1265–1278. doi: 10.1016/0016-7037(80)90087-3

595 Lorrain et al., 2005. Strong kinetic effects on Sr/Ca rations in the calcitic bivalve *Pecten maximus*.
596 Geology 33: 965–968. doi: doi.org/10.1130/G22048.1

597 Lutz, 1984. Paleoecological implications of environmentally controlled variation in molluscan
598 shell microstructure, Geobios 17: 93–99. doi:10.1016/S0016-6995(84)80161-8

599 Marali et al., 2017a. Reproducibility of trace element time-series (Na/Ca, Mg/Ca, Mn/Ca, Sr/Ca,
600 and Ba/Ca) within and between specimens of the bivalve *Arctica islandica*–A LA-ICP-MS line
601 scan study. Palaeogeogr. Palaeoclimatol. Palaeoecol. 484: 109–128. doi:
602 10.1016/j.palaeo.2016.11.024

603 Marali, et al., 2017b. Ba/Ca ratios in shells of *Arctica islandica*—Potential environmental proxy
604 and crossdating tool. Palaeogeogr. Palaeoclimatol. Palaeoecol. 465: 347–361. doi:
605 10.1016/j.palaeo.2015.12.018

606 Milano et al., 2017a. The effects of environment on *Arctica islandica* shell formation and
607 architecture. Biogeosciences 14: 1577–1591. doi:10.5194/bg-14-1577-2017

608 Milano et al., 2017b. Changes of shell microstructural characteristics of *Cerastoderma edule*
609 (Bivalvia) - A novel proxy for water temperature. Palaeogeogr. Palaeoclimatol. Palaeoecol. 465:
610 395–406. doi:10.1016/j.palaeo.2015.09.051

611 Nehrke and Nouet, 2011. Confocal Raman microscope mapping as a tool to describe different
612 mineral and organic phases at high spatial resolution within marine biogenic carbonates: case
613 study on *Nerita undata* (Gastropoda, Neritopsina). Biogeosciences 8: 3761–3769.
614 doi:10.5194/bg-8-3761-2011

615 Nishida et al., 2015. Thermal dependency of shell growth, microstructure, and stable isotopes in
616 laboratory-reared *Scapharca broughtonii* (Mollusca: Bivalvia). Geochem. Geophys.
617 Geosystems 16: 2395–2408. doi:10.1002/2014GC005684

618 Nishida et al., 2012. Seasonal changes in the shell microstructure of the bloody clam, *Scapharca*
619 *broughtonii* (Mollusca: Bivalvia: Arcidae). Palaeogeogr. Palaeoclimatol. Palaeoecol. 363–364,
620 99–108. doi:10.1016/j.palaeo.2012.08.017

621 Nürnberg et al., 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy
622 for water mass temperatures. Geochim. Gosmochim. Acta., 60: 803–814. doi: 10.1016/0016-
623 7037(95)00446-7

624 O'Neil and Gillikin, 2014. Do freshwater mussel shells record road-salt pollution? Sci. Rep. 4: 7168.
625 doi: 10.1038/srep07168

626 Poulain et al., 2015. An evaluation of Mg/Ca, Sr/Ca, and Ba/Ca ratios as environmental proxies in
627 aragonite bivalve shells. Chem. Geol. 396: 42–50. doi: 10.1016/j.chemgeo.2014.12.019

628 Purroy et al., 2018. Drivers of shell growth of the bivalve, *Callista chione* (L. 1758)–Combined
629 environmental and biological factors. Mar. Environ. Res. 134:138–149. doi:
630 10.1016/j.marenvres.2018.01.011

631 Purton et al., 1999. Metabolism controls Sr/Ca ratios in fossil aragonitic mollusks. Geology 27:
632 1083-1086. doi: 10.1130/0091-7613(1999)027%3C1083:MCSCRI%3E2.3.CO;2

633 Rosenheim et al., 2004. High resolution Sr/Ca records in sclerosponges calibrated to temperature
634 in situ. Geology 32: 145–148. doi: 10.1130/G20117.1

635 Rucker and Valentine, 1961. Salinity response of trace element concentration in *Crassostrea*
636 *virginica*. Nature, 190: 1099. doi:10.1038/1901099a0

637 Schöne et al., 2013. Crystal fabrics and element impurities (Sr/Ca, Mg/Ca, and Ba/Ca) in shells of
638 *Arctica islandica*—Implications for paleoclimate reconstructions. Palaeogeogr. Palaeoclimatol.
639 Palaeoecol. 373: 50–59. doi:10.1016/j.palaeo.2011.05.013

640 Schöne et al., 2011. Sr/Ca and Mg/Ca ratios of ontogenetically old, long-lived bivalve shells
641 (*Arctica islandica*) and their function as paleotemperature proxies. Palaeogeogr.
642 Palaeoclimatol. Palaeoecol. 302: 52–64. doi: 10.1016/j.palaeo.2010.03.016

643 Schöne et al., 2010. Effect of organic matrices on the determination of the trace element chemistry
644 (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (*Arctica islandica*)—Comparison of ICP-
645 OES and LA-ICP-MS data. Geochem. J. 44: 23–37. doi: 10.2343/geochemj.1.0045

646 Soldatov et al., 2013. Qualitative composition of carotenoids, catalase and superoxide dismutase
647 activities in tissues of bivalve mollusc *Anadara inaequivalvis* (Bruguiere, 1789), J. Evol.
648 Biochem. Phys., 49: 3889–398. doi:10.1134/S0022093013040026

649 Stecher et al., 1996. Profiles of strontium and barium in *Mercenaria mercenaria* and *Spisula*
650 *solidissima* shells. Geochim. Gosmochim. Acta. 60: 3445–3456. doi: 10.1016/0016-
651 7037(96)00179-2

652 Stemmer and Nehrke, 2014. The distribution of polyenes in the shell of *Arctica islandica* from
653 North Atlantic localities: a confocal Raman microscopy study. J. Molluscan Stud. 80: 365–
654 370, https://doi.org/10.1093/mollus/eyu033

Strasser et al., 2008. Temperature and salinity effects on elemental uptake in the shells of larval and juvenile softshell clams *Mya arenaria*. Mar. Ecol. Prog. Ser. 370, 155–169. doi: 10.3354/meps07658

Surge and Walker, 2006. Geochemical variation in microstructural shell layers of the southern quahog (*Mercenaria campechiensis)*: Implications for reconstructing seasonality. Palaeogeogr. Palaeoclimatol. Palaeoecol. 237: 182–190. doi: /10.1016/j.palaeo.2005.11.016

Tan Tiu, 1988. Temporal and spatial variation of shell microstructure of *Polymesoda caroliniana* (Bivalvia: Heterodonta). Am. Malacol. Bull. 6: 199–206.

Tan Tiu and Prezant, 1989. Temporal variation in microstructure of the inner shell surface of *Corbicula fluminea* (Bivalvia: Heterodonta), Am. Malacol. Bull. 7: 65–71.

Tan Tiu and Prezant, 1987. Shell microstructural responses of *Geukensia demissa granosissima* (Mollusca: Bivalvia) to continual submergence, Am. Malacol. Bull. 5: 173–176.

Thébault et al., 2009. Barium and molybdenum records in bivalve shells: Geochemical proxies for phytoplankton dynamics in coastal environments? Limnol. Oceanogr. 54: 1002–1014. doi: 10.4319/lo.2009.54.3.1002

Toland et al., 2000. A study of sclerochronology by laser ablation ICP-MS. J. Anal., At. Spectrom. 15: 1143–1148. doi: 10.1039/b002014l

Urey et al., 1951. Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States. Bull. Geol. Soc. Am. 62: 399–416. doi: 10.1130/0016-7606(1951)62[399:MOPATO]2.0.CO;2

675 Vander Putten et al., 2000. High resolution distribution of trace elements in the calcite shell layer
676 of modern *Mytilus edulis*: Environmental and biological controls. Geochim. Gosmochim.
677 Acta. 64: 997–1011. doi: 10.1016/S0016-7037(99)00380-4

678 Wanamaker and Gillikin. 2018. Strontium, magnesium, and barium incorporation in aragonitic
679 shells of juvenile *Arctica islandica*: Insights from temperature controlled experiments. Chem.
680 Geol. doi: 10.1016/j.chemgeo.2018.02.012.

681 Wanamaker et al., 2008. Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve
682 calcite for *Mytilus edulis*: implications for paleotemperature reconstructions. Geo-Mar. Lett.
683 28: 359–368. doi: 10.1007/s00367-008-0112-8

684 Winter, 1978. A review on the knowledge of suspension-feeding in lamellibranchiate bivalves,
685 with special reference to artificial aquaculture systems. Aquaculture 13: 1–33. doi:
686 10.1016/0044-8486(78)90124-2.

687 Zhao et al., 2017a. Insights from sodium into the impacts of elevated pCO_2 and temperature on
688 bivalve shell formation. J. Exp. Mar. Biol. Ecol. 486: 148–154. doi:
689 10.1016/j.jembe.2016.10.009

690 Zhao et al., 2017b. Controls on strontium and barium incorporation into freshwater bivalve shells
691 (*Corbicula fluminea*). Palaeogeogr. Palaeoclimatol. Palaeoecol. 465: 386–394. doi:
692 10.1016/j.palaeo.2015.11.040

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