Mussel shells of *Mytilus edulis* as Bioarchives of the Distribution of Rare Earth Elements and Yttrium in Seawater and the Potential Impact of pH and Temperature on their Partitioning Behaviour

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Abstract

Mussel shells are potential bioarchives of proxies for changes of the physico-chemical conditions in the bivalve's habitat. One such proxy is the distribution of the Rare Earths and Yttrium (REY) in seawater, as REY speciation in seawater is sensitive to pH and temperature variations, due to the impact of these parameters on the activity of CO_3^{2-} in seawater. We present a new protocol for sample preparation and determination of ultratrace concentrations of REY in bulk bivalve shells (comprised of calcite and aragonite), that includes sample treatment with NaOCl followed by REY separation and preconcentration. The data obtained was used to calculate REY partition coefficients between bulk shells of *Mytilus edulis* and ambient seawater, and acquired results were then used to investigate the potential effects of pH and temperature on REY partitioning.

Shells of *Mytilus edulis* mussels from the North Sea show consistent shale-normalized ("SN") REY patterns that increase from the light REY to the middle REY and decrease from the middle REY to the heavy REY. Despite being different to the general seawater REY_{SN} pattern, the shells still display distinct REY features of seawater, such as a negative Ce_{SN}

anomaly and small positive Y_{SN} and Gd_{SN} anomalies. Apparent REY partition coefficients between shells and seawater ($_{app}D_{Tot.REY}^{shell/seawater}$) are low and decrease strongly from the light REY (4.04 for La) to the heavy REY (0.34 for Lu). However, assuming that only the free REY^{3+} are incorporated into the shell, $_{mod}D_{FreeREY3+}^{shell/seawater}$ values are higher and rather similar for all REY (102.46 for La; 113.44 for Lu), but show a slight maximum at Tb (199.18). Although the impact of vital effects such as REY speciation in a mussel's extrapallial fluid from which the carbonate minerals precipitate, cannot be quantified yet, it appears that *M. edulis* shells are bioarchives of some REY features of seawater.

We modelled the REY_{SN} patterns of a hypothetical mussel shell at pH 8.2 and 7.6 and at temperatures of 25°C and 5°C, assuming that only free REY^{3+} are incorporated into the carbonate's crystal lattice and that vital effects do not obliterate the REY signal of the shells. The results suggest that with lower pH, REY concentrations in shells increase, but with little effect on the shape of the REY_{SN} patterns, while a temperature change has an impact on the REY_{SN} pattern, but only minor effects on REY concentrations. Hence, after additional calibration studies, the REY systematics in mussel shells may become a valuable proxy for paleo-pH and ocean acidification.

1. Introduction

Mussels and mussel shells have increasingly gained importance as bioarchives of proxies that record physico-chemical changes in their marine or freshwater habitat (Bau et al. 2010, Gillikin et al. 2006a, Merschel and Bau 2015, Puente et al. 1996, van der Putten et al. 2000, Scourse et al. 2006, Sturesson 1976, Wanamaker et al. 2008). The chemical composition of bivalve shells is known to contain a record of their past growth, based on the sequential deposition of layers of mineralized material during their lifetime (Lindh et al. 1988, Weiner 2008, Wilbur and Saleuddin 1983). Hence, mussel shells may be valuable high-resolution bioarchives of past marine, estuarine, fluviatile and limnic conditions.

Trace elements such as the rare earths and yttrium (REY) have been shown to be useful indicators of environmental change (Bau and Dulski 1996, Bau et al. 2010, Bolhar et al. 2004, Kulaksız and Bau 2013, Lee et al. 2003, Möller et al. 2000, Murray et al. 1990, Nothdurft et al. 2004, Tepe et al. 2014, Viehmann et al. 2014, Webb and Kamber 2000, Wyndham et al. 2004). The REY are a group of elements that are similar in atomic structure and chemical properties and hence behave coherently in natural systems. Their speciation in seawater and the distinct REY patterns exhibited by different geological materials make them very useful as geochemical proxies of oceanic change (Byrne 2002, Byrne and Miller 1985).

Other trace elements have also been shown to be incorporated into the shells during annual layer formation and are assumed to be essentially immobile (Lindh et al. 1988). Stable isotope studies of mussel shells and particularly of *Mytilus edulis* corroborate the use of shells as paleoceanographic bioarchives (Gillikin et al. 2006b, Wanamaker et al. 2006, 2007).

Various mussel species have already been used as environmental indicators to monitor pollution and bioavailability of (micro)contaminants (Liang et al. 2004, Lindh et al. 1988, Merschel and Bau 2015, Puente et al. 1996, Sturesson 1976, Wagner and Boman 2004, Zuykov et al. 2013) . However, the focus so far has often been on major and minor elements such as Mg and Sr, while REY data for mussel shells are still rather scarce and underrepresented. Given the growing importance of mussels in proxy development for the ocean-climate system including ocean acidification, there is an ample need to better understand these organisms and how they may be used as bioarchives of trace elements and their isotopes.

Numerous studies have provided insights into the composition of ancient seawater and the evolution of the environmental system based on the distribution of rare earth elements (REEs) in chemical sediments (Alexander et al. 2009, Alibert and McCulloch 1993, Bau et al. 1997, Bau and Alexander 2006, 2009, Derry and Jacobsen 1990, Nothdurft et al. 2004, Viehmann et al. 2014, 2015; and references therein). Similarly, other studies have demonstrated the potential of bivalve shells to track the environmental conditions they were exposed to (Bau et al. 2010, Dunca et al. 2005, Heinemann et al. 2011, Klein et al. 1996, McCoy et al. 2011, Thébault et al. 2009, Weiner 2008) . Bau et al. (2010) have shown that the positive Eu anomalies in the REY distribution patterns of shells of marine *Bathymodiolus* mussels can be used as tracers for hidden or fossil high-temperature hydrothermal systems, while Merschel and Bau (2015) demonstrated that shells of freshwater *Corbicula* mussels may be used to study the bioavailability of anthropogenic REE microcontaminants. This already gives a hint that mussel shells archive certain REY signatures of the environment in which they grow. However, not much has been done to evaluate how accurately mussel shells reflect REY patterns of seawater and what impacts their partitioning behavior.

In this study, we approached this issue via an *in situ* culture experiment using Blue Mussels, *Mytilus edulis,* belonging to the family Mytilidae under the phylum Molluscae. This species is endemic in the Northern Hemisphere and can be found in littoral and sublittoral zones. Blue Mussels are tolerant to wide temperature and salinity ranges (Seed 1992), making them good model organisms to study the aquatic environment. The *M. edulis* used in our study were cultured offshore with no contact to the ocean floor, hence avoiding any contamination from porewater or resuspended sediment.

We compared the REY distribution in *M. edulis* mussel shells and ambient seawater with the aim to (i) establish a sample preparation and analytical protocol that allows for the reliable and reproducible determination of the ultralow REY concentrations in mussel shells, (ii) investigate which REY species is incorporated into the shells of *M. edulis*, and (iii) illustrate and provide hints as to how such shells may reflect the REY characteristics of ambient seawater and how they can be used as environmental proxies. Results from this study show that mussel shells can serve as bioarchives of the REY distribution in their habitat and can thus provide the basis for using mussel shells as bioarchives that host geochemical proxies for paleoceanographic environmental reconstructions.

2. Materials and Methods

2.1 Samples and Sites

The mussels for this study originate from three locations in the North Sea along the coast of the German Bight, namely; (a) nearshore in the *Jade Bay* (JD) (53° 35’ 05’’ N; 008° 09’ 14’’ E), (b) offshore at the lighthouse *Roter Sand* (RS) near the entrance of the Weser estuary (53° 51’ 00’’ N; 008° 04’ 20’’ E) and (c) offshore west of the island of Sylt at the *ODAS* site (OD) (Messpfahl Süd/Southern pole: 54° 59’ 36’’ N; 007° 54’ 46’’ E) (*Fig. 1*). The mussels at *Jade Bay* and *Roter Sand* settled on suspended artificial spat collectors (harnesses made from polypropylene ropes and plastic binders) while those at the *ODAS* site grew wild on a steal pile from a 25 years old research platform of the Federal Maritime and Hydrographic Agency (BSH), formerly used to fix oceanographic measuring instruments. The testing areas were specifically selected and designed to have the mussels grow on suspended artificial substrate, which eliminates any potential contribution of porewater- or sediment-derived REY. The mussels from *Jade Bay* and *Roter Sand* were approximately 18 months old, while those from the *ODAS* site were approximately 24 months old.

2.2 Shell Preparation

Eight to eleven mussels from each site were pooled together based on the sampling site and date. The mussels were lyophilized and the soft tissue was removed leaving the shells intact. The obtained shell sizes ranged from 40 to 55 mm.

The shells obtained from the *ODAS* site were categorized in different sample pools (ODAS I-III and ODAS IV-VIII) to evaluate two different protocols for the removal of the periostracum, i.e. of the outer organic layer that covers the shell surface (Bellotto and Miekely

2007). For the ODAS I-III shell pools, the shells were soaked in NaOCl overnight before the shells were rinsed several times with de-ionized water to remove remaining NaOCl and then air-dried. For the ODAS IV-VIII pools, the shells were put in an oven and the periostracum was then removed using a spatula. This difference in sample preparation does not affect the analytical results (*Fig. 2*), and hence the method using NaOCl is strongly recommended, because it is much more convenient and efficient, less time consuming and minimizes potential contamination.

The bulk carbonate shells of each individual pool were then crushed in an agate mortar and homogenized. One and half grams of each shell powder were digested for 2 h at 90°C in 30 ml of 5 M Suprapur®HNO_3 (Carl Roth GmBH + Co.KG, Germany) in pre-cleaned Teflon beakers covered by small Teflon plates. After two hours, the beakers were uncovered and the sample solutions were evaporated to incipient dryness. The residues were dissolved in 25 ml of 0.5 M HNO_3 (Carl Roth GmBH + Co.KG, Germany), and filtered into a small polyethylene bottles using an acid-cleaned 0.2 μm cellulose acetate filter and syringe. The international reference standard JLs-1 (a Permian limestone from Japan) was used as the certified reference material, because it is similar in composition to the carbonate shell matrix and contains low REY concentrations.

A separation and preconcentration procedure (Bau et al. 2010), adapted from a method used to determine REY in seawater and freshwater (Shabani et al. 1992, Bau and Dulski 1996) was utilized owing to the low REY concentrations and potential matrix problems that may occur due to the Ca-rich shell matrix. A 12 mL aliquot of the digested sample solution was diluted in 500 mL de-ionized water, acidified to a pH value between 1.8 and 2.0 with Suprapur® HCl (Merck KGaA, Germany), and subsequently spiked with 0.5 ml of a 100 ppb Tm solution to monitor the recovery rates of the REY during the preconcentration procedure, while the remaining solution was set aside and labelled as 'digest'. A 15 mL aliquot of each filtered, acidified and spiked sample was labelled as 'original' and set aside to be used to determine the reference concentration of the Tm spike.

Each shell sample solution was then passed through a C^{18} cartridge (Waters, Sep-Pak® Classic C^{18}, single use) pre-loaded with a 2-ethylhexyl phosphate ester (Merck KGaA, Germany), in order to quantitatively retain the REY. Each cartridge was then 'washed' with 0.01 M Suprapur® HCl (Merck KGaA, Germany) to remove remaining matrix elements like major alkali and alkali earth elements before the REY were eluted using 40 ml of 6 M Suprapur® HCl (Merck KGaA, Germany). The eluate was then evaporated in a Teflon beaker to incipient dryness, and the residue was eventually dissolved in 10 ml of 0.5 M Suprapur® HNO_3 (Carl Roth GmBH + Co.KG, Germany).

A 100 ppb internal standard consisting of Ru, Re and Bi was added to each sample solution. Procedural blanks and the JLs-1 reference standard were processed along with the samples for analytical quality control.

2.3 Water Sample Preparation

A 1000 mL sample of North Sea water from the ODAS area was filtered using a filter tower mounted with 0.2 µm membrane filters (Sartorius AG, Germany). The pH of the filtrate was adjusted to 2.0 using Suprapur® HCl (Merck KGaA, Germany). Furthermore, 0.4 mL of a 100 ppb Tm solution was added to monitor the recovery rates of the REY during the subsequent separation and preconcentration procedure. 20 mL of this solution was set aside as 'original' to determine the Tm reference concentration. A separation and preconcentration procedure similar to the one used for the shell samples was then employed (see *Sect. 2.2*).

2.4 Analysis

The sample solutions were analyzed for REY using an inductively coupled plasma mass spectrometer, ICP-MS (Perkin-Elmer/Sciex ELAN DRC-e), at Jacobs University Bremen. As 0.5 g of 100 ppb Tm had been added as an internal standard to each sample solution to evaluate REY recovery during pre-concentration, Tm data for the shells is not reported.

2.5 Analytical Quality Assessment

To validate the method, the analytical precision was determined by applying our analytical procedure of sample digestion, REY separation and preconcentration, and measurement by ICP-MS to multiple aliquots (n=4) of homogenized *M. edulis* shells that were milled together to form a single large sample pool, and to an aliquot of the international reference standard JLs-1 which is a REY-poor Permian limestone from Japan.

It is common practice to present REY data normalized to Post-Archean Australian Shale, PAAS (subscript "$_{SN}$"; PAAS after McLennan 1989) . The REY_{SN} patterns for the multiple aliquots of homogenized *M. edulis* shells for the quality assessment are presented in *Fig. 3*. Anomalies of Ce_{SN}, Gd_{SN} and Y_{SN} have been quantified using *Eq. (1a-c).*

$$Ce_{SN}\text{ anomaly} = Ce_{SN} / (2Pr_{SN} - Nd_{SN}) \qquad \textbf{(1a)}$$

$$Gd_{SN}\text{ anomaly} = Gd_{SN} / (0.33Sm_{SN} + 0.67\,Tb_{SN}) \qquad \textbf{(1b)}$$

Y_{SN} anomaly = Y_{SN} / Ho_{SN} ***(1c)***

Precision (*Fig. 3*), expressed as relative standard deviation (RSD) from the average, is <4% for Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Mo, Er and Yb, <9% RSD for Lu and <34% RSD for La. The reason for the high RSD for La is unclear. Except for La, however, the reproducibility is excellent and falls within the symbol size used in *Fig. 3*. The analytical accuracy of the applied method was established from the JLs-1 values obtained and compared to published reference values from Dulski (2001), and did not show any significant systematic difference between our data and published values (*Fig. 3*).

2.6 Modelling of the Speciation of REY

To get a better insight as to how REY behave during their incorporation in the shells of *M. edulis*, the inorganic speciation of REY in North Sea seawater at the ODAS site was modelled complementing previous work on the REE speciation in seawater by Byrne et al. (1988) by including Y. Following Byrne et al. (1988) and Millero (1992), modelling was done for pH 8.2 and pH 7.6 at 25°C and 5°C using the Hyperquad Simulation and Speciation 2009 (HySS2009) modelling software. The inorganic speciation of REY was modelled for REY^{3+} (as the free uncomplexed REY), $REY(OH)^{2+}$, $REY(F)^{2+}$, $REY(Cl)^{2+}$, $REY(SO_4)^{+}$, $REY(CO_3)^{+}$, $REY(CO_3)_2^{-}$ and $REY(HCO_3)^{+}$. Stability constants and ligand concentrations were obtained from Byrne et al. (1988), and Millero (1992). The percentage of the REY complexes relative to the total REY concentration was calculated using *Eq. (2)* (where the brackets denote the dissolved concentration in seawater).

$$\%\ \text{Complex of Total REY} = \frac{\{\text{REY Complex}\}}{\{\text{Total REY}\}} \times 100$$ ***Eq. (2)***

3. Results

3.1 REY in *Mytilus edulis* shells and ambient seawater from the *ODAS* site

All REY_{SN} data for the mussel shells from *ODAS*, *Jade* and *Roter Sand* are summarized in *Fig. 4*. The *M. edulis* shells from the North Sea have Nd concentrations of 8.0 μg kg^{-1} (mean for *ODAS*, 1σ: 0.0017), 6.1 μg kg^{-1} (*Jade Bay*) and 8.7 μg kg^{-1} (*Roter Sand*), respectively. The REY_{SN} patterns of the shells from the three different sites are also very similar and increase from the light REY (LREY) to the middle REY (MREY) and decrease from the MREY to the heavy REY (HREY) (*Fig. 4*). All patterns display negative Ce_{SN} (0.70) and positive Gd_{SN} (1.49) and Y_{SN} (1.70) anomalies (*Fig. 4*).

The Nd concentration in the North Sea water sample from the *ODAS* site is 32 pM and its REY_{SN} pattern is a “typical” seawater pattern with depletion of LREY relative to HREY, positive Y_{SN} anomaly, and a negative Ce_{SN} anomaly (*Fig. 4*). The characteristic small anthropogenic positive Gd_{SN} anomaly present in the southern North Sea (Kulaksiz and Bau, 2007) is also seen in the *ODAS* seawater sample.

3.2 REY Speciation in North Sea Seawater

As previously shown for seawater in general (Byrne et al. 1988, Cantrell and Byrne 1986, Millero 1992), the mono- and di-carbonate complexes ($REY(CO_3)^+$ and $REY(CO_3)_2^-$, respectively) are the dominant inorganic REY complex species in North Sea seawater of temperature 25°C at pH 8.2 (*Fig. 5a*). Only a small fraction (<5%) of each REY occurs as free REY^{3+}, but this percentage increases to 13.5% (for La) when the pH is reduced to 7.6. As pH decreases, $REY(CO_3)^+$ complexes increase at the expense of $REY(CO_3)_2^-$ complexes (*Fig. 5b*). Yttrium displays a very similar speciation to Ho (*Fig. 5*), conforming the similarity between these two geochemical twins. The modelled percentages of free REY^{3+} available in the North Sea seawater at 5°C for the two pH conditions can be found in the online supplementary material provided.

4. Discussion

4.1 Partitioning of REY

Apparent bulk distribution coefficients of REY between *M. edulis* shells and ambient seawater, ${}_{app}D_{Tot.REY}^{shell/seawater}$, may be calculated from *Eq. (3)*:

$$_{app}D_{\mathrm{Tot.REY}}\,\mathrm{shell/seawater} = \frac{\left({}^{[\mathrm{REY}]}/_{[\mathrm{Ca}]} \right)^{\mathrm{Shell}}}{\left({}^{\{\mathrm{Total\ REY}\}}/_{\{\mathrm{Ca}\}} \right)^{\mathrm{Seawater}}} \qquad \textbf{\textit{Eq. (3)}}$$

where;

Ca in seawater = 0.01 mol/l;

Ca in shells = 10 mol/l.

This equation has previously been used to calculate the distribution coefficients of trace elements between the two major polymorphs of calcium carbonate namely calcite and aragonite, and ambient seawater (e.g., Akagi et al. 2004, Sholkovitz and Shen 1995). Shells of *M. edulis* are known to be bimineralic, i.e. composed of the two polymorphs of Ca carbonate: calcite and aragonite (e.g., Lorens and Bender 1980). The apparent distribution coefficients of REY between the shells and seawater from the *ODAS* site are shown in *Fig. 6*. The $_{app}D_{\mathrm{Tot.REY}}{}^{\mathrm{shell/seawater}}$ of our shells reveal fractionation with a preferential uptake of LREY as compared to HREY from seawater into the carbonate shell.

Certain differences and similarities are observed when published field and experimental apparent bulk partition coefficients are compared with our results (*Fig. 7)*. A clear separation with regard to partition coefficients for calcite and aragonite can be observed in the field observations, showing that $_{app}D_{\mathrm{Tot.REY}}{}^{\mathrm{shell/seawater}}$ values for corals of aragonitic composition vary between approximately 1 and 10 (Akagi et al. 2004, Sholkovitz and Shen 1995), while those of calcitic composition such as foraminifera, microbialites and other marine calcites, are much higher and range between 70 and 1656 (Palmer 1985, Parekh et al. 1977, Webb and Kamber 2000) . Laboratory experiments exploring the partitioning of REE and Y between calcite or aragonite and aqueous solutions have also been carried out to elucidate the incorporation process. Terakado and Masuda (1988) obtained values ranging between 2.5 to ~ 10 for calcite and ~ 2.5 to ~ 5 for aragonite. Zhong and Mucci (1995) on the other hand found much higher partition coefficients for their experimental calcite, ranging from 4169 (Pr) to 794 (Lu) and fractionation between the LREE and HREE. This pattern is quite similar to that of the *M. edulis* shells and ambient North Sea seawater determined in our study, despite much lower absolute values (between 4.23 for La and 0.17 for Lu). A study of *Bathymodiolus puteoserpentis* mussels that lived close to a high-temperature hydrothermal system (Bau et al. 2010) indicate partition coefficient values which define a pattern showing preferential incorporation of the MREY and a decrease towards the HREY with a maximum at Eu.

Comparisons of the ${}_{app}D_{Tot.REY}^{shell/seawater}$ pattern of our *M. edulis* shells shown in *Fig. 6* with the REY speciation in North Sea seawater shown in *Fig. 5a*, and of the REY_{SN} patterns of the ODAS shells with the free $REY^{3+}{}_{SN}$ patterns of North Sea seawater (Fig. 8) suggest that (i) free REY^{3+} may be the REY species which are actually removed from seawater and incorporated into the *M. edulis* shell, and that (ii) scavenging of REY carbonate complexes and formation of ternary surface-complexes may only play a minor role. Based on this hypothesis, we recalculated the apparent partition coefficients following *Eq. (4)*, using only the concentrations of free REY^{3+} in North Sea seawater instead of the *total* REY concentrations:

$$ {}_{mod}D_{FreeREY3+}\text{shell/seawater} = \frac{\left({[REY]}/{[Ca]} \right)^{Shell}}{\left({\{Free\ REY^{3+}\}}/{\{Ca\}} \right)^{Seawater}} $$

Eq. (4)

The resulting new pattern of distribution coefficients (modelled mean ${}_{mod}D_{FreeREY3+}^{shell/seawater}$) (*Fig. 6*), show preferential incorporation of the MREY and suggest that Ce is not taken up to the same extent as its redox-insensitive REY neighbors. However, all other REY anomalies that are present in the shells and in ambient seawater have disappeared, indicating only minor fractionation of neighboring REY during removal from seawater.

Incorporation of REY into $CaCO_3$ is assumed to occur through the coupled substitution of a REY^{3+} plus a charge-balancing monovalent cation for two Ca^{2+} ions in the calcite's crystal structure (Elzinga et al. 2002, Zhong and Mucci 1995), due to the similarity of the ionic radii of REY^{3+} and Ca^{2+}. Since the ionic radius of Nd^{3+} is most similar to that of Ca^{2+}, it may be expected that patterns of REY partition coefficients show a maximum at Nd and decrease slightly towards the lighter and heavier REY. However, the maximum in *Fig. 6* for the modelled partition coefficient, ${}_{mod}D_{FreeREY3+}^{shell/seawater}$, occurs at Tb, suggesting that additional factors besides ionic size also affect the incorporation of REY into the carbonate shells of *M. edulis*.

Judging from *Fig. 7* and considering that *M. edulis* shells are bimineralic, it would be desirable to study REY distributions and partition coefficients for calcite and aragonite individually, and not for bulk mussel shells. However, the ultralow REY concentrations and the intimate association of the two carbonate minerals are severe limitations that prevent such data to be determined. Thus, we have to accept that we are restricted to REY data for bulk shell carbonate until microanalytical techniques such as Laser-Ablation ICP-MS have become more sensitive.

In any case, the calculation of an apparent partition coefficient between a mussel shell and ambient seawater is a severe simplification, of course. From what is currently known, it appears that the shell of a bivalve does not directly precipitate from seawater, but from the extrapallial fluid (EPF) of the mussel, which is secreted from the epithelial cells of the bivalve's mantle (Wilbur 1972). The speciation of the REY in the EPF, therefore, will also affect REY incorporation into Ca carbonate. Although the exact chemical composition of the EPF is not known, the combination of elevated concentrations of organic compounds such as (amino)carboxylic acids, in the EPF (Misogianes and Chasteen 1979, Weiner 1979) and high stability constants of REY complexes with such carboxylic acids (e.g., Martell and Smith 1974) renders it very likely that REY speciation in the EPF is rather different from REY speciation in seawater, and that this "vital" effect will affect REY incorporation into the shell. Available thermodynamic data, however, suggest that carboxylic acids are often characterized by REY stability constants that strongly increase from the LREY to the HREY (Byrne and Kim 1990, Martell and Smith 1974) and thus may produce similar LREY-HREY fractionation between the available REY^{3+} species in the EPF as the (di)carbonate complexes produced in seawater.

As the exact chemical composition of the EPF is not known, it is currently impossible to decide whether the decrease of the REY partition coefficients between *M. edulis* shells and ambient seawater with decreasing REY ionic radius is controlled by the REY speciation in seawater or by the REY speciation in the EPF. Nevertheless, in the following we will address the impact of seawater pH and temperature on REY partitioning, assuming that REY speciation in the EPF is of minor importance, because this will reveal whether or not the REY distribution in *M. edulis* shells *a priori* has any potential to serve as a pH or temperature proxy.

4.2 Impact of temperature and pH on REY patterns in *Mytilus edulis* shells

Environmental parameters such as pH and temperature affect the speciation of REY in seawater via their impact on the activity of CO_3^{2-}, and hence the amount of free REY^{3+} available for uptake by the mussels (*Fig. 5*). The REY signature of mussel shells, therefore, might be an indicator for pH and/or temperature changes in a mussel's habitat, unless this proxy is obliterated by the vital effects discussed earlier.

Using the partition coefficients calculated from *Eq. (4)* and the concentrations of free REY^{3+} in seawater at pH 7.6 and 8.2 (as calculated using the HySS software), we modelled from *Eq. (5)* the REY concentrations and REY_{SN} pattern of a hypothetical *M. edulis* shell that grew in seawater of pH 7.6 and 8.2 and at temperatures of 25°C and 5°C, respectively (*Fig. 9*).

$$[REY]_{Shell} = mod D_{FreeREY3+}\,\text{shell/seawater} \times \left({\{\text{Free REY}^{3+}\}}/{\{Ca\}} \right) \times [Ca]_{Shell} \qquad \textbf{\textit{Eq. (5)}}$$

where;

Ca in seawater = 0.01 mol/l;

Ca in shells = 10 mol/l.

At a given temperature, the shape of the resulting REY_{SN} patterns of such hypothetical shells are very similar at both pH values (*Fig. 9*), but due to higher availability of free REY^{3+} in seawater at pH 7.6, more REY are incorporated into the Ca carbonate at pH 7.6 as compared to pH 8.2. In contrast, at a given pH, a temperature change results in slightly different REY_{SN} patterns (particularly between the MREY and HREY), but has an only minor impact on overall REY concentrations (*Fig. 9*). Hence, it appears that absolute REY concentrations may have the potential to be used as a pH proxy, whereas REY distribution patterns are more sensitive to temperature changes.

5. Conclusion

A new and efficient protocol for sample preparation and determination of REY concentrations in bivalve shells was established. This quick and clean method includes sample treatment with NaOCl followed by REY separation and preconcentration.

The shells of *Mytilus edulis* used in our study demonstrate the potential of using bivalve shells as bioarchives of proxies for changes in the physico-chemical conditions in the bivalve's habitat. All shells from three different sites in the southern North Sea show distinct REY_{SN} distribution patterns that increase from the LREY to the MREY and decrease from the MREY to the HREY. Despite the REY_{SN} patterns of the shells being different to that of general seawater, the shells still exhibited distinct signatures of the seawater they grew in, such as small positive Y_{SN} and Gd_{SN} anomalies and a negative Ce_{SN} anomaly. Apparent partition coefficients ${}_{app}D_{Tot.REY}^{shell/seawater}$ of REY between the shells and seawater are low and decrease strongly from the LREY to the HREY. Comparison of ${}_{app}D_{Tot.REY}^{shell/seawater}$ patterns to the REY speciation in the North Sea seawater suggest that the free REY^{3+} may be the most likely REY species that are actually removed from seawater and incorporated into the *M. edulis* shell and that scavenging of REY carbonate complexes and formation of ternary surface-complexes may only play a minor role.

Although the impact of vital effects and particularly that of REY speciation in the extrapallial fluid from which the carbonate minerals precipitate cannot be quantified yet, we demonstrate in this study that mussel shells like those of *M. edulis* can still be used as bioarchives of some REY features of seawater. Following our assumptions that only the free REY^{3+} are incorporated into the carbonate's crystal lattice and further modelling of the REY_{SN} patterns of a hypothetical mussel shell grown at pH of 8.2 and 7.6 and at temperatures of 25°C and 5°C reveals that with lower pH, REY concentrations in shells increase, but with little effect on the shape of the REY_{SN} patterns, while a temperature change has an impact on the REY_{SN} pattern, but only minor effects on absolute REY concentrations. The absolute REY concentrations in shells of *M. edulis* may thus have the potential to be used as a pH proxy, whereas REY_{SN} distribution patterns of the shells may rather be used as a temperature proxy.

Our findings open up doors to better understand how bivalves incorporate trace elements like REY into their shells and how these shells can be used to extract information about their habitat. Since change in physico-chemical conditions like pH and temperature affect REY speciation in seawater due to their impact on the activity of CO_3^{2-}, further research to calibrate these changes may turn the REY distribution into a valuable proxy for paleo-pH and past ocean acidification. However, in order to successfully develop REY systematics into a quantitative temperature and/or pH proxy, the impact of the EPF and other vital effects needs to be assessed, for example by studying *M. edulis* mussels cultured under controlled pH and temperature conditions. Additionally, before applying the REY distribution in fossil shells as a paleoproxy, future studies need to investigate the potential impact of diagenesis.

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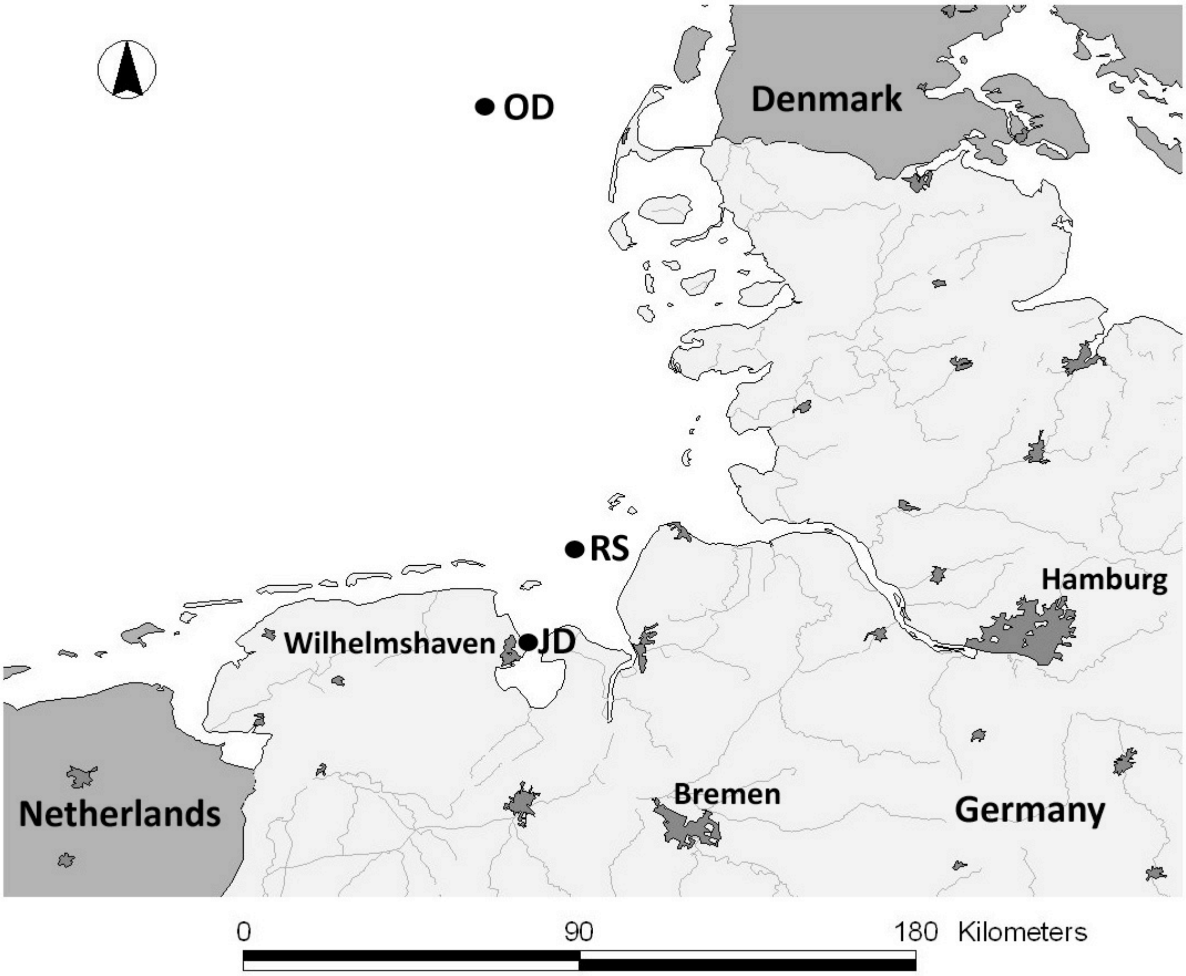

Figure 1: Map of the German Bight showing the sampling locations of *Mytilus edulis* mussels from the offshore sites *ODAS* (OD), *Jade Bay* (JD), and *Roter Sand* (RS) (after Brenner et al. 2009).

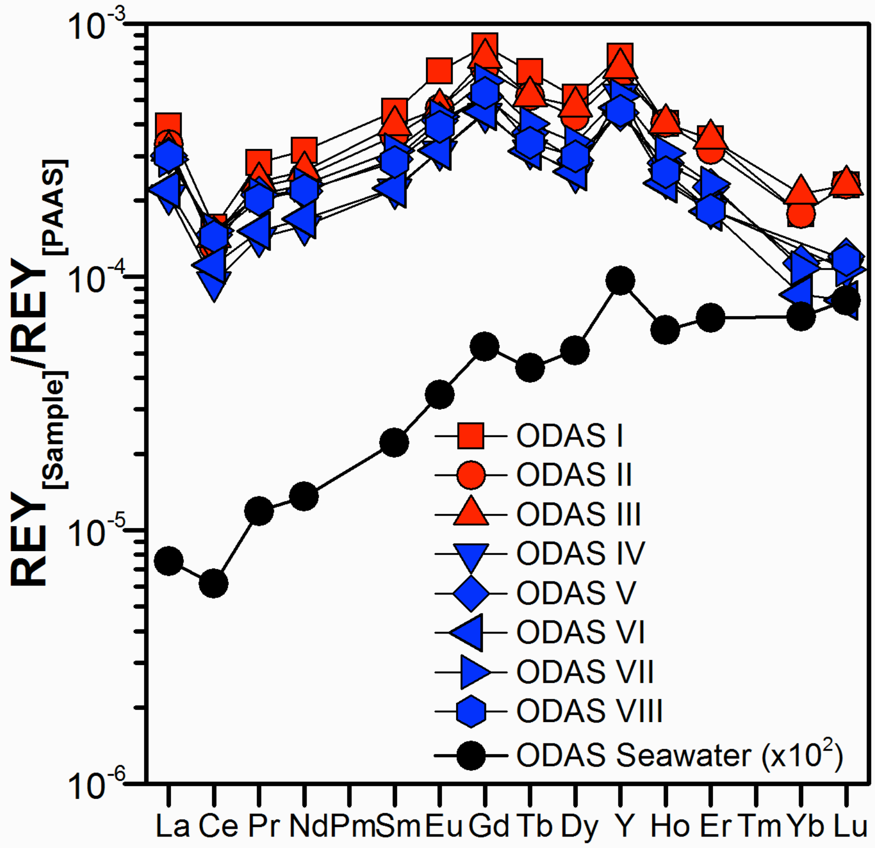

Figure 2: REY_{SN} patterns of the ODAS seawater and of all pools of *Mytilus edulis* shells from the ODAS site (ODAS I to III shell pools were treated with NaOCl; ODAS IV to VIII shell pools were heated and had their periostracum manually removed). Note the similarity of all REY_{SN} patterns.

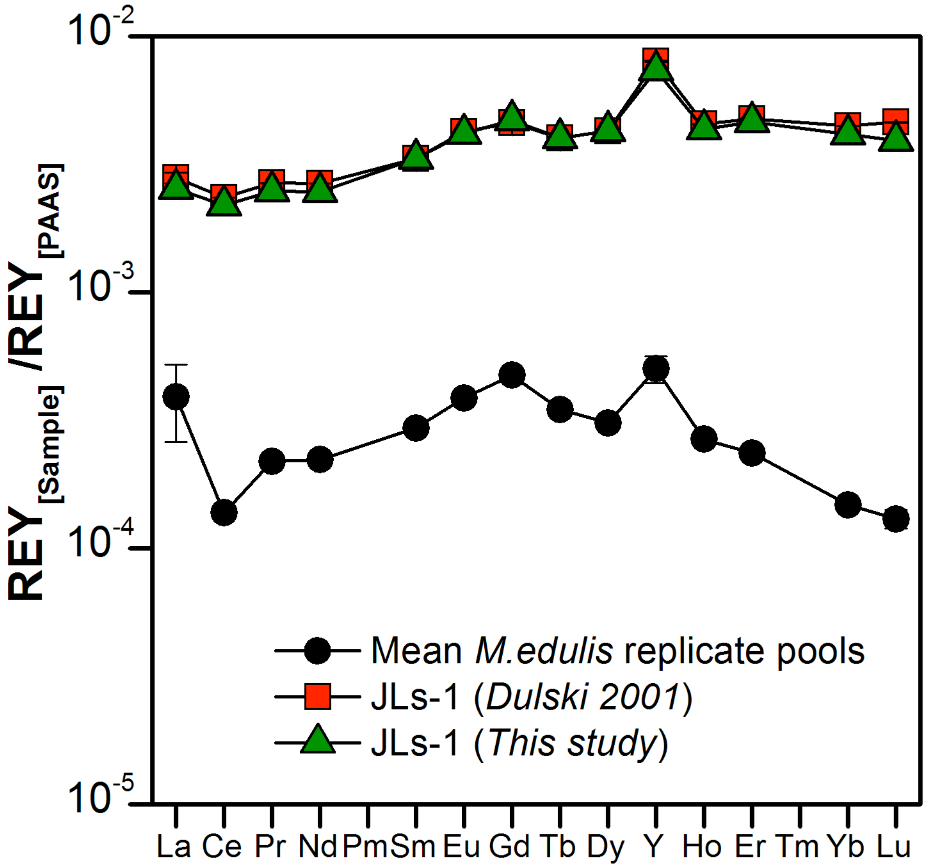

Figure 3: REY_{SN} patterns of the 4 replicate pools of *Mytilus edulis* shells determined in our study and of international reference standard JLs-1 (a Permian limestone from Japan; data from our study and from Dulski 2001) used for analytical quality assessment during method development. Note that except for La and Y, error bars (1 δ) are smaller than the symbol size.

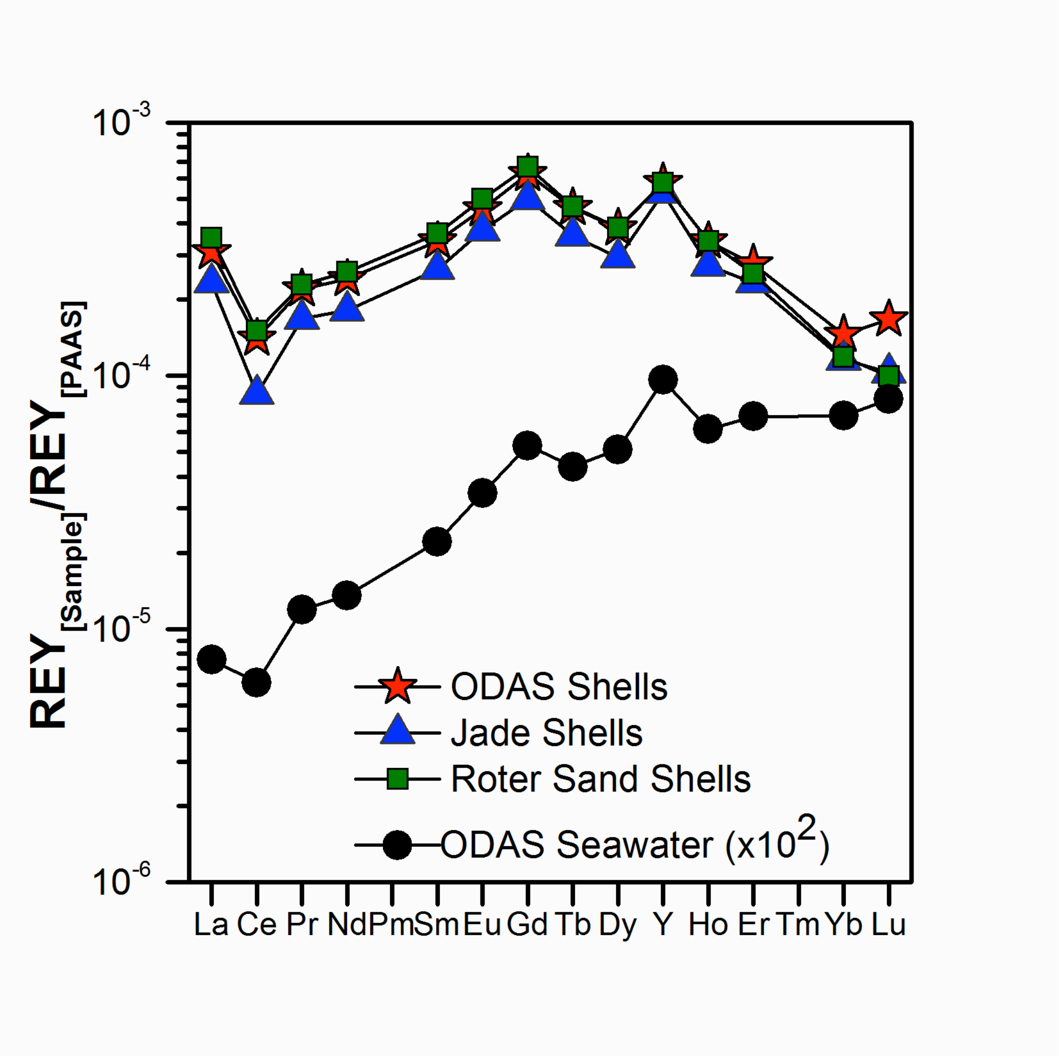

Figure 4: REY_{SN} patterns of *Mytilus edulis* shells from different sites in the North Sea in comparison to seawater from the ODAS site. Note the close similarity of all REY_{SN} patterns regardless of their origin.

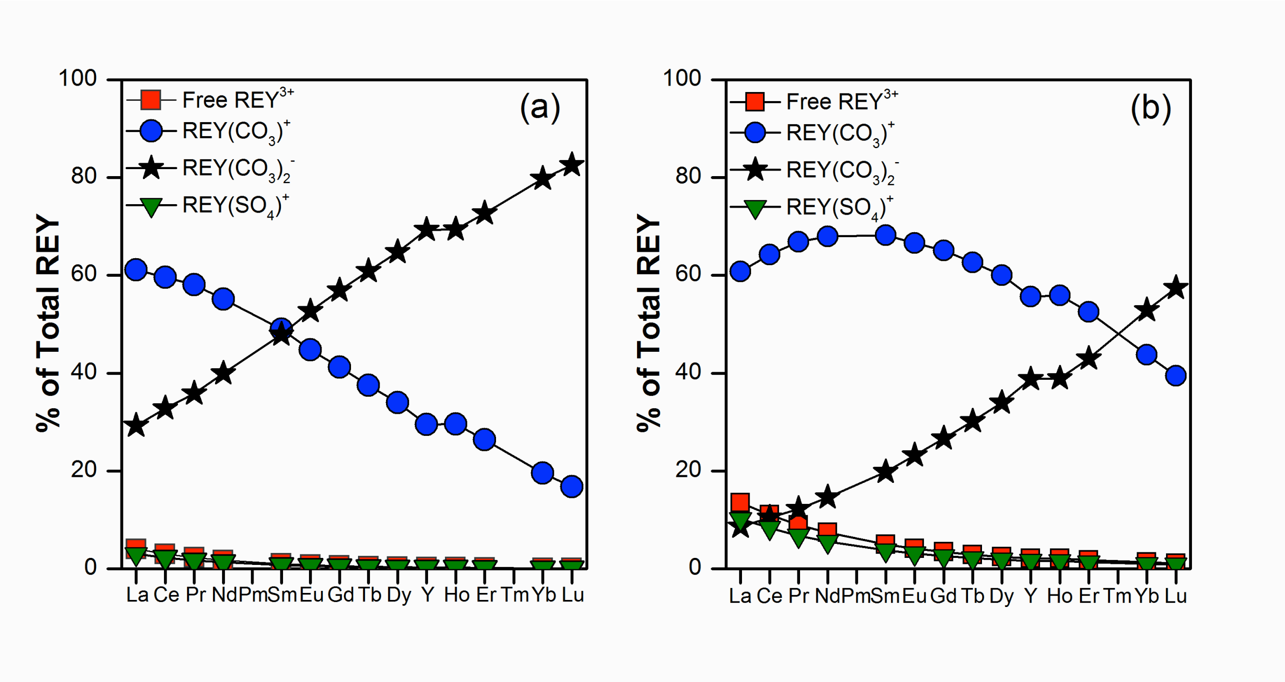

Figure 5: REY speciation in the North Sea water at 25°C for *(a)* pH 8.2 and *(b)* pH 7.6 (as modelled using HySS2009).

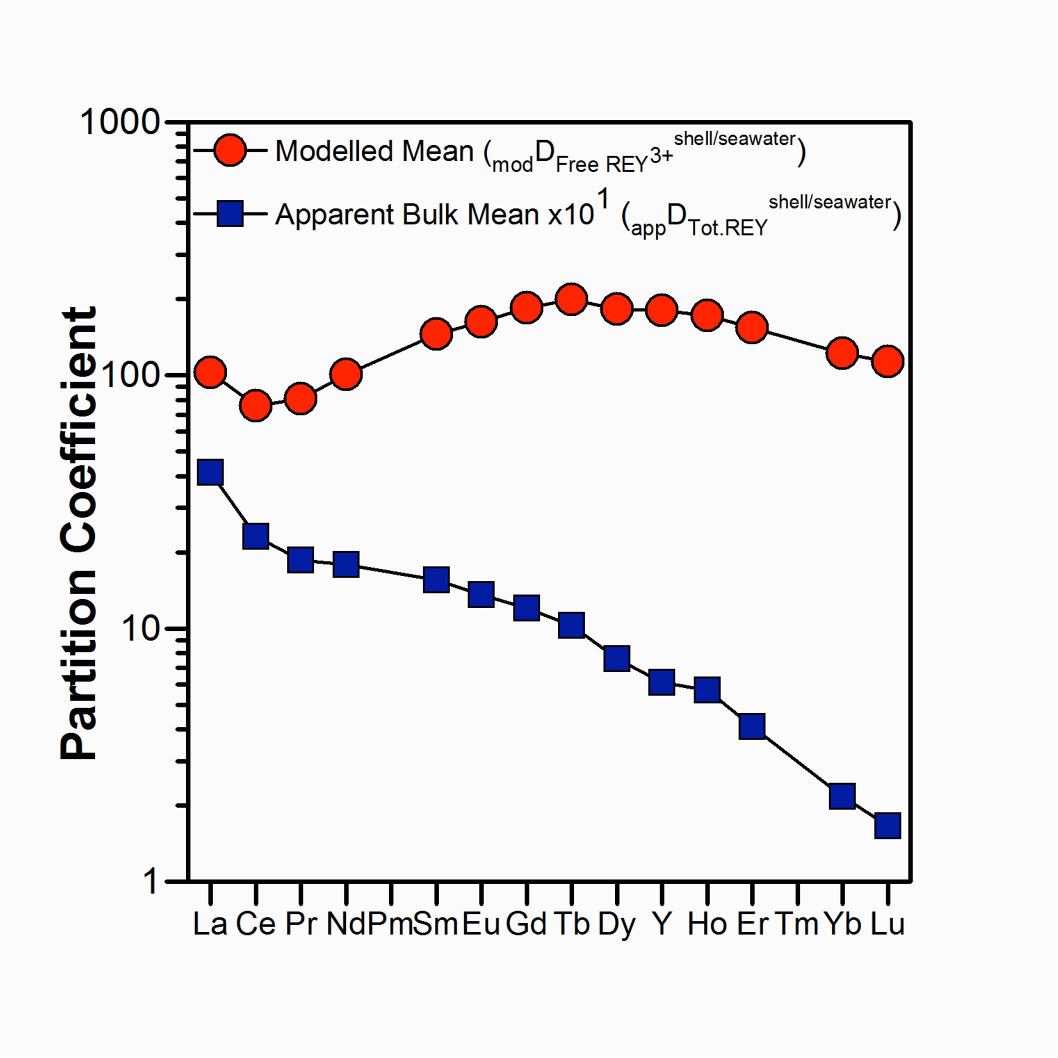

Figure 6: Mean apparent REY partition coefficients for *M. edulis* shells from the ODAS site and total REY in ambient seawater ($_{app}D_{Tot.REY}^{shell/seawater}$), and modelled mean apparent REY partition coefficients for *M. edulis* shells from the ODAS site and free REY^{3+} in ambient seawater ($_{mod}D_{FreeREY3+}^{shell/seawater}$).

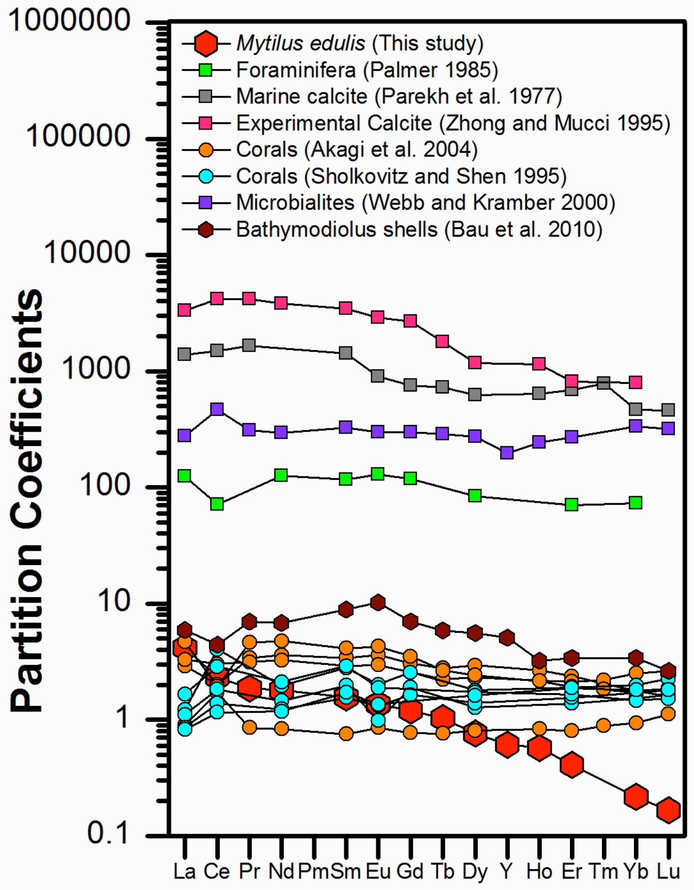

Figure 7: REY partition coefficients for different marine carbonates and ambient seawater (field studies and laboratory experiments).

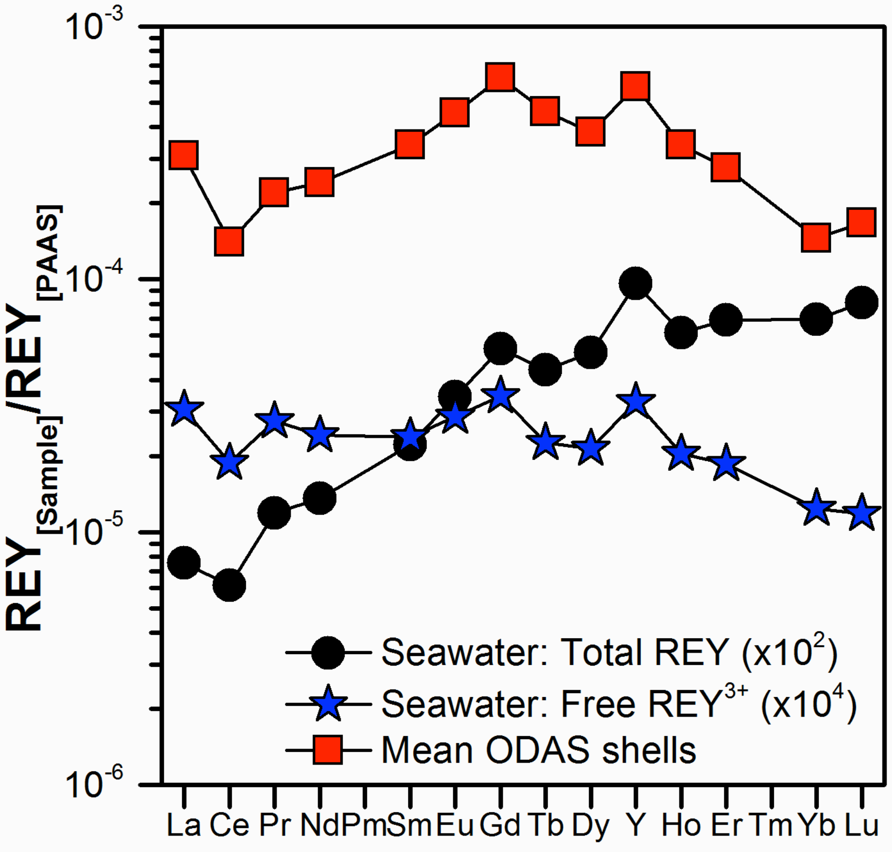

Figure 8: Total REY_{SN} and free $REY^{3+}{}_{SN}$ patterns of North Sea water at the ODAS site and the average REY_{SN} pattern of the ODAS shells.

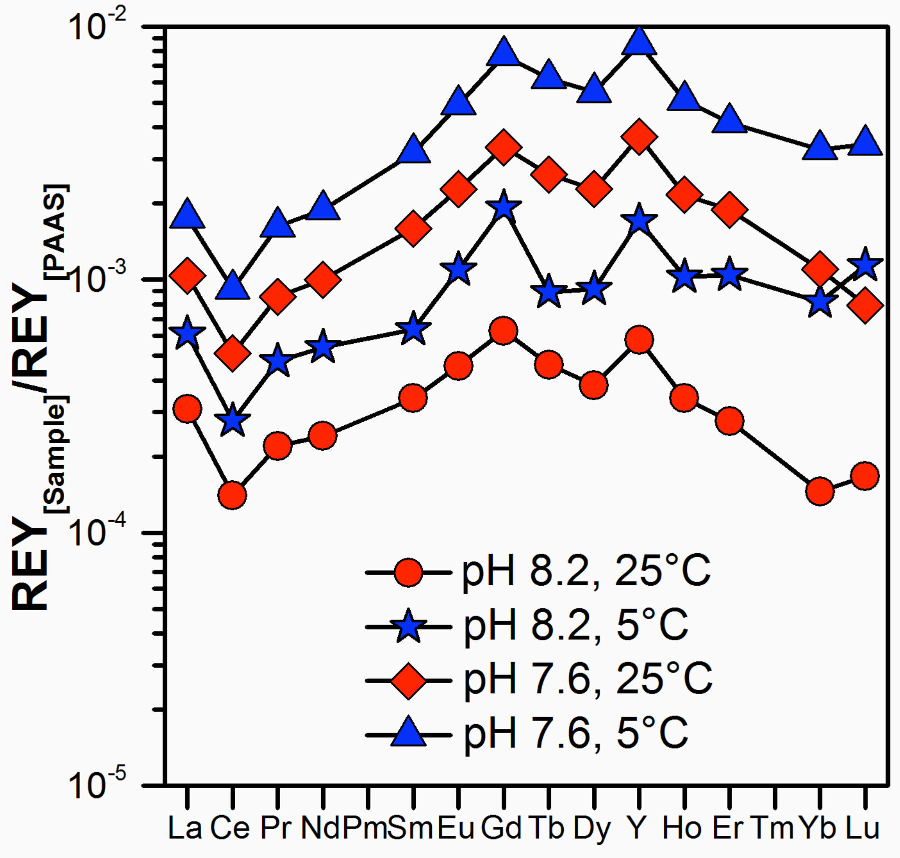

Figure 9: Modelled REY_{SN} patterns of hypothetical *M. edulis* shells from the ODAS site for different pH and temperature conditions in ambient seawater.