

## Lanthanum anomalies as fingerprints of methanotrophy

X. Wang<sup>1,4</sup>, J.-A. Barrat<sup>2,3\*</sup>, G. Bayon<sup>4</sup>, L. Chauvaud<sup>3</sup>, D. Feng<sup>1</sup>



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### Abstract



Methane is an important greenhouse gas whose emissions into the oceans and atmosphere are regulated by relatively unconstrained anaerobic and aerobic microbial processes. The aerobic pathway for methane oxidation is thought to be largely dependent upon the use of rare earth elements (REE), but to date the effects of this process on their abundances in bacteria or in organisms living in symbiosis with methanotrophs remain to be evaluated. Here we show that deep sea chemosynthetic mussels prospering at methane seeps display distinctive lanthanum enrichments linked to the enzymatic activities of their symbionts. These results demonstrate that methanotrophy is able to fractionate efficiently REE distributions in organisms and possibly in the environment. Lanthanum anomalies recorded in ancient sediments are potential chemical fossils that could be used in the geological record for tracking early evidence of microbial life.

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### Introduction

Until recently, REEs were assumed to have no manifest biological function. The discovery of a REE dependent enzyme involved in the metabolism of aerobic methanotrophic bacteria has radically changed this view (Pol *et al.*, 2014; Semrau *et al.*, 2018; Cotruvo, 2019), showing that these elements could be essential for microbial life. Methanotrophic bacteria first convert methane to methanol. This first step of aerobic methane oxidation is followed by the degradation of methanol into formaldehyde using methanol dehydrogenase enzymes. These enzymes are either Ca dependent (MxaF type) or light REE dependent (XoxF type) (Skovran *et al.*, 2011). However, the latter XoxF type seems to be more frequently used by marine bacteria (Ramachandran *et al.*, 2015; Taubert *et al.*, 2015), hence suggesting that REEs could play a previously unsuspected and important role in the development of marine ecosystems relying on aerobic methanotrophic symbioses.

At ocean margins, areas of active methane seepage at the seafloor (or cold seeps) typically host abundant macrofaunal communities, which derive the vast majority of their nutrition from symbiotic chemotrophic microbes hosted in their gills. The dominant biogeochemical reaction at cold seeps is the anaerobic oxidation of methane (AOM) typically coupled with sulphate reduction, a process that is mediated by a consortium of anaerobic methanotrophic archaea and sulphate reducing bacteria (Boetius *et al.*, 2000). While a substantial fraction of the macrofauna at methane seeps relies on anaerobic microbial

symbionts (mostly using dissolved sulphide, such as tubeworms and clams), mussels are instead commonly associated with aerobic methanotrophic symbionts. In this study, we analysed a series of well characterised shellfish samples from two active seepage sites (Haima and Site F) located in the South China Sea (Feng *et al.*, 2015, 2018; Liang *et al.*, 2017). Our sampling includes both methanotrophic (*Gigantidas platifrons* and *Gigantidas haimaensis*) and thiotrophic mussels (*Bathymodiolus adulooides*), in addition to other bivalves (clams) associated with sulphur oxidising bacteria (*Calyptogena marissinica*). For comparison, additional thiotrophic clams from the Nankai Trough (Fiala-Médioni *et al.*, 1993), and a series of heterotrophic blue mussels (*Mytilus edulis*) devoid of any chemotrophic symbionts from coastal waters of France were also analysed (Table S-1). Methods are described in Supplementary Information.

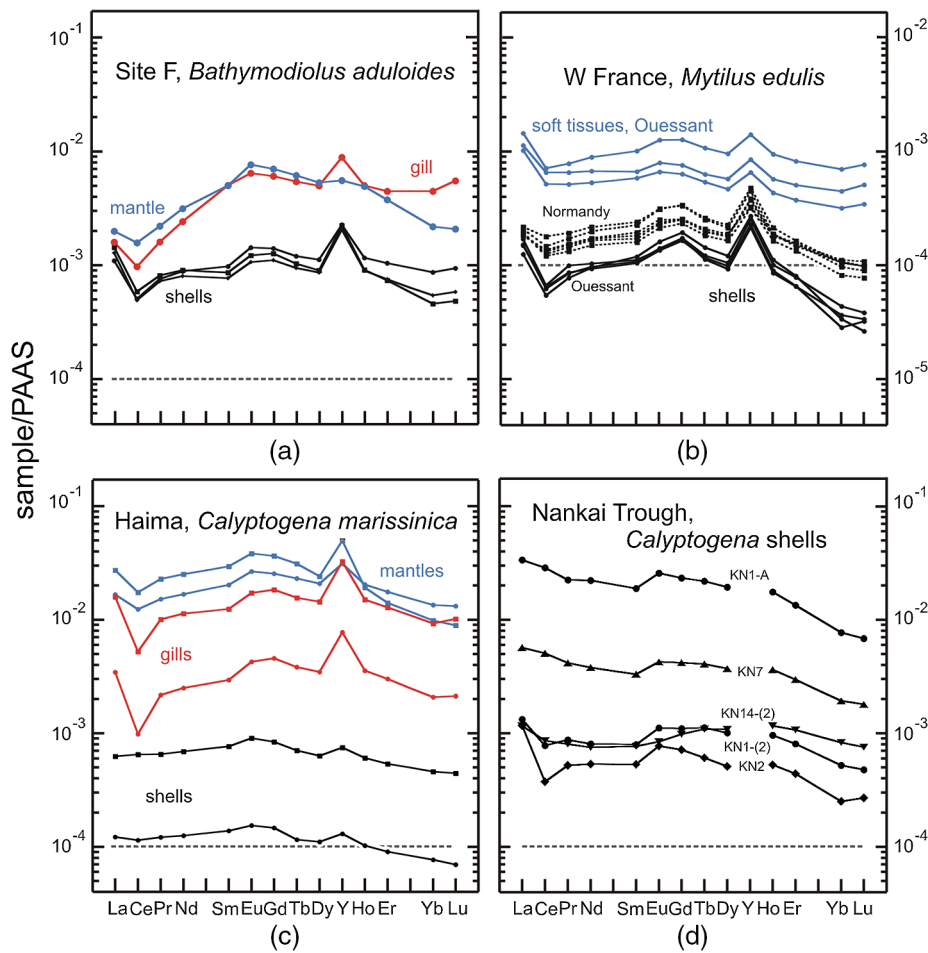
### Results and Discussion

The abundances of REEs in shellfish samples are low, but highly variable, in both shells and soft tissues (Figs. 1, 2; Tables S-2–8). Most samples display positive yttrium and negative cerium anomalies respectively, which represent conspicuous features of seawater and marine-derived materials such as biogenic carbonates. A striking feature of our results is that methanotrophic mussels display shale normalised distribution patterns that strongly differ from the other studied shellfish samples. Compared to the thiotrophic shellfish samples, the shells and soft tissues (feet, mantles) of methanotrophic mussels are

1. Shanghai Engineering Research Center of Hadal Science and Technology, College of Marine Sciences, Shanghai Ocean University, Shanghai 201306, China
2. Université de Bretagne Occidentale, Brest, CNRS, UMR 6538 (Laboratoire Géosciences Océan), Institut Universitaire Européen de la Mer (IUEM), Place Nicolas Copernic, 29280 Plouzané, France
3. Université de Bretagne Occidentale, Brest, CNRS, UMR 6539 (Laboratoire des Sciences de l'Environnement Marin), LIA BeBEST, Institut Universitaire Européen de la Mer (IUEM), Place Nicolas Copernic, 29280 Plouzané, France
4. IFREMER, Marine Geosciences Unit, F-29280 Plouzané, France

\* Corresponding author (email: barrat@univ-brest.fr)





**Figure 1** REE + Y patterns normalised to Post Archean Australian Shale (PAAS; Pourmand *et al.*, 2012) for thiotrophic shellfish from cold seeps and for heterotrophic *Mytilus edulis* from France. The grey dashed line corresponds to the  $10^{-4} \times$  PAAS level. Notice that the *Bathymodiolus* shells are ten times more REE richer than the *Mytilus* ones.

characterised by often large positive lanthanum anomalies ( $La/La^* = 2.0\text{--}5.9$ ; Figs. 2–4, see Supplementary Information for the calculations of the anomalies). Furthermore, their gills exhibit spectacular enrichment in light REEs (Figs. 2, 4).

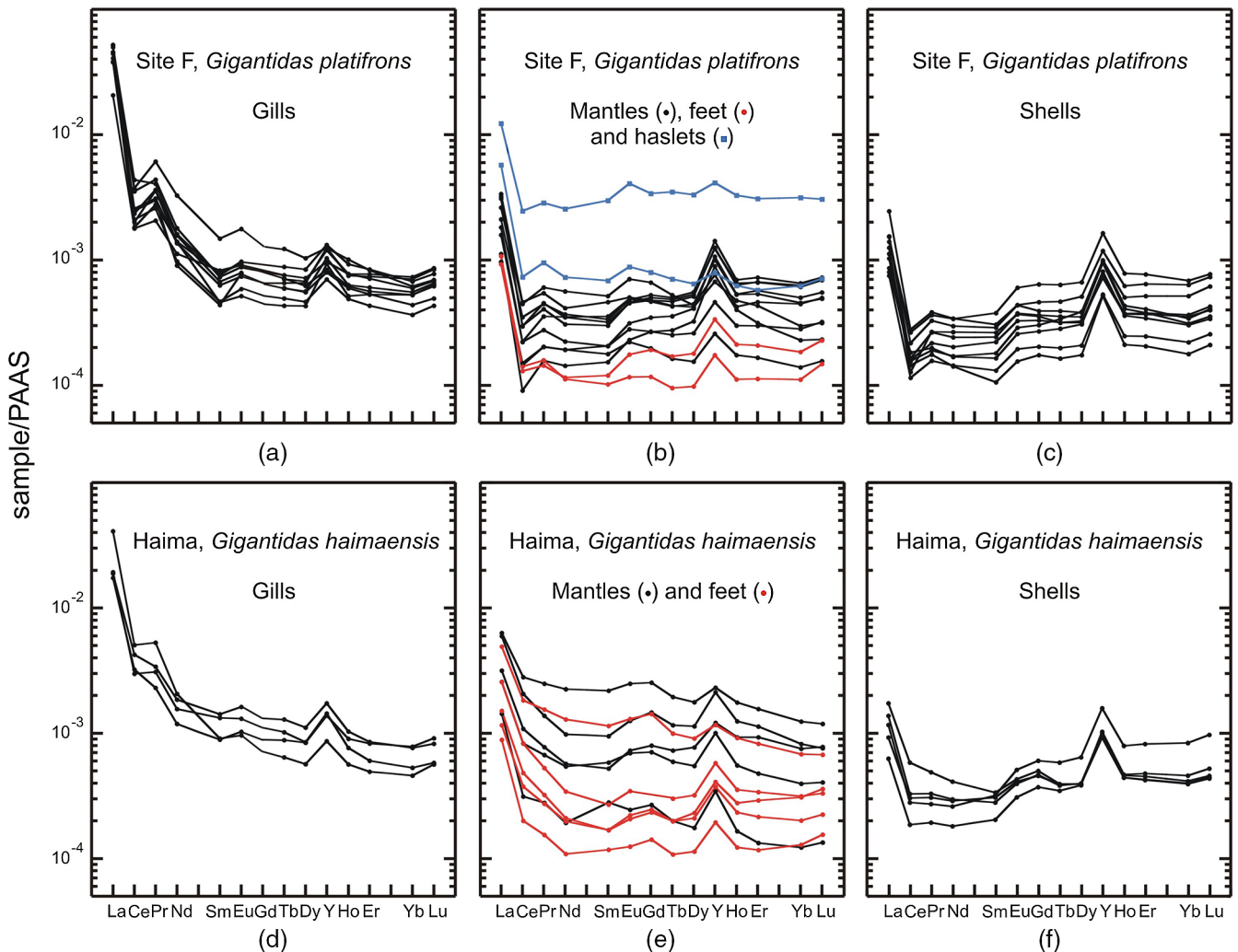
To a large extent, the shale normalised distribution patterns of REEs in the studied shellfish samples are controlled by variable source contributions to the different organs or tissues. In the case of heterotrophic shellfish from coastal areas, the REEs contained in the soft tissues are mainly derived from suspended particles with a reduced contribution from seawater (Akagi and Edanami, 2017). Shells result from the activity of the mantle epithelium inside specific internal liquid (Wheeler, 1992). Consequently, their REE distribution patterns directly reflect the composition of the mantle, with additional potential inputs from the fluids associated with carbonate secretion. In any case, the corresponding REE patterns differ significantly from typical seawater signatures, more closely resembling those of the soft tissues, dominated by inputs from filtered suspended particles.

Overall, heterotrophic (Bau *et al.*, 2010; Ponnurangam *et al.*, 2016; Akagi and Edanami, 2017; Le Goff *et al.*, 2019) and thiotrophic shellfish both display comparable REE features. The shells of thiotrophic mussels *Bathymodiolus aduloides* and of coastal mussels *Mytilus edulis* (Bau *et al.*, 2010; Ponnurangam *et al.*, 2016 and this work, Table S-7) exhibit very similar REE patterns, albeit being characterised by different REE abundances (Fig. 1). Gill and mantle samples also display similar patterns, both characterised

by light REE depletions [ $(La/Sm)_{sn} = 0.32\text{--}0.40$ ] and moderately positive La anomalies ( $La/La^* = 1.83\text{--}2.26$ ). In agreement with previous studies (Akagi and Edanami, 2017), soft tissues are enriched in REEs compared to shells. Like previous mussels, the REE patterns of thiotrophic *Calyptogena* display mixed features between those of terrigenous sediments and seawater. Importantly, all these samples plot within the range of  $La/La^*$  values for seawater (Fig. 3). The same conclusion also applies to the dominantly thiotrophic shellfish collected at hydrothermal fields along oceanic ridges (Bau *et al.*, 2010). Their shells exhibit patterns largely influenced by the composition of hydrothermal fluids but never show any particular lanthanum excesses, suggesting that these animals and their symbionts do not require light REEs (Fig. 3).

The situation for methanotrophic shellfish is clearly different (Figs. 3, 4). Their gills show exceptional enrichments in light REEs, which, to the best of our knowledge, have never been reported for any natural sediments, seafloor rocks or authigenic phases. Other soft tissues and shells also exhibit significant lanthanum enrichments, as well as marked lanthanum anomalies, with  $La/La^*$  ratios (up to 5) that largely exceed the highest values measured in South China Sea water ( $La/La^* = 2.5$ ; Alibo and Nozaki, 2000). To date, the exchange and recycling of REEs between the different organs of the studied molluscs has never been investigated. However, the exceptional La anomalies reported here cannot be explained by the contribution from any hypothetical fluid or sediment sources. If this was the case,





**Figure 2** REE + Y patterns normalised to PAAS (Pourmand *et al.*, 2012) for methanotrophic mussels from South China Sea cold seeps.

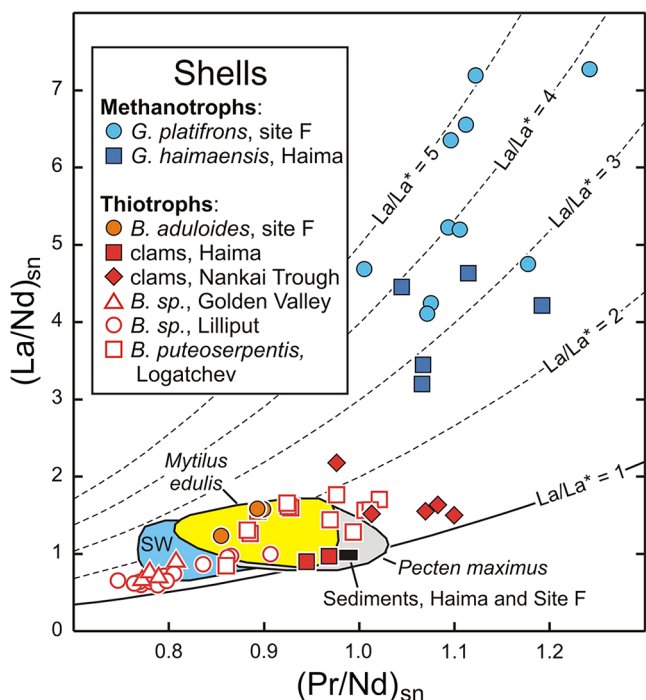
thiotrophic clams and mussels sampled from the same sites would have also displayed similar La enrichments. Therefore, the specific REE features observed in the methanotrophic shellfish samples are best explained by biological processes. It is very important to note that the gills show the highest enrichments in light REEs and lanthanum abundances, because this is precisely where the methanotrophic symbionts are hosted in these mussels (Barry *et al.*, 2002; Yu *et al.*, 2019). Therefore, the enzymatic activity of the methanotrophic symbionts is the most plausible cause for the selective accumulation of light REEs in the *Gigantidas* gills, and the large lanthanum anomalies in the other soft tissues and recorded in the shells.

An immediate question concerns the presumed source of the light REEs hosted in the mussel gills. Marine sediments are significantly enriched in REEs and contain about 10 million times more lanthanum than seawater, and as such could possibly contribute to the observed light REEs enrichment of the gills, *via* possibly an unrecognised process of remineralisation by the gill cells or by the symbionts. However, it is more likely that most of the light REEs contained in the gills are derived from the fluids filtered by the shellfish. Considering an average mass for *Gigantidas* gill of  $\sim 0.7$  g (on a dry basis), we can estimate that it contains about  $1.9 \mu\text{g/g}$  lanthanum at Site F, and  $1 \mu\text{g/g}$  at Haima. This corresponds to the quantity of lanthanum contained in about 275 litres of seawater at Site F (or 14.4 litres of pore waters; Himmler *et al.* 2013) and 145 litres of sea water at

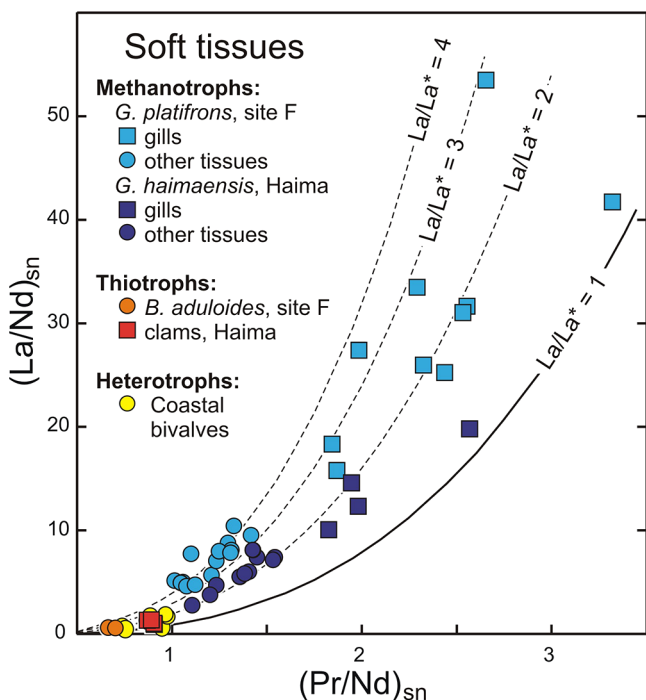
Haima (or 7.6 litres of pore waters). Whatever the exact composition of the involved fluids, these volumes are very small compared to the volume of water that a mussel can filter every day:  $\sim 180$  litres, estimated assuming a mussel with 3 g of soft tissues on a dry basis and a rate similar to that of *Mytilus edulis* (Blayne *et al.*, 1989). Based on these considerations, it is very likely that the methanotrophic symbionts hosted in gills can access the light REEs they need from the seawater filtered by the shellfish, without invoking more complex uptake processes. This raises the question as to whether methanotrophic activity at cold seeps could have an impact on the oceanic REE budget, at least in the water column surrounding the seepage sites. We can estimate that about  $72 \text{ m}^3$  of water is filtered every day by one square metre of a typical mussel bed with 400 animals. And if we limit our estimate to the amount of lanthanum contained only by gills, the same square metre at Site F contains as much lanthanum as  $110 \text{ m}^3$  of seawater. At present, we do not know what proportion of dissolved REEs in the fluids filtered by molluscs are actually taken up by the symbionts, nor what proportions of these are transferred to the organs of the shellfish. However, the biological activity (including not only the mussels that dominate the biomass, but all the other methanotrophs that use XoxF-type enzymes) must necessarily have an impact on the chemistry of seawater near cold seeps. The capability of methanotrophic bacteria to affect the local distribution of REEs in seawater has already been observed in the Gulf of Mexico. Following the



Deepwater Horizon well blowout in 2010, the release of massive methane plumes in the water column was accompanied by substantial removal of light REEs, interpreted as the result of intense methanotrophic activity (Shiller *et al.*, 2017).



**Figure 3**  $(La/Nd)_{sn}$  vs.  $(Pr/Nd)_{sn}$  plot for the shells from various shellfish from cold seeps (this work), from Atlantic hydrothermal sites (Golden Valley, Lilliput and Logatchev; Bau *et al.*, 2010), heterotrophic shellfish (*Mytilus edulis* and *Pecten maximus*; Bau *et al.*, 2010; Ponnuram *et al.*, 2016; Le Goff *et al.*, 2019 and this work). Fields for seawater (SW; Alibo and Nozaki, 2000) and sediments from Haima and Site F (this work) are shown for comparison.



**Figure 4**  $(La/Nd)_{sn}$  vs.  $(Pr/Nd)_{sn}$  plot for the soft tissues prepared from various shellfish from cold seeps, and various heterotrophic coastal bivalves (Akagi and Edanami, 2016 and this work).

Our findings could have implications for the understanding of other lanthanum excesses previously identified in seawater and various marine precipitates, but remaining unexplained so far. However, this element was assumed to be more stable than other light REEs during complexation in seawater (de Baar *et al.*, 1985) or could be released from suspended barite particles (Grenier *et al.*, 2018). In marine precipitates, lanthanum anomalies are common features that are usually inferred to be largely inherited from seawater (e.g., Bau and Dulski, 1996; Kamber and Webb, 2001), but for which no clear explanation has been proposed. Our data demonstrate unambiguously that positive lanthanum anomalies can be generated by the enzymatic activity of methanotrophs, hence providing the possibility of promising applications in various fields of research. Importantly, in future studies, the presence of marked lanthanum anomalies or any other large light REE enrichments in fossil shells could be used as proxies for past methanotrophic activity. Methanotrophic organisms have been present since the Archean, but their detection in the geological record, and in the sedimentary archives of the early Earth oceans are still the subject of intense debate (Thomazo *et al.*, 2009; Knoll *et al.*, 2016). In particular, lanthanum anomalies are frequent in ancient microbial carbonates and banded iron formations (Bau and Dulski, 1996; Kamber and Webb, 2001; Planavsky *et al.*, 2010), which may point towards a putative REE dependent microbiological origin. Revisiting the REE geochemistry of these ancient rocks could provide fresh insights into the emergence and diversification of life.

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### Additional Information

Supplementary Information accompanies this letter at <http://www.geochemicalperspectivesletters.org/article2019>.



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