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## Rare earth elements as new biogeochemical proxies in deep-sea mussels

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1 **Rare Earth Elements as new**  
2 **biogeochemical proxies in deep-sea mussels**

3 By

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## 27 **Abstract**

28 We report on the abundances of REE in a comprehensive suite of shells of deep-sea  
29 chemosynthetic mussels from hydrothermal vents and cold seeps. Except for mussel shells  
30 from oceanic hydrothermal sites that often show extremely pronounced Eu anomalies  
31 ((Eu/Sm)<sub>sn</sub>=2-200), and abundances for this element that can occasionally exceed 200 ng/g,  
32 REE concentrations are usually low and typically between 10<sup>-4</sup> and 10<sup>-3</sup> times the shale  
33 reference.

34 In addition to exhibiting commonly high Eu anomalies, mussel shells from  
35 hydrothermal vents are depleted in light-REE and heavy-REE compared to reference shales  
36 (e.g., Pr/Sm)<sub>sn</sub><1, (Tb/Yb)<sub>sn</sub>=1-6). These features are inherited from hydrothermal fluids.  
37 Mussel shells from cold seeps have very different REE concentrations, which also reflect the  
38 compositions of the waters they filter: their (Eu/Sm)<sub>sn</sub> ratios are much lower (<2) and are  
39 much less heavy-REE fractionated ((Tb/Yb)<sub>sn</sub> =0.5-2.7). Furthermore, the REE distributions  
40 show a clear dichotomy between thiotrophic and methanotrophic mussels. The latter show  
41 marked enrichments in light-REE and even sometimes La enrichments much larger than those  
42 classically observed in deep-sea waters, leading to huge positive La anomalies (La/La\*>10).  
43 These light-REE enrichments are likely related to REE-dependent methanol dehydrogenase  
44 enzymes used by the symbionts that these mussels host in their gills. These data show that  
45 REE chemistry is a promising tool to study chemosynthetic faunas living near hydrothermal  
46 vents or cold seeps. Furthermore, REE chemistry, coupled with stable isotopes, should reveal  
47 the footprint of aerobic methanotrophy in carbonates formed in cold seeps, but also  
48 potentially in ancient sediments.

49  
50

## 51 **Key words:**

52 rare earth elements, bivalve, shell chemistry, hydrothermal system, cold seep, methanotrophy

53

## 55 **1/ Introduction**

56 Over the last fifty years, rare earth elements (REEs) have become one of the most  
57 studied groups of elements by geochemists, finding applications in all fields, from the  
58 petrogenesis of rocks, terrestrial or not, to the origin of the first condensed solids in the solar  
59 system, through rock dating and isotope tracing (e.g., Henderson, 1984). In marine  
60 geochemistry, REEs and their isotopes have been also extensively studied since they allow,  
61 among other things, the characterization of the different water masses composing the oceans  
62 and their circulation (e.g., Elderfield, 1988). For decades, it has been assumed that these  
63 elements do not participate in any biochemical cycle and therefore that their distributions at  
64 the Earth's surface are not impacted by life. Nature is certainly more complex. Notable  
65 biological effects of the REEs have been observed in plants, and REEs are even used as  
66 fertilizers (Tommasi et al., 2021). Furthermore, some species can accumulate large amounts  
67 of these elements, as exemplified by the fern *Dicranopteris linearis* (Ozaki et al., 1997). More  
68 recently, it was established that light REEs are vital for aerobic methanotrophic and  
69 methylotrophic bacteria that play an essential role in global carbon cycling (e.g., Pol et al.,  
70 2014; Semrau et al., 2018; Cotruvo, 2019). Methanotrophic bacteria first convert methane to  
71 methanol, which is subsequently degraded into formaldehyde using Ca-dependent (MxaF  
72 type) or REE-dependent (XoxF type) methanol dehydrogenase enzymes (Skovran *et al.*,  
73 2011). Ramachandran and Walsh (2015) and Taubert et al. (2015) have shown that XoxF type  
74 enzymes are more frequently used by marine bacteria. These recent findings clearly indicate  
75 the ability of micro-organisms to fractionate and redistribute REE, emphasizing the  
76 importance of life in the REE cycling in Earth's surface environments. For instance, there is  
77 growing evidence that bacterial activity and chemosynthetic ecosystems can influence the  
78 REEs distribution in seawater, at least locally. During the blowout of the Deepwater Horizon  
79 well (Gulf of Mexico), the depletion of light REE in a submerged plume of methane-rich  
80 water was explained by an uptake of these elements by methanotrophic bacteria (Shiller et al.,  
81 2017). More recently, light REE depletions were observed in seawater from the Sargasso Sea,  
82 at depths between approximately 200 and 500 m, ascribed to the activity of methanotrophic or  
83 methylotrophic microorganisms (Meyer et al., 2021). Therefore, methanotrophic bacteria, at  
84 least those using enzymes containing REEs, display distinctive REE distributions, as do  
85 animals living in symbiosis with them.

86           Within this scope, deep-sea fauna associated with black smokers and cold seeps are  
87 ideal targets to evaluate the distinctive chemical signatures developed by organisms. In these  
88 environments, reduced sulfur compounds, methane and/or hydrogen fuel primary productivity  
89 sustained by the symbiotic bacteria that form here the basis of the trophic web (Cavanaugh et  
90 al., 1981, 1987; Felbeck, 1981; Childress et al., 1986; Petersen et al., 2011), which are vital  
91 for many thiotrophic or methanotrophic animals. Recently, two investigations of  
92 chemosynthetic fauna have provided direct support for an active consumption of light-REE  
93 associated with aerobic microbial oxidation of methane at cold seeps. Bayon et al. (2020a)  
94 determined trace element abundances along a one-meter-long chitin tube of a siboglinid worm  
95 (*Escarpia southwardae*) collected at the Regab pockmark (Congo margin), identifying  
96 distinctive light REE enrichments that were attributed to methanotrophic activity in the  
97 branchial plume region of the worm. Additionally, similar light-REE enrichments were found  
98 in the gills of methanotrophic mussels collected at seeps from the South China Sea, but not in  
99 thiotrophic clams or mussels from the same sites (Wang et al., 2020). Because gills are the  
100 organs hosting the methanotrophic symbionts in mussels, Wang et al. (2020) proposed that  
101 these light-REE enrichments were related to aerobic methanotrophy. In that latter study, other  
102 soft tissues and the shell of studied methanotrophic mussels, while displaying lower light-  
103 REE abundances, also show higher positive La anomalies relative to seawater or pore water.

104           Rare earth element data for deep-sea shells are still sparse. To date, mussels from only  
105 three hydrothermal vent fields (Bau et al., 2010) and two cold seeps (Wang et al., 2020) have  
106 been investigated. Here we present a comprehensive review for the distribution of REE in  
107 mussel shells from deep-sea chemosynthetic environments, which combine previously  
108 published literature data (Bau et al., 2010; Wang et al., 2020) and new results obtained on a  
109 suite of deep-sea mussels from both submarine methane seeps and hydrothermal vents (Fig.  
110 1). The aim of this study was to further investigate the factors influencing the REE  
111 distribution in marine mollusks and assess the degree to which REE abundances in shells can  
112 be used to monitor the activity of methanotrophic symbiotic bacteria.

113

## 114 **2/ Sampling**

115           Forty-five mussel shells belonging to ten different species were analyzed here, of  
116 which nine reside in the genus *Bathymodiolus*. Application of DNA analyses to newly  
117 discovered species has led to the assignment of the intensively studied *Bathymodiolus*

118 *childressi* to the genus *Gigantidas* (Xu et al., 2019). Twenty-five shell samples, belonging to  
119 nine species of *Bathymodiolus*, were sourced from the Scripps Institution of Oceanography  
120 Benthic Invertebrate Collection (SIO-BIC) in La Jolla (California, USA). Our sampling  
121 includes both thiotrophic and methanotrophic species. Descriptive data for each sample (site,  
122 depth, species, museum catalog number, etc.) are given in Supplementary Table 1.

123

## 124 **2.1/ Hydrothermal vents**

125 We analyzed three shells from three different sites in the Pacific Ocean for this study,  
126 which complement the dataset obtained by Bau et al. (2010) on thiotrophic mussels from the  
127 Mid-Atlantic Ridge. Two mussels (*Bathymodiolus thermophilus*) originate from the East  
128 Pacific Rise (SIO-BIC M16462) and the Rose Garden vent field (SIO-BIC M8223) located  
129 east of the Galápagos Islands, which was among the first submarine hydrothermal sites  
130 discovered (e.g., Corliss and Ballard, 1977). The third shell (*Bathymodiolus septemdierum*;  
131 SIO-BIC M15353) comes from the Nifonea vent field (Schmidt et al., 2017), located within  
132 the caldera of a large shield-type volcano of the Vate Trough, a young extensional rift in the  
133 New Hebrides back-arc (Vanuatu, SW Pacific).

134

## 135 **2.2/ Cold seeps**

136 We analyzed samples from seven seeps located in three different regions: the Gulf of  
137 Mexico, the western margin of Costa Rica, and Melanesia.

138 The Gulf of Mexico seeps (Brine Pool, Bush Hill, and West Florida Escarpment) have  
139 been extensively studied both geologically and biologically (Paull et al., 1984, 1985; Martens  
140 et al. 1991; Chanton et al., 1993), and were the basis for seminal observations on  
141 chemoautrophic life, including the demonstration of methane-based symbiosis between  
142 animals and intracellular bacteria (Childress et al., 1986). The mussels of these sites are  
143 particularly well characterized, and the reader is referred to the numerous papers concerning  
144 in particular their symbionts or their isotopic compositions allowing analysis of their diet  
145 (e.g., Duperron et al., 2007; Macavoy et al., 2008; Riekenberg et al., 2018; Feng et al., 2018).  
146 Twenty *Gigantidas childressi* shells from Brine Pool and Bush Hill, and one *Bathymodiolus*  
147 *heckerae* shell from West Florida Escarpment (SIO-BIC M8018), were analyzed. *Gigantidas*  
148 *childressi* is associated with only one type of methanotrophic symbionts, and consumes  
149 primarily methane. The metabolism of *Bathylomodius heckerae*, predominantly

150 methanotrophic, is however potentially much more complex as its gills harbor a greater  
151 diversity of symbionts, including one methanotrophic, one methylotrophic, and two  
152 thiotrophic polytypes (Duperron et al., 2007). However,  $\delta^{13}\text{C}$  values of these mussels confirm  
153 that seep hydrocarbons represent the dominant carbon source in their soft tissues (Cavanaugh  
154 et al., 1987; Paull et al., 1989; Duperron et al., 2007).

155  
156 Cold seeps at the continental margin offshore Costa Rica harbor a varied fauna  
157 (Sahling et al., 2008; Levin et al., 2012, 2015), including mytilid species whose distribution  
158 shows some stratification with water depth (McCowin et al., 2020): *Bathymodiolus*  
159 *billschneideri* (~1400–1900 m), *B. earlougheri* (~1000–1900 m), and *B. nancyschneiderae*  
160 (1000–1100 m). Furthermore, *Bathymodiolus thermophilus*, a thiotrophic mussel typically  
161 encountered at hydrothermal vents along the Galápagos Rift Zone and the East Pacific Rise  
162 (e.g., Kenk and Wilson, 1985), has been also identified in this area (McCowin et al., 2020).  
163 These four species are all thiotrophic (Levin et al., 2012; Brzechffa and Goffredi, 2021). A  
164 total of twenty shells from three seeps (Jaco Scar, Mound 12, and Parrita Seep) were analyzed  
165 (SIO-BIC specimens listed in Table S-1).

166 Two mussel shells (*Bathymodiolus edisonensis*, SIO-BIC M18361; and *B.*  
167 *anteumbonatus*, SIO-BIC M15360) from a cold seep in the vicinity of Edison Seamount (New  
168 Ireland Basin, Melanesia, Herzig et al., 1998) complement our sampling of methane seep  
169 mollusks. Mussels from this location have been described by von Cosei and Janssen (2008),  
170 but to the best of our knowledge, the nature of their symbionts and their isotopic compositions  
171 are unknown. The abundance of methane at these sites (Herzig et al., 1998), as well as the  
172 similarities of these shells to *Gigantidas childressi* from Gulf of Mexico, strongly suggest that  
173 they are methanotrophic (von Cosei and Janssen, 2008).

174

### 175 **3/ Analytical procedures**

176 Periostracum was removed from the shells by scraping using a clean steel blade. The  
177 determination of the REE abundances follows the procedure designed by Barrat et al. (2020).  
178 About 150–200 mg of shell were precisely weighted, spiked with Tm, and dissolved with  
179  $\text{HNO}_3$ . REE and Y were separated using ion-exchange chromatographic columns loaded with  
180 about 1 ml DGA resin. The measurements were performed on a Thermo Scientific  
181 ELEMENT XR™ spectrometer located at the “Pôle Spectrométrie Ocean”, Institut

182 Universitaire Européen de la Mer (IUEM), Plouzané (France). The reader is referred to  
183 previous papers where our analytical methodology for carbonate samples (Barrat et al., 2020),  
184 calibration, isobaric interference corrections and calculation of concentrations based on Tm  
185 addition are extensively described, and in which reference values for many international rock  
186 standards are reported (e.g., Barrat et al., 1996, 2012, 2016; Charles et al., 2021).

187 Measured REE concentrations were normalized to the Post Archean Australian Shale  
188 (PAAS; Pourmand et al., 2012), adjusted to standard results obtained in our laboratory (Barrat  
189 et al., 2020). The La, Ce, and Gd anomalies were calculated using the  $La/La^*$ ,  $Ce/Ce^*$ ,  
190  $Gd/Gd^*$  ratios, where  $X^*$  is the extrapolated concentration for a smooth PAAS-normalized  
191 REE pattern and  $X_{sn}$  is the concentration of element X normalized to PAAS:  $La_{sn}^* =$   
192  $Pr_{sn}^3/Nd_{sn}^2$ ,  $Ce_{sn}^* = Pr_{sn}^2/Nd_{sn}$ ,  $Gd_{sn}^* = Tb_{sn}^2/Dy_{sn}$ . Replicate measurements obtained for two  
193 carbonate standards (CAL-S and BEAN) during the analytical sessions are given in  
194 Supplementary Table 1, together with reference values from the literature. The precision  
195 (RSD) on measured abundances and elemental ratios was generally better than 3 %. Note that  
196 there is no analytical bias between the REE concentrations presented here and those obtained  
197 by Wang et al. (2020), which were obtained using the same procedure in our laboratory; or  
198 those from Bau et al. (2010) obtained at Jacobs University (Bremen), which are also of very  
199 high quality. However, Y concentrations obtained in Bremen on different standards (e.g., Bau  
200 et al., 2010, 2014, 2018) were systematically lower than those obtained in Brest (e.g., Barrat  
201 et al., 2012, 2020, Charles et al., 2021). This calibration bias was corrected here by  
202 multiplying all Y concentrations obtained by Bau et al. (2010) by 1.10.

203

## 204 **4/ Results**

205 Sixty-six shell analyses were obtained here (supplementary Table 1), greatly  
206 increasing the number of available data for deep-sea mussels. These shells are very poor in  
207 REEs. Only rarely did concentrations of an element exceed 100 or 200 ng/g (e.g. Eu for some  
208 samples from hydrothermal vents). PAAS-normalized REY patterns from the available data  
209 set (Bau et al., 2010; Wang et al., 2020 and this work) are given in Figures 2-5 and provide an  
210 overview of the diversity of compositions of these shells.

211

212

213



## 214 **4.1/ Hydrothermal vent mussels**

215 As shown by Bau et al. (2010), the shells of hydrothermal vent mussels have REY  
216 patterns showing large positive Eu anomalies, inherited from hydrothermal fluids (Fig. 2). In  
217 this regard, the shells analyzed here are no exception. The East Pacific shells show identical  
218 patterns (Fig. 2b), except for Eu, with the EPR sample having an Eu-anomaly similar to those  
219 from Logatchev (Fig. 2a) and the Rose Garden sample similar to those from Golden Valley  
220 (Fig. 2c) obtained by Bau et al. (2010). Both samples are also marked by positive Y and  
221 negative Ce anomalies that are unquestionably the fingerprint of seawater. Two samples of  
222 the same shell from Nifonea were analyzed and the results are almost identical, suggesting a  
223 homogeneity of the latter (Fig. 2e). The patterns obtained show positive Eu anomalies lower  
224 than for the two other samples from Pacific, as well as negative anomalies in Ce and positive  
225 in Y quite pronounced. These patterns are similar to those of Lilliput (Fig. 2b). The less  
226 pronounced Eu anomalies here could be explained by the composition of hydrothermal fluids,  
227 whose Eu anomalies are much lower than those of typical oceanic hydrothermal fluids  
228 (Schmidt et al., 2017), but also by a more important contribution of ambient seawater than for  
229 the other sites.

230

## 231 **4.2/ Cold seep mussels**

### 232 **4.2.1/ Thiotrophic mussels**

233 Only three thiotrophic mussel shells from the China Sea (Site F, Fig. 1) had been  
234 analyzed before this study (Wang et al., 2020). Here we studied a much larger series of such  
235 mussels collected from three sites located in western Costa Rica. Four species of mussels  
236 were analyzed. For these three sites, whatever the species considered, the REY-patterns are  
237 quite similar (Fig. 3), and comparable in abundance and shape to those of *Bathymodiolus*  
238 *aduloides* collected in the China Sea (Fig. 4 b). Unlike shells from hydrothermal sites, these  
239 shells display low  $(\text{Eu}/\text{Sm})_{\text{sn}}$  ratios (=1.21-1.92). The shape of their REY patterns resembles  
240 those of marine carbonates (e.g., Northdurft et al., 2004). The patterns are characterized by  
241 marked negative Ce anomalies ( $\text{Ce}/\text{Ce}^*= 0.34\text{-}0.77$  for Costa Rica samples, and  $0.77\text{-}0.81$  for  
242 China Sea samples) and positive Y anomalies ( $\text{Y}/\text{Ho}= 51\text{-}80$  for Costa Rica samples, and  $50\text{-}$   
243  $64$  for China Sea samples). They also display positive anomalies in La ( $\text{La}/\text{La}^*=1.9\text{-}3.0$ ) in  
244 the range of seawater values, and apparent positive anomalies in Gd ( $\text{Gd}/\text{Gd}^*=1.1\text{-}1.37$ ).

245

#### 246 **4.2.1/ Methanotrophic mussels**

247         The two *Bathymodiolus* shells from the Edison Seamount display lower REE than the  
248 *Gigantidas* from the China Sea (Wang et al., 2020), but their patterns show quite similar  
249 characteristics (Fig. 4). Two fragments per shell were analyzed, and the results are very  
250 consistent, suggesting that both shells do not show marked heterogeneities for REE and Y. As  
251 expected, they show positive anomalies in Y ( $Y/Ho=59.8-69.5$ ) and negative in Ce  
252 ( $Ce/Ce^*=0.39-0.46$ ), but also striking La anomalies ( $La/La^*=7.9-11.9$ ). Wang et al. (2020)  
253 previously showed that the shells of methanotrophic mussels from the China Sea display  
254 exceptionally high positive anomalies in La ( $La/La^*=2.5-5.1$ ). Those measured in Edison  
255 Seamount shells are even higher, and to the best of our knowledge, represent the most  
256 pronounced La anomalies ever measured to date in natural environments not affected by  
257 emerging micropollutants. In addition, these shells also show apparent anomalies in Gd  
258 ( $Gd/Gd^*=1.26-1.77$ ).

259         Different REY patterns were obtained for mussels from the Gulf of Mexico (Fig. 5):

260         -The patterns of *Gigantidas* shells from Brine Pool and Bush Hill show distinctive  
261 light REE enrichments, moderate negative Ce anomalies, variable Gd anomalies  
262 ( $Gd/Gd^*=0.88-1.53$ ), as well as usual seawater-like positive Y anomalies ( $Y/Ho=43.8-72.5$ ).  
263 Their La anomalies ( $La/La^*=1.73-2.70$ ) are not as high as those discussed above, being  
264 comparable to typical marine values. Mussels from these two sites display similar REE  
265 concentrations, showing that the brines at Brine Pool do not affect the REE composition of  
266 shells. Note that the mussels at this site do not live in direct contact with the brines but within  
267 at ~10-cm-thick layer of seawater immediately overlying the brine pool or the surficial  
268 sediment at the pool edge. However, a sample from Brine Pool (BP7a) stands out from other  
269 shells by displaying higher heavy REE contents. We analyzed a second fragment from the  
270 same shell (BP7b), and obtained results that are closer to the rest of studied samples,  
271 suggesting that the first sub-sample may have been contaminated by impurities. Alternatively,  
272 the range of shell composition could be larger than suggested by the other samples.

273         -Two fragments of the same *Bathymodiolus heckeriae* shell from the West Florida  
274 Escarpment were analyzed, with identical results (Fig. 5c). The corresponding patterns are  
275 different from those of other methanotrophic mussels. They do not show notable enrichments  
276 in light REE like those obtained from the Brine Pool or Bush Hill *Gigantidas*, nor large

277 positive La anomalies like the methanotrophic mussels from China Sea or Melanesia. Their  
278  $La/La^*$  (=2.35-2.40) are in the range of seawater values. They are similar to those of  
279 thiotrophic mussels (e.g., those from Costa Rican seeps, Fig. 3), with similar negative Ce and  
280 positive Y anomalies ( $Ce/Ce^*=0.39-0.41$ ) and positive Y ( $Y/Ho=55.2-56.3$ ).

## 281 **5/ Discussion**

### 282 **5.1/ REE characteristics of hydrothermal versus cold seep mussels**

283 Notable differences are apparent between the REE concentrations of mussel shells  
284 from hydrothermal vents and those from cold seeps. Bau et al. (2010) showed that mussel  
285 shells living in the vicinity of Atlantic hydrothermal vents exhibit characteristic REY patterns,  
286 with large positive Eu anomalies [ $(Eu/Sm)_{sn}$  up to 5, and  $>2$  in most cases], which are  
287 inherited from hydrothermal fluids. The analyses obtained here confirm this observation,  
288 showing that all shells from submarine hydrothermal vents display positive Eu anomalies. In  
289 contrast, significant positive Eu anomalies are totally absent in shells from cold seeps (Fig. 6),  
290 which exhibit  $(Eu/Sm)_{sn}$  ratios with little variability (between 1.1 to 1.9). Furthermore,  
291 'hydrothermal' shells show much more variable and generally much higher REE  
292 concentrations than those from cold seeps, as illustrated by the histograms of Sm  
293 concentrations (Fig. 7). Their patterns also sometimes show enrichments in middle REE  
294 compared to heavy REE, which can be quantified with  $(Tb/Yb)_{sn}$  ratios frequently exceeding  
295 2 (Fig. 8). These mid-REE enrichments, combined with presence of significant positive Eu  
296 anomalies, reflect the contribution of hydrothermal fluids with REY distributions very  
297 different from seawater abundances (Bau and Dulski, 1999; Douville et al., 1999, Schmidt et  
298 al., 2007, 2017; Craddock et al. (2010) ; Cole et al., 2014), with no evidence for any presumed  
299 biological effect.

300

### 301 **5.2/ Differences between thiotrophic and methanotrophic mussels at cold seeps**

302 At cold seeps, the REE distributions in mussel shells appear, at least in part, to reflect  
303 some characteristics of the surrounding water that they filter (Fig. 8, Fig. 9). Thiotrophic  
304 mussels have REE ratios falling in the range of seawater and pore water values. Some  
305 samples, however, have slightly higher  $(Tb/Sm)_{sn}$  ratios than pore waters, but we do not  
306 consider this discrepancy to be very significant since the fluid composition at the sites where  
307 our shells originate is not known. As for the mussels from hydrothermal sites, there is no  
308 evidence for measurable imprint of any biochemical process involving REE for these animals.

309           The situation is quite different for methanotrophic mussels. While their heavy-REE  
310 ratios are similar to those for seawater or pore waters (Fig. 8), light-REE abundances depart  
311 substantially from both seawater and pore water values (Fig. 9). They are, in most cases,  
312 much more enriched in light-REE than waters, but also more than oil that can be present in  
313 some seeps like those of the Gulf of Mexico (Smrzka et al., 2019). As mentioned above,  
314 Wang et al (2020) showed that the shells of methanotrophic mussels at methane seeps from  
315 the South China Sea display high La anomalies relative to nearby thiotrophic mussel species,  
316 clams and seawater. The decoupling of La from other trivalent REE at cold seeps has been  
317 interpreted as reflecting the enzymatic activity of associated methanotrophic symbionts, hence  
318 indicating aerobic methanotrophy. The new data obtained in this study provide further support  
319 to this hypothesis. First, the thiotrophic mussel shells are not particularly enriched in light-  
320 REE, and do not show positive La anomalies exceeding usual seawater values, confirming  
321 that the enzymatic activity of their symbionts has no notable effect on these elements. On the  
322 other hand, the REY patterns obtained for methanotrophic mussel shells are much more  
323 variable than those associated with thiotrophic species. Light REE provide a clear-cut  
324 separation between most thiotrophic and methanotrophic mussels (Figs. 8 and 9). Except for  
325 one sample that will be discussed later, methanotrophic mussels show light-REE enrichments  
326 (illustrated by  $(Pr/Sm)_{sn}$  or  $(Pr/Nd)_{sn}$  ratios, Figs. 8 and 9) commonly accompanied by even  
327 more pronounced La enrichments (illustrated by the ratios  $(La/Nd)_{sn}$  and  $La/La^*$ , Fig. 9). The  
328 range of measured light-REE enrichments or La anomalies cannot be explained by local or  
329 environmental factors because it far exceeds the range of values known for sediments and  
330 marine waters (e.g., Fig. 8 and 9). In agreement with Wang et al. (2020), these enrichments  
331 are most likely related to the enzymatic activity of methanotrophic symbionts hosted by the  
332 gills of these mussels. It is logical to link them to the degradation of methane to methanol and  
333 then to formaldehyde, since REE-dependent (XoxF type) methanol dehydrogenase enzymes  
334 are used by these organisms (e.g., Skovran et al., 2011; Semrau et al., 2018; Cotruvo et al.,  
335 2019). The large variability of REY patterns obtained for methanotrophic shells suggests that  
336 the use of light-REE by these organisms differs from one species to another. For example,  
337 some animals generate very large La anomalies in their shells (e.g., *Gigantidas platifrons*  
338 from China Sea or *Bathymodiolus* from Edison Seamount), whereas others display only light  
339 REE enrichments, with La anomalies similar to those in seawater (e.g., *Gigantidas childressi*  
340 from Gulf of Mexico). These differences, which are extremely marked, necessarily reflect  
341 different needs in light-REE for the enzymatic activity of these animals. It should be noted,  
342 however, that the transfer of these elements from the gills (which are the seat of symbiont

343 activity) to the mantle (which synthesizes the shell) has never been considered until now, and  
344 that REE fractionation during this transfer cannot be ignored.

345 One sample, a *Bathymodiolus heckeræ* shell from the West Florida Escarpment (Gulf  
346 of Mexico), differs from all other methanotrophic mussel shells analyzed to date (Wang et al.,  
347 2020 and this study) in that it lacks significant light REE enrichment, and has an anomaly in  
348 La within the range of conventional marine values. This sample even shows REE distributions  
349 very similar to those of thiotrophic mussel shells (Fig. 5c). Note that these particular REE  
350 signatures associated with *Bathymodiolus heckeræ* cannot be related to analytical artefacts  
351 because two separate shell fragments were analyzed, both returning similar results  
352 (Supplementary Table 1). Clearly, the symbionts of this mussel did not require light-REE to  
353 degrade methane. It is logical to assume that they do not use REE-dependent (XoxF type)  
354 methanol dehydrogenase enzymes, but rather Ca-dependent (MxaF type) methanol  
355 dehydrogenase enzymes. However, only one shell of this species was available for our study.  
356 It would be appropriate at this stage to analyze other samples from this site, and obviously to  
357 identify the enzymes used by the mussel symbionts of this species.

358

### 359 **5.3/ Gd anomalies in mussels from cold seeps**

360 An unexpected result of our study concerns the presence of marked positive Gd  
361 anomalies in many cold seep shells (Fig. 10), whereas those from hydrothermal vents show a  
362 much narrower range of Gd/Gd\* values.

363 Gd anomalies are a common feature of REE sorption in the presence of strong solution  
364 complexation, for example with carbonate (dominant in the ocean) or with organic ligands  
365 (e.g., Quinn et al., 2006; Christenson and Schijf, 2011). Gd/Gd\* ratios on the order of 1.2 are  
366 typically encountered in the marine environment. The cold seep mussels investigated here  
367 typically display Gd/Gd\* ratios exceeding 1.2. We are confident that these anomalies do not  
368 correspond to analytical artifacts resulting from biased correction of interferences produced  
369 by Ce and Pr oxides. Our procedure effectively corrects for these interferences (e.g., Charles  
370 et al., 2021), and the results obtained on standards during the course of the study demonstrate  
371 the quality of our analyses (Supplementary Table 1). Moreover, these anomalies were  
372 confirmed through multiple measurements of different fragments of the same shells, and  
373 cannot possibly relate to inappropriate normalization values, because similar Gd/Gd\* values  
374 are calculated by normalizing abundances to other shale references or to chondrites (e.g.,

375 Barrat et al., 2012; Bau et al., 2018, not shown). We are also confident that measured Gd  
376 excesses in this study were not caused by an artefact related to the calculation of Gd  
377 anomalies. Here, Gd anomalies have been calculated by extrapolating Gd\* using Tb and Dy  
378 concentrations, but similar results are obtained when Gd\* is interpolated between Sm and Tb  
379 (not shown).

380 Positive Gd anomalies have been measured in mollusk shells living in waters polluted  
381 by Gd-based contrast agents (GBCA) used for medical imaging by magnetic resonance (e.g.,  
382 Merschel and Bau, 2015; Ponnurangam et al., 2016; Akagi and Edanami, 2017; Le Goff et al.,  
383 2019). Even if this pollution is now common in coastal waters worldwide, it is highly unlikely  
384 that anthropogenic Gd pollution affects the deep sea at which our samples were collected. We  
385 tentatively propose that these Gd anomalies are the result of either biological fractionation or  
386 that they reflect the composition of the water filtered by the mussels. Since Gd anomalies are  
387 present in the shells of both thiotrophic and methanotrophic animals, they are probably not the  
388 result of any specific enzymatic activity related to nutritional symbiosis. It is possible that  
389 cold seep mussels fractionate Gd for an undiscovered use in other biological functions. One  
390 may wonder why these anomalies are not ubiquitous features of bivalve shells, including in  
391 thiotrophic species encountered at hydrothermal vents, where mussel shells lack the marked  
392 Gd anomalies. As an alternative, Gd anomalies could be inherited from water filtered by  
393 mussels. Recently, Möller et al. (2021) reported high Gd/Gd\* ratios in natural,  
394 uncontaminated, spring waters and hypersaline waters (e.g., Dead Sea). Gd/Gd\* ratios close  
395 to 1.7 or higher are unknown in seawaters and oceanic hydrothermal fluids. What about  
396 methane-rich fluids from cold seeps? There are limited REE data for methane-rich fluids at  
397 cold seeps (e.g., Himmler et al., 2013; Lemaitre et al., 2014). However, some of their  
398 characteristics can be inferred from the composition of the carbonates they deposit (e.g.,  
399 Smrzrka et al., 2020; Bayon et al., 2020a). To date, it has not been convincingly shown that  
400 cold seeps carbonates can exhibit such anomalies, making it more than hypothetical that they  
401 are present in cold seeps fluids. The origin and significance of the Gd anomalies observed in  
402 cold seeps mussel shells is not yet understood.

403

## 404 **6/ Conclusions and perspectives**

405 In this study, we compared REE abundances in deep-water mussel shells from various  
406 hydrothermal and cold seep systems worldwide, confirming that animals living at

407 hydrothermal sites display compositional shell characteristics of surrounding fluids such as  
408 positive Eu anomalies (Bau et al., 2010). The REE distribution in bivalve shells at submarine  
409 methane seeps provides a window into the enzymatic activity of associated symbionts. Unlike  
410 thiotrophic mussels, methanotrophic species whose symbionts use light-REE to degrade  
411 methane, show characteristic light-REE enrichments in their shells. Our results confirm that  
412 only methanotrophic mussels display distinctive positive La anomalies, but selective La  
413 enrichments are not systematic to all species analyzed. The degree of light REE enrichment in  
414 shells probably depends on the distribution pattern of hosted symbionts and associated  
415 enzymatic activities, suggesting regional variability. For instance, only shells from western  
416 Pacific sites displayed strong positive La anomalies in this study. At this stage, it is unclear  
417 whether this characteristic relates to the presence of specific symbiont lineages. Future  
418 investigations of other mussel species from additional sites combined with symbiont  
419 characterization will be required to address this issue. In future studies, REE concentrations in  
420 abyssal mussel shells could be used to distinguish methanotrophic from thiotrophic species,  
421 hence serving as tools for identifying their general enzymatic pathway. This novel approach  
422 could be also applied to the sedimentary record and the investigation of fossil shells in ancient  
423 seep deposits, providing of course that diagenesis or any other post-depositional processes  
424 have not modified their REE abundances. Overall, these findings open up important  
425 multidisciplinary perspectives in biology, Earth and environmental sciences, requiring further  
426 improvements in our understanding of the REE biogeochemistry and inventory in organisms,  
427 and their role in enzymatic activities and metabolic pathways. Additional work will be also  
428 needed to assess the impact of life on REE cycling in the ocean, something that has been  
429 largely overlooked until now. Our new data reinforce the idea that methanotrophic organisms,  
430 at least those using REE-based enzymes, can leave a distinctive chemical fingerprint in rocks,  
431 in particular in methane-derived carbonates at cold seeps. Some of these authigenic  
432 carbonates contain organic-rich aragonitic cements exhibiting high light-REE enrichments  
433 (Zwicker et al., 2018). So far, these cements have been interpreted as fossil biofilms  
434 (Hagemann et al., 2013), but it is very tempting to see here the imprint of aerobic  
435 methanotrophic activity. Finally, we draw the attention of the large geochemical community  
436 studying ancient marine sedimentary formations of Precambrian age, to the interest of  
437 carrying out detailed investigations coupling REE to other conventional biogeochemical  
438 proxies such as stable isotopes in order to detect the emergence of aerobic methanotrophy on  
439 Earth.

440

441

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457

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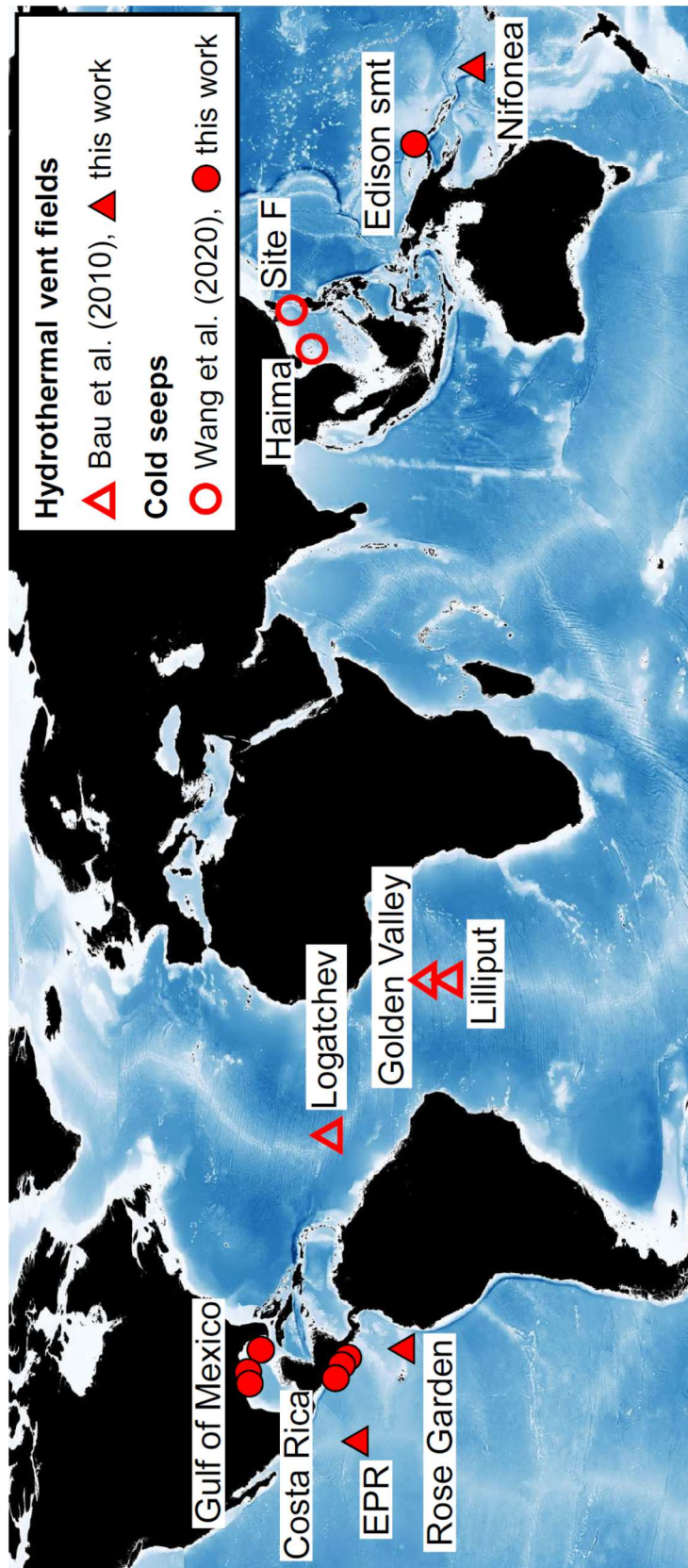
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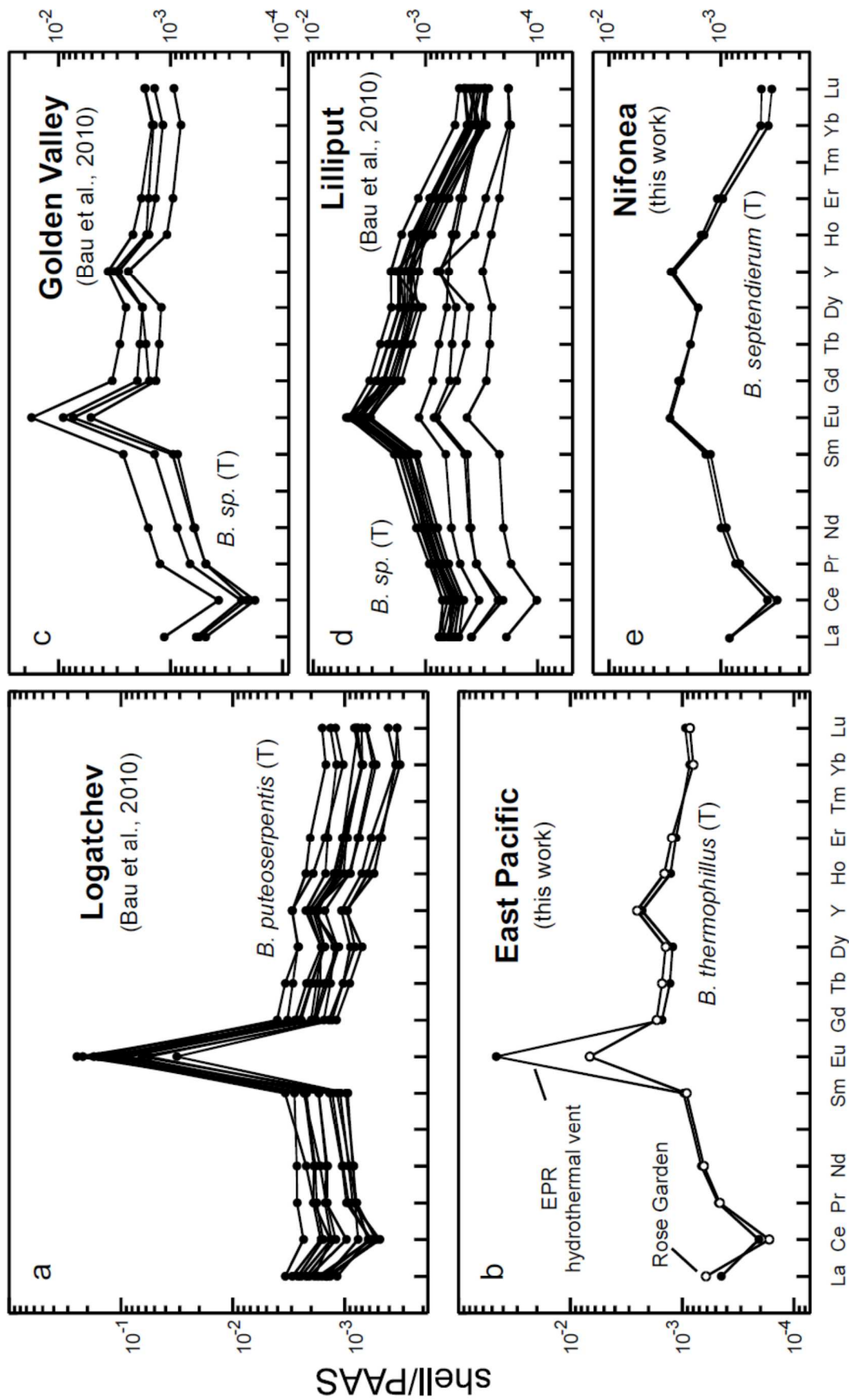
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Figure 1. World map and location of the hydrothermal vents and cold seeps where mussels have been analyzed for REE and Y abundances (Bau et al., 2010; Wang et al., 2020; this study).

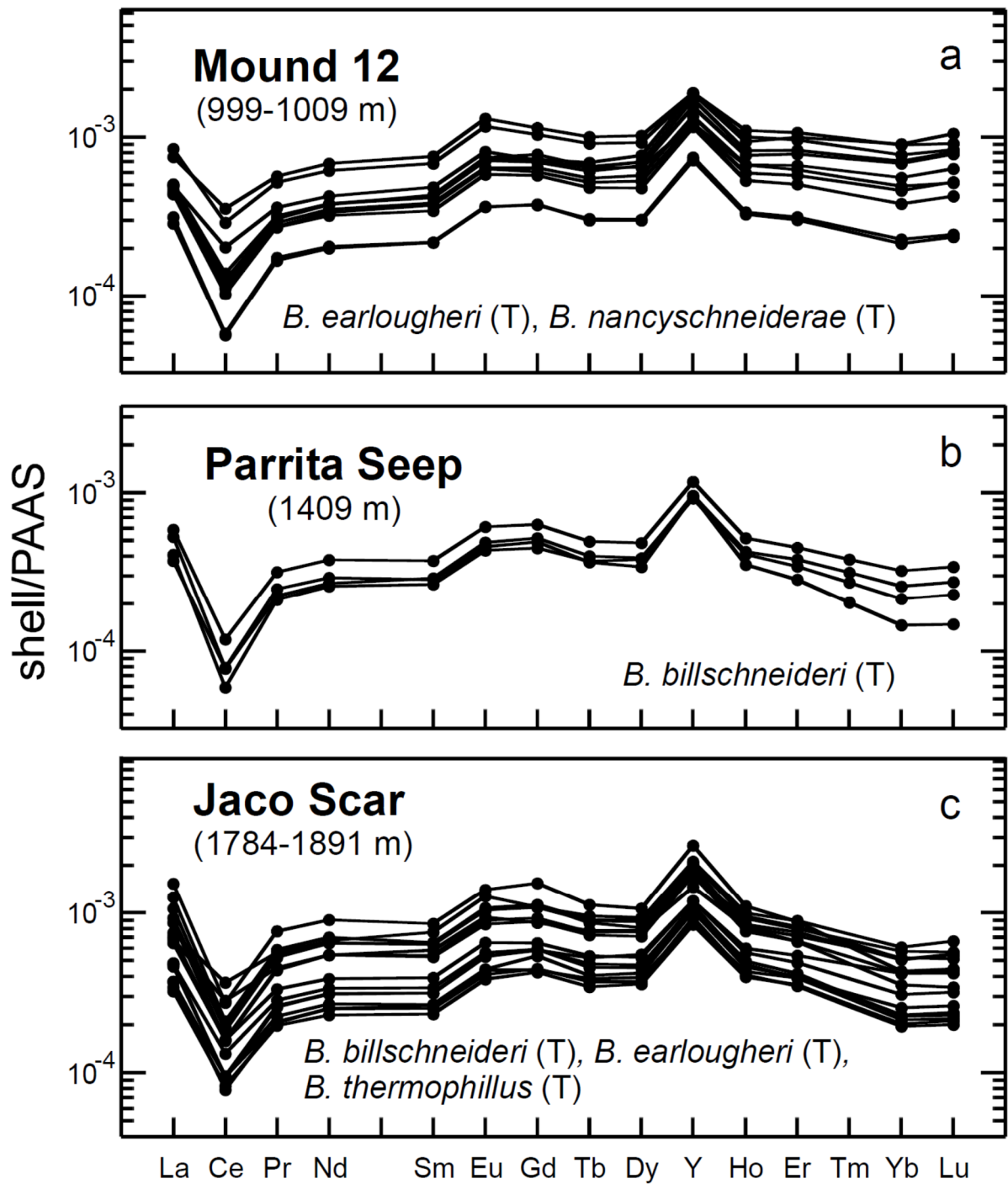


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Figure 2. PAAS-normalized REY patterns for shells of thiotrophic (T) mussels from hydrothermal vents.

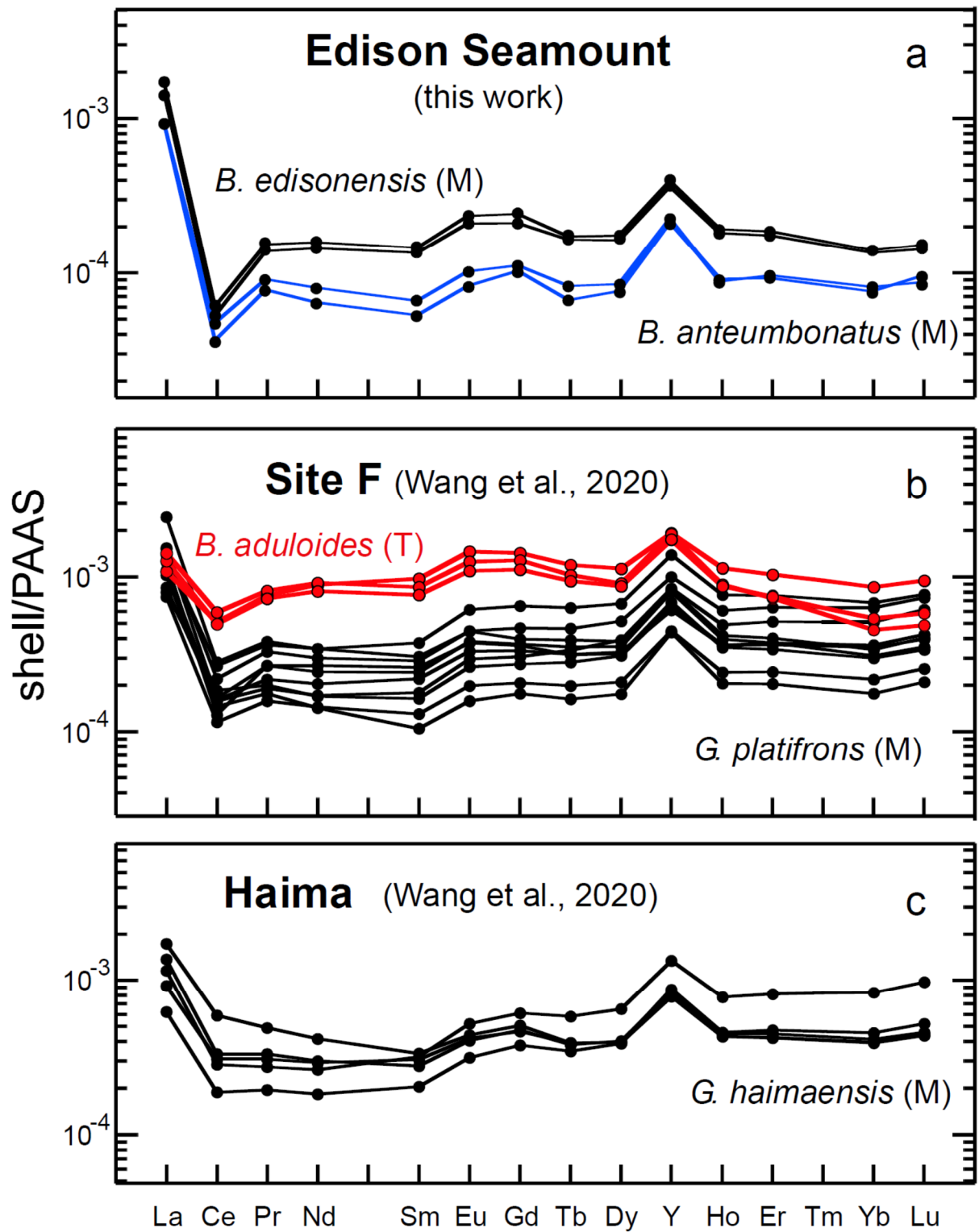


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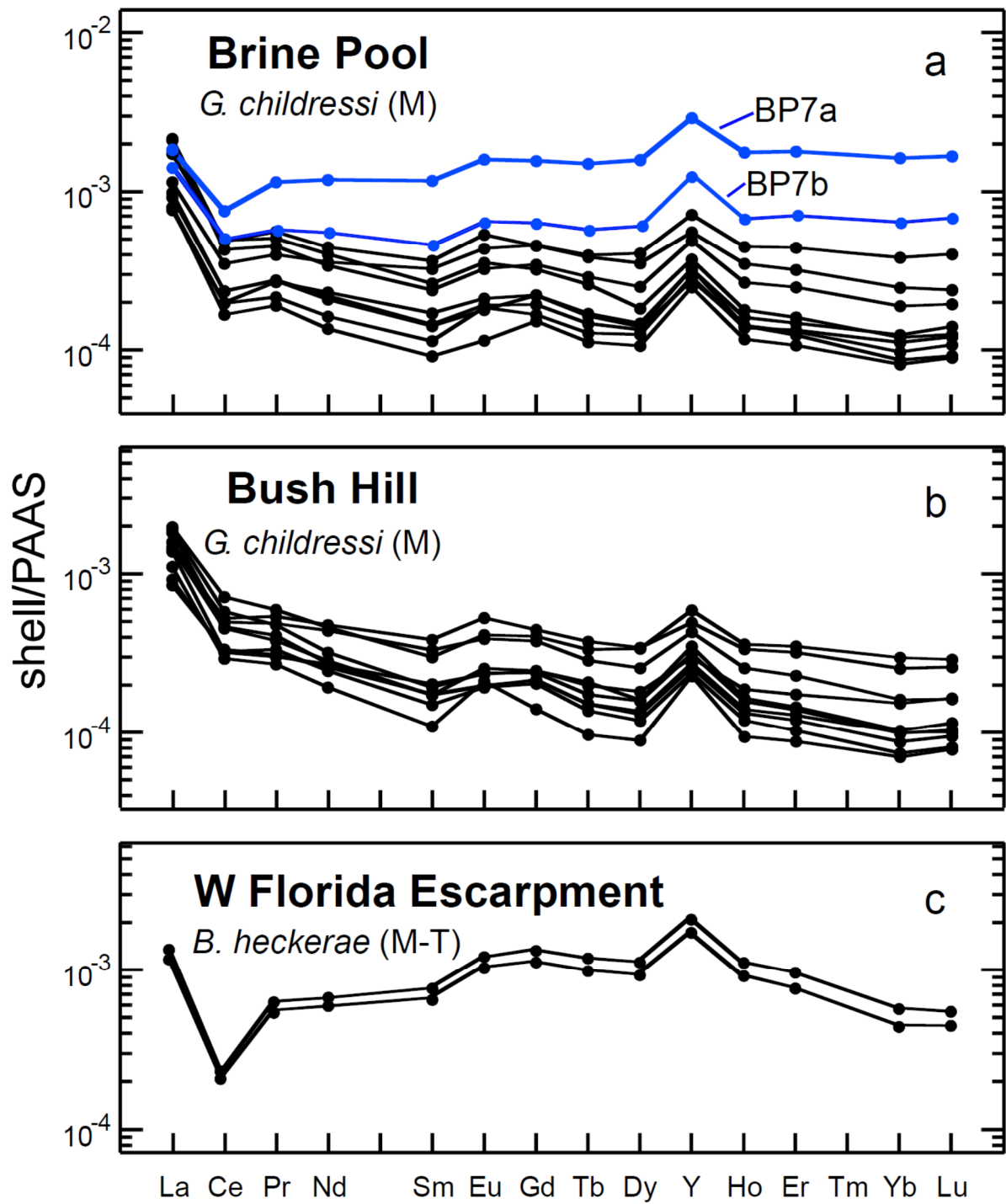
Figure 3. PAAS-normalized REY patterns for mussel shells from vents located west of Costa Rica. Here, all the mussels are thiotrophic (T).



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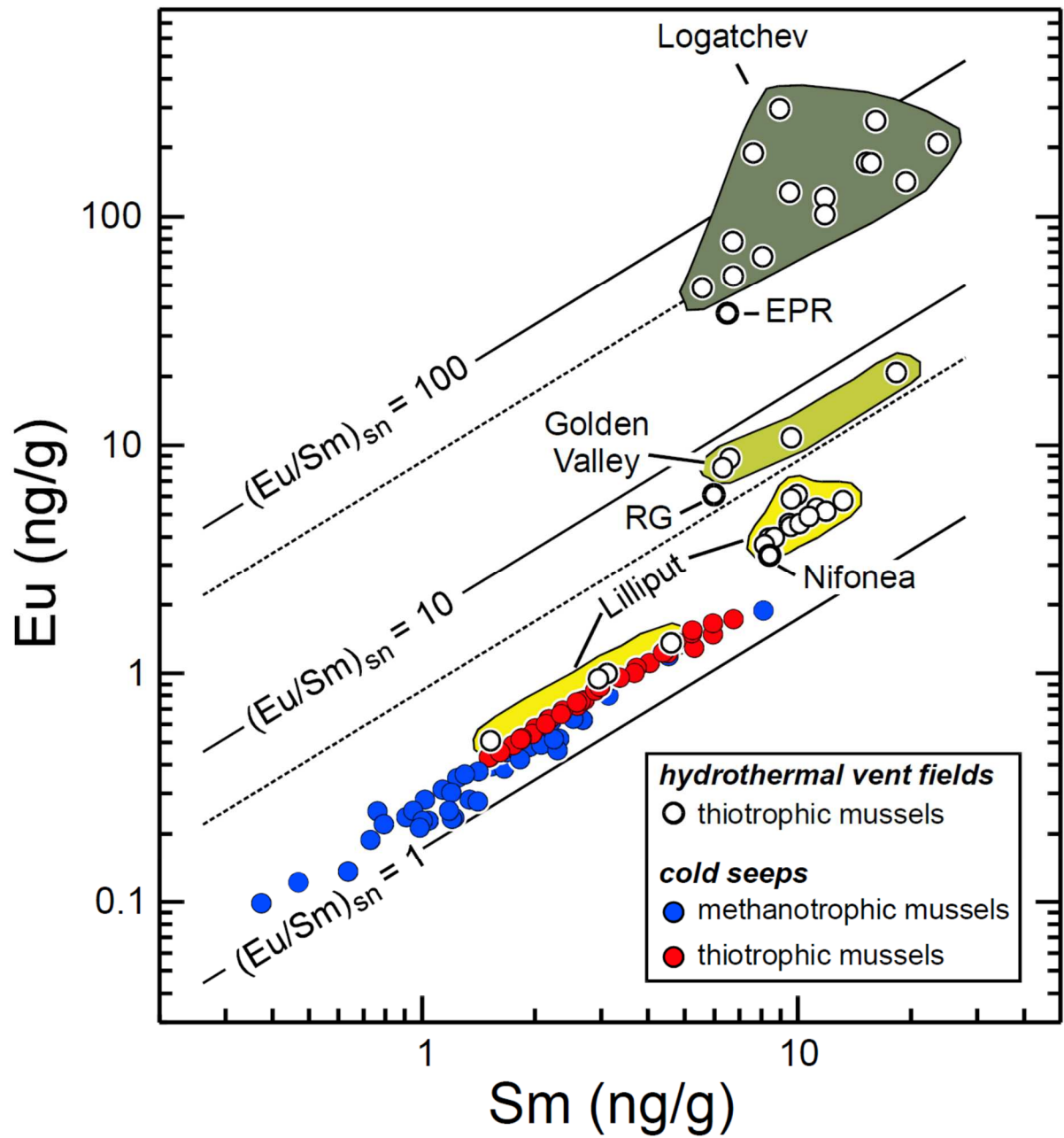
Figure 4. PAAS-normalized REY patterns for mussel shells from cold seeps located in the China Sea (site F and Haima, Wang et al., 2020), and from Edison Seamount (this work). *Bathymodiolus aduloides* mussels are thiotrophic (T). The others are methanotrophic (M).





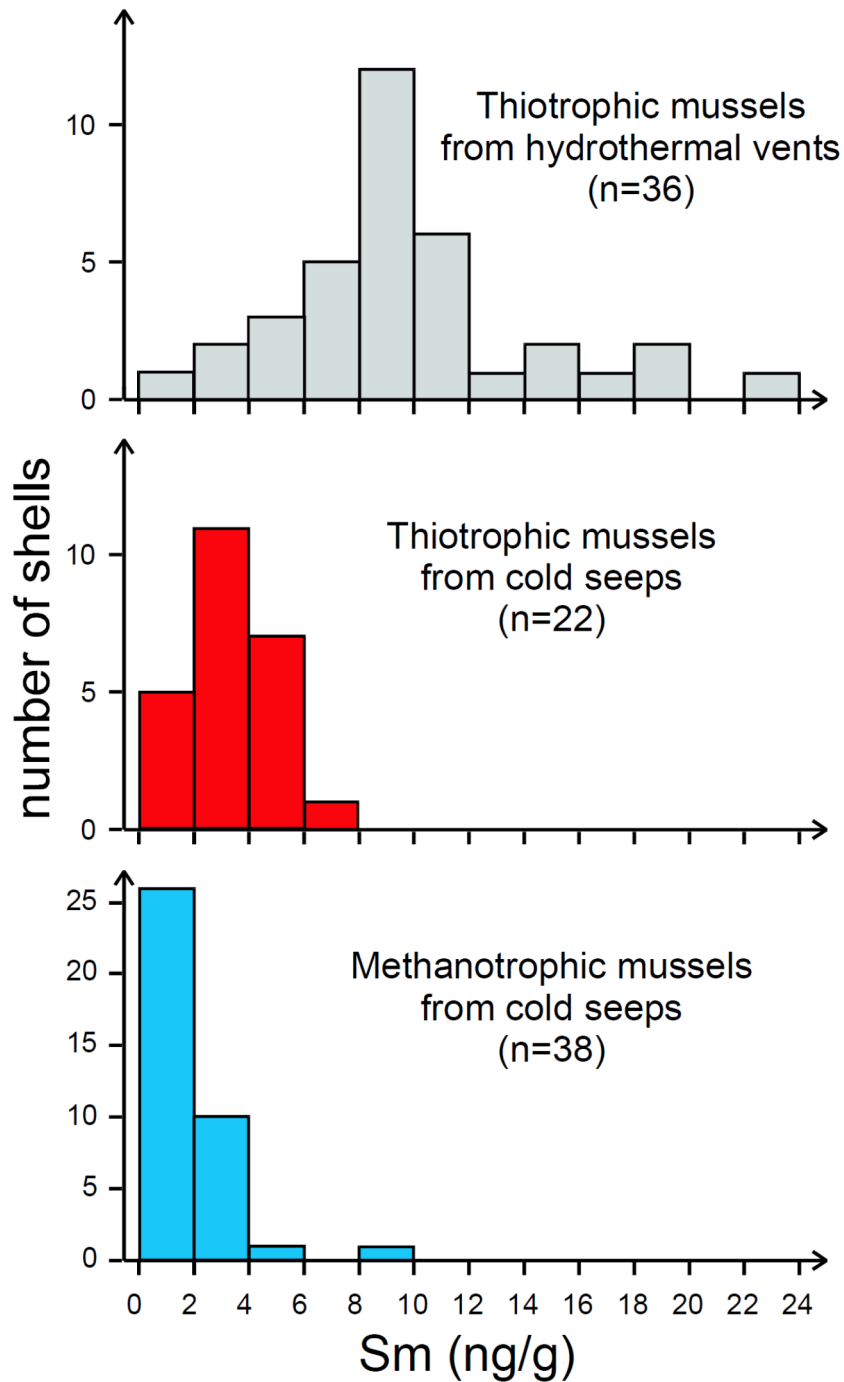
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Figure 5. PAAS-normalized REY patterns for mussel shells from the Gulf of Mexico (M: methanotrophic; T: thiotrophic).



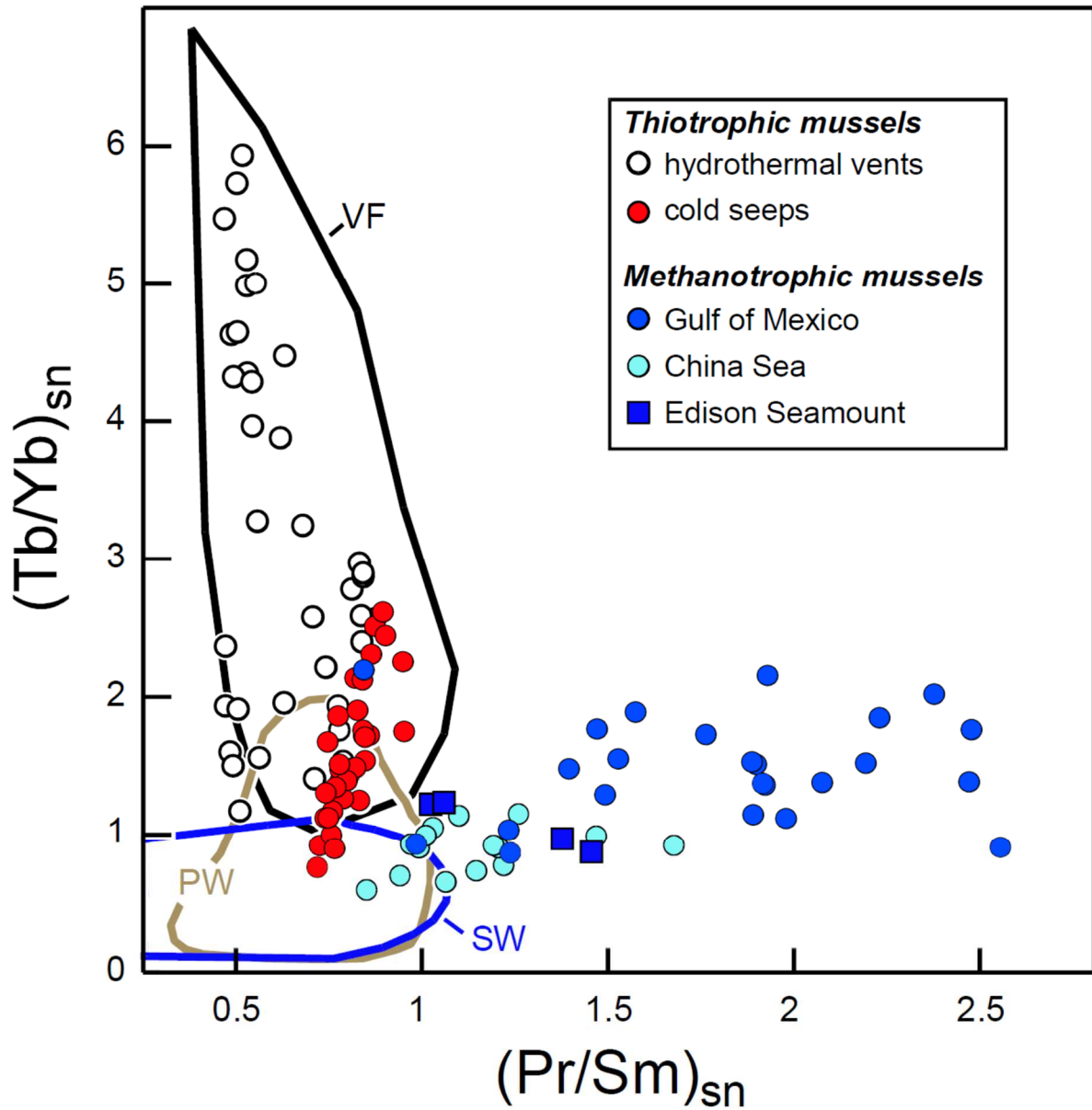
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Figure 6. Eu vs. Sm plot for mussel shells from hydrothermal vents and cold seeps (data from Bau et al., 2010, Wang et al., 2020, and this study).



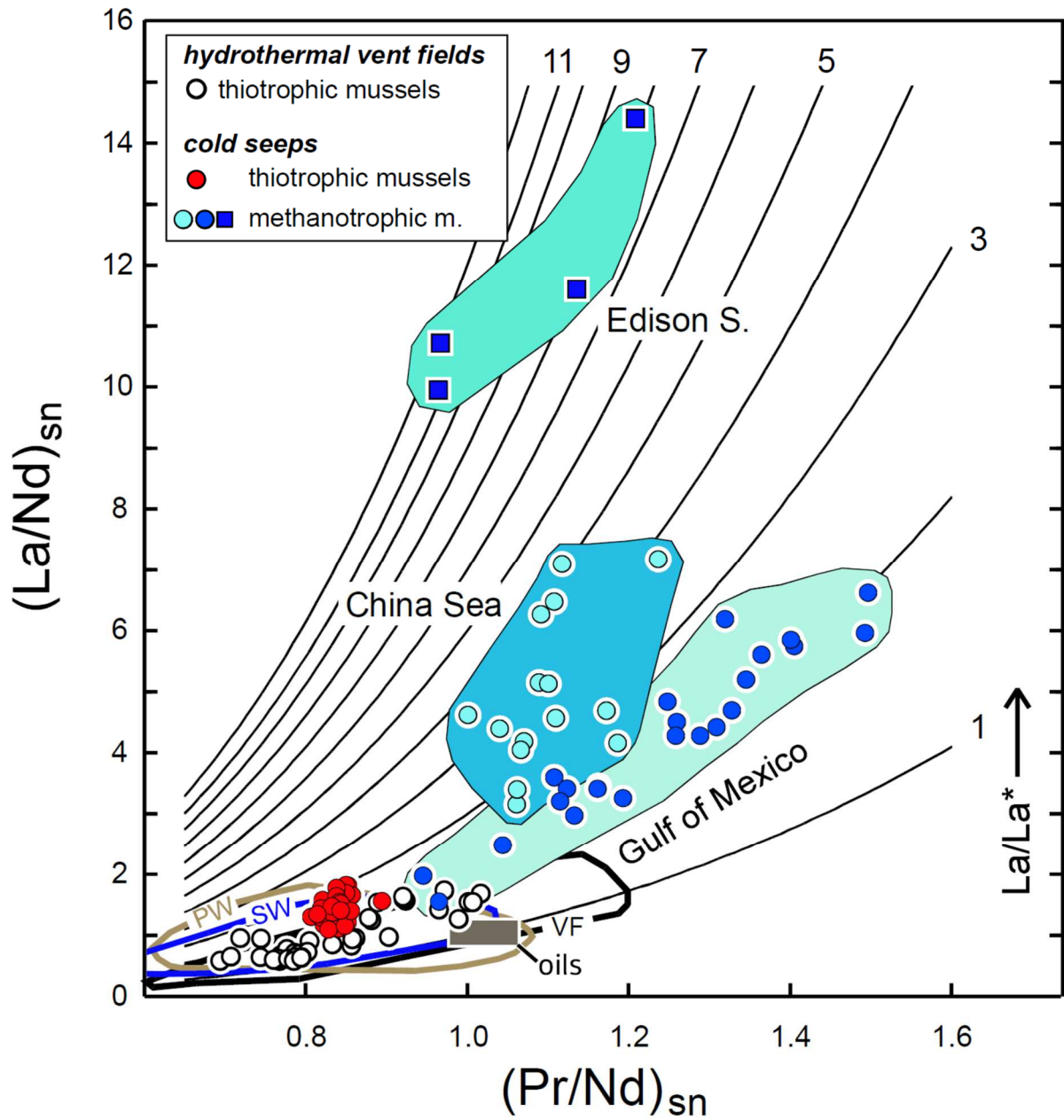
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Figure 7. Histograms of Sm abundances in mussel shells from hydrothermal vents and cold seeps (data from Bau et al., 2010, Wang et al., 2020, and this study). When two fragments of the same shell were analyzed, we used their average here.



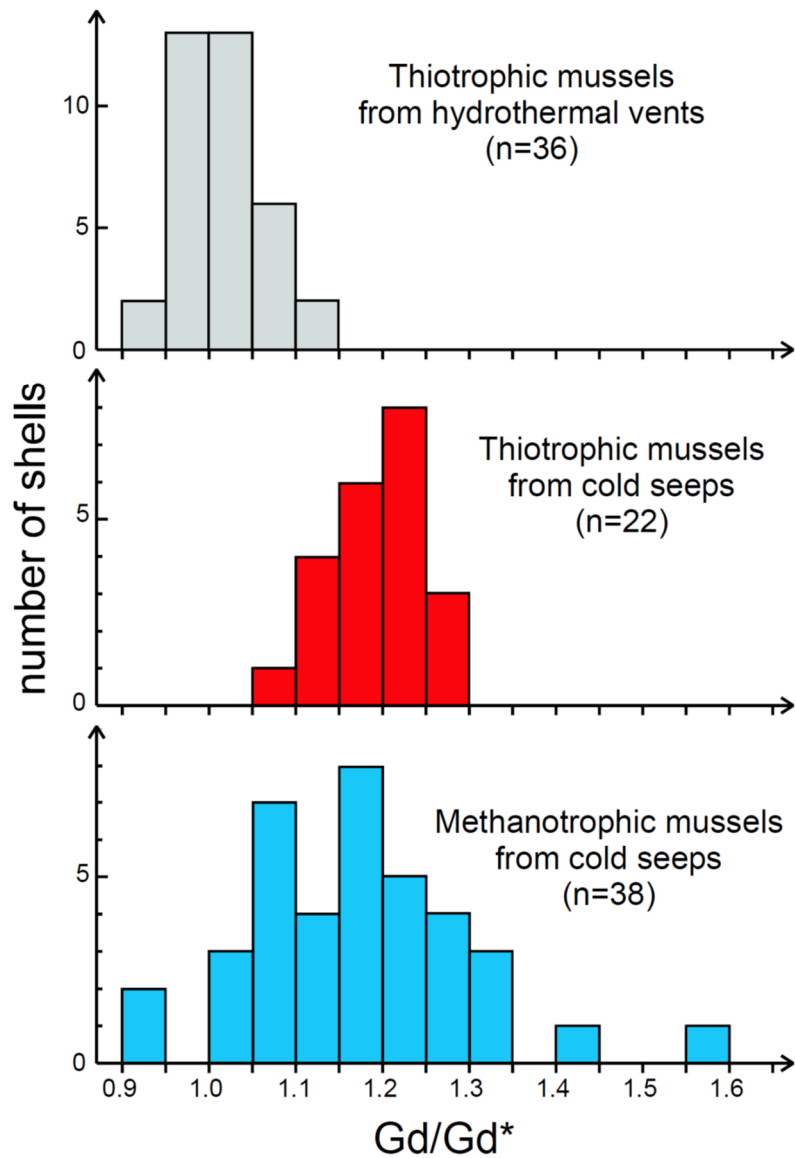
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Figure 8.  $(Tb/Yb)_{sn}$  vs.  $(Pr/Sm)_{sn}$  plot for mussels shells (data from Bau et al., 2010, Wang et al., 2020, and this study). Fields for seawater (SW), pore waters (PW) and vent fluids (VF) are drawn from a database of results of the literature (see Bayon et al. (2020b) for the references for seawaters and pore waters, and Bau and Dulski (1999, Douville et al. (1999), Schmidt et al. (2007, 2017), Craddock et al. (2010), and Cole et al. (2014) for vent fluids).



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Figure 9.  $(La/Nd)_{sn}$  vs.  $(Pr/Nd)_{sn}$  plot for mussels (data from Bau et al., 2010, Wang et al., 2020, and this study). Fields for seawater (SW), pore waters (PW) and vent fluids (VF) are drawn from a database of results of the literature (see figure 8 for the references). Oils from seeps from the southern Gulf of Mexico are shown for comparison (Smrzka et al., 2019).



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Figure 10. Histograms of Gd/Gd\* ratios in mussel shells from hydrothermal vents and cold seeps (data from Bau et al., 2010, Wang et al., 2020, and this study). When two fragments of the same shell were analyzed, we used their average here.