



Differential tissue accumulation in the invasive Manila clam, *Ruditapes philippinarum*, under two environmentally relevant lanthanum concentrations

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Abstract Among the environmental emerging concern rare earth elements, lanthanum (La) is one of the most common and reactive. Lanthanum is widely used in numerous modern technologies and applications, and its intense usage results in increasing discharges into the environment, with potentially deleterious consequences to earthlings. Therefore, we exposed the important food resource and powerful monitoring tool Manila clam to two environmentally relevant concentrations of La ($0.3 \mu\text{g L}^{-1}$ and $0.9 \mu\text{g L}^{-1}$) for 6 days, through water, to assess the bioaccumulation pattern in the gills, digestive gland, and remaining body. The La bioaccumulation was measured after

1 (T1), 2 (T2), and 6 (T6) days of exposure. Lanthanum was bioaccumulated after 2 days, and the levels increased in all tissues in a dose-dependent manner. When exposed to $0.3 \mu\text{g L}^{-1}$, the enrichment factor pattern was gills > body > digestive gland. However, when exposed to $0.9 \mu\text{g L}^{-1}$, the pattern appears to change to gills > digestive gland > body. Tissue partitioning appears to be linked with exposed concentration: In higher exposure levels, digestive gland seems to gain importance, probably associated with detoxification mechanisms. Here, we describe for the first time La bioaccumulation in these different tissues in a bivalve species. Future studies dealing with the bioaccumulation and availability of La should connect them with additional water parameters (such as temperature, pH, and major cations).

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Introduction

Most high technology equipment relies on rare earth elements (REE) to be manufactured, such as electric and hybrid vehicles, smartphones, and digital cameras (Wall, 2014). REE are also applied in low carbon energy technologies like nuclear, solar, eolic, bioenergy, carbon capture and storage, and electricity grids (Wall, 2014). The rising demand for up-to-date electronic equipment engenders enormous quantities of e-waste. Insufficient public knowledge on its recycling accoupled with inefficient recycling methodologies culminates in REE build-up in the environment (Tansel, 2017), highlighting the urgent need to study their speciation, availability, and ecotoxicological behavior and impacts. This has also led to REE being considered contaminants of environmental emerging concern.

REE are naturally present in small concentrations (from thousands of μg to tens of ng per gram); however, near mining and industrial locations, these can increase up to hundreds of times (Liang et al., 2014). Rare earth elements enter aquatic ecosystems through domestic and industrial wastewater discharges and leaching of REE enriched soils. Although REE exists in very low concentrations in non-contaminated seawater (in the pg L^{-1} range; Wang & Yamada, 2007), they are known to be bioaccumulated by marine organisms (i.e., squids, krill, Nautilus) (Palmer et al., 2006; Pernice et al., 2009).

Lanthanum (La) is the first REE, with the major atomic radius is of the most reactive amidst them (Herrmann et al., 2016). Lanthanum is an essential catalyst for oil refineries and is fundamental for alloy making, alkali-resistant glass, hydrogen storage, battery electrodes, and camera lenses (Sanghera & Aggarwal, 1998). The complex lanthanum carbonate is applied in medicine, to treat end-stage kidney disease, as it is capable to captivate excess phosphate in the blood (Albaaj & Hutchison, 2005). With increasing production and demand, it is expected that La availability in the environment will augment. The aquatic La speciation is powerfully affected by pH

and other cations (Herrmann et al., 2016; Moermond et al., 2001). In seawater, La carbonates complexes and free ions are the dominant species, while sulfates, humic complexes, and phosphates are a slight amount (Moermond et al., 2001).

Bivalves are well known as indicators of pollution, which brands them useful for monitoring studies due to their known ability to accumulate contaminants in their tissues. The Manila clam, *Ruditapes philippinarum*, is broadly distributed. Although presenting a long-life cycle, reaches maturity at ~ 30 mm in shell length, before 1 year old (Moura et al., 2018). Given that this species is a worldwide commercially important food resource can reach great economic worth. The Manila clam is the most cultured clam species, representing approximately 25% of global mollusk production in 2018 (Sofia, 2018). It also demonstrates a high propensity to bioaccumulate pollutants (e.g. carbon nanoparticles, drugs, trace metals), under both natural and laboratory conditions (De Marchi et al., 2017; Won et al., 2016).

Within this framework, the purpose of this study was to assess the bioaccumulation ability of the Manila clam to two different La concentrations. The chosen environmentally realistic La exposure concentrations fall within the levels quantified in a European wastewater outlet (Brito et al., 2018): low La $0.3 \mu\text{g L}^{-1}$ and high La $0.9 \mu\text{g L}^{-1}$. The bioaccumulation was assessed by analyzing the La content present in the gills, digestive gland, and remaining body (containing the siphon, adductor muscle, mantle, and foot and hereafter called body).

Materials and methods

Sampling

Manila clam (*Ruditapes philippinarum*) specimens were collected in a single sampling event in July 2018, during the low tide, in a subtidal zone at Ria de Aveiro, Portugal (SW Europe). After sampling, specimens were transported to the MARE-FCUL aquaculture facilities and depurated for 7 days under a 12-h light/12-h dark photoperiod. Individuals were kept in filtered ($0.35 \mu\text{m}$, Harmsco, Florida, USA) and UV-irradiated (Vecton 600, TMC Iberia) natural seawater with parameters as those measured in the sampling event: salinity = 35 ± 0.1 (V2 refractometer,

TMC Iberia), temperature = 16.2 ± 0.1 °C (TFX 430 Precision Thermometer, WTW GmbH), and pH = 8.19 ± 0.03 (SevenGo pro™, Mettler Toledo).

Experimental design

One hundred and fifty Manila clam individuals were randomly assigned to fifteen 5-L glass tanks, with continuously aerated, filtered, and UV-irradiated natural seawater directly pumped from the ocean ($38^{\circ}70'99.7''N$ and $9^{\circ}48'69.2''W$), into three treatments: control (La = $0 \mu\text{g L}^{-1}$), low La concentration (La = $0.3 \mu\text{g L}^{-1}$), and high La concentration (La = $0.9 \mu\text{g L}^{-1}$). In each sampling event, 10 clams were randomly collected from the 5 replicates of each treatment. The temperature was kept constant by immersing the experimental tanks in a bath with submerged heaters (V₂Therm 200 W aquarium heater, TMC Iberia) and chillers (HC-250A, Hailea). Dissolved La levels were warranted through the addition of a La solution in the seawater. This solution was prepared with a LaCl₃ standard solution (Merck) diluted in filtered ultra-pure water (18.2 MΩ, Milli-Q, Merck). The water was renewed daily, at the same time, for the three treatments to keep dissolved inorganic carbon speciation, due to bacterial activity minor, and afterwards, the respective La solution was added, in the exposed treatments. Water aliquots were sampled every hour, for the first 12 h of the first exposure, to evaluate the variation of dissolved La levels. Individuals were fed with a commercially concentrated mixture of green and brown marine phytoplankton—Reef Phytoplankton™, Seachem—every day, 1 h before water change to minimize the potential removal of La.

Clams were sampled before the beginning of the trial (T0) and after 1 (T1), 2 (T2), and 6 (T6) days of exposure to La.

Lanthanum quantification

To quantify the total dissolved La levels ($\mu\text{g L}^{-1}$), water triplicates were filtrated ($0.45 \mu\text{m}$, MF-Millipore™, Merck) and acidified (2% Ultrapur® HNO₃).

Clams were dissected into gills, digestive gland, and remaining body and kept at -80 °C until further analyses. Lanthanum concentrations were determined in freeze-dried, grounded, and homogenized gills,

digestive gland, and remaining body, after digestion with nitric acid (HNO₃, distilled, 65% v/v) and hydrogen peroxide (H₂O₂, Suprapur®, 30% v/v) in accordance to Raimundo et al. (2013). All labware was previously decontaminated with HNO₃ (20%) for 48 h and rinsed with ultra-pure water (18.2 MΩ, Milli-Q, Merck).

The concentration of La was determined in a quadrupole ICP-MS (Thermo Elemental, X-Series), and the experimental ICP-MS parameters for La determinations are detailed in Caetano et al. (2009). ¹¹⁵In was the internal standard (Merck, CertiPUR®) chosen. The ¹³⁹La was the isotope selected for the quantification that has minimum isobaric and polyatomic interferences. Quality control (QC) solutions were run every 20 samples. The coefficients of variation for counts ($n=5$) were lower than 2%, and a 5-point calibration from 0.005 to 5 ppm was used for quantification. Accumulation results are given in milligram per kilogram of dry weight tissue (mg Kg^{-1} , dw).

To control the analytical quality of the method, three procedural blanks were prepared, using the same analytical procedure described above, and included within each batch of 20 samples. Blanks accounted for less than 1% of the total La concentration in all the samples. Additionally, a certified reference material BCR 668 (muscle of *Mytilus edulis*) was included within each batch of 20 samples to evaluate the accuracy of all analytical procedures, and the obtained values were consistent with the certified ones.

Lanthanum enrichment factor

To estimate the La specific affinity for the body, the digestive gland, and the gill, the enrichment factor was determined as the quotient of the median La levels in exposed and control samples, for T1, T2, and T6, according to Pereira et al. (2015).

Seawater carbonate system

Temperature (°C), salinity, and pH were measured four times for each treatment, in each sampling day (T0, T1, T2, and T6). The total alkalinity (TA) and pH total scale (pHT) were used to calculate the specifics of the seawater carbonate system (e.g., $p\text{CO}_2$, HCO_3^- , ΩCa , ΩAr), using the CO2SYS software.

Statistical analyses

Kolmogorov–Smirnov and Levene’s tests were used to test data for normality and equality of variances, and non-compliance of these parametric assumptions led to the practice of the Mann–Whitney non-parametric test. All pairwise differences in La concentrations between treatments within and between sampling times were assessed. Differences between La concentrations in different body parts for each treatment were also tested.

Given that the water was renewed entirely daily, the seawater carbonate system was measured every sampling day in experimental water representative of only that day. For that reason, the differences between each seawater physicochemical parameter were tested only between treatments and not between sampling days.

Statistical analyses were performed in STATISTICA™ 12 software (Statsoft, Inc., Tulsa, OK 74,104, USA), using a significance level of $p < 0.05$.

Results

Dissolved lanthanum levels

The total dissolved La levels ($\mu\text{g L}^{-1}$) in the water samples collected every hour, for 12 h, after La spike is presented in Supplemental Table 1. Average concentrations in the first 12 h were $0.25 \pm 0.10 \mu\text{g L}^{-1}$ in the low exposure concentration treatment and $0.84 \pm 0.09 \mu\text{g L}^{-1}$ in the higher exposure concentration treatment.

In the present study, the La levels quantified in the spiked water stabilized after 6 h, for the lower La concentration, and remained stable for the following period (Supplemental Table 1). This period of non-equilibrium was lower for the higher spiked concentration. The average dissolved concentration was very similar to the desired exposure concentration for both exposed treatments, and its presence was assured by adding the same concentration to a renewed medium every day. The constancy of the total dissolved La concentration points that minor precipitation may have occurred.

Lanthanum accumulation

Median and ranges of La concentrations (mg Kg^{-1} , dry weight) in the three studied parts, namely, body, digestive gland, and gills, are presented in Table 1.

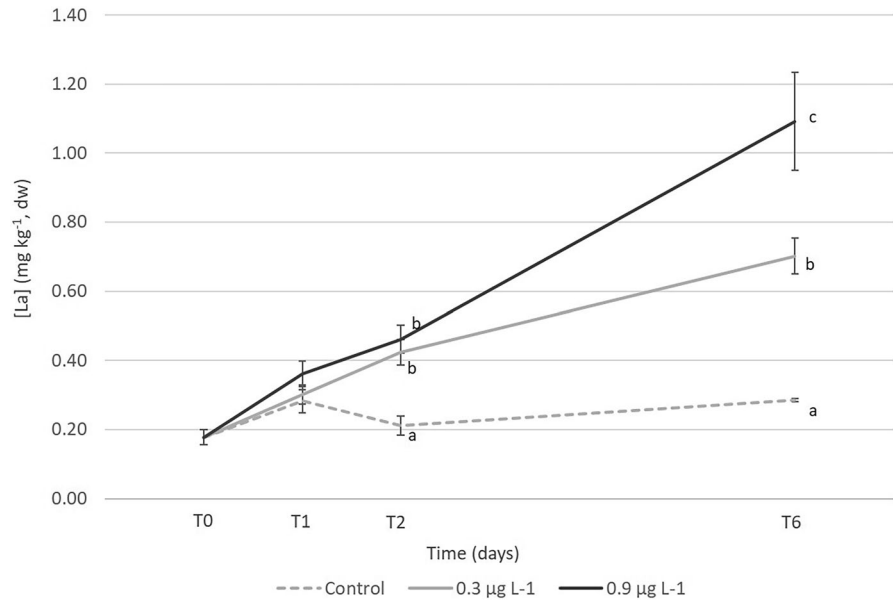
Concentrations of La (mg Kg^{-1} , dw) in the Manila clams’ body are presented in Fig. 1.

Levels on the control treatment varied in a narrow range between 0.18 and 0.28 mg Kg^{-1} , without significant differences between sampling times ($p > 0.05$, statistical differences shown in Supplemental Table 2a, b, and c, Online Resources 1, 2, and 3). The concentration of La found in the body of both exposed treatments increased with time. For the low La treatment ($0.3 \mu\text{g L}^{-1}$), median levels of La in the body reached their lowest at T1 (0.30 mg Kg^{-1}) and its highest at T6 (0.70 mg Kg^{-1}). The same trend occurred in the bodies of the Manila clams exposed to high La ($0.9 \mu\text{g L}^{-1}$), with concentrations ranging from 0.36 mg Kg^{-1} at T1 to 1.1 mg Kg^{-1} at T6.

Table 1 Median and ranges of La concentration (mg Kg^{-1} , dry weight) in Manila clams’ body, digestive gland, and gills after 0 (T0), 1 (T1), 2 (T2), and 6 (T6) days of exposure to control ($0 \mu\text{g L}^{-1}$), low ($0.3 \mu\text{g L}^{-1}$), and high ($0.9 \mu\text{g L}^{-1}$) La concentration, through water

Time	N	Treatment	Body (mg Kg^{-1} , dry weight)	Digestive gland	Gills
T0	10	Control	0.18 (0.14–0.29)	0.22 (0.17–0.26)	0.29 (0.25–0.34)
T1	10	Control	0.28 (0.27–0.38)	0.51 (0.44–0.59)	0.49 (0.42–0.56)
		Low La	0.30 (0.20–0.37)	0.54 (0.29–0.68)	0.63 (0.36–0.80)
		High La	0.36 (0.29–0.43)	0.69 (0.42–0.86)	0.66 (0.52–0.93)
T2	10	Control	0.21 (0.20–0.29)	0.34 (0.32–0.36)	0.37 (0.37–0.42)
		Low La	0.42 (0.28–0.59)	0.61 (0.53–0.88)	0.78 (0.70–0.94)
		High La	0.46 (0.44–0.64)	0.71 (0.49–1.2)	0.82 (0.48–1.1)
T6	10	Control	0.28 (0.28–0.30)	0.48 (0.46–0.50)	0.34 (0.31–0.36)
		Low La	0.70 (0.34–0.82)	1.0 (0.72–1.7)	1.5 (0.70–1.7)
		High La	1.1 (0.52–1.7)	1.9 (1.5–2.6)	1.8 (1.1–2.9)

Fig. 1 Concentrations of lanthanum (mg Kg⁻¹, dry weight) in Manila clams' body under control (0 µg L⁻¹), low La (0.3 µg L⁻¹), and high La (0.9 µg L⁻¹) at different sampling times (T0, T1, T2, and T6). Values represent medians ± SE. Different letters represent significant differences between treatments within each sampling time (*p* < 0.05). For additional significant differences, see Supplemental Table 2



One day of exposure to La (T1) was insufficient to display significant differences between the three treatments (*p* > 0.05); however, significant differences between the control and both exposure concentrations were found on the second day (T2, *p* = 0.032 and *p* = 0.037, respectively). The accumulation of La in the body did not show significant differences between the two different exposure concentrations after 2 days of exposure (*p* = 0.213). Nevertheless, at T6, the three treatments were significantly different among them (*p* < 0.05).

The same trend was observed for the digestive gland (Fig. 2).

At T1, no significant differences were shown between treatments (*p* > 0.05), with La values varying from 0.51 mg Kg⁻¹ in the control, 0.54 mg Kg⁻¹ in low La, and to 0.69 mg Kg⁻¹ in high La concentrations. After 2 days of exposure (T2), accumulation was evident, with the control digestive glands

exhibiting significantly lower concentrations than the ones registered in digestive glands exposed to 0.3 µg L⁻¹ (*p* = 0.020) and 0.9 µg L⁻¹ of La (*p* = 0.008). As expected, the highest accumulation value occurred after 6 days of exposure (T6) and in the treatment with higher La concentration (1.9 mg Kg⁻¹, dw). This level was significantly different from their control counterpart (*p* = 0.037) and also from the digestive glands exposed to low La (*p* = 0.023).

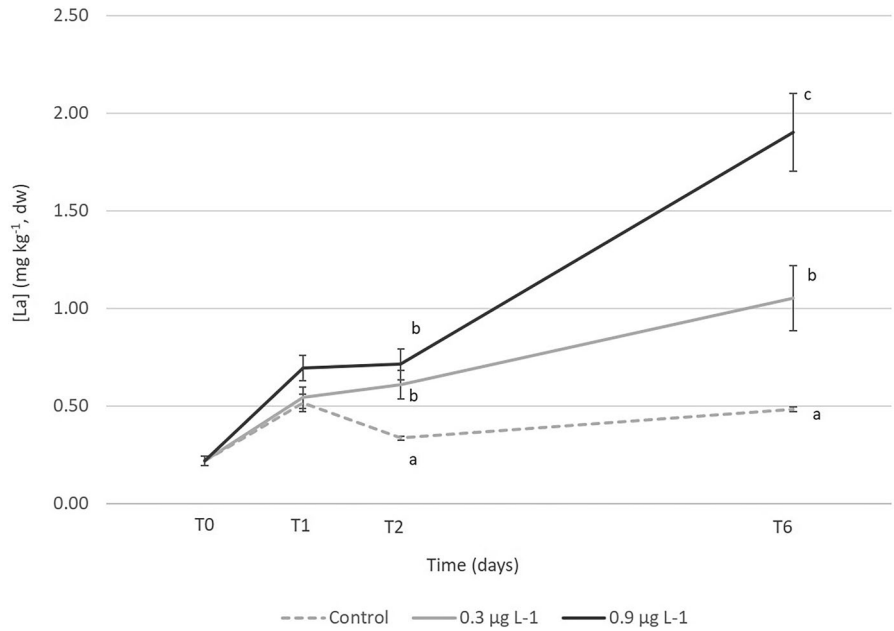
Lanthanum concentrations in the Manila clams' gills are shown in Fig. 3.

The accumulation of La in the gills increased steadily over time till day 6 (T6). As found in the other tissues, 1 day of exposure was not sufficient to induce measurable La bioaccumulation. Nevertheless, after 2 days of exposure, the low and high La treatments differed from the control (*p* = 0.028, for both La treatments). At T6, this significant difference between the control and the exposed

Table 2 Seawater physicochemical parameters and specifics of the seawater carbonate system for the three treatments (median ± SD)

Treatment	Temperature (°C)	Salinity	pH	Total Alkalinity (µmol/kg SW)	pCO ₂ (µatm)	HCO ₃ ⁻ (µmol/kg SW)	OH ⁻ (µmol/kg SW)	ΩCa	ΩAr
Control	16.1 ± 0.1	35 ± 0.1	8.2 ± 0.03	2570 ± 382	318 ± 53	1981 ± 325	4.1 ± 0.44	5.8 ± 0.75	3.7 ± 0.48
Low La (0.3 µg L ⁻¹)	16.3 ± 0.1	35 ± 0.1	8.2 ± 0.03	2213 ± 361	259 ± 59	1690 ± 308	4.2 ± 0.27	5.0 ± 0.71	3.2 ± 0.46
High La (0.9 µg L ⁻¹)	16.2 ± 0.1	35 ± 0.1	8.2 ± 0.03	2460 ± 355	308 ± 62	1956 ± 312	4.1 ± 0.52	4.9 ± 0.67	3.2 ± 0.43

Fig. 2 Concentrations of lanthanum (mg Kg⁻¹, dry weight) in Manila clams’ digestive gland under control (0 µg L⁻¹), low La (0.3 µg L⁻¹), and high La (0.9 µg L⁻¹) at different sampling times (T0, T1, T2, and T6). Values represent medians ± SE. Different letters represent significant differences between treatments within each sampling time (*p* < 0.05). For additional significant differences see Supplemental Table 2



treatments was upheld, but the exposed treatments did not show a significant difference between them (*p* = 0.298).

La enrichment factor

The highest enrichment factor occurred after 6 days of exposure in the gills, for both La concentrations

(Fig. 4), and it was greater, for all body parts, at the high La concentration. When exposed to 0.3 µg L⁻¹ La, the highest enrichment factor was observed in the gills, followed by the body and ultimately the digestive gland. However, even though not significantly (*p* > 0.05), when exposed to 0.9 µg L⁻¹ La, the accumulation pattern appears to change, and the highest enrichment factor was observed in the gills,

Fig. 3 - Concentrations of lanthanum (mg Kg⁻¹, dry weight) in Manila clams’ gills under control (0 µg L⁻¹), low La (0.3 µg L⁻¹), and high La (0.9 µg L⁻¹) at different sampling times (T0, T1, T2, and T6). Values represent medians ± SE. Different letters represent significant differences between treatments within each sampling time (*p* < 0.05). For additional significant differences, see Supplemental Table 2

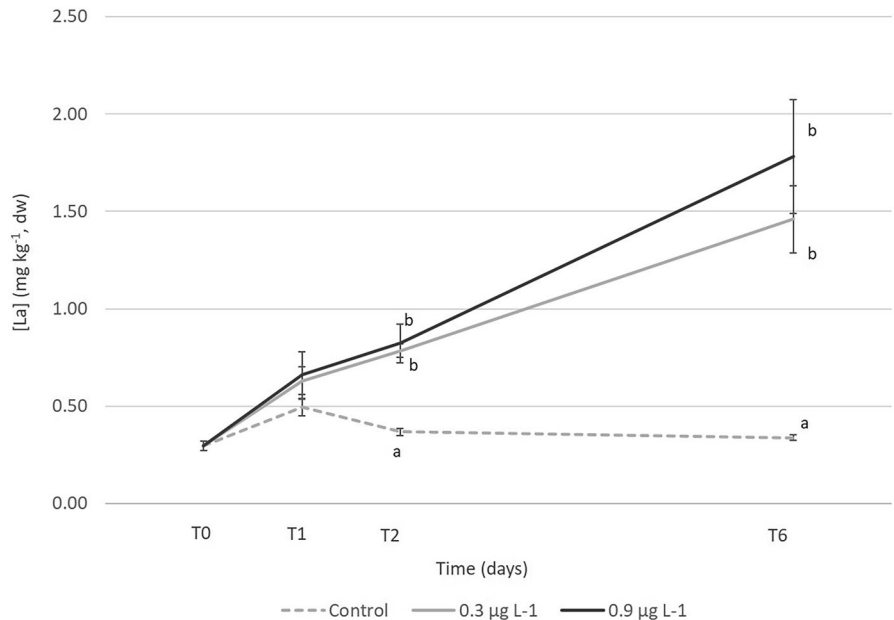
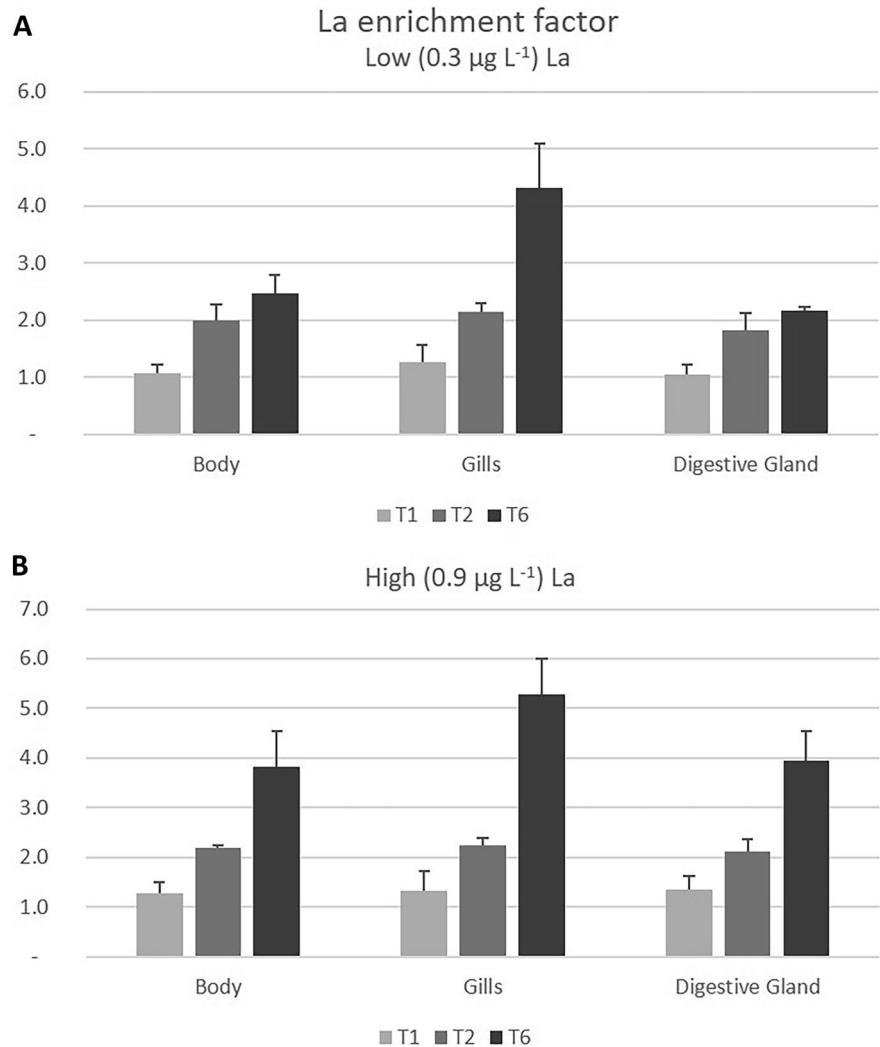


Fig. 4 Enrichment factor for the body, gills, and digestive gland of the Manila clam for the three exposed sampling times, T1, T2, and T6. Values represent medians \pm SE



followed by the digestive gland and ultimately the body.

Seawater carbonate system

Seawater physicochemical parameters and specifics of the seawater carbonate system are presented in Table 2.

The temperature, salinity, and pH were kept stable for the three treatments (control, $0.3 \mu\text{g L}^{-1}$ La and $0.9 \mu\text{g L}^{-1}$ La), and the total alkalinity was 2570 ± 382 ($\mu\text{mol/kg SW}$) in the control treatment and slightly lower for the added lanthanum treatments: 2213 ± 361 ($\mu\text{mol/kg SW}$) for the low La treatment and 2460 ± 355 for the high La treatment.

Nevertheless, the studied water parameters (TA, pCO_2 , HCO_3^- , OH^- , ΩCa , and ΩAr) remained comparable between treatments ($p > 0.05$).

Discussion

The availability of REE in the environment is increasing, and they are becoming worldwide-ranging contaminants. Their behavior has been described in the water, particulate matter, sediments, and organisms living in rivers, estuaries, and oceans (e.g. Akagi & Edanami, 2017). Nevertheless, studies evaluating the availability and bioaccumulation of La under-laboratory conditions are still scarce. To the best of our knowledge, this is the

first study on the differential tissue accumulation of La in a bivalve species. Therefore, any comparison with the literature is hampered and can only be achieved with other chemical elements that may have distinct toxicokinetics or other species.

One day of exposure to La, at both concentrations, was insufficient to trigger accumulation in the three studied body parts. The ability of bivalves to reduce filtration rates when exposed to pollutants is well known (e.g., Almeida et al., 2015). This is achieved by valve closing and diminishing the respiration and filtration rates, to avoid pollutant accumulation. Our results showed that this could have happened upon La exposure. Pinto et al. (2019) exposed the mussel *M. galloprovincialis* to different levels of La (0, 0.1, 1, 10 mg/L), and after measuring accumulation in the whole soft body, they found that as La exposure concentration increased, the bioconcentration factor (the ratio between the concentration in the organisms and the water) decreased. In fact, just after 1 day of exposure to La, the exposed body parts showed very similar values to the control ones, which may corroborate the hypothesis that this species presents an ability to deal with La in a short-time frame. This ability seems to be very limited as after 2 days of exposure accumulation occurred in all body parts. However, at this point, we did not find significant differences between the body parts exposed to low and high La concentrations, which could still be due to this ability to reduce respiration and filtration, and therefore pollutant accumulation. In the face of this adaptation to La-induced environmental stress, bivalves may not get adequate energy from feeding, which may impair other physiological processes, such as growth and reproduction. Future studies should further investigate this ability by means of, for example, quantification of stress biomarkers in the first days of exposure to the same range of environmental realistic La concentrations.

The La bioaccumulation partly depends on the element bioavailability that is influenced by a complex array of factors, such as water hardness, alkalinity, dissolved organic carbon, and pH. Furthermore, the bioaccumulation is influenced by the physicochemical characteristics of the exposed organisms (Herrmann et al., 2016). On another side, bioaccumulation will depend on the uptake and elimination kinetics, and such processes may be species-specific, and on the biologically active fractions of the element (Khan et al., 2017). Cánovas et al. (2020)

studied REE exposure in aquatic organisms using diffusive gradient in thin films (DGT) and observed that element retention in DGTs was related to total concentration in water (dissolved + particulate), suggesting that a relation between the DGT-labile concentration and the accumulated levels in organisms exists. These findings corroborate our results since a dose-dependent accumulation was found, which relates to the total and labile concentration in water. In fact, our research showcase the great ability of the environmentally realistic La concentrations to be accumulated in a swift timeframe. Additionally, Bonnail et al. (2017) showed a *Corbicula fluminea*'s REE uptake proportional to the pollution degree, and the REE geochemical signature of the environment was preserved in their soft tissue. Furthermore, Cánovas et al. (2020) showed that in brackish seawater, REE-CO₃ complexes prevail over free ions, Cl⁻, F⁻, Sc⁻, and O/OH⁻ complexes. Nevertheless, the CO₃ complexes showed the lowest contribution for La (~60% of all species), in comparison to the remaining REE (up to 96% of all species).

In our study, the body presented the lowest accumulation values, which is consistent with previous ecotoxicological studies with other metals on the same species. Jang et al. (2009) exposed the Manila clam to different concentrations of cadmium (Cd) and described the gills as the tissue with the higher accumulation, followed by the digestive gland and ultimately the residual tissues. Furthermore, Liu et al. (2017) investigated the tissue-specific bioaccumulation of heavy metals (Cr, Cu, Hg, Zn, As, Cd, and Pb) in the Manila clam demonstrating that the visceral masses tended to accumulate more efficiently than the muscle. The gills have been indicated as a temporary target organ for pollutants in bivalves that are later transferred to the digestive organs, such as the digestive gland (Guo-Qing & Dong-Feng, 2016). In our study, the gills and the digestive gland accumulated similar quantities of La. However, in the gills, no significant difference was discerned between the two exposed treatments at T6. The La accumulation similarity in both the gills and the digestive gland may be related to the short exposure period used in this trial (6 days). Perhaps in a long exposure experiment a clear difference between the gills, a key interface for the uptake of contaminants from the water, and the digestive gland, a vital detoxification tissue, may occur. Won

et al. (2016) characterized the target organs (gill, mantle, digestive gland, siphons, adductor muscle, and foot) of the Manila clam by exposing them to copper (Cu) and lead (Pb) and described a linear uptake and that the order of accumulation rate in laboratory exposure was not concomitant with that of the field study, suggesting that different routes of metal uptake and exposure duration may induce distinct partitioning of metals in *R. philippinarum*. Hence, in order to better understand the La uptake, we suggest that more than three datasets (T1, T2, and T6) should be applied, with particular emphasis on the first days due to this bivalve species ability to cope with La exposure. More studies on La toxicity should be carried out, and this study provides key information on which future studies will build upon.

Focusing attention on the enrichment factors calculated in the present study, they were higher in the gills, followed by the body and ultimately the digestive gland for the Manila clams exposed to $0.3 \mu\text{g L}^{-1}$ La. However, when the exposure concentration tripled, the enrichment factor was higher in the gills followed by the digestive gland and ultimately the body. It could be interesting to see if this changed accumulation pattern would become more noticeable with a higher concentration exposure. Considering this, we suggest that future studies perform these trials with an increased gap between the environmentally realistic exposure concentrations studied. Additionally, the Manila clam presented an increase in La concentration level when exposed to a higher concentration, and this might have increased even more if a higher concentration was applied.

If the contamination route used in our study would be through the diet (i.e., by adding La to the concentrated marine phytoplankton used to feed the Manila clams), the results might likely be different. The gills would probably reach lower La enrichment factors than the digestive gland, attending that the contamination vehicle would not pass directly by them. This highlights the importance of differential tissue accumulation studies, and the organ specificity of La accumulation should be further investigated.

Here, the accumulation increased in all body parts till T6, and therefore, an extension of the exposure duration should also happen in upcoming studies as Figueiredo et al. (2018) found that the accumulation of La peaked and decreased afterwards even in a

continuously exposed medium, in freshwater glass eels (*Anguilla anguilla*). Unfortunately, we lost the 6-day elimination phase samples and were unable to process them and therefore advise researchers to evaluate the elimination rate of this element in future studies.

The increased usage of modern electronic technologies, and other activities, is rising the availability of La in the aquatic environment, which is in turn being accumulated by commercially important food resources. The information on the toxicity of La is unfortunately scarce and still quite puzzling. Therefore, we also suggest that future studies should look into its effects, equally on a cellular, tissue, and individual level as we must adopt a holistic approach to better understand REE toxicology and its effects on species sustainability and human health.

Conclusion

The findings described in this article revealed that environmentally realistic concentrations of La ($0.3 \mu\text{g L}^{-1}$ and $0.9 \mu\text{g L}^{-1}$) were not bioaccumulated by *R. philippinarum* in the first 24 h of exposure. Nevertheless, on the second day of exposure, accumulation occurred in the three studied body parts (digestive gland, gills, and remaining body). The accumulation was upheld until the sixth day of exposure and occurred in a dose-dependent manner. Having into consideration that the studied concentrations represent environmentally realistic concentrations, like the ones present at a European wastewater outlet, the results highlight the bivalve propensity to accumulate La in its' tissues. Owing to the limited information about these emerging contaminants, the evidence added by this study is of key importance from an ecological and food safety point of view.

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Availability of data The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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