1 **Mg/Ca from mussel shells rather than δ^{18}O as a promising temperature proxy for**
2 **hydrothermal vent ecosystems**
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22 **Abstract**
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24   Minor and trace metal fluctuations in biogenic carbonates have been demonstrated to
25 be important potential environmental tracers in coastal areas, but remained poorly studied in
26 deep-sea environments. For the first time, this study assesses the use of Mg/Ca signal as a
27 thermometer proxy in hydrothermal vent ecosystems together with the analysis of oxygen
28 stable isotope composition. *Bathymodiolus azoricus* and *B. thermophilus* mussels were
29 collected at three hydrothermal vent fields from the mid-Atlantic ridge (Rainbow, Menez
30 Gwen) and the East Pacific Rise (EPR 9°50N), presenting contrasted temperature and
31 chemical habitat conditions. The variation of Mg/Ca was analysed via LA-ICP-MS and is
32 revealed suitable to reconstruct temperature variations in these ecosystems, presumably due to
33 stable Mg compositions of the seawater surrounding hydrothermal systems. Temperature
34 anomalies inferred from Mg/Ca ratios can be good tracers of fluid pulses. Important fluid
35 emissions appear however to generate major growth cessation in shell mineralization
36 Temperatures inferred from IRMS δ^{18}O analyses systematically underestimated the measured
37 values in the environment. The isotopic disequilibrium is likely due to pH fluctuations in the
38 mussel habitat, in the vicinity of vent fluid discharges, and/or interactions with the symbiotic
39 chemosynthetic bacteria. Those results will first benefit to the ecological study of deep-sea
40 mussels, but also provide a promising contribution for the study of the environmental
41 dynamic in hydrothermal systems at short (daily) to long-term (pluri-annual) scales, recorded
42 in the calcite material of bivalves.
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44 **Keywords**
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1. Introduction

As they build carbonate structures from ions sampled from the surrounding environment, mollusc shells are used as a natural probe of past and present climate and environmental parameters (Dodd, 1965; Klein et al., 1996; Huyghe et al., 2015). The shells are formed by incremental growth, preventing the destruction of previously secreted parts, which allows the evaluation of the environmental variations throughout the life of an organism (Schöne, 2008). Historically, this type of study started with the measurements of oxygen isotope ratios ($\delta^{18}O$; Urey, 1947), which are commonly used as a proxy for seasonal temperature reconstructions in a variety of environments such as coastal (Klein et al., 1996; Lartaud et al., 2010a; Welsh et al., 2011) and pelagic (Urey et al., 1951; Schöne et al. 2005a) settings. However, several limitations have been identified for the use of this proxy. First, $\delta^{18}O$ in shells, not only changes with temperature, but also with the seawater $\delta^{18}O$ composition ($\delta^{18}O_w$), which covaries with salinity, and is not properly constrained particularly in coastal environments (Rohling, 2000). Second, strong disequilibrium between seawater and biogenic carbonates has been observed in numerous taxa (Urey et al., 1951; Aharon, 1991; Daëron et al., 2019). Although these disequilibria, called 'vital effects' (*i.e.*, resulting from kinetic and physiological impacts during biomineralization), appear limited in mollusc biominerals (Aharon, 1991; Welsh et al., 2011), strong isotopic anomalies have been recently highlighted in juvenile oyster shells, likely due to kinetic effects during this period of rapid growth (Huyghe et al., 2020), and for deep-sea oysters (Wisshak et al., 2009; Huyghe et al., 2022).

As alternative proxies for temperature, elemental ratios (*i.e.*, Mg/Ca in calcite and Sr/Ca in aragonite) have been investigated and salinity fluctuations appear to have a weak impact, if any (Vander Putten et al., 2000; Mouchi et al., 2013). However, differences in Mg uptake from natural sites or aquarium-based rearing experiments have been reported for mytilids (Klein et al., 1996; Vander Putten et al., 2000; Freitas et al., 2008; Wanamaker et al., 2008; Tanaka et al., 2019), oysters (Surge and Lohmann, 2008; Mouchi et al., 2013; Tynan et al., 2017) and pectinids (Freitas et al., 2012). In coastal waters, although some Mg/Ca-temperature relationships appear very similar depending on the environmental settings (Mouchi et al., 2018), it has been reported that Mg/Ca variations in seawater caused by rapid salinity changes and Mg supply from continental rock alteration can mask the temperature effect on the Mg uptake in shell carbonates (Dodd, 1965; Wanamaker et al., 2008). Vital effects may also impact elemental uptake (Carré et al., 2006; Schöne et al., 2011; Mouchi et al., 2013).

In deep-sea environments, few studies were dedicated to the analyses of metal concentrations in calcifying materials. Most focus has been on investigations in cold-water coral skeletons, where Mg/Ca ratio has been demonstrated to be an unreliable paleothermometer proxy (Lopez-Correa et al., 2010; Robinson et al., 2014). Although potentially in the vicinity of hydrothermal systems, those corals are not directly associated with vent fluids in contrast to chemosynthetic bivalve species such as hydrothermal mussels. The geochemical proxies studied in bivalve shells from hydrothermal vents focused on stable isotopes, such as the $\delta^{13}C$ to reconstruct chemosynthetic pathways (Nedoncelle et al., 2014) or carbon sources (Naraoka et al., 2008; Lietard and Pierre, 2009; Lartaud et al., 2010b, 2011), and the use of minor and trace elements for the characterization of heavy metals present in the mixing zone (Kádár et al., 2005; Cravo et al., 2008) or the study of metal detoxification processes (Demina et al., 2013). The studies thus focused on trace metals typically associated with hydrothermal systems (*e.g.*, Fe, Mn, Zn, Cu, Cd), and other classical elements used in shallow-water biological archives were left behind, particularly Mg/Ca as end-member

100 hydrothermal fluids are severely depleted in magnesium (Corliss et al., 1979; Charlou et al.,
101 2002). Although no precise calibrations were performed, previous works from Rio et al.
102 (1988) suggest that the Mg/Ca and Sr/Ca ratios in hydrothermal bivalve shells are consistent
103 with those of the ambient seawater. Shirai et al. (2008) deeply studied the distribution of
104 Mg/Ca in *Bathymodiolus* shells but they focused on the inner aragonite nacreous layer, which
105 is generally not used for environmental reconstruction in shallow-water species. Indeed,
106 sclerochemistry in bivalve shells is mostly performed on the outer calcite prismatic layer, an
107 area associated with higher growth rates leading to better time-resolution sampling.
108   Mytilids from the genus *Bathymodiolus* are among the most successful colonizers of
109 vent environments and often dominate the biomass of hydrothermal communities (Van Dover,
110 2000). *Bathymodiolus* habitat is characterized by fluctuation of the environmental parameters,
111 including pH, oxygen and temperature, consecutively to hydrothermal fluid mixing with
112 background cold-seawater (Le Bris and Duperron, 2010; Le Bris et al. 2019). The
113 temperatures range classically from strictly deep seawater temperature (i.e., 2-4°C) to fluid-
114 related (reaching 10-15°C), associated to the influence of tides on the deep-sea current regime
115 and fluid vent flux (Johnson et al., 1994; Tivey et al., 2002; Nedoncelle et al., 2015; Lelièvre
116 et al. 2017). This range seems to be maintained for long time periods, only disrupted by
117 occasional anomalies (Scheirer et al., 2006; Khripounoff et al., 2008; Mullineaux et al., 2012).
118   The purpose of this paper is to investigate the potential of using Mg/Ca and $\delta^{18}O$ in
119 deep-sea mussel shells from hydrothermal vents. Particularly, the study focuses on the
120 variations of these proxies during growth, to investigate the record of the vent dynamics.
121 Using *Bathymodiolus azoricus* (von Cosel et al., 1999) and *B. thermophilus* (Kenk & Wilson,
122 1985) growing at five contrasting temperature vent habitats from three sites, the evolution of
123 Mg/Ca was measured by LA-ICP-MS and $\delta^{18}O$ by IRMS and compared to the temperature
124 monitored in the mussel habitat.
125
126   **2. Methods**
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128   2.1. Sampling area
129
130   Mussel shells were collected at three hydrothermal vent sites from the Mid-Atlantic
131 Ridge (MAR) and the East Pacific Rise (EPR): Rainbow (36°13.8'N-33°54.1'W, 2275 m),
132 Menez Gwen (37°50.6'N-31°31.2'W, 815 m) and EPR 9°50'N (09°47.3'N-104°17.0'W,
133 2513m). Rainbow end-member fluids formed in ultramafic basement are characterized by
134 high methane and hydrogen contents but also high metal concentrations. Menez Gwen fluids
135 are also characterized by methane and hydrogen gases but depleted in metals (Charlou et al.,
136 2002). Due to lower depths, the average temperature in the mussel habitat is higher at Menez
137 Gwen (10.9°C) than at Rainbow (3.7°C) (Desbruyères et al., 2001). EPR vent fluids exhibit
138 important free sulphide but low methane and hydrogen concentrations.
139   Live *B. azoricus* from Rainbow were collected during the MoMARDREAM cruise
140 (CNRS, R/V L'Atalante, August-September 2008) using the Victor 6000 ROV and then
141 dissected onboard. Two areas of collection were selected as described in Nedoncelle et al.
142 (2014): Z1 zone, which corresponds to mussels living at the basis of a sulphide structure
143 about 1 meter-high in the immediate vicinity of a small spire-type active chimney; and Z3
144 zone, which harbours a higher density of mussels and is located further away from the
145 chimney on the top of the rocky structure at ~1 m above Z1 (Fig. 1).
146   At Menez Gwen, live *B. azoricus* shells were collected during the MenezMAR cruise
147 (MARUM, R/V Meteor, September-October 2010) using the Quest 4000 ROV. Mussels were
148 sampled at Cage Site (CS), from a small discretely distributed mussel patch at the top of a

149 hydrothermal area presenting some active diffuse vents, and along the flank of the White
150 Flame (WF) site fully colonized by mussels and dominated by a large active white smoker
151 (Fig. 1).
152   The live *B. thermophilus* from EPR 9°50N were collected during the MESCAL-1
153 cruise (UPMC, R/V *L'Atalante*, April-May 2010) with the Nautile submersible in a ~50 m^2
154 mussel bed at V-Vent site (MES), a diffuse vent area on the basaltic seafloor (Fig. 1) (see
155 Nedoncelle et al., 2013 for more details).
156
157   2.2. Shell preparation and observation
158
159   *Bathymodiolus* shells are constituted of an aragonite lower (inner) layer and a calcite
160 upper (outer) layer (Carter, 1990; Nedoncelle et al., 2013). Geochemical analyses were
161 performed on the calcite upper layer, since calcite is less sensitive to alteration and dissolution
162 than aragonite in those environments, and preserves the pristine signal from diagenetic
163 alterations (Lutz et al., 1994). Shells were sectioned along the maximum growth axis and
164 mounted on epoxy blocks. Sections were ground flat using 180, 400 and 800 grit powder with
165 distilled water and then polished using Al_2O_3 powder at 3, 1 and 0.3 µm. A Mutvei treatment
166 (Schöne et al., 2005b) was used to reveal growth structures (Fig. 2A). Major growth lines
167 were identified as growth lines present both in the upper calcitic and lower aragonitic layers,
168 often accompanied with shell distortion on the external surface (Schöne and Giere, 2005).
169 Part of the data were from Nedoncelle et al. (2014).
170   During the MenezMAR cruise, shells from Cage Site at Menez Gwen were stained
171 with calcein following the protocol described in Nedoncelle et al. (2013) and recovered after
172 10 days. Despite the success of staining (100% of the shells revealed the calcein line), no
173 growth was observed during the 10 days mark and recapture period (Supplementary Figure
174 S1), contrary to the *B. thermophilus* used in Nedoncelle et al. (2013).
175
176   2.3. LA-ICP-MS analyses
177
178   Four shells from Rainbow (1 for Zone 1 and 3 for Zone 3, with sizes ranging from 34
179 to 52 mm), four shells from Menez Gwen vent sites (2 for Cage Site and 2 for White Flame,
180 with shell sizes from 46 to 84 mm), and two shells from EPR 9°50N (sizes of 154 and 170
181 mm) were analysed for Mg abundances. Before elemental analyses, the sections were
182 repolished to remove the Mutvei solution and prevent any influence of this treatment on the
183 chemical analyses. The shell sections were analysed by Laser Ablation-Inductively Coupled-
184 Plasma-Mass Spectrometry (LA-ICP-MS) with a Compex102 193 nm excimer laser (Lambda
185 Physik) coupled to a sector field ICP-MS (Thermofinnigan Element XR) at Montpellier
186 University (AETE-ISO platform of the OSU OREME). Measured elements were ^{24}Mg and
187 ^{43}Ca as the internal standard to control the ablated volume. The laser was operated at a
188 repetition rate of 7 Hz and an energy density of 12 J cm^{-2}. Each analysis was done as a single
189 spot using a spot size of 51 µm (Fig 2B) after a pre-ablation time of 1 second for surface
190 cleaning and corresponds to a total acquisition time of 180 seconds with the first 120 seconds
191 as background acquisition (gas blank). Data were normalized using the ^{43}Ca signal (internal
192 standard) according to Longerich et al. (1996) and calibrated using NIST SRM 612 glass
193 reference material (values after Pearce et al., 1997). Reproducibility of the values in the shell
194 samples was also tested using the USGS MACS-3 carbonate reference material. Both
195 reference materials were analysed every 10 measurements. Precision based on the analysis of
196 the reference materials was approximately ±5%.
197

198   2.4. Temperature reconstruction from Mg/Ca

199

200   Values of Mg/Ca were converted into temperatures using the equation from Vander
201 Putten et al. (2000), established on the shell of the shallow water mussel *Mytilus edulis*:

202

203   Mg/Ca (mmol. mol^{-1}) = 0.70 * T(°C) – 0.63 (r = 0.91)

204

205 This equation was chosen among the different equations existing for bivalve shells as it was
206 established with a Mytilid species, as *Bathymodiolus* (Mikkelsen et al., 2006), with a similar
207 shell microstructure (*i.e.*, fibrous prismatic calcite layer) in the area investigated (Carter,
208 1980; Machado et al., 2009; Génio et al., 2012; Checa et al., 2014). Additionally, in the
209 course of this study, the Mg/Ca measurement was performed with LA-ICP-MS, similarly to
210 Vander Putten et al. (2000). This contrasts with the other temperature-Mg/Ca relationships
211 published for *M. edulis* which were acquired by ICP-AES analyses (Klein et al., 1996;
212 Wanamaker et al., 2008).

213

214   2.5. Oxygen isotope ratios

215

216   Shells were placed in a peroxide solution (H_2O_2 3.4%) during 6 to 10 h at 60°C to
217 remove the periostracum and then rinsed with demineralized water before sampling the calcite
218 outer layer for the oxygen isotopes ($\delta^{18}O$) analysis (see Nedoncelle et al., 2014). Shells were
219 then drilled every 1 mm from the ventral margin to the hinge area along the maximum growth
220 axis, with a Dremel device equipped with a 0.3 mm drill bit. The nacreous aragonite
221 underlayer was carefully avoided to preclude any mineralogical effect on the isotopic values
222 as aragonite is comparatively to calcite enriched in ^{18}O by 0.6 ‰ (Tarutani et al., 1969).
223   The collected calcium carbonate powders were acidified in 100% H_3PO_4 at 90°C
224 under vacuum. The CO_2 produced was collected and analysed using a VG Instruments
225 Isoprime mass spectrometer. Isotopic data are reported in the conventional delta (δ) notation
226 relative to the Vienna Pee Dee Belemnite (V-PDB). The standard deviation for $\delta^{18}O$ values
227 was ± 0.1 ‰.
228 Seawater samples directly above the mussel bed were additionally collected during the dives
229 for mussel collection at EPR 9°50N (MES) and Rainbow (Z1 and Z3). 20 mL of *in situ* water
230 were sampled in airtight titanium syringes and analysed following the method of Pierre et al.
231 (1994). The analyses were performed at the Institut d'Écologie et des Sciences de
232 l'Environnement de Paris at Grignon. Seawater oxygen isotope compositions ($\delta^{18}O_w$) were
233 obtained after CO_2-H_2O equilibration using an Isoprime mass spectrometer coupled to a
234 Gilson X222 Micromass Aquaprep. Standard error for $\delta^{18}O_w$ was 0.15 ‰ and values
235 expressed relative to SMOW reference.

236

237   2.6. Temperature reconstruction from $\delta^{18}O$

238

239   Based on the thermodynamic model for synthetic and biogenic carbonates,
240 temperatures can be estimated from shell $\delta^{18}O$ and $\delta^{18}O_w$ measured in each habitat. We used
241 the equation of Kim & O'Neil (1997):

242

243   1000 x ln(α) = 18.03 x (10^3 x T^{-1}) - 32.42

244

245   where α represents the isotope fractionation factor:

246

247    $\alpha = (1000 + \delta^{18}O_{shell}) / (1000 + \delta^{18}O_w)$

248

249   In this last equation, both $\delta^{18}O_{shell}$ and $\delta^{18}O_w$ are expressed in ‰ SMOW. $\delta^{18}O_{shell}$ were
250   converted from V-PDB to SMOW according to the equation of Coplen et al. (1983).

251

252   As no $\delta^{18}O_w$ were measured from habitats at Menez Gwen, and considering its proximity with
253   Rainbow, we used the mean $\delta^{18}O_w$ value of Rainbow for temperature reconstructions from
254   shells of Menez Gwen.

255
256   2.7. Temperature probes

257
258   *In situ* temperature measurements in mussel habitats were acquired at discrete
259   locations in Rainbow (on both Z1 and Z3 areas), based on continuous records over 5 minutes
260   for each analysis using a glass Ag/AgCl electrode coupled to a thermocouple (MICREL,
261   France) (Fig. 1). Discrete measurements were also acquired at Menez Gwen (White Flame)
262   by a temperature probe fixed to the sampling nozzle of the Kiel In Situ Pumping System
263   (KIPS) (Meier et al., 2016; raw data pers. comm. C. Vidoudez). At Menez Gwen (Cage Site)
264   and EPR 9°50N (V-Vent), autonomous probes (S2T6000, NKE) were used above the
265   collected mussels (Fig. 1). The autonomous probes were deployed during 10 days at Menez
266   Gwen (*i.e.*, during the staining experiment) on the mussel bed studied and recovered at the
267   time of mussel sampling, with an acquisition every 5 min during this period. The deployment
268   protocol of autonomous probes at EPR 9°50 was described in Nedoncelle et al. (2015). The
269   probes monitored the habitat conditions during 10 days with a frequency of 2 measurements
270   per hour.

271
272   2.8. Statistical analysis

273
274   Comparisons of isotopic and Mg compositions between shells were performed using
275   Matlab (R2022a; www.mathworks.com) by applying the Kruskal and Wallis test (KW)
276   followed by a *post hoc* test to discriminate the outlying groups (Tukey's honestly significant
277   difference procedure; HSD). The KW test is used for comparison of more than two samples to
278   infer if at least one sample is different from the others, whereas the HSD test determines
279   which sample is different from the others. Before using this non-parametric test, the non-
280   respect of the normality and homoscedasticity conditions were checked using the
281   Kolmogorov-Smirnov and Levene tests, respectively. Shell Mg/Ca were compared using the
282   correlation test of Spearman.

283
284   **3. Results**

285
286   3.1. Mg/Ca analysis

287
288   The Mg/Ca values range from 1.8 to 11.1 mmol.mol^{-1} (average of 3.1 ± 1.1 mmol.mol^{-1}) in shells from Rainbow, from 3.3 to 19.5 mmol.mol^{-1} (average of 6.8 ± 3.4 mmol.mol^{-1}) in
290   shells from Menez Gwen, and from 2.8 to 4.5 mmol.mol^{-1} (average of 3.4 ± 0.4 mmol.mol^{-1})
291   in shells from EPR 9°50N (Fig. 3). We observe highly significant differences in shell Mg/Ca

292 between sites (Kruskal and Wallis test, p<0.01). Similarly, we note significant differences
293 between habitats within both Menez Gwen and Rainbow (Kruskal and Wallis test, p<0.01).
294
295   The temperatures for Rainbow, estimated from Mg/Ca in the shells, range from 3.5 to
296 16.7°C (average of 5.3 ± 1.5°C). These calculated temperatures are quite stable (variation of
297 ~1°C) with the exceptions of one very localized peak at 10.1°C in the shell Z3-116 and two
298 thin peaks above 12°C in the shell Z3-131 (Fig. 3). For each shell, these values are close
299 similar to temperatures measured in the mussel habitat, showing an average of 5.6 ± 0.9°C in
300 Z1 mussel habitat and 4.8±0.4°C in Z3 habitat (Table 1, Fig. 3).
301
302   The estimated temperatures from the Menez Gwen shells range from 5.6 to 28.8°C
303 (average of 10.7 ± 4.8°C) (Fig. 3). The two shells from Cage Site exhibit the same pattern
304 with a slight variability in the estimated temperatures (between 8.4 and 13.5°C) before a
305 strong increase at the end of the deployment period (close to the shell ventral margin), with
306 temperatures over 25°C. We observe a similar pattern at White Flame for shell WF-24, which
307 shows a relatively stable estimated temperature (between 7.0 and 8.9°C) before an increase up
308 to 16.4°C at the end of the profile. However, shell WF-20 does not exhibit such an increase of
309 estimated temperature, which remains between 5.6 and 8.0°C across the transect.
310   For EPR 9°50N, both shells exhibit the same relatively stable estimated temperatures,
311 ranging from 4.9 to 6.9°C and from 4.9 to 7.4°C, for MES6 and MES10, respectively.
312
313   The shell Z3-131, from Rainbow vent site, displays two major growth anomalies,
314 close similar to the major growth lines (based on the description of major growth line made
315 by Schöne and Giere (2005) in *Bathymodiolus brevior* and by Nedoncelle et al. (2014) in *B.
316 azoricus* shells). These growth breaks are associated with the two peaks of Mg/Ca (Fig. 3).
317 For both, the Mg/Ca values increased just before the occurrence of the major growth line
318 (following the direction of growth), reaching the maximum values in the major growth line,
319 and fall to the baseline values after. Two major growth lines were also detected in Z3-116 and
320 one of these (M1, see Fig. 2) corresponds to a Mg/Ca peak (Fig. 3).
321   At Menez Gwen, the two shells from Cage Site, as well as shell specimen WF24 from
322 White Flame, displayed a significant increase in Mg/Ca at the end of the profile (Fig. 3), but
323 no major growth line was clearly observed. Nevertheless, the staining experiment revealed an
324 absence of mineralization during the last 10 days of life (Supplementary Figure S1), likely
325 suggesting the formation of a growth stop (i.e., a major growth line) at this period. Thus
326 Mg/Ca increase would anticipate the growth line formation as observed for Z3-116 and Z3-
327 131 (Fig. 3). No major growth lines were detected in the shells from EPR 9°50N.
328
329   3.2. Oxygen stable isotope ratios
330
331   The measured $\delta^{18}O_w$ at Rainbow range from 0.05‰ (SMOW) at Z3 to 0.27‰
332 (SMOW) at Z1. Due to the lack of data in mussel beds at Menez Gwen and its proximity to
333 Rainbow vent field, we assume a mean value of 0.16‰ (SMOW) for mussel habitats at
334 Menez Gwen. The measurements in water surrounding mussels from EPR 9°50N gives a
335 $\delta^{18}O_w$ value of -0.44‰ (SMOW).
336   Shells from Rainbow present $\delta^{18}O$ values between -1.37 and 4.12‰ (VPDB), and
337 exhibit significant differences between (p<0.01) and within habitats (Z1-7 different from Z1-1
338 and Z1-4: p<0.01; Z3-131 different from A3-LIV4 and Z3-118: p<0.01). Shells from Menez
339 Gwen range from 2.52 to 3.91‰. A significant difference between shells between (p<0.01)
340 and within each habitat is also observed (WF-24 different from WF-20 and WF-22: p<0.01;

341 CS-4 different from CS-7: p<0.01). $\delta^{18}O$ of shells from EPR 9°50N range from 2.21 to
342 4.27‰, and show no significant difference between shells from this site (p=0.57). We also
343 note a significant difference between sites (p<0.01).
344   Reconstructed temperatures from each shell are presented in Figure 4. The estimated
345 temperatures are mostly lower than those measured on site (Fig. 4; Table 2). At Rainbow, two
346 shells from Zone 1 present estimated temperatures lower than the measured *in situ*
347 temperature range (mean estimates at 2.3°C and 2.9°C respectively, while mean measured
348 temperature is 5.6°C), along with some values below 0°C, and only one shell (Z1-7) harbors
349 reconstructed values in agreement with measured temperatures (mean estimated temperature:
350 5.2°C; Fig. 4). For the Zone 3 of Rainbow, except for two values from one shell (A3-LIV4)
351 which give estimated temperatures within the range of measured temperatures, the rest of
352 reconstructed temperatures are below the minimum recorded temperature, with some negative
353 values. All estimated temperatures from shells of Menez Gwen are deeply lower than the
354 minimum measured on site, with occasionally negative reconstructed temperatures at Cage
355 Site.
356   Only one value of estimated temperatures from shells of EPR 9°50N is above 0°C. All
357 others are highly below the measured temperatures.
358
359   **4.   Discussion**
360
361   4.1. Mg/Ca of bivalve shells as a possible thermometer proxy in hydrothermal settings
362
363   Within an error of ~1°C, the skeletal Mg/Ca ratios in *B. azoricus* and *B. thermophilus*
364 allow a reliable reconstruction of the average temperatures measured in the mussel habitat of
365 the investigated vent sites, from the Atlantic and Pacific ridges (Table 1; Fig. 3). Although
366 established with a shallow-water mussel species, the equation from Vander Putten et al.
367 (2000) appears suitable to estimate temperatures using Mg/Ca ratios of deep-sea mussel
368 shells. This is presumably due to (i) similar mineralogical and microstructural shell
369 organisation of the two Mytilid groups (Génio et al., 2012; Checa et al., 2014) and (ii) the
370 specificity of geochemical conditions in these ecosystems. Far from the main continental
371 inputs of Mg, the mussels used a stable supply of Mg from seawater to form their shells as the
372 hydrothermal fluids are depleted in Mg (Charlou et al., 2002). Based on this assertion, the
373 distribution coefficient of Mg^{2+} into the calcite material is strongly influenced by temperature
374 changes. Thus, the difference observed between shells from the vent sites rely mainly to the
375 different temperature conditions.
376   As it was reported for shallow-water mussel species (Lorens and Bender, 1980), the
377 Mg/Ca ratio does not change in relation to growth variations in *Bathymodiolus* shells. An
378 ontogenic trend in Mg/Ca was described for different bivalve species (Freitas et al., 2005;
379 Schöne et al., 2011; Mouchi et al., 2013) and could explain the slightly highest values
380 observed in specimen Z3-131 (Fig. 3), the smallest shell, compared to other shells from
381 Rainbow. But this 'age effect' should have a lower influence in regard to temperature as
382 specimen CS9 from Menez Gwen is in the same size range than the specimens from Rainbow,
383 with clearly higher Mg/Ca ratios, and shells from EPR 9°50N exhibit similar values to shells
384 from Rainbow while three times bigger. A better ontogenic growth profile for this species is
385 however needed as growth rates can change according to the local environmental conditions
386 (Nedoncelle et al., 2013).
387   Except for rare peaks, the reconstructed temperatures are quite stable at Rainbow
388 (within ~1°C oscillation), which is consistent with the measurements done at this site. At

389   Menez Gwen – Cage Site habitat, before the large increase at the end of both shell profiles
390   close to the ventral margin, that might be related to a specific process (see below), the
391   estimated temperatures show more variability, with ~5°C range. This variability is higher
392   compared to our measurements done at this site during 10 days, but close to the temperature
393   changes observed on long-term monitoring at Lucky Strike, the closest known site to Menez
394   Gwen, and could refer to the lunar and two lunar periodicities identified there (Sarrazin et al.,
395   2014). The lunar-related temperature dynamic is classically observed in *Bathymodiolus*
396   habitats (Khripounoff et al., 2008; Nedoncelle et al., 2015). Similarly, at White Flame, the
397   reconstructed temperatures (i.e., 5.6-8.9°C when final increase of WF-24 shell is avoided)
398   should reflect the environmental periodicity of temperatures. The range of values are however
399   slightly underestimated compared to the discrete measured temperature from this study during
400   the MenezMAR cruise (8.4-20.4°C), but are close similar to the minimum recorded from
401   other studies on this vent site (i.e., 7.2°C; Colaço et al., 1998).
402   Short-term peaks of Mg/Ca are however observed in some shells, and seems to be
403   related to the formation of major growth breaks (called major growth lines in Schöne and
404   Giere, 2005). The corresponding estimated temperatures increase reaches up to 17°C at
405   Rainbow and 29°C at Menez Gwen, consistent with the maximum values measured at these
406   sites (Table 1; Sarradin et al., 1999; Sarrazin et al., 2020). The maximum temperature
407   recorded in the major growth lines can be however slightly overestimated due to possible high
408   Mg concentrations in the shell organic matrix (Rosenberg and Hughes, 1991; Takesue et al.,
409   2008; Lartaud et al., 2010b; Pérez-Huerta et al., 2020). Formation of shell organic matrix in
410   biogenic carbonates is rich in sulphur, either as sulphated polysaccharides or S-rich
411   aminoacids, and for some species distribution of Mg correlates with S variability (Lorens and
412   Bender, 1980; England et al., 2006; Foster et al., 2008). Some shells (WF-24, CS-6, CS-9, Z1-
413   4) display a rise of Mg/Ca in the most recent shell parts (near collection date). This increase
414   may correspond to the current formation of major growth lines, as we noted an absence of
415   shell growth from calcein labels 10 days prior collection (Supplementary Figure S1).
416
417   4.2. Oxygen isotope ratios from hydrothermal vent mussels are unreliable temperature proxies

418
419   Contrary to Mg/Ca, δ^{18}O-inferred temperatures are underestimated, with values generally near
420   or below 0°C, which are unrealistic in these settings. Only one shell from Z1 presents
421   reconstructed values close to the temperature measurements. All other shells from the
422   different sites and habitats present reconstructed temperatures strongly lower than the
423   minimum *in situ* temperature (Fig. 4). The measured values of $\delta^{18}O_w$, required to convert shell
424   δ^{18}O into temperatures, may be a source of uncertainty if this parameter fluctuates outside the
425   sampling period. Based on Kim and O'Neil (1997) equation, the $\delta^{18}O_w$ value expected to
426   reconstruct accurate temperatures from shell δ^{18}O can be inferred. The expected values at
427   both sites of Rainbow (0.55 and 0.71‰ at Z1 and Z3, respectively) are slightly higher than
428   those measured (0.05‰ and 0.27‰, respectively), but within the known $\delta^{18}O_w$ from
429   Rainbow, which linearly ranges from 0‰ (seawater) to ~1‰ (hydrothermal fluid) (Andreani
430   et al., 2014). However, such $\delta^{18}O_w$ values would correspond to a mixing of >50% of end-
431   member fluid, leading to a temperature of ~180°C if we assume temperature of hydrothermal
432   fluids reaching 365°C at Rainbow (Charlou et al., 2002), which is unlikely for an area suitable
433   for mussels. The expected $\delta^{18}O_w$ values at Menez Gwen (White Flame and Cage Site habitats)
434   and EPR 9°50N are substantially higher (1.91, 1.97 and 1.74‰, respectively). These values
435   are largely above the current measured or estimated values for this site (i.e., 0.16 and -0.44‰
436   at Menez Gwen and EPR 9°50N, respectively). They also exceed $\delta^{18}O_w$ reports from Menez
437   Gwen hydrothermal end-member (1.17±0.06‰) (Jean-Baptiste et al., 1997) but are close to

438   the higher values recorded in the hydrothermal fluids from EPR 9-10°N (0.6 to 1.9 ‰)
439   (Shanks et al., 1991). Still, the corresponding conditions for such $\delta^{18}O_w$ values surrounding
440   mussels would reach a mixing with > 90% of end-member, thus a temperature of ~347°C if
441   we assume temperature of hydrothermal fluids reaching 390°C at EPR 9°50N (Proskurowski
442   et al., 2008), far above the tolerance limit values of temperature for *Bathymodiolus* observed
443   experimentally (i.e., 25-30°C, Boutet et al., 2009). A wrong estimation of $\delta^{18}O_w$ cannot
444   therefore explain the discrepancy in reconstructed temperatures from shell $\delta^{18}O$.
445   Based on measured temperatures and $\delta^{18}O_w$ at sampling sites, the expected value of shell $\delta^{18}O$
446   at equilibrium with the environment was done using the equation of Kim & O'Neil (1997),
447   and compared to the median shell $\delta^{18}O$ values. The resulting difference corresponds to the
448   oxygen isotope disequilibrium (Table 3). *Bathymodiolus azoricus* from Rainbow are closer to
449   isotopic equilibrium (i.e., mean disequilibrium of 0.28‰ and 0.66‰ for Z1 and Z3 habitats,
450   respectively) than the shells from the other sites (disequilibrium of 1.75 ‰ for *B. azoricus*
451   shells from White Flame and 1.81 ‰ for the ones from Cage Site, and 2.18‰ for *B.*
452   *thermophilus* from EPR 9°50N). Such disequilibrium could be related to the influence of pH
453   on oxygen isotope composition in biogenic carbonates (Usdowski et al. 1991; Kim et O'Neil
454   1997; Zeebe 1999). Particularly at hydrothermal vents, pH can greatly change with time and
455   location, leading to a range of pH of 6.2 to 8 in *Bathymodiolus* habitats on the MAR and the
456   EPR (Desbruyères et al. 2001; Kadar et al., 2005; Nedoncelle et al., 2015). Although bivalves
457   can regulate their internal pH, it is estimated that this process is lower compared to other
458   calcifying species such as corals, foraminifera and coralligenous algae, with an up-regulation
459   limited to 0.5 pH unit (Crenshaw & Neff, 1969; Ip et al., 2006; McConnaughey & Gillikin,
460   2008).
461   Using the model described by Zeebe (2007), we reconstructed potential oxygen isotope
462   disequilibrium induced by several pH scenarios (Table 4), in order to evaluate the impact of
463   this parameter on shell $\delta^{18}O$. To do this, we considered salinity to be 34.7 at the EPR and 35
464   at the MAR vent sites (Haalboom et al., 2020). The oxygen isotope disequilibrium was tested
465   for different pH scenarios and relative to a maximum pH of 8 (i.e., pH of deep seawater,
466   Nedoncelle et al., 2015). At Rainbow, the oxygen isotope disequilibrium estimated in Table 3
467   can be explained by relatively restricted decrease in pH values compared to deep seawater. A
468   fractionation of 0.2 to 0.3 at Z1 habitat is consistent with pH values of 7.7±0.3 measured in
469   *Bathymodiolus* habitats at this site (Desbruyères et al. 2001). The disequilibrium observed at
470   Z3 habitat however requires a mean pH of 7.2, which appears low compared to the
471   *Bathymodiolus* environment. A stronger decrease in pH would be necessary to cause the high
472   isotopic disequilibrium observed at both Menez Gwen and EPR 9°50N. Although a pH
473   decrease (values up to 6.7) has been observed at Menez Gwen (Desbruyères et al. 2001) and
474   could explain a disequilibrium of 1.43 (Table 4), closer but still below to the ones described in
475   Menez Gwen habitats (Table 3), it is unlikely mussels experienced such low pH values
476   continuously. Other discrete measures in *Bathymodiolus* habitats from Menez Gwen exhibited
477   pH values of 6.9 (Kadar et al., 2005), leading to a disequilibrium of ~1 while the current
478   fractionation is >1.7. Thus another factor would explain the high $\delta^{18}O$ values observed in
479   mussel shells from this site. Similarly, at EPR 50°N continuous measurements show that pH
480   range between 6.9 to 8, leading to a maximum disequilibrium of 1.05 (Table 4). To reach an
481   oxygen isotope disequilibrium >2 as observed in Table 3, pH should decrease to 6.5, which is
482   not consistent with the living conditions of this species there. It is thus unlikely that pH
483   mainly controls shell $\delta^{18}O$ at Menez Gwen and EPR 9°50N.
484   Another potential influencing factor of stable isotope composition in shells is chemosynthesis,
485   as already shown for $\delta^{13}C$ of *Bathymodiolus* (Nedoncelle et al., 2014). The sulfur-oxidizing
486   symbionts provide CO_2 fixation in the Calvin-Benson cycle by the use of the RuBisCo
487   enzyme. This enzyme favors light carbon incorporation in the organic tissues (Robinson &

488 Cavanaugh 1995; Fiala-Médioni et al. 2002), resulting in an enriched ^{13}C availability in the
489 extrapallial cavity and thus in the shell. As they use carbon from methane rather than
490 dissolved inorganic carbon from seawater as source of carbon, methanotroph symbionts have
491 lower effect on the ^{13}C used for mineralization, leading to produce shells closer to isotopic
492 equilibrium (Nedoncelle et al., 2014). Such effect on δ^{18}O was however never described for
493 hydrothermal vent shells, although RuBisCo was also shown to induce oxygen isotope
494 fractionation in cyanobacteria, photosynthetic bacteria and high-level plants, in favor of ^{16}O
495 (Guy et al. 1993; Helman et al. 2005). An offset on oxygen isotopes occurs during microbial
496 oxidation of sulfide (Markovic et al., 2016), which can lead to higher δ^{18}O values for the
497 water pool used by the host organisms. The sulfo-oxidizing symbiont activity thus might
498 additionally explain the positive disequilibrium measured on δ^{18}O of shells. Interestingly,
499 contrary to the shells from Rainbow, shells from Menez Gwen (partly) and EPR 9°50N
500 (mostly) have δ^{18}O values far from the isotopic equilibrium (Table 3), likely corresponding to
501 a higher proportion of sulfo-oxidizing symbionts. This is consistent with the known
502 repartition of symbionts in these mussel populations. *Bathymodiolus thermophilus* from EPR
503 9°50N rely only on sulfo-oxidizing symbionts and *B. azoricus*, which can harbor a dual
504 symbiosis of sulfo-oxidizing and methanotrophs, exhibit a higher contribution to sulfo-
505 oxidizing bacteria at Menez Gwen compared to Rainbow (Duperron et al., 2006; Halary et al.,
506 2008; Nedoncelle et al., 2014).
507
508   **5. Conclusion**

509
510   Investigation of the Mg/Ca-temperature relationship in hydrothermal mussel shells
511 reveals this proxy as a promising paleotemperature tracer for deep-sea species. This is the first
512 suitability report of this proxy in deep-sea ecosystems, and although limited to hydrothermal
513 vents, numerous applications of Mg/Ca analyses can be listed both with ecological and
514 geological perspectives. Estimation of temperatures based on shell material will provide
515 complementary tools to the direct measurements made by *in situ* probes, useful for the
516 reconstruction of temperature changes at hydrothermal vents both over short-term (daily) and
517 long-term (pluri-annual) scales. Although this relationship needs to be investigated deeper in
518 details, deep-sea mussels, as they inhabit various ecosystems (e.g., hydrothermal vents, cold
519 seeps) seem to be a promising archive of long-term trends in deep-seawater temperatures. As
520 such, they would give precious information for oceanographic current modifications, as well
521 as monitoring of fluid emissions.
522   On the contrary, δ^{18}O provides under-estimated temperatures. Although more study
523 should be undertaken to explain this observation, pH variations on site may explain a
524 substantial part of the isotopic disequilibrium. Still, we hypothesize that the type of symbiotic
525 chemosynthetic bacteria has a strong impact on the oxygen isotope disequilibrium of their
526 host's shell.
527   Finally, the results also show great benefits in the ecological study of vent mussels,
528 particularly to determine temperature variations in the habitat and identify the response of
529 biodiversity to thermal anomalies. This will give clues to better describe the ecological
530 features of those species living in inaccessible habitats.
531

547

548 **Appendix A. Supplementary Material**

549

550 **Data Availability**
551 https://doi.org/10.5281/zenodo.14716571

552

553 **References**

554

555 Aharon P. (1991) Recorders of reef environment histories: Stable isotopes in corals, giant
556   clams and calcareous algae. *Coral Reefs* **10**, 71-90.
557 Andreani M., Escartin J., Delacour A., Ildefonse B., Godard M., Dyment J., Fallick A.E.,
558   Fouquet Y. (2014) Tectonic structure, lithology, and hydrothermal signature of the
559   Rainbow massif (Mid-Atlantic Ridge 36°14′N). *Geochemistry, Geophysics, Geosystems*
560   **15(9)**, 3543–3571.
561 Boutet I., Tanguy A., Le Guen D., Piccino P., Hourdez S., Legendre P., Jollivet D. (2009)
562   Global depression in gene expression as a response to rapid thermal changes in vent
563   mussels. *Proc R Soc Lond B* **276**, 3071–3079.
564 Carré M., Bentaleb I., Bruguier O., Ordinola E., Barret N. T., Fontugne M. (2006)
565   Calcification rate influence on trace element concentrations in aragonitic bivalve shells:
566   Evidence and mechanisms. *Geochim. Cosmochim. Acta* **70**, 4906–4920.
567 Carter J. G. (1980) Guide to bivalve shell microstructures. *In* Skeletal growth of aquatic
568   organisms (eds. D. C. Rhoads and R. A. Lutz). Plenum Press, New York. pp. 645–673.
569 Carter J. G. (1990) Evolutionary significance of shell micro- structure in the Palaeotaxodonta,
570   Pteriomorphia and Isofilibranchia. *In*: Skeletal Biomineralization: Patterns, Processes, and
571   Evolutionary Trends (ed J. G. Carter), vol. I. Van Nostrand Reinhold, New York, USA, pp.
572   135–296.
573 Charlou J. L., Donval J. P., Fouquet Y., Jean-Baptiste P., Holm N. (2002) Geochemistry of
574   high H2 and CH4 vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal
575   field (36°14'N, MAR). *Chem. Geol.* **191**, 345–359.
576 Checa A. G., Pina C. M., Osuna-Mascaro A. B., Harper E. M. (2014) Crystalline organization
577   of the fibrous prismatic calcitic layer of the Mediterranean mussel *Mytilus*
578   *galloprovincialis*. *Eur. J. Mineral.* **26**, 495–505.
579 Colaço A., Desbruyères D., Comtet T., Alayse A.M. (1998) Ecology of the Menez Gwen
580   hydrothermal vent field (Mid-Atlantic Ridge/Azores Triple Junction). *Cah. Biol. Mar*. **39**,
581   237-240.
582 Coplen T. B., Kendall C., Hopple J. (1983) Comparison of stable isotope reference samples.
583   *Nature* **302** (5905), 236–238.
584 Corliss J. B., Dymond J., Gordon L. I., Edmond J. M., von Herzen R. P., Ballard R. D., Green
585   K., Williams D., Bainbridge A., Crane K., van Andel T. H. (1979) Submarine thermal
586   springs on the galapagos rift. *Science* **203**, 1073–83.

587 Cravo A., Foster P., Almeida A., Bebianno M. J., Company R. (2008) Metal concentrations in
588   the shell of *Bathymodiolus azoricus* from contrasting hydrothermal vent fields on the mid-
589   Atlantic ridge. *Mar. Environ. Res.* **65**, 338–348.
590 Crenshaw M. A. and Neff J. M. (1969) Decalcification at the mantle-shell interface in
591   molluscs. *Am. Zool.*, **9**, 881–885.
592 Daëron M., Drysdale R.N., Peral M., Huyghe D., Blamart D., Coplen T.B., Lartaud F.,
593   Zanchetta G. (2019) Most Earth-surface calcites precipitate out of isotopic equilibrium.
594   *Nat. Comm.* **10**, 429.
595 Demina L. L., Holm N. G., Galkin S. V, Lein A. Y. (2013) Some features of the trace metal
596   biogeochemistry in the deep-sea hydrothermal vent fields (Menez Gwen, Rainbow, Broken
597   Spur at the MAR and 9°50'N at the EPR): a synthesis. *J. Mar. Syst.* **126**, 94–105.
598 Desbruyères D., Biscoito M., Caprais J. C., Colaço A., Comtet T., Crassous P., Fouquet Y.,
599   Khripounoff A., Le Bris N., Olu K., Riso R., Sarradin P. M., Segonzac M., Vangriesheim
600   A. (2001) Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic
601   Ridge near the Azores plateau. *Deep Sea Res. I* **48**, 1325–1346.
602 Dodd J. R. (1965) Environmental control of strontium and magnesium in *Mytilus. Geochim.*
603   *Cosmochim. Acta* **29**, 385–398.
604 Duperron S., Bergin C., Zielinski F., Blazejak A., Pernthaler A., McKiness Z. P., DeChaine
605   E., Cavanaugh C. M., Dubilier N. (2006) A dual symbiosis shared by two mussel species,
606   *Bathymodiolus azoricus* and *Bathymodiolus puteoserpentis* (Bivalvia: Mytilidae), from
607   hydrothermal vents along the northern Mid-Atlantic Ridge. *Environ. Microbiol.* **8**, 1441–
608   1447.
609 England J., Cusack M., Lee M. R. (2006) Magnesium and sulphur in the calcite shells of two
610   brachiopods, *Terebratulina retusa* and *Novocrania anomala*. Lethaia **40**, 2–10.
611 Fiala-Médioni A., McKiness Z. P., Dando P., Boulegue J., Mariotti A., Alayse-Danet A. M.,
612   Robinson J. J., Cavanaugh C. M. (2002) Ultrastructural, biochemical, and immunological
613   characterization of two populations of the mytilid mussel *Bathymodiolus azoricus* from the
614   Mid-Atlantic Ridge: evidence for a dual symbiosis. *Mar. Biol.* **141**, 1035–1043.
615 Foster L. C., Finch A. A., Allison N., Andersson C., Clarke L. J. (2008) Mg in aragonitic
616   bivalve shells: Seasonal variations and mode of incorporation in *Arctica islandica. Chem.*
617   *Geol.* **254**, 113–119.
618 Freitas P., Clarke L. J., Kennedy H., Richardson C., Abrantes F. (2005) Mg/Ca, Sr/Ca, and
619   stable-isotope (δ^{18}O and δ^{13}C) ratio profiles from the fan mussel *Pinna nobilis*: Seasonal
620   records and temperature relationships. *Geochemistry Geophys. Geosystems* **6**, Q04D14.
621 Freitas P. S., Clarke L. J., Kennedy H. A., Richardson C. A. (2008) Inter- and intra-specimen
622   variability masks reliable temperature control on shell Mg/Ca ratios in laboratory- and
623   field-cultured *Mytilus edulis* and *Pecten maximus* (bivalvia). *Biogeosciences* **5**, 1245–
624   1258.
625 Freitas P. S., Clarke L. J., Kennedy H., Richardson C. A. (2012). The potential of combined
626   Mg/Ca and δ^{18}O measurements within the shell of the bivalve *Pecten maximus* to estimate
627   seawater δ^{18}O composition. *Chemical Geology* **291**, 286–293.
628 Génio L., Kiel S., Cunha M. R., Grahame J., Little C. T. S. (2012) Shell microstructures of
629   mussels (Bivalvia: Mytilidae: Bathymodiolinae) from deep-sea chemosynthetic sites: do
630   they have a phylogenetic significance? *Deep Sea Res. I* **64**, 86–103.
631 Guy R. D., Fogel M. L., Berry J. A. (1993) Photosynthetic fractionation of the stable isotopes
632   of oxygen and carbon. *Plant. Physiol.* **101**, 37–47.
633 Haalboom S., Price D. M., Mienis F., van Bleijswijk J. D. L., de Stigter H. C., Witte H. J.,
634   Reichart G.-J., Duineveld G. C. A. (2020) Patterns of (trace) metals and microorganisms in
635   the Rainbow hydrothermal vent plume at the Mid-Atlantic Ridge. *Biogeosciences*. **17**,
636   2499-2519.

637 Halary S., Riou V., Gaill F., Boudier T., Duperron S. (2008) 3D FISH for the quantification
638   of methane- and sulphur-oxidizing endosymbionts in bacteriocytes of the hydrothermal
639   vent mussel *Bathymodiolus azoricus*. *ISME J.* **2**, 284–292

640 Helman Y., Barkan E., Eisenstadt D., Luz B. and Kaplan A. (2005) Fractionation of the three
641   stable oxygen isotopes by oxygen-producing and oxygen-consuming reactions in
642   photosynthetic organisms. *Plant. Physiol.* **138**, 2292–2298.

643 Huyghe D., Lartaud F., Emmanuel L., Merle D., Renard M. (2015) Palaeogene climate
644   evolution in the Paris Basin from oxygen stable isotope ($\delta^{18}O$) compositions of marine
645   molluscs. *J. Geol. Soc. London.* **172**, 576–587.

646 Huyghe D., Emmanuel L., de Rafélis M., Renard M., Ropert M., Labourdette N., Lartaud F.
647   (2020) Oxygen isotope disequilibrium in the juvenile portion of oyster shells biases
648   seawater temperature reconstructions. *Estuar. Coast Shelf Sci.* **240**, 106777.

649 Huyghe D., Daëron M., de Rafelis M., Blamart D., Sébilo M., Paulet Y.-M., Lartaud F.
650   (2022) Clumped isotopes in modern marine bivalves. *Geochimica et Cosmochimica Acta*
651   **316**, 41–58.

652 Ip Y. K., Loong A. M., Kiong K. C., Wong W. P., Chew S. F., Reddy K., Sivalonganathan B.,
653   Ballantyne J. S. (2006) Light induces an increase in the pH of and a decrease in the
654   ammonia concentration in the extrapallial fluid of the giant clam *Tridacna squamosa*.
655   *Physiol. Biochem. Zool.* **79**, 656–664.

656 Jean-Baptiste P., Charlou J. L., Stievenard M. (1997) Oxygen isotope study of mid-ocean
657   ridge hydrothermal fluids: Implications for the oxygen-18 budget of the oceans,
658   *Geochimica Cosmochimica Acta* **61**, 2669–2677.

659 Johnson K. S., Childress J. J., Beelher C. L., Sakamoto C. M. (1994) Biogeochemistry of
660   hydrothermal vent mussel communities: the deep-sea analogue to the intertidal zone. *Deep*
661   *Sea Res. I* **41**, 993–1011.

662 Kádár E., Costa V., Martins I., Santos R. S., Powell J. J. (2005) Enrichment in Trace Metals
663   (Al, Mn, Co, Cu, Mo, Cd, Fe, Zn, Pb and Hg) of Macro-Invertebrate Habitats at
664   Hydrothermal Vents Along the Mid-Atlantic Ridge. *Hydrobiologia* **548**, 191–205.

665 Kenk V. C. and Wilson B. R. (1985) A new mussel (Bivalvia, Mytilidae) from hydrothermal
666   vents, in the Galapagos Rift zone. *Malacologia.* **26** (1-2): 253-271.

667 Khripounoff A., Vangriesheim A., Crassous P., Segonzac M., Lafon V., Warén A. (2008)
668   Temporal variation of currents, particulate flux and organism supply at two deep-sea
669   hydrothermal fields of the Azores Triple Junction. *Deep Sea Res. I* **55**, 532–551.

670 Kim S. T. and O'Neil J. R. (1997) Equilibrium and nonequilibrium oxygen isotope effects in
671   synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475.

672 Klein R. T., Lohmann K. C., Thayer C. W. (1996) Bivalve skeletons record sea-surface
673   temperature and $\delta^{18}O$ via Mg/Ca and $^{18}O/^{16}O$ ratios. *Geology* **24**, 415–418.

674 Lartaud F., Emmanuel L., de Rafélis M., Ropert M., Labourdette N., Richardson C.A., Renard
675   M. (2010a). A latitudinal gradient of seasonal temperature variation recorded in oyster
676   shells from the coastal waters of France and The Netherlands. *Facies* **56**, 13-25.

677 Lartaud F., de Rafelis M., Oliver G., Krylova E., Dyment J., Ildefonse B., Thibaud R., Gente
678   P., Hoisé E., Meistertzheim A. L., Fouquet Y., Gaill F., Le Bris N. (2010b) Fossil clams
679   from a serpentinite-hosted sedimented vent field near the active smoker complex Rainbow
680   (MAR, 26°13N): insight into the biogeography of vent fauna. *Geochemistry Geophys.*
681   *Geosystems* **11**, Q0AE01.

682 Lartaud F., Little C. T. S., de Rafelis M., Bayon G., Dyment J., Ildefonse B., Gressier V.,
683   Fouquet Y., Gaill F., Le Bris N. (2011) Fossil evidence for serpentinization fluids fueling
684   chemosynthetic assemblages. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 7698–7703.

685 Le Bris N., Duperron S. (2010) Chemosynthetic communities and biogeochemical energy
686   pathways along the Mid-Atlantic Ridge: the case of *Bathymodiolus azoricus*. *Geophys*

687   *Monogr* **188**, 409–429.
688   Le Bris N., Yücel M., Das A., Sievert S.M., LokaBharathi P., Girguis P.R. (2019)
689   Hydrothermal energy transfer and organic carbon production at the deep seafloor.
690   *Frontiers in Marine Science* **5**, 531.
691   Lelièvre Y., Legendre P., Matabos M., Mihaly M., Lee R. W., Sarradin P. M., Arango C. P.,
692   Sarrazin J. (2017) Astronomical and atmospheric impacts on deep-sea hydrothermal vent
693   invertebrates. *Proc. R. Soc. – Biol. Sci.* **284**, 20162123.
694   Lietard C. and Pierre C. (2009) Isotopic signatures (δ^{18}O and δ^{13}C) of bivalve shells from
695   cold seeps and hydrothermal vents. *Geobios* **42**, 209–219.
696   Longerich H. P., Gunther D., Jackson S. E. (1996) Elemental fractionation in laser ablation
697   inductively coupled plasma mass spectrometry. *Fresenius J. Anal. Chem.* **355**, 538–542.
698   Lopez-Correa M., Montagna P., Vendrell-Simon B., McCulloch M., Taviani M. (2010) Stable
699   isotopes (δ^{18}O and δ^{13}C), trace and minor element compositions of Recent scleractinians
700   and Last Glacial bivalves at the Santa Maria di Leuca deep-water coral province, Ionian
701   sea. *Deep Sea Res. II* **57**, 471–486.
702   Lorens R. B. and Bender M. L. (1980) The impact of solution chemistry on *Mytilus edulis*
703   calcite and aragonite. *Geochim. Cosmochim. Acta* **44**, 1265–1278.
704   Luther G. W., Gartman A., Yücel M., Madison A. S., Moore T. S., Nees H. A., Nuzzio D. B.,
705   Sen A., Lutz R. A., Shank T. M., Fisher C. R. (2012) Chemistry, temperature, and faunal
706   distributions at diffuse-flow hydrothermal vents: comparison of two geologically distinct
707   ridge systems. *Oceanography* **25**, 234–245.
708   Lutz R. A., Kennish M. J., Pooley A. S., Fritz L. W. (1994) Calcium carbonate dissolution
709   rates in hydrothermal vent fields of the Guaymas Basin. *J. Mar. Res.* **52**, 969–982.
710   Machado J., Lopes-Lima M., Damasceno-Olivera A., Colaço A., Andrade J., Silva D.,
711   Jiménez-Lopez C., Rodriguez-Navarro A., Checa A. (2009) The influence of hydrostatic
712   pressure on shell mineralization of *Anodonta cygnea*: a comparative study with a
713   hydrothermal vent bivalve *Bathymodiolus azoricus*. *J. Shellfish Res.* **28**, 899–904.
714   Markovic S., Paytan A., Li H., Wortmann U.G. (2016) A revised seawater sulfate oxygen
715   isotope record for the last 4 Myr. *Geochim. Cosmochim. Acta* **175**, 239-251.
716   McConnaughey T. A. and Gillikin D. P. (2008) Carbon isotopes in mollusk shell carbonates.
717   *Geo-Mar. Lett.*, **28**, 287–299.
718   Meier D. V., Bach W., Girguis P. R., Gruber-Vodicka H. R., Reeves E. P., Richter M.,
719   Vidoudez C., Amann R., Meyerdierks A. (2016) Heterotrophic *Proteobacteria* in the
720   vicinity of diffuse venting. *Env. Microbiol.* **18**, 4348-4368.
721   Mikkelsen P. M., Bieler R., Kappner I., Rawlings T. A. (2006) Phylogeny of Veneroidea
722   (Mollusca: Bivalvia) based on morphology and molecules. *Zool. J. Linn. Soc.* **148**, 439–
723   521.
724   Mills S. W., Mullineaux L.S., Tyler P. A. (2007) Habitat Associations in Gastropod Species at
725   East Pacific Rise Hydrothermal Vents (9°50′N). *The Biological Bulletin* **212**, 185–194.
726   Moore, T. S., Shank, T. M., Nuzzio, D. B., Luther, G. W. (2009) Time-series chemical and
727   temperature habitat characterization of diffuse flow hydrothermal sites at 9°50'N East
728   Pacific Rise. *Deep-Sea Research II* **56**, 1616–1621.
729   Mouchi V., de Rafelis M., Lartaud F., Fialin M., Verrecchia E. (2013) Chemical labelling of
730   oyster shells used for time-calibrated high-resolution Mg/Ca ratios: a tool for estimation of
731   past seasonal temperature variations. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **373**, 66–
732   74.
733   Mouchi V., Briard J., Gaillot S., Argant T., Forest V., Emmanuel L. (2018) Reconstructing
734   environments of collection sites from archaeological bivalve shells: Case study from
735   oysters (Lyon, France). J. *Archaeol. Sci. Reports* **21**, 1225–1235.

736 Mullineaux L. S., Le Bris N., Mills S. W., Henri P., Bayer S. R., Secrist R. G., Siu N. (2012)
737   Detecting the influence of initial pioneers on succession at deep-sea vents. *PloS One* **7**,
738   e50015.
739 Naraoka H., Naito T., Yamanaka T., Tsunogai U. and Fujikura K. (2008) A multi-isotope
740   study of deep-sea mussels at three different hydrothermal vent sites in the northwestern
741   Pacific. *Chem. Geol.* **255**, 25–32.
742 Nedoncelle K., Lartaud F., de Rafelis M., Boulila S., Le Bris N. (2013) A new method for
743   high-resolution bivalve growth rate studies in hydrothermal environments. *Mar. Biol.* **160**,
744   1427–1439.
745 Nedoncelle K., Le Bris N., de Rafelis M., Labourdette N., Lartaud F. (2014) Non-equilibrium
746   fractionation of stable carbon isotopes in chemosynthetic mussels. *Chem. Geol.* **387**, 35–
747   46.
748 Nedoncelle K., Lartaud F., Contreira Pereira L., Yücel M., Thurnherr A. M., Mullineaux L.,
749   Le Bris N. (2015) *Bathymodiolus* growth dynamics in relation to environmental
750   fluctuations in vent habitats. *Deep Sea Res.* I **106**, 183–193.
751 Pearce N. J. G., Perkins W.T., Westgate J. A., Gorton M. P., Jackson S. E., Neal C. R.,
752   Chenery S. P. (1997) A compilation of new and published major and trace element data for
753   NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostandard Newsletters*
754   **21**, 115-144.
755 Pérez-Huerta A., Walker S. E., Cappelli C. (2020) In situ geochemical analysis of organics in
756   growth lines of Antarctic scallop shells: implications for sclerochronology. *Minerals* **10**,
757   529.
758 Pierre C., Vangriesheim A., Laube-Lenfant E. (1994) Variability of water masses and of
759   organic production-regeneration systems as related to eutrophic, mesotrophic and
760   oligotrophic conditions in the northeast Atlantic ocean. *Journal of Marine Systems* **5**, 159–
761   170.
762 Proskurowski G., Lilley M. D., Olson E. J. (2008) Stable isotopic evidence in support of
763   active microbial methane cycling in low-temperature diffuse flows vents at 9°50'N East
764   pacific Rise. *Geochimica et Cosmochimica Acta* **72**, 2005–2023.
765 Rio M., Renard M., Roux M., Clauser S., Davanzo F., Herrerra Duvault Y. (1988)
766   Composition chimique et isotopique des tests de bivalves des sources hydrothermales
767   océaniques. *Bull. Soc. Geol. Fr.* **9**, 151–159.
768 Robinson J. J. and Cavanaugh C. M. (1995) Expression of form I and form II ribulose-1,5-
769   bisphosphate carboxylase/oxygenase (RuBsiCo) in chemoautotrophic symbioses:
770   Implications for the interpretation of stable carbon isotope ratios. *Limnology and
771   Oceanography* **40**, 1496–1502.
772 Robinson L. F., Adkins J. F., Frank N., Gagnon A. C., Prouty N., Roark E. B., van de Flierdt
773   T. (2014) The geochemistry of deep-sea coral skeletons: a review of vital effects and
774   applications for palaeoceanography. *Deep Sea Res. II* **99**, 184–198.
775 Rohling E. J. (2000) Paleosalinity: confidence limits and future applications. *Mar. Geol.* **163**,
776   1-11.
777 Rosenberg G. D. and Hughes W. W. (1991) A metabolic model for the determination of shell
778   composition in the bivalve mollusc, *Mytilus edulis*. *Lethaia* **24**, 83–96.
779 Sarradin P. M., Caprais J. C., Riso R., Kerouel R., Aminot A. (1999) Chemical environment
780   of the hydrothermal mussel communities in the Lucky Strike and Menez Gwen vent fields,
781   Mid-Atlantic Ridge. *Cah. Biol. Mar.* **40**, 93–104.
782 Sarrazin J., Cuvelier D., Peton L., Legendre P., Sarradin P. M. (2014) High-resolution
783   dynamics of a deep-sea hydrothermal mussel assemblage monitored by the EMSO-Açores
784   MoMAR observatory. *Deep Sea Res. I* **90**, 62–75.

785 Sarrazin J., Portail M., Legrand E., Cathalot C., Laes A., Lahaye N., Sarradin P.M., Husson
786   B. (2020) Endogenous versus exogenous factors: What matters for vent mussel
787   communities? *Deep-Sea Research* **160**, 103260.
788 Scheirer D. S., Shank T. M., Fornari D. J. (2006) Temperature variations at diffuse and
789   focused flow hydrothermal vent sites along the northern East Pacific Rise. *Geochemistry*
790   *Geophys. Geosystems* **7**, Q03002.
791 Schöne B. R. (2008) The curse of physiology-challenges and opportunities in the
792   interpretation of geochemical data from mollusk shells. *Geo-Mar Lett* **28**, 269–285.
793 Schöne B. R. and Giere O. (2005) Growth increments and stable isotope variation in shells of
794   the deep-sea hydrothermal vent bivalve mollusk *Bathymodiolus brevior* from the North Fiji
795   Basin, Pacific Ocean. *Deep Sea Res. I* **52**, 1896–1910.
796 Schöne B. R., Pfeiffer M., Pohlmann T., Siegismund F. (2005a) A seasonally resolved
797   bottom-water temperature record for the period 1866-2002 based on shells of *Arctica*
798   *islandica* (Mollusca, North Sea). *Int J Climatol* **25**, 947-962.
799 Schöne B. R., Dunca E., Fiebig J., Pfeiffer M. (2005b) Mutvei's solution: an ideal agent for
800   resolving microgrowth structures of biogenic carbonates. *Palaeogeogr. Palaeoclimatol.*
801   *Palaeoecol.* **228**, 149–166.
802 Schöne B. R., Zhang Z., Radermacher P., Thébault J., Jacob D., Nunn E., Maurer A. F. (2011)
803   Sr/Ca and Mg/Ca ratios of ontogenetically old bivalve shells (*Arctica islandica*) and their
804   function as paleotemperature proxies. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **302**, 52–
805   64.
806 Shanks W.C., III, Bohlke J.K., Seal R.R. II (1991) Stable isotopes studies of vent fluids, 9-
807   10°N East Pacific Rise: Water- rock interaction and phase separation. *EOS* **72**, 48.
808 Shirai K., Takahata N., Yamamoto H., Omata T., Sasaki T., Sano Y. (2008) Novel analytical
809   approach to bivalve shell biogeochemistry: a case study of hydrothermal mussel shell.
810   *Geochem. J.* **42**, 413–420.
811 Surge D. and Lohmann K. C. (2008) Evaluating Mg/Ca ratios as a temperature proxy in the
812   estuarine oyster, *Crassostrea virginica*. *J. Geophys. Res. Biogeosciences* **113**, G2001.
813 Takesue R. K., Bacon C. R., Thompson J. K. (2008) Influences of organic matter and
814   calcification rate in trace elements in aragonitic estuarine bivalve shells. *Geochim.*
815   *Cosmochim. Acta* **72**, 5431–5445.
816 Tanaka K., Okaniwa N., Miyaji T., Murakami-Sugihara N., Zhao L., Tanabe K., Schöne B.R.,
817   Shirai K. (2019) Microscale magnesium distribution in shell of the Mediterranean mussel
818   *Mytilus galloprovincialis*: An example of multiple factors controlling Mg/Ca in biogenic
819   calcite. *Chem. Geol.* **511**, 521-532.
820 Tarutani T., Clayton R. N., Mayeda T. K. (1969) The effect of polymorphism and magnesium
821   substitution on oxygen isotope fractionation between calcium carbonate and water.
822   *Geochim. Cosmochim. Acta* **33**, 987–996.
823 Tivey M. K., Bradley A. M., Joyce T. M., Kadko D. (2002) Insights into tide-related
824   variability at seafloor hydrothermal vents from time-series temperature measurements.
825   *Earth Planet. Sci. Lett.* **202**, 693–707.
826 Tynan S., Opdyke B. N., Walczak M., Eggins S., Dutton A. (2017) Assessment of Mg/Ca in
827   *Saccostrea glomerata* (the Sydney rock oyster) shell as a potential temperature record.
828   *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **484**, 79–88.
829 Usdowski E., Michaelis J., Böttcher M. E., Hoefs J. (1991) Factors for the oxygen isotope
830   equilibrium fractionation between aqueous and gaseous CO_2, carbonic acid, bicarbonate,
831   carbonate, and water (19°C) *Zeitschrift für Physikalische Chemie* **170**, 237–249.
832 Von Cosel R., Comtet T., Krylova E. M. (1999) *Bathymodiolus* (Bivalvia: Mytilidae) from
833   hydrothermal vents from hydrothermal vents on the Azores triple junction and the
834   Logatchev hydrothermal field. *The Veliger*, **42(3)**, 218-248.

835 Vander Putten E., Dehairs F., Keppens E., Baeyens W. (2000) High resolution distribution of
836   trace elements in the calcite shell layers of modern *Mytilus edulis*: Environmental and
837   biological controls. *Geochim. Cosmochim. Acta* **64**, 997–1011.
838 Wanamaker A. D. J., Kreutz K. J., Wilson T., Borns H. W. J., Introne D. S., Feindel S. (2008)
839   Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve calcite for *Mytilus*
840   *edulis*: implications for paleotemperature reconstructions. *Geo-Mar Lett* **28**, 359–368.
841 Welsh K., Elliot M., Tudhope A., Ayling B., Chapell J. (2011) Giant bivalves (*Tridacna*
842   *gigas*) as recorders of ENSO variability. *Earth Planet Sci Lett* **307**, 266-270.
843 Wisshak M., Lopez Correa M., Gofas S., Salas C., Taviani M., Jakobsen J., Freiwald A.
844   (2009) Shell architecture, element composition, and stable isotope signature of the giant
845   deep-sea oyster *Neopycnodonte zibrowii* sp. N. from the NE Atlantic. *Deep-Sea Research I*
846   **56**, 374–407.
847 Zeebe R. E. (1999) An explanation of the effect of seawater carbonate concentration on
848   foraminiferal oxygen isotopes. *Geochim. Cosmochim. Acta* **63**, 2001–2007.
849 Zeebe, R. E. (2007) An expression for the overall oxygen isotope fractionation between the
850   sum of dissolved inorganic carbon and water. *Geochem. Geophys. Geosyst*. **8**, Q09002,
851   doi:10.1029/2007GC001663.
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855 **Tables**

856

857 Table 1: Reconstructed temperatures from shell Mg/Ca compared to measured temperatures

858 on study sites.

859

860 Table 2: Reconstructed temperatures from shell $\delta^{18}O$ compared to measured temperatures on

861 study sites.

862

863 Table 3: Shell oxygen isotope disequilibrium based on measured temperature and seawater

864 $\delta^{18}O$. The indicated value of $\delta^{18}O$ disequilibrium is the difference between shell $\delta^{18}O$ and

865 expected shell $\delta^{18}O$ at equilibrium.

866

867 Table 4: Impact of pH on $\delta^{18}O$ disequilibrium. The model of Zeebe (2007) was used to

868 reconstruct the α parameter from the thermodependent equation of Kim & O'Neil (1997),

869 from salinity (S), temperature (T) and various pH scenarios. Theoretical $\delta^{18}O$ from potential

870 pH values were then calculated from the equation using α and the measured $\delta^{18}O_w$.

871 **Figures**
872
873 Figure 1: Sampling areas for the three hydrothermal vent sites. At Rainbow, shells were
874 collected from two micro-habitats: Z1, where mussels lived close to the active chimney (in
875 black), and Z3, corresponding to a denser mussel patch ~1m above (© Ifremer –
876 MoMARDREAM). At Menez Gwen, shells originated from two locations: White Flame (WF)
877 and Cage Site (CS). For CS, the shells collected are the ones below the red autonomous probe
878 (© Marum – MenezMAR). At EPR 9°50N, shells were collected in the V-Vent area (here
879 called MES), around the yellow autonomous probe (© Ifremer – MESCAL-1).
880
881 Figure 2: Photographs of cross section of a *B. azoricus* shell at the ventral margin highlighting
882 the details of the microstructures and growth increments revelation under Mutvei treatment
883 (A), and the position of the LA-ICP-MS samplings (B). d.o.g. is direction of growth.
884
885 Figure 3: Shell Mg/Ca and corresponding estimated temperatures. For each habitat, recorded
886 temperature ranges are indicated by the red dashed lines and the average temperature in red
887 dotted lines (this study; EPR: Nedoncelle et al., 2015). The historical temperature ranges,
888 measured at each hydrothermal site, are indicated by the blue dashed lines (Rainbow:
889 Desbruyères et al., 2001; Sarrazin et al., 2020; Menez Gwen: Colaço et al., 1998; Sarradin et
890 al., 1999; Desbruyères et al., 2001; Sarrazin et al., 2020; EPR 9°50N: Mills et al., 2007;
891 Moore et al., 2009; Luther et al., 2012). The red arrows correspond to the positions of major
892 growth lines.
893
894 Figure 4: Shell $\delta^{18}O$ and corresponding estimated temperatures. For each habitat, recorded
895 temperature ranges are indicated by the red dashed lines and the average temperature in red
896 dotted lines (this study; EPR: Nedoncelle et al., 2015). The historical temperature ranges
897 measured at each hydrothermal site are indicated by the blue dashed lines (Rainbow:
898 Desbruyères et al., 2001; Sarrazin et al., 2020; Menez Gwen: Colaço et al., 1998; Sarradin et
899 al., 1999; Desbruyères et al., 2001; Sarrazin et al., 2020; EPR 9°50N: Mills et al., 2007;
900 Moore et al., 2009; Luther et al., 2012).
901