

May 25th, 2014

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Dear Editor and Referees,

We enclosed a revised version of our manuscript. Please see the responses below. We express our deep appreciation to editor and referees of your time and energy to the task of improving our manuscript.

Sincerely yours,

Toshihiro Yoshimura

Japan Agency for Marine-Earth Science and Technology (JAMSTEC) 2-15, Natsushima-cho,
Yokosuka-city, Kanagawa, 237-0061, Japan

phone: +81.46.867.9783

e-mail: yoshimurat@jamstec.go.jp

1 P. 1614, L. 25: This sentence should probably have a reference.

2 → We added some papers as suggested; Corrège, T.: Sea surface temperature and salinity
3 reconstruction from coral geochemical tracers, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*,
4 232, 408–428, 2006; Henderson, G. M.: New oceanic proxies for paleoclimate. *Earth*
5 *Planet. Sci. Lett.*, 203, 1–13, 2002.

6

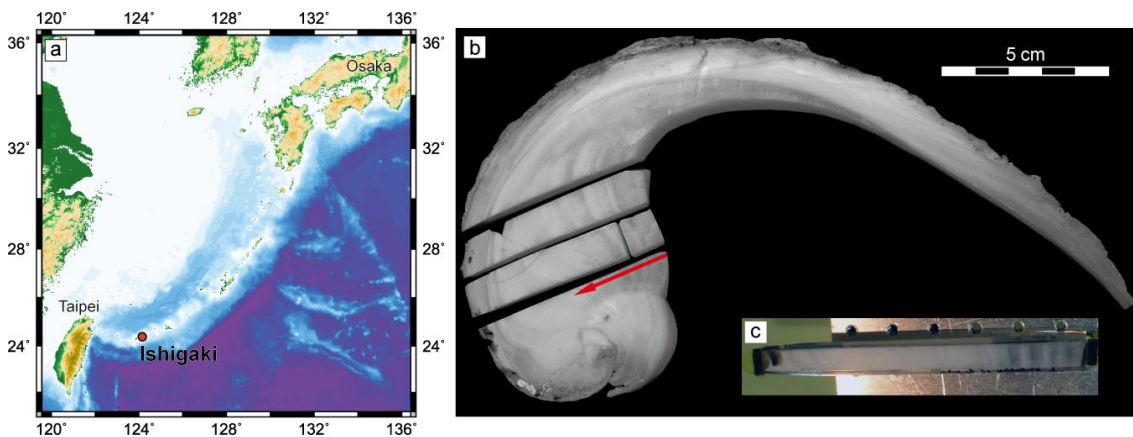
7 P. 1615, L. 14: Why is insolation singled out here? Is there preexisting evidence that
8 insolation per se is relevant, or are the authors essentially implicating seasonality? If
9 the latter is the case, I suggest revising this sentence.

10 → We changed the sentence. Please see the MS.

11

12 P. 1616, L. 25: A locality map would be a valuable addition to the manuscript.

13 → Locality map was added as Figure 1a.



14

15

16 P. 1617, L. 2-3: This statement should be referenced.

17 → We added a reference; Pätzold, J., Heinrichs, J. P., Wolschendorf, K., and Wefer, G.:
18 Correlation of stable oxygen isotope temperature record with light attenuation profiles in
19 reef-dwelling *Tridacna* shells. *Coral Reefs*, 10, 65-69, 1991.

20

21 P. 1617, P. 5: The authors should provide more detail regarding polishing?

22 → The cutting plane was polished using #1200, #2000 and #6000 3M Imperial polishing
23 paper. The polished slab was cleaned ultrasonically and dried at room temperature. We
24 added these information in Material and Methods.

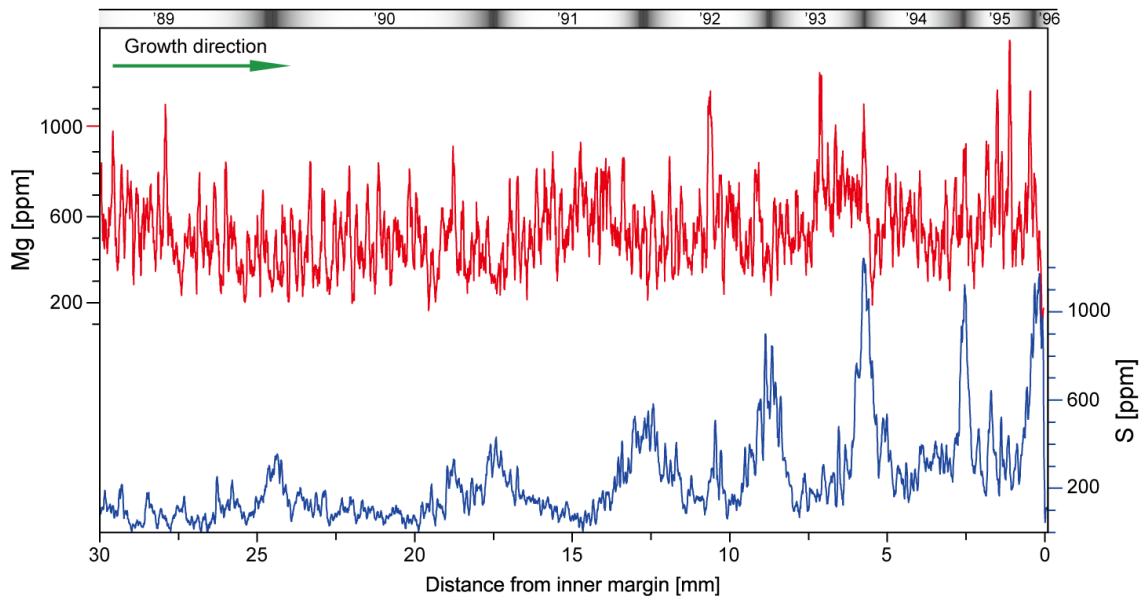
25

26 P. 1619, L. 13-14: These references should be mentioned earlier (see comment above).

27 → We added the references in Introduction.

28

1 P. 1619, L. 26-27: While I understand what the authors are inferring about growth rates,
2 this information is not explicitly showed in Figure 4. Perhaps this can be developed
3 more completely.
4 → We changed Figure 4.



5
6

7 P. 1620, L. 1-12: This is somewhat speculative. Can additional support (experimental
8 or literature) be provided that would bolster this hypothesis?

9 → A time-series measurement of calcifying fluid chemistry is the best to support the results.
10 But, to the authors' knowledge, such data are limited. To determine the CO_3^{2-}
11 concentration of calcifying fluid, the state of aqueous carbonate system can be
12 constrained by parameters, pH, total alkalinity and dissolved inorganic carbon. The pH
13 proxies of extrapallial fluid such as boron isotope ratio (Heinemann et al. 2012) probably
14 give further information to justify the ontogenetic change of calcifying fluid chemistry.
15 The $\delta^{11}\text{B}$ of *Mytilus edulis* shows no trend with seawater pH but appears to represent pH
16 of extrapallial fluid; Heinemann, A., Fietzke, J., Melzner, F., Böhm, F., Thomsen, J.,
17 Garbe-Schönberg, D., & Eisenhauer, A. (2012). Conditions of *Mytilus edulis*
18 extracellular body fluids and shell composition in a pH-treatment experiment: Acid-base
19 status, trace elements and $\delta^{11}\text{B}$. *Geochemistry, Geophysics, Geosystems*, 13(1).
20 doi:10.1029/2011GC003790.

21

22 Because the data presented here are from a single specimen, it is difficult to evaluate the
23 reproducibility of the patterns presented here (i.e., ontogenetic changes in elemental
24 concentrations).

25 → The synchrotron measurements provide some interesting features of element distributions
26 and chemical speciation in bivalve shell carbonate, but unfortunately machine time of
27 synchrotron facility is very limited. We are going on performing measurements. We

1 added related sentence that individual variability of shell growth partly exert systematic
2 control on sulfate concentration in *H. Hippopus*.
3

1 **Original comments**

2

3 **M. Cusack (Referee #1)**

4

5 This manuscript deals with sulfate, S-amino acids and Mg in the giant clam, *H. hippopus*.
6 These chemical species are considered in terms of environmental conditions and, importantly
7 in the context of ontogeny. The strength of the manuscript is the fact

8 that it deals with these important chemical species in meticulous detail in the environmental
9 and ontogenetic context, providing novel insight for both. The fact that the Mg concentration
10 correlates with sulfur amino acids, rather than sulfate, seems to contrast with some calcite
11 biominerals where there is a correlation between Mg and sulfate and the Mg is a true lattice
12 component. This is likely to all relate to the crystallography and the relative ease with which
13 Mg can substitute for Ca in the calcite lattice with both Mg and sulfate being incorporated
14 into calcite more readily at high precipitation rates. It is interesting to note the annual
15 fluctuations in sulfate concentration and the fact that their amplitude increases with age. This
16 contrasts with the concept of sulfate incorporation increasing with precipitation rate. The
17 authors present interesting discussion on this phenomenon by way of the chemistry of
18 calcifying fluids indicating that this may be a useful means of considering the influence of
19 environmental change on calcifying fluid chemistry. The authors provide fascinating data,
20 giving the reader a lot to think about along with an ontogenetic and environmental framework
21 in which to consider these data.

22

23

24 **Anonymous Referee #2**

25

26 **General Comments**

27 This study examines S and Mg element profiles from the shells of the giant clam *Hippopus*
28 *hippopus*, which were collected in Ishigaki-Jima, Japan. The main goal of the study was to
29 document ontogenetic trends, with the ultimate goal of understanding elemental partitioning
30 during biomineralization. The authors document elemental concentrations in both organic and
31 inorganic phases from a single specimen. Several specific hypotheses regarding the
32 mechanisms and controls on S and Mg variations are proposed. These ideas will likely serve
33 to motivate more detailed future experiments/monitoring studies. Because the data presented
34 here are from a single specimen, it is difficult to evaluate the reproducibility of the patterns
35 presented here (i.e., ontogenetic changes in elemental concentrations).

36

37 **Specific comments**

38 P. 1614, L. 25: This sentence should probably have a reference.

1 P. 1615, L. 14: Why is insolation singled out here? Is there preexisting evidence that
2 insolation per se is relevant, or are the authors essentially implicating seasonality? If
3 the latter is the case, I suggest revising this sentence.

4 P. 1616, L. 25: A locality map would be a valuable addition to the manuscript.

5 P. 1617, L. 2-3: This statement should be referenced.

6 P. 1617, P. 5: The authors should provide more detail regarding polishing?

7 P. 1619, L. 13-14: These references should be mentioned earlier (see comment above).

8 P. 1619, L. 26-27: While I understand what the authors are inferring about growth rates,
9 this information is not explicitly showed in Figure 4. Perhaps this can be developed
10 more completely.

11 P. 1620, L. 1-12: This is somewhat speculative. Can additional support (experimental
12 or literature) be provided that would bolster this hypothesis?

13

14

1 **Fluctuations of sulfate, S-bearing amino acids and** 2 **magnesium in a giant clam shell**

3

4 **T. Yoshimura^{1,*}, Y. Tamenori², H. Kawahata¹, A. Suzuki³**

5 [1]{Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha,
6 Kashiwa, Chiba 277-8564, Japan }

7 [2]{Japan Synchrotron Radiation Research Institute/SPring-8, 1-1-1 Kouto, Sayo 679-5198,
8 Japan }

9 [3]{Geological Survey of Japan, National Institute of Advanced Industrial Science and
10 Technology, Tsukuba Central 7, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8567, Japan }

11 [*]{now at: Japan Agency for Marine-Earth Science and Technology 2-15, Natsushima-cho,
12 Yokosuka-city, Kanagawa, 237-0061, Japan }

13 Correspondence to: T. Yoshimura (yoshimurat@jamstec.go.jp)

14

15 **Abstract**

16 We used micro-X-ray fluorescence combined with X-ray photoabsorption spectroscopy to
17 investigate speciation-specific sulfur profiles in the inner shell layer of a giant clam
18 (*Hippopus hippopus*). The sulfate, S-bearing amino acids, and total sulfur profiles indicated
19 that inorganic sulfate was the dominant component in the shell of this bivalve. Sulfur profiles
20 in the inner shell layer showed clear annual fluctuations that varied by more than one order of
21 magnitude, from <50 to 1420 ppm, and sulfate and total sulfur maxima became higher with
22 age, whereas no ontogenetic trend was noticeable in the profile of S-bearing amino acids. A
23 changes in the carbonate ion concentration in the calcifying fluid would suggest that an
24 ontogenetic increase in the relative activity of sulfate ions to carbonate ions in the calcifying
25 fluid affects sulfate concentrations in the shells. These results suggest that trace sulfur profiles
26 in the shell of the giant clam may reflect both cyclic shell growth related to environmental
27 factors such as insolation and temperature and ontogenetic changes of the calcifying fluid
28 chemistry mediated by physiological processes. The observed S profile implies a clear change
29 in calcifying fluid chemistry towards less alkaline condition with age. Magnesium

1 fluctuations suggested that Mg was incorporated into the shells at high growth rates during
2 warm seasons. The spectrum of Mg *K*-edge XANES and comparison of Mg and S-bearing
3 amino acids profiles indicated that a pronounced effect of the organic fraction or disordered
4 phases were observed in aragonitic shell of *H. hippopus* rather than regulated substitution into
5 the aragonite crystal lattice.

6

7 **1 Introduction**

8 The characteristic variations of element profiles in biogenic calcium carbonates are widely
9 used for identifying differences in biological and physicochemical processes associated with
10 element partitioning (Henderson 2002; Corrège 2006; Cusack and Freer 2008). Long-lived
11 bivalves such as reef-dwelling giant clams are geologically important producers of
12 biominerals that provide long-term records (up to ~100 years) of environmental conditions in
13 tropical and sub-tropical oceans (e.g., Watanabe et al. 2004; Aubert et al. 2009). Previous
14 studies have analyzed the element profiles of divalent cations and Sr/Ca ratios, the primary
15 objective being to evaluate them as environmental proxies (e.g., Elliot et al. 2009; Sano et al.
16 2012; Yan et al. 2013).

17 Sulfate is the third most abundant ion in seawater, and sulfur concentrations in biogenic
18 CaCO₃ range from several hundred to several thousand ppm (e.g., Busenberg & Plummer
19 1985). To date, however, little attention has been paid to seasonal intrashell sulfur variations.
20 The dynamics of sulfur incorporation into biogenic carbonates is of interest in relation to
21 environmental and physiological parameters (Berry 1998; Yoshimura et al. 2013a). In the
22 inner shell layer, sulfur shows clear annual first-order fluctuations, with lower sulfur
23 concentrations being associated with periods of faster growth. Thus, trace sulfur profiles in
24 aragonitic shells may reflect cyclic changes of shell growth related to ~~insolation or other~~
25 [environmental](#) factors that influence shell growth (Yoshimura et al. 2013a).

26 Sulfur is an important component of organic compounds, but the chemical form of sulfur in
27 bivalve shells is still poorly understood. The chemical environment of sulfur has been studied
28 in biogenic carbonates (Cuif et al. 2003, 2008; Cusack et al. 2008; Dauphin et al. 2008), and
29 the sulfate concentration in biogenic carbonates has been proposed as an indicator of skeletal
30 organic materials (Lorens & Bender 1980; Cuif et al. 2003; Takesue et al. 2008), but
31 inorganic sulfate is also present in CaCO₃ (e.g., Kitano et al. 1975; Takano 1985; Kralj et al.
32 2004; Kontrec et al. 2004). Because of the complexity of organic compounds associated with

1 CaCO₃ biomineralization processes (e.g., Cusack and Freer 2008), both organic and inorganic
2 sulfur potentially contribute to the total sulfur concentration of shell carbonates.

3 The Mg content of the shell aragonite might also reflect in part biological contamination with
4 organic material and adsorption phenomena during crystal precipitation rather than
5 physicochemically regulated substitution into the aragonite crystal lattice. The Mg/Ca profiles
6 of both marine and freshwater aragonitic species shows large interspecimen differences with a
7 minor contribution from the variation of ambient environmental factors (e.g., Takesue et al.,
8 2008; Schöne et al., 2010; Izumida et al., 2011), suggesting that the Mg/Ca ratios of shell
9 aragonite may reflect biological factors rather than regulated substitution into the aragonite
10 crystal lattice. In a giant clam *Tridacna gigas*, Elliot et al. (2009) observed seasonal Mg/Ca
11 oscillations superimposed on an ontogenetic trend with increasing values and increasing
12 amplitude with age. However, to our knowledge no detailed comparison regarding the
13 covariation of organic materials with Mg concentrations in the bivalve shell are available.

14 X-ray photoabsorption spectroscopy (XAS) is a molecular-scale spectroscopy technique that
15 yields electronic and geometric structural information about the element of interest (Stöhr
16 1992). Sulfur and magnesium *K*-edge XAS has also been applied to the characterization of the
17 chemical environment (e.g., Pingitore et al. 1995; Morra et al. 1997; Finch and Allison, 2008;
18 Foster et al., 2008; Cusack et al. 2008; Yoshimura et al. 2013a). XAS can be used in
19 combination with X-ray fluorescence with a focused X-ray beam (i.e., micro-X-ray
20 fluorescence; μ -XRF) to determine the speciation-specific spatial element distribution in
21 biogenic carbonates and to generate time series of trace-element profiles in biogenic CaCO₃
22 with high lateral resolution.

23 In this study, we investigated sulfur and magnesium element profiles in a reef-dwelling clam
24 collected from Ishigaki-jima, Japan. Our aim was to use μ XRF/XAS measurements to assess
25 ontogenetic trends in the variability of speciation-specific sulfur and magnesium profiles.
26 Clarification of how sulfur and magnesium are hosted in the shell of a giant clam will provide
27 insight into the mechanisms of minor/trace element incorporation into bivalve shells, and we
28 expect this knowledge to be useful in environmental and biological studies of present and past
29 reef environments.

30

1 2 Materials and Methods

2 We collected a live specimen of the strawberry clam *Hippopus hippopus* (Fig. 1) from
3 Ishigaki Island, Japan (24°33'N, 124°16'E), which lies in the subtropical northwestern Pacific
4 Ocean (Fig. 1). The average temperature and annual precipitation at the collection site are
5 24.0 °C and 2061 mm, respectively (Japan Meteorological Agency;
6 <http://www.data.jma.go.jp/obd/stats/etrn/index.php>). *Hippopus hippopus* has an aragonitic
7 shell that consists of an outer and an inner layer (Pätzold et al. 1991). The valves of our
8 specimen were first cut along the maximum growth axis and then sliced into 5-mm-thick slabs
9 with a diamond saw. The cut surfaces were then polished using #1200, #2000 and #6000 3M
10 Imperial polishing paper. The polished slab was cleaned ultrasonically and dried at room
11 temperature.

12 All X-ray experiments were carried out by using the B-branch of the soft X-ray
13 photochemistry beamline (BL27SU) at the SPring-8 synchrotron radiation facility. A two-
14 dimensional (2D) approach was used for partial fluorescence yield measurements in the soft
15 X-ray region (Tamenori et al. 2011). The X-ray beam was focused on a 15- μm -diameter spot
16 on the sample by Kirkpatrick-Baez mirrors. The photon flux on the focal point was 1×10^{11}
17 Ph s^{-1} at 2500 eV. In the $\mu\text{-XRF}$ analysis, X-ray fluorescence signals were detected with a
18 single-element silicon drift detector. The sample holder was mounted on a motorized XYZ
19 stage. During analysis, the intensity of the incoming photon beam (I_0) was monitored by
20 measuring the drain current on the surface of a post-focusing mirror. All measurements were
21 performed under vacuum to avoid absorption and scattering of the incident and fluorescent X-
22 rays by air.

23 The $\mu\text{-XRF/XAS}$ chemical mapping measurements were made at a fixed excitation energy
24 based on the characteristic resonance peak energies determined by XAS analysis (2473 and
25 2482 eV). Total sulfur profiles were obtained at 2600 eV, which is >100 eV above the sulfur
26 *K*-edge energy region. In addition to the $\mu\text{-XRF/XAS}$ analysis data, full XRF data were also
27 obtained at each mapping point during the $\mu\text{-XRF/XAS}$ analysis, and elemental imaging data
28 were extracted from the XRF data set. Although the energy of the excitation X-ray beam was
29 set to the sulfur *K*-edge energy region, all energetically accessible elements were
30 simultaneously excited by the incident photon beam. The element concentrations were
31 calculated by measuring carbonate reference materials J Cp-1 and J Ct-1, which were obtained
32 from the Geological Survey of Japan (Okai et al. 2004). These reference materials were

1 prepared from a modern coral skeleton (JCp-1) and a Holocene fossil bivalve shell (JCt-1).
2 The relative standard deviation of S and Mg signal intensities, based on 10 replicate
3 measurements of carbonate reference material JCp-1 with an acquisition time of 1 s at 2480.0
4 eV, was 1.3 and 2.3%, respectively.

5 For Mg *K*-edge XANES measurements, powdered *H. hippopus* shell were fixed with
6 conductive double-sided carbon tape onto an aluminum sample holder. The results were
7 recorded as fluorescence-yield (FY) spectra with a SDD (Tamenori et al., 2011). The selected
8 energy range for Mg XANES measurements was 1300–1350 eV with an energy step of 0.2
9 eV and an acquisition time of 4 s.

10

11 **3 Results and Discussion**

12 **3.1 Sulfur speciation profiles**

13 The *K*-edge X-ray absorption near-edge structure (XANES) spectra of sulfur exhibit several
14 features that differ among inorganic and organic reference compounds (Fig. 2), and the main
15 peaks of the spectra show large shifts toward higher energies with increasing oxidation state.
16 The main peak of the amino acids cysteine and methionine, which have an H-S-R and an R-S-
17 R bond, respectively, is at around 2473 eV. The μ -XRF/XAS mapping result at 2473 eV thus
18 represents an organic sulfur, S-bearing amino acid (S-AA) signal. The XANES spectra of
19 various sulfate compounds exhibit a pronounced peak at around 2482 eV (Fig. 2).

20 In our specimen, both the S-AA and sulfate profiles exhibited clear cyclic fluctuations, but the
21 phases of the seasonal cycle differed (Fig. 3). The sulfate profile showed a pronounced
22 ontogenetic increase in the amplitude and maximum of each cycle, but no ontogenetic trend
23 was observed in the S-AA profile. The lack of a consistent S-AA pattern suggests that
24 temperature and body size had only a minor effect on the amino acid content of the shell of
25 this giant clam. Comparison of the S-AA, sulfate, and total sulfur profiles showed that sulfate
26 and total sulfur (Fig. 3) fluctuated synchronously. Given that inorganic sulfate is the dominant
27 component of the *H. hippopus* shell (Yoshimura et al. 2013a), the total sulfur profile reflects
28 changes in the concentration of inorganic sulfate. Total sulfur concentrations in the specimen
29 showed pronounced first-order fluctuations that varied by more than one order of magnitude,
30 from <50 to 1420 ppm (Fig. 4). Yoshimura et al. (2013a) reported that the annual cycles in
31 sulfur profiles suggest that inorganic sulfate is incorporated into the shell at a higher rate

1 during the winter. On contrary, the low total sulfur values in aragonitic shells associated with
2 high shell growth during summer.

3 The SO_4^{2-} distribution coefficient in abiotic calcite is kinetically controlled, and sulfate
4 incorporation by calcite tends to increase with the calcite precipitation rate (Busenberg &
5 Plummer 1985), then the sulfur content of biogenic carbonates may thus be proportional to
6 the rate of shell growth. However, in clamshells, sulfate concentration peaks do not
7 correspond to shell growth rate maxima. For this reason, Yoshimura et al. (2013a) proposed
8 that there are physiological controls on the calcifying fluid chemistry. The shell growth rate of
9 a giant clam shell is higher when insolation is increased (Watanabe et al. 2004; Aubert et al.
10 2009). Supplementary sources of nutrients from symbiotic algae may also maintain the
11 characteristic fast growth of tropical clamshells. Translocated photosynthates produced by
12 zooxanthellae by phototrophy are the most important source of energy in clams; this source
13 provides sufficient carbon for the growth and metabolic requirements of giant clams (e.g.,
14 Klumpp & Griffiths 1994). Because higher insolation during summer promotes higher rates of
15 photosynthesis by symbiotic algae, enhanced nutrient production may partly explain the high
16 summer growth rates of clamshells (Watanabe & Oba 1999). During periods of faster growth,
17 the concentration of carbonate ions (CO_3^{2-}) in calcifying fluids is increased and, at the same
18 time, the increased calcium carbonate saturation state induces higher calcification rates.

19 Total sulfur profile showed a clear ontogenetic increase in the S maximum of each cycle,
20 whereas the shell thickening rate of *H. hippopus* decreased as the clam became older (Fig. 4).
21 Because the S content increased with age, we inferred that the sulfate content of the *H.*
22 *hippopus* shell is governed primarily by physiological controls on the calcifying fluid
23 chemistry. A positive correlation between the seasonal skeletal growth rate and changes in the
24 carbonate ion concentration in the calcifying fluid would suggest that an ontogenetic increase
25 in the relative activity of sulfate ions to carbonate ions in the calcifying fluid affects sulfate
26 concentrations in the shells. Decreases in CO_3^{2-} concentrations with age, corresponding to
27 lower growth rates at older ages, would positively influence sulfate incorporation into CaCO_3
28 by increasing the relative activity of sulfate ions to carbonate ions in the calcifying fluid.
29 These results implies a clear change in calcifying fluid chemistry towards less alkaline
30 condition with age. Therefore, the ontogenetic effect in the S profiles of the shell of the giant
31 clam may be due to changes in the relative ionic strength of sulfate and carbonate ions. **To**
32 **determine the CO_3^{2-} concentration, the state of aqueous carbonate system can be constrained**

1 by parameters, pH, total alkalinity and dissolved inorganic carbon. The pH proxies of
2 extrapallial fluid such as boron isotope ratio (Heinemann et al. 2012) would give further
3 information to justify the ontogenetic change of calcifying fluid chemistry. Since the presence
4 of SO_4^{2-} in a fluid is known to inhibit CaCO_3 crystal growth (Busenberg & Plummer 1985), a
5 giant clam may try to reduce the SO_4^{2-} in calcifying solutions to facilitate rapid calcification
6 especially during young age.

7 Interpretations relating the mode of shell growth to environmental and physiological changes
8 have focused on the total sulfur concentration. Large seasonal and ontogenetic changes in
9 shell sulfur concentrations may record cyclic changes in the calcifying fluid chemistry; these
10 changes may be related to environmental factors (e.g., insolation and temperature) as well as
11 to ontogenetic changes in bivalve physiology and individual variability of shell growth. The
12 results of our speciation-specific analysis suggest that bivalve shells can serve as archives of
13 aqueous chemistry of calcifying fluids. Moreover, these results suggest that sulfate
14 concentrations would be a useful proxy for evaluating the response of calcifying fluid
15 chemistry to ocean acidification.s

16 **3.2 Magnesium chemical environment and profile**

17 The stacked spectra of *H. hippopus* and Mg-bearing compounds are shown in Fig. 2. The
18 most pronounced peaks were at around 1313 eV for *H. hippopus* shell aragonite and other
19 carbonates, calcite and dolomite (Fig. 2). For reference materials, some peaks were evident in
20 the post-edge region. However, the overall shape of the Mg-XANES spectrum for *H.*
21 *hippopus* was featureless (Fig. 2), which arises either from the organic materials or the highly
22 disordered phase (Finch and Allison, 2008; Foster et al., 2008). XANES results did not permit
23 us to discriminate between organic and disordered Mg phase in aragonitic shells.

24 Magnesium profile showed a relatively irregular pattern and higher frequency of periodicity
25 than the sulfur profile (Fig. 3, Fig. 4). Mg concentrations in the specimen varied from 150 to
26 >1000 ppm and it tend to be somewhat higher at low sulfur concentrations during high growth
27 seasons (Fig. 4). This is consistent with earlier findings that Mg/Ca ratios of inner layer of *T.*
28 *gigas* is inversely correlated to the oxygen isotope profile and thus positively correlated with
29 temperature (Elliot et al., 2009). Moreover, the Mg fluctuations are to some extent correlated
30 with S-AA (Fig. 3). A positive correlation was found between Mg and S-AA (correlation

1 coefficient $r=0.400$, $p<0.001$, Student's t-test). The incorporation of Mg into aragonitic shells
2 could be interpreted as the result of the influence of biologically mediated controls.

3 The distribution of organic matrices across the shell has been proposed as the controlling
4 factor responsible for the large annual fluctuation of magnesium. Foster et al. (2008) found
5 that the highest Mg/Ca ratios of *A. islandica* were typically present in the annual growth
6 bands, and that Mg/Ca variations among growth bands were not significant in most specimens.
7 Moreover, Mg-XANES studies reported that Mg in biogenic aragonite is not incorporated into
8 the aragonite crystal lattice but instead occurs, inter alia, in organic material, disordered
9 phases, and amorphous CaCO_3 (Finch and Allison, 2008; Foster et al., 2008). A large
10 biological controls on Mg incorporation is consistent with earlier findings for other giant
11 clams. For example, the Mg/Ca ratios of a giant clam *T. gigas* showed heterogeneous
12 distribution across the different shell layers and seasonal Mg/Ca oscillations superimposed on
13 an increasing trend with age (Elliot et al., 2009). In the present case, the distribution patterns
14 of Mg more plausibly reflect fluctuations of biological controls as previously proposed.

15 The Mg content of the *H. hippopus* shell aragonite also reflect in part biological
16 contamination with organic material or adsorption phenomena during crystal precipitation
17 rather than thermodynamic substitution into the aragonite crystal lattice. The discrepancies
18 between Mg and S-AA could be interpreted as the result of the simultaneous influence of
19 inorganically driven controls, such as temperature and growth rate. Because specific functions
20 of organic molecules involved in structural organization and the mode of biomineralization
21 have been proposed, the observed annual Mg fluctuation can have implications for the
22 properties of shell crystallization.

23

24 **Acknowledgements**

25 We express our appreciation to Naohiko Ohkouchi of Japan Agency for Marine-Earth Science
26 and Technology. We also acknowledge interactive discussions by Maggie Cusack and an
27 anonymous reviewer. This study was conducted with the approval of the SPring-8 Proposal
28 Review Committee (Proposal 2012A1556, 2012B1116 and 2013B1340).

29

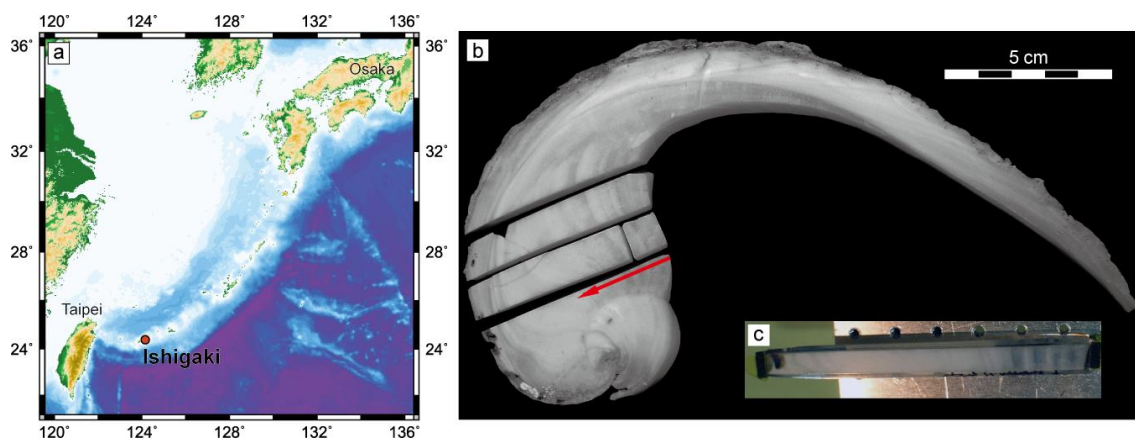
1 **References**

- 2 Aubert, A., Lazareth, C. E., Cabioch, G., Boucher, H., Yamada, T., Iryu, Y., and Farman, R.:
3 The tropical giant clam *Hippopus hippopus* shell, a new archive of environmental conditions
4 as revealed by sclerochronological and $\delta^{18}\text{O}$ profiles, *Coral Reefs*, 28, 989–998, 2009.
- 5 Berry, J. N.: Sulfate in foraminiferal calcium carbonate: investigating a potential proxy for sea
6 water carbonate ion concentration, PhD Thesis, Massachusetts Institute of Technology, 1998.
- 7 Busenberg, E., Plummer, L. N.: Kinetic and thermodynamic factors controlling the
8 distribution of SO_3^{2-} and Na^+ in calcites and selected aragonites, *Geochim. Cosmochim. Acta*,
9 49, 713–725, 1985.
- 10 [Corrège, T.: Sea surface temperature and salinity reconstruction from coral geochemical](#)
11 [tracers, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 232, 408–428, 2006.](#)
- 12 Cuif, J. P., Dauphin, Y., Doucet, J., Salome, M., and Susini, J.: XANES mapping of organic
13 sulfate in three scleractinian coral skeletons, *Geochim. Cosmochim. Acta*, 67, 75–83, 2003.
- 14 Cuif, J. P., Dauphin, Y., Farre, B., Nehrke, G., Nouet, J., and Salomé, M.: Distribution of
15 sulphated polysaccharides within calcareous biominerals suggests a widely shared two-step
16 crystallization process for the microstructural growth units, *Miner. Mag.*, 72, 233–237, 2008.
- 17 Cusack, M., and Freer, A.: Biomineralization: elemental and organic influence in carbonate
18 systems, *Chem. Rev.*, 108, 4433–4454, 2008.
- 19 Cusack, M., Dauphin, Y., Cuif, J. P., Salomé, M., Freer, A., and Yin, H.: Micro-XANES
20 mapping of sulphur and its association with magnesium and phosphorus in the shell of the
21 brachiopod, *Terebratulina retusa*, *Chem. Geol.*, 253, 172–179, 2008.
- 22 Dauphin, Y., Ball, A. D., Cotte, M., Cuif, J. P., Meibom, A., Salomé, M., Susini, J., and
23 Williams, C. T.: Structure and composition of the nacre-prism transition in the shell of
24 *Pinctada margaritifera* (Mollusca, Bivalvia), *Anal. Bioanal. Chem.*, 309, 1659–1669, 2008.
- 25 Elliot, M., Welsh, K., Chilcott, C., McCulloch, M., Chappell, J., and Ayling, B.: Profiles of
26 trace elements and stable isotopes derived from giant long-lived *Tridacna gigas* bivalves:
27 Potential applications in paleoclimate studies, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 280,
28 132–142, 2009.

- 1 Finch, A. A., and Allison, N.: Mg structural state in coral aragonite and implications for the
2 paleoenvironmental proxy, *Geophys. Res. Lett.*, 35:L08704, 2008.
- 3 Foster, L. C., Finch, A. A., Allison, N., Andersson, C., and Clarke, L. J.: Mg in aragonitic
4 bivalve shells: Seasonal variations and mode of incorporation in *Arctica islandica*, *Chem.*
5 *Geol.*, 254, 113–119, 2008.
- 6 Heinemann, A., Fietzke, J., Melzner, F., Böhm, F., Thomsen, J., Garbe-Schönberg, D., and
7 Eisenhauer, A.: Conditions of *Mytilus edulis* extracellular body fluids and shell composition
8 in a pH-treatment experiment: Acid-base status, trace elements and $\delta^{11}\text{B}$, *Geochem. Geophys.*
9 *Geosys.*, 13, doi:10.1029/2011GC003790, 2012.
- 10 Henderson, G. M.: New oceanic proxies for paleoclimate. *Earth Planet. Sci. Lett.*, 203, 1–13,
11 2002.
- 12 Izumida, H., Yoshimura, T., Suzuki, A., Nakashima, R., Ishimura, T., Yasuhara, M., Inamura,
13 A., Shikazono, N., and Kawahata, H.: Biological and water chemistry controls on Sr/Ca,
14 Ba/Ca, Mg/Ca and $\delta^{18}\text{O}$ profiles in freshwater pearl mussel *Hyriopsis* sp., *Palaeogeogr.*
15 *Palaeoclimatol. Palaeoecol.*, 309, 298-308, 2011.
- 16 Kitano, Y., Okumura, M., and Idogaki, M.: Incorporation of sodium, chloride and sulfate with
17 calcium carbonate, *Geochem. J.*, 9, 75–84, 1975.
- 18 Klumpp, D. D., and Griffiths, C. C.: Contributions of phototrophic and heterotrophic nutrition
19 to the metabolic and growth requirements of four species of giant clam (Tridacnidae), *Mar.*
20 *Ecol. Prog. Ser.*, 115, 103–115, 1994.
- 21 Kontrec, J., Kralj, D., Brečević, L., Falini, G., Fermani, S., Noethig-Laslo, V., and
22 Mirosavljević, K.: Incorporation of inorganic anions in calcite, *Eur. J. Inorg. Chem.*, 2004,
23 4579–4585, 2004.
- 24 Kralj, D., Kontrec, J., Brečević, L., Falini, G., and Nöthig-Laslo, V.: Effect of inorganic
25 anions on the morphology and structure of magnesium calcite, *Chem. Eur. J.*, 10, 1647–1656,
26 2004.
- 27 Lorens, R. B., and Bender, M. L.: The impact of solution chemistry on *Mytilus edulis* calcite
28 and aragonite, *Geochim. Cosmochim. Acta*, 44, 1265–1278, 1980.

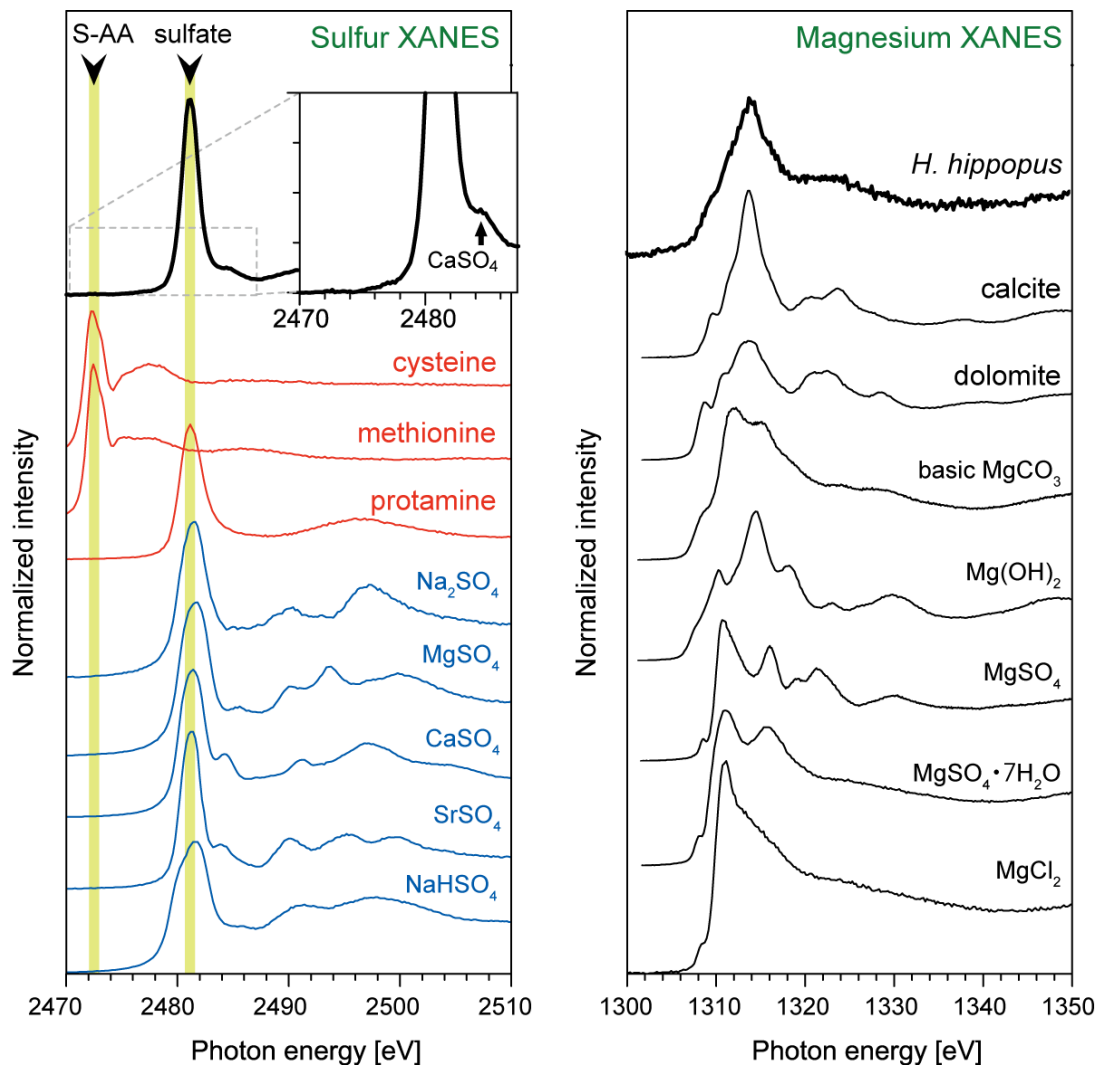
- 1 Morra, M. J., Fendorf, S. E., and Brown, P. D.: Speciation of sulfur in humic and fulvic acids
2 using X-ray absorption near-edge structure (XANES) spectroscopy, *Geochim. Cosmochim.*
3 *Acta*, 61, 683–688, 1997.
- 4 Okai, T., Suzuki, A., Terashima, S., Inoue, M., Nohara, M., Kawahata, H., and Imai, N.:
5 Collaborative analysis of GSJ/AIST geochemical reference materials JCp 1 (Coral) and JcT 1
6 (Giant Clam), *Geochemistry*, 38, 281–286, 2004. (in Japanese)
- 7 [Pätzold, J., Heinrichs, J. P., Wolschendorf, K., and Wefer, G.: Correlation of stable oxygen
8 isotope temperature record with light attenuation profiles in reef-dwelling *Tridacna* shells.
9 *Coral Reefs*, 10, 65-69, 1991.](#)
- 10 Pingitore, Jr. N. E., Meitzner, G., and Love, K. M.: Identification of sulfate in natural
11 carbonates by X-ray absorption spectroscopy, *Geochim. Cosmochim. Acta*, 59, 2477–2483,
12 1995.
- 13 Sano, Y., Kobayashi, S., Shirai, K., Takahata, N., Matsumoto, K., Watanabe, T., Sowa, K.,
14 and Iwai, K.: Past daily light cycle recorded in the strontium/calcium ratios of giant clam
15 shells, *Nature Comm.*, 3, doi:10.1038/ncomms1763, 2012.
- 16 Schöne, B. R., Zhang, Z., Jacob, D. E., Gillikin, D. P., Tütken, T., Garbe-Schönberg, D.,
17 McConnaughey, T., and Soldati, A.: Effect of organic matrices on the determination of the
18 trace element chemistry (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (*Arctica*
19 *islandica*)—Comparison of ICP-OES and LA-ICP-MS data, *Geochem. J.*, 44, 23–37, 2010.
- 20 Stöhr, J.: *NEXAFS Spectroscopy*. Springer, Berlin, 1992.
- 21 Takano, B.: Geochemical implications of sulfate in sedimentary carbonates, *Chem. Geol.*, 49,
22 393–403, 1985.
- 23 Takesue, R. K., Bacon, C. R., and Thompson, J. K.: Influences of organic matter and
24 calcification rate on trace elements in aragonitic estuarine bivalve shells, *Geochim.*
25 *Cosmochim. Acta*, 72, 5431–5445, 2008.
- 26 Tamenori, Y., Morita, M., and Nakamura, T.: Two-dimensional approach to fluorescence
27 yield XANES measurement using a silicon drift detector, *J. Synchrotron Rad.*, 18, 747–752,
28 2011.

- 1 Watanabe, T., and Oba, T.: Daily reconstruction of water temperature from oxygen isotopic
2 ratios of a modern *Tridacna* shell using a freezing microtome sampling technique, *J. Geophys.*
3 *Res.*, 104, 20,667–20,674, 1999.
- 4 Watanabe, T., Suzuki, A., Kawahata, H., Kan, H., and Ogawa, S.: A 60-year isotopic record
5 from a mid-Holocene fossil giant clam (*Tridacna gigas*) in the Ryukyu Islands: physiological
6 and paleoclimatic implications, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 212, 343–354, 2004.
- 7 Yan, H., Shao, D., Wang, Y., and Sun, L.: Sr/Ca profile of long-lived *Tridacna gigas* bivalves
8 from South China Sea :A new high-resolution SST proxy, *Geochim. Cosmochim. Acta*, 112,
9 52–65, 2013.
- 10 Yoshimura, T., Tamenori, Y., Suzuki, A., Nakashima, R., Iwasaki, N., Hasegawa, H., and
11 Kawahata, H.: Element profile and chemical environment of sulfur in a giant clam shell:
12 insights from μ -XRF and X-ray absorption near-edge structure, *Chem. Geol.*, 352, 170–175,
13 2013a.
- 14 Yohimura, T., Tamenori, Y., Iwasaki, N., Hasegawa, H., Suzuki, A. and Kawahata, H.:
15 Magnesium *K*-edge XANES spectroscopy of geological standards, *J. Synchrotron Rad.*, 20,
16 734–740, 2013b.
- 17



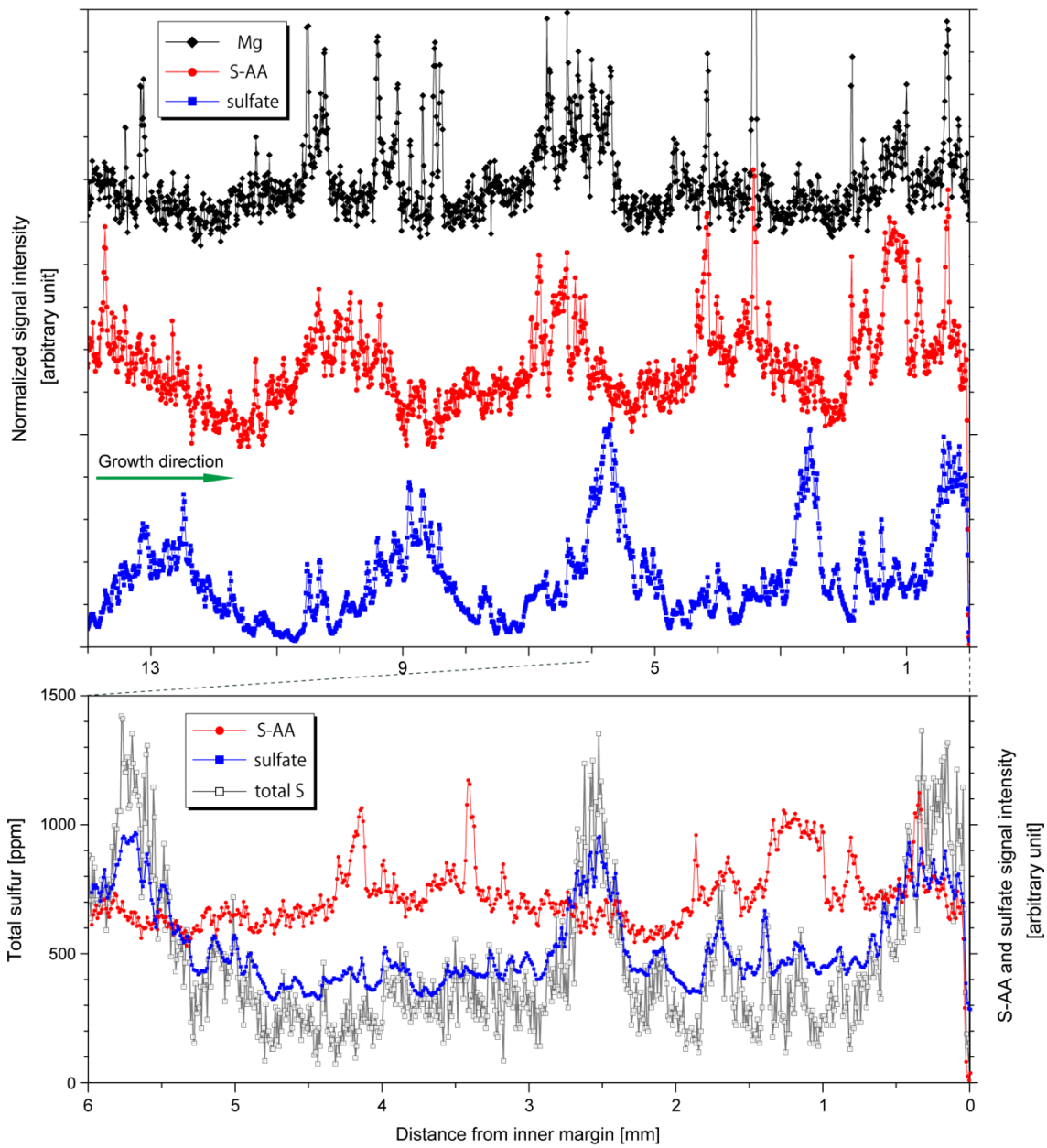
1
2 **Fig. 1.** (a) Map of the location of Ishigaki Island, Japan. (b) A slab of the *Hippopus hippopus*
3 specimen. A polished slab obtained from the inner shell layer was used for μ -XRF/XAS
4 measurements. The red arrow indicates the measurement transect. (c) The polished slab was
5 fixed in an aluminum sample holder with conductive double-sided carbon tape and inserted
6 into the vacuum chamber.

7



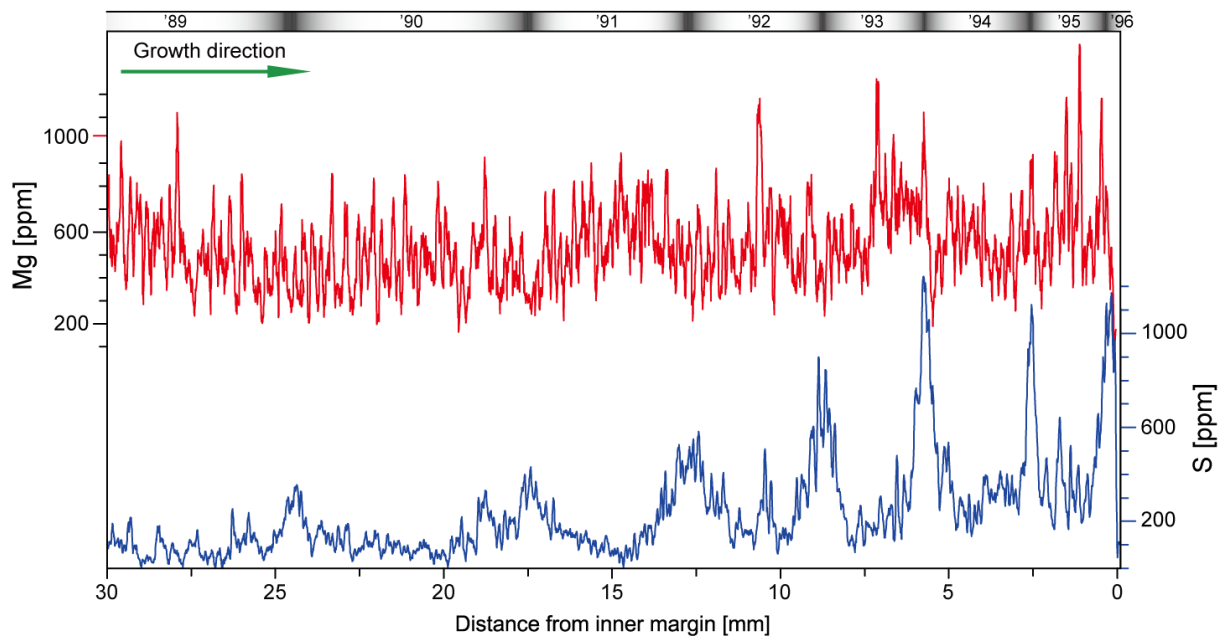
1
 2 **Fig. 2.** S (left) and Mg (right) *K*-edge XANES spectra of a giant clam shell aragonite (*H.*
 3 *hippopus*) and reference materials. Micro-XANES of marine clam shell aragonite (*H.*
 4 *hippopus*) performed at focused mode on points at S maxima. The XANES spectra have been
 5 published previously (Yoshimura et al., 2013a, 2013b). The μ -XRF/XAS measurements were
 6 made at a fixed excitation energy based on the characteristic resonance peak energies of S-
 7 bearing amino acids (2473 eV) and sulfate (2482 eV).

8



1
2
3
4
5
6
7

Fig. 3. (Upper panel) Profiles of two different sulfur species, S-bearing amino acids (S-AA, red) and sulfate (blue), and Mg in the inner shell layer of *H. hippopus*, obtained by the μ -XRF/XAS method at 10- μ m measurement intervals. (Lower panel) Total sulfur concentration measured at 8- μ m intervals compared with sulfur speciation profiles. The cyclic fluctuation of total sulfur correlated well with that of sulfate.



1

2 **Fig. 4.** Changes in the moving average trends (15-point smoothing window) of magnesium
 3 and total sulfur in the inner shell layer of *H. hippopus*, obtained by the μ -XRF/XAS method at
 4 8- μ m intervals. The spatial resolution of μ -XRF allows the variations of the Mg and S content
 5 to be examined at a daily timescale (300-850 points per year).

6