Supporting Information

Scallop shells as geochemical archives of phytoplankton-related ecological processes in a temperate coastal ecosystem

Julien Thébault*1, Aurélie Jolivet1,2, Matthieu Waeles1, Hélène Tabouret2, Sophie Sabarot1,3, Christophe Pécheyran3, Aude Leynaert1, Klaus Peter Jochum4, Bernd R. Schöne5, Lukas Fröhlich5, Valentin Siebert1, Erwan Amice1 and Laurent Chauvaud1

* Corresponding author: julien.thebault@univ-brest.fr
Supplementary Methods

SM1 - Description of the methods used to analyze environmental parameters in seawater

**Chlorophyll a and phytoplankton species**

Water samples for Chl a determination were filtered with glass fiber filters (GF/F Whatman). Chl a was extracted in 6 mL of 90 % acetone and kept in the dark at 4°C for 12 h. Samples were then centrifuged and fluorescence was measured with a Turner Design fluorometer. The equation of [Lorenzen (1966)](https://www.jstor.org/stable/774866) was used to calculate the Chl a concentration. Phytoplankton species determination was performed on bottom water samples preserved in Lugol’s iodine solution ([Edler & Elbrächter, 2010](https://www.jstor.org/stable/42980286)). All taxa were then identified and counted by examination on an inverted microscope.

**Particular organic carbon**

Water samples collected for POC analyses were filtered with pre-combusted (450°C for 4 h) Whatman GF/F filters and placed in a stove at 60°C. Filters were then analyzed for their POC concentration by the combustion method, using a CHN elemental analyzer (Thermo Fisher Flash EA 1112).

**Nutrients**

DSi (silicate Si(OH)$_4$) was analyzed in water samples filtered through Nuclepore membrane filters (47 mm diameter), and then stored at 4°C in the dark. Nitrate concentration was measured in water samples filtered with pre-combusted Whatman GF/F filters (25 mm diameter), then frozen at -20°C. DSi and NO$_3^-$ concentrations were later measured by the colorimetric method on a Technicon automatic Analyzer II and semi-automatic analyzer, respectively ([Tréguer & Le Corre, 1975](https://www.jstor.org/stable/42980286)).
Dissolved and particulate molybdenum

DMo and PMo species were separated following filtration of seawater on 0.45-µm mixed cellulose ester filters (HATF Millipore). DMo was measured on UV-irradiated aliquots using differential pulse adsorptive voltammetry at a static mercury drop electrode (Metrohm 663 VA model) following the method developed by Quentel et al. (1992). An Ag/AgCl (supra-pure 3 M KCl) electrode and a platinum electrode were used as the reference and the auxiliary electrodes, respectively. After dilution of 4 mL of sample in ultra-pure water (final volume 20 mL), fulvic acid (R1S101F-IHSS) and 1,10 phenanthroline (Fluka) were added at concentrations of 150 mg L\(^{-1}\) and 0.10 mmol L\(^{-1}\), respectively. The solution was then de-aerated for 10 min with nitrogen and a potential of -0.25 V (vs Ag/AgCl/KCl, 3 M) was applied under stirring for 60 s allowing the adsorption of the Mo(VI)-FA-phen complex at the mercury drop and its subsequent reduction to Mo(V)-FA-phen. After a 10 s equilibration time, stripping was performed as follows: sweeping from -0.15 V to -0.75 V, pulse time: 50 ms, pulse rate: 2 s\(^{-1}\), step potential: 2 mV, pulse amplitude: 50 mV. DMo concentrations were determined after three standard additions of a Mo(VI) solution.

The reliability of the method was checked by five analyses of a NASS-5 (National Research Council Canada) certified reference seawater. Obtained concentration was 94±4 nmol L\(^{-1}\) (certified value: 100±10 nmol L\(^{-1}\)). PMo was measured after digestion of the filters in Teflon vials with 4 mL of 69 vol% nitric acid (ultratrace quality). Aliquots of 700 µL were diluted to 14 mL with ultra-pure deionized water and the measurements were then conducted on a quadrupole mass spectrometer coupled with induced plasma (ICP-Q-MS Thermo Scientific X-Series 2) at Pôle Spectrométrie Océan (PSO, Plouzané, France).
SM2 - Description of the methods used to analyze Mo:Ca and Li:Ca ratios in shells

SN-ICP-MS methodology used at PSO

Using a hand-held micro-drilling device equipped with a 300-µm tungsten carbide drill bit, small aliquots of shell calcite were collected on the upper surface of the left valve of the shells, along the axis of maximum growth. One stria was milled every 3 striae, a sampling strategy corresponding to approx. two calcite samples per week of shell growth (sub-weekly resolution). Sample preparation and analyses were performed at the Pôle Spectrométrie Océan (Plouzané, France). All samples were prepared in a class 10000 clean laboratory. Ultra-pure deionized water (resistivity = 18.2 MΩ.cm) was used for material cleaning and acid dilutions. Nitric acid solutions (commercial grade, Merck) were purified by distillation in sub-boiling silica glass stills (Quartex). All material (polypropylene centrifuge tubes, disposable pipette tips, etc.) was pre-cleaned using 5 % HNO₃ and rinsed with ultra-pure deionized water.

A known weight of each shell sample (average weight = 121 µg) was transferred into a pre-cleaned polypropylene centrifuge tube, dissolved in 2 % HNO₃, and spiked with a known amount (about 7 µL) of a mono-elemental thulium solution (Tm concentration = 77.9 ng g⁻¹). Thulium was used as an internal standard to correct short- and long-term instrumental drift (see Bayon et al. (2009) for detailed information on this method). External calibration was performed using an in-house multi-element solution prepared from certified stock solutions. This calibration solution was prepared so that it closely matched the calcium carbonate matrix and elemental composition of mollusk shells.

Elemental concentrations were measured on a Thermo Electron Element2 high-resolution inductively coupled plasma mass spectrometer equipped with an ASX 260 auto-sampler (CETAC Technologies). Solutions were introduced via a Teflon nebulizer and a Peltier cooled cyclonic spray chamber. The Element2 was equipped with a glass injector and a set of nickel sampler and skimmer cones. Along the course of this study, plasma power ranged between 1270 and 1310 W and argon flow rates were 16 L min⁻¹ (cooling gas), 0.54-0.65 L min⁻¹ (auxiliary gas), and 0.95-1.35 L min⁻¹.
(nebulizer gas). The Element2 was operated in low resolution (m/Δm = 400) and measured isotopes were $^7$Li, $^{97}$Mo and $^{43}$Ca (among other elements not presented in this article). Concentrations were calculated using the Tm addition method. Details on the calculations can be found in Bayon et al. (2009). Briefly, for each sample, elemental concentrations were calculated using the sample mass, the amount of Tm added, and by calibrating the raw data acquired during the measurement session against the unspiked (no added Tm) in-house multi-element solution, run after every 5 samples.

Precision (degree of reproducibility) and accuracy (degree of veracity) of our procedure were controlled through analyses of a certified reference material purchased from the National Research Council of Canada (FEBS-1: red snapper Lutjanus campechanus sagittal otolith; certified values in Sturgeon et al., 2005). Repeated measurements of this reference material yielded a precision (relative standard deviation) of 0.76 %. Li concentration in FEBS-1 was slightly underestimated with an average value of 0.250±0.002 mg kg$^{-1}$ (mean ± standard deviation), compared with the recommended value of 0.305±0.044 mg kg$^{-1}$. No certified or reference Mo values were available at the time of PSO analyses.

**LA-ICP-MS methodology used at MPIC**

Prior to LA-ICP-MS analyses, the left valve of each specimen was hand-cleaned with a soft brush and subsequently immersed in an ultrasonication bath for 3 minutes (Elma ultrasonic cleaning unit, model Elmasonic S30). To fit into the ablation chamber of the laser system, the shells were cut along the axis of maximum growth using a hand drill equipped with a 150-µm thin diamond coated saw (Komet – Dental Gebr. Brasseler GmbH & Co. KG; Art.-No.: 6911H. 104.220). After cutting, the shell slabs were soaked for 1 minute in acetic acid (10 %) to remove surface contamination and afterwards rinsed with deionized water. Prior to ablation, each sample was pre-ablated with a spot size of 100 µm to remove fouling and contamination.
LA-ICP-MS analyses were performed at the Max Planck Institute for Chemistry (Mainz, Germany) using a NewWave Research UP-213 Nd:YAG laser ablation system with He as an initial carrier gas (quality 5.0, flow rate 0.57 L min⁻¹), coupled to a Thermo Fisher Element 2 single collector sector-field ICP-MS (at low-mass resolution mode) using Ar as an initial carrier gas (quality 5.0, flow rate 0.77 L min⁻¹). Analyzed isotopes were ⁷Li, ⁴³Ca and ⁹⁷Mo, besides other isotopes not presented in this study. The laser operated in line-scan mode with a laser spot size of 80 × 600 µm at a speed of 5 µm s⁻¹ and a repetition rate of 10 Hz with a laser energy density of 15.8 J cm⁻². To ensure the daily resolution of the data, ablation for each measurement was performed on the outer shell surface following the outline of individual daily increments. The obtained signal intensities were averaged for each ablation. Daily-resolved time series were measured following the direction of growth starting from one of the first stria formed after winter growth cessation to the ventral margin.

The synthetic silicate glass NIST SRM 612 (Jochum et al., 2011) was used as an external standard and ⁴³Ca as an internal standard, assuming a Ca content of 38 wt%. The data reduction was done using a Microsoft Excel spreadsheet, following the calculations published by Longerich et al. (1996) and Jochum et al. (2011, 2007). Based on the 3σ criterion, the standard deviation of the blank signal (15 seconds prior to sample ablation) was used to calculate the detection limits. On average, the calculated detection limits were \(3.3 \times 10^{-3}\) mmol mol⁻¹ for ⁷Li and \(3.6 \times 10^{-2}\) µmol mol⁻¹ for ⁹⁷Mo. In addition, the synthetic carbonate powder pellet USGS MACS-3 was used as quality control. Blind measurements of the MACS-3 material provided on average 52.8±1.6 µg g⁻¹ for Li, about 10 µg g⁻¹ lower than the published reference value (62.9 µg g⁻¹) and 1.66±0.2 µg g⁻¹ for Mo that differed about 0.4 µg g⁻¹ from the given literature value (1.21 µg g⁻¹). Reference values were provided by the GeoReM database (http://georem.mpch-mainz.gwdg.de/; last access: 22 February 2021). Measured deviations from the reference value were potentially induced by the changing ablation behavior caused by the varying size of the particles in the MACS-3 pellet leading to differences in ionization.
LA-ICP-MS methodology used at IPREM

Scallop shells were analysed at IPREM (Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials, Pau, France) using an IR 1030 nm femtosecond laser (Alfamet-Novalase, France) in conjunction with an ELAN DRC II ICP-MS (Perkin Elmer). The LA-ICP-MS was tuned daily for the best signal sensitivity while keeping complete particle atomisation. This was achieved by adjusting the plasma conditions so that U/Th ratio of 1 ± 0.05 on NIST SRM 612 (NIST, USA). The ICP-MS operating conditions were similar as those described by Tabouret et al. (2012). The ablation strategy consisted in linear raster 2D scan (beam diameter: 17 µm; speed: 5 µm s⁻¹; repetition rate: 1000 Hz), resulting in a 100 × 500 µm transect applied at the surface of the shell every third daily stria from the winter growth cessation to the ventral margin. Analyzed isotopes were \(^{7}\text{Li}, \(^{43}\text{Ca}, \(^{95}\text{Mo}\) nd \(^{97}\text{Mo}\).

External calibrations were performed using synthetic silicate glasses NIST SRM 614, NIST SRM 612 and NIST SRM 610. Analytical accuracy for Li was achieved with the fish otolith FEBS-1 (Sturgeon et al., 2005). Variation in ablation yield was checked using \(^{43}\text{Ca}\) as an internal standard for each ablation; the instrumental drift was followed using \(^{103}\text{Rh}\) in the nebulisation solution. Data processing and quantification were performed using a VBA Macro developed at the IPREM. Limits of detection were calculated based on a 3\(\sigma\) criterion, where \(\sigma\) is the standard deviation for 30 seconds measurements of the blank signal. Calculated detection limits were \(3.2 \times 10^{-2}\) µmol mol\(^{-1}\) for \(^{7}\text{Li}\), \(0.66 \times 10^{-3}\) µmol mol\(^{-1}\) for \(^{95}\text{Mo}\) and \(0.22 \times 10^{-3}\) µmol mol\(^{-1}\) for \(^{97}\text{Mo}\). FEBS-1 analyses provided on average 0.037±0.011 µmol mol\(^{-1}\) for Li that is very close from published reference value (0.044±0.006 µmol mol\(^{-1}\)). No certified or reference Mo values were available at the time of IPREM analyses.
SM3 - Description of the method used to analyze Li and Mo content in soft tissues

Powders were digested in Teflon vials with a mixture of 4 mL of 69 vol% nitric acid and 1 mL of 30 vol% hydrogen peroxide (reactants were of ultra-trace quality), heated at 105°C for 4 h under a fume hood. Aliquots of 700 µL were then diluted to 14 mL with ultra-pure deionized water (resistivity = 18.2 MΩ.cm). A set of six calibration solutions with increasing Li and Mo concentrations was prepared in 2.5% nitric acid. Digested tissues, calibration solutions and certified reference materials were finally analyzed on a quadrupole mass spectrometer coupled with induced plasma (Thermo Scientific X-Series 2) at PSO. Obtained Mo values, all expressed in micrograms of element per gram of the dry weight of tissues (µg g^{-1}), were 1.48±0.06 (n=5), 3.44±0.19 (n=5) and 0.30±0.03 (n=5), for DOLT-5, TORT-3 and DORM-4, respectively. These values were in line with the certified values (1.41±0.22 and 3.44±0.12, for DOLT-5 and TORT-3 respectively) and in line with the indicative value of DORM-4 (0.29). In the case of Li, certified or indicative values are not provided, but our measurements of the certified reference materials were of satisfactory reproducibility. Obtained Li values, all expressed in µg g^{-1}, were 0.076±0.004 (n=5), 1.09±0.03 (n=5) and 0.29±0.04 (n=5) for DOLT-5, TORT-3 and DORM-4, respectively.
**Supplementary Visuals**

**Supplementary Table S1:** Information on *Pecten maximus* specimens collected alive in the Bay of Brest that were chemically analyzed.

| Shell ID (curator) | Short ID (this study) | Date of collection | Sampling locality | Age class | Analytical method | Laboratory       |
|--------------------|-----------------------|--------------------|-------------------|-----------|-------------------|------------------|
| RBC1 10 2011 004   | A                     | 24/10/2011         | Lanvéoc           | I         | SN-ICP-MS         | PSO, Plouzane, FR |
| RBC1 10 2011 021   | B                     | 24/10/2011         | Lanvéoc           | I         | SN-ICP-MS         | PSO, Plouzane, FR |
| RBC1 10 2011 022   | C                     | 24/10/2011         | Lanvéoc           | I         | SN-ICP-MS         | PSO, Plouzane, FR |
| RBC1 08 2011 022   | D                     | 30/08/2011         | Lanvéoc           | I         | LA-ICP-MS         | MPIC, Mainz, DE  |
| RBC1 08 2011 023   | E                     | 30/08/2011         | Lanvéoc           | I         | LA-ICP-MS         | MPIC, Mainz, DE  |
| RBC1 08 2011 024   | F                     | 30/08/2011         | Lanvéoc           | I         | LA-ICP-MS         | MPIC, Mainz, DE  |
| RBC1 10 2011 033   | G                     | 24/10/2011         | Lanvéoc           | I         | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC1 10 2011 034   | H                     | 24/10/2011         | Lanvéoc           | I         | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC1 10 2011 035   | J                     | 24/10/2011         | Lanvéoc           | I         | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 01 2012 002   | K                     | 05/01/2012         | Roscanvel         | II        | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 01 2012 006   | L                     | 05/01/2012         | Roscanvel         | II        | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 01 2012 008   | M                     | 05/01/2012         | Roscanvel         | II        | LA-ICP-MS         | IPREM, Pau, FR   |

Shells analyzed for their 1st full year of growth

Shells analyzed for their 2nd full year of growth

| Shell ID (curator) | Short ID (this study) | Date of collection | Sampling locality | Age class | Analytical method | Laboratory       |
|--------------------|-----------------------|--------------------|-------------------|-----------|-------------------|------------------|
| RBC2 10 2011 003   | N                     | 17/10/2011         | Lanvéoc           | II        | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 10 2011 004   | P                     | 17/10/2011         | Lanvéoc           | II        | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 10 2011 005   | R                     | 24/10/2011         | Lanvéoc           | II        | LA-ICP-MS         | IPREM, Pau, FR   |
| RBC2 10 2011 006   | S                     | 24/10/2011         | Lanvéoc           | II        | LA-ICP-MS         | IPREM, Pau, FR   |
Supplementary Figure S1: Average daily shell growth rate after the 1\textsuperscript{st} winter growth check, measured in scallop shells analyzed for Mo:Ca and Li:Ca ratios (mean + 95 % upper confidence interval). Green vertical bar denotes the main spring phytoplankton bloom.

Supplementary Figure S2: Springtime variations of the Aulne River water discharge (Châteaulin gauging station), and seawater temperature and salinity at Lanvéoc (average over the entire water column).
Supplementary References

Bayon, G., J.-A. Barrat, J. Etoubleau, M. Benoit, C. Bollinger, and S. Révillon. 2009. Determination of rare earth elements, Sc, Y, Zr, Ba, Hf and Th in geological samples by ICP-MS after Tm addition and alkaline fusion. Geostand. Geoanal. Res. 33: 51-62.

Edler, L., and M. Elbrächter. 2010. The Utermöhl method for quantitative phytoplankton analysis, p. 13-20. In B. Karlson, C. Cusack and E. Bresnan [eds.], Microscopic and molecular methods for quantitative phytoplankton analysis. Intergovernmental Oceanographic Commission Manuals and Guides, UNESCO.

Jochum, K. P., B. Stoll, K. Herwig, and M. Willbold. 2007. Validation of LA-ICP-MS trace element analysis of geological glasses using a new solid-state 193 nm Nd:YAG laser and matrix-matched calibration. J. Anal. At. Spectrom. 22: 112-121.

Jochum, K. P., and others. 2011. Determination of reference values for NIST SRM 610–617 glasses following ISO guidelines. Geostand. Geoanal. Res. 35: 397-429.

Longerich, H. P., S. E. Jackson, and D. Günther. 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. 11: 899-904.

Lorenzen, C. J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep-Sea Res. Oceanogr. Abstr. 13: 223-227.

Quentel, F., C. Elleouet, and C. Madec. 1992. Synergic effect of fulvic acids on the differential pulse adsorptive voltammetry of the Mo(VI) phenanthroline complex. Electroanalysis 4: 707-711.

Sturgeon, R. E., and others. 2005. Certification of a fish otolith reference material in support of quality assurance of trace element analysis. J. Anal. At. Spectrom. 20: 1067-1071.
Tabouret, H., S. Pomerleau, A. Jolivet, C. Pécheyran, R. Riso, J. Thébault, L. Chauvaud, and D. Amouroux. 2012. Specific pathways for the incorporation of dissolved barium and molybdenum into the bivalve shell: An isotopic tracer approach in the juvenile great scallop (Pecten maximus). Mar. Environ. Res. 78: 15-25.

Tréguer, P., and P. Le Corre. 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'Auto-Analyseur II Technicon, 2nd ed. Laboratoire d'Oceanographie Chimique, Université de Bretagne Occidentale, Brest.