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Bioaccumulation of lithium isotopes in mussel soft tissues and implications for coastal environments

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13 ABSTRACT

14 Lithium production has dramatically increased over the past decade, and first cases of
15 environmental Li pollution have been recently reported in urban and in mining regions. While
16 elevated Li concentrations may be toxic for living organisms, tools to monitor Li in the
17 environment have not yet been developed. Consequently, its impact on key biota and human
18 health is still poorly known. The present laboratory-based study shows that the soft tissue of blue
19 mussels (*Mytilus edulis*) can be used to quantify Li contamination in coastal waters. Stable Li
20 isotope ratios ($^7\text{Li}/^6\text{Li}$) measured in these soft tissues correlate positively with seawater Li
21 concentrations and show precisely the threshold above which mussels shift their depuration
22 mechanism. Combined with other data from the natural environment, the experimental results
23 have profound implications for the fate of coastal ecosystems and shellfish consumption living
24 under high Li environmental level. We also highlight the need to develop innovative tools to
25 extract Li from wastewaters before its release into rivers and, ultimately, the ocean.

26 KEYWORDS

27 Bivalves, Lithium, Isotopic tools, Emerging contaminant, Fractionation, Concentration effects

Introduction

Lithium (Li) occurs naturally at trace levels in the environment. Consequently, it is also present in living organisms, and some studies have even suggested that Li should be recognized as an essential element¹. However, as observed for other metals^{2,3}, Li is toxic and can eventually be lethal beyond a specific threshold⁴⁻⁶. Elevated levels of Li in the aquatic environment can be due to natural inputs, mainly from rock erosion⁷, but they can also be related to anthropogenic activities. There is an increasing demand of Li from high-tech industries producing Li-rich batteries for laptops, mobiles, and other electronic devices worldwide. The Li production has increased from 23 to 33 kt.yr⁻¹ during 2010–2017⁸⁻¹⁰, and the demand for Li is expected to follow the greatest growth trajectory among all metals by 2025⁹. Li contamination has already been observed in groundwater and river water with close proximity to mining sites¹¹, as well as in municipal waters of a modern and densely populated metropolitan area¹². This is a worrying problem as the various protocols of wastewater depuration appear to be inefficient¹² at removing Li because of the high mobility of Li and its poor ability to adsorb onto particle surfaces¹³ (Li was used as an aquifer water flux tracer^{14,15}). In addition, there is no major Li sink in estuaries and, therefore, dissolved Li is conservatively delivered to littoral waters and to the ocean^{16,17}. Overall, there is a growing concern on the fate of global Li increase in the environment, especially the risk of this emerging aquatic pollution on organisms and humans that consume them.

Li monitoring in the marine environment is not commonly performed as compared to other trace metals¹⁸⁻²⁰. For instance, mussel-based biomonitoring programs²¹⁻²³ do not include Li in the

chemicals analyzed, even if mussels, along with other shellfish and bivalves, contain more Li than any other species from higher trophic levels²⁴. The paucity of information on the process of Li accumulation in marine coastal organisms needs to be addressed to better understand the response, the fate, and the risk of Li contamination in the marine environment, as well as for potential human health implications.

To better comprehend the biological mechanisms at play, it is possible to measure precisely the natural variations of Li stable isotope ratios (⁶Li and ⁷Li, abundances of 7.59% and 92.41%, respectively). Stable isotopes of trace metals are valuable tools to investigate subtle physiological changes in organisms^{25–28}. However, Li isotopes in marine organisms were, so far, not used due to technical limitations related to low Li levels in tissues²⁹. Yet, being able to measure and track Li isotopes in this manner (1) will help understand how they are affected by homeostatic processes and elevated Li levels in the environment, and (2) will also benefit paleo-oceanographic studies wherein Li has proven utility. Indeed, Li isotopes are now widely used to reconstruct past ocean and climate by analyzing fossil carbonates formed by calcifying organisms^{30–34}. However, the role and impact of biological processes (‘vital effects’) on these biogenic minerals still remain an open question^{35–38}, which leads to a high level of uncertainties concerning Earth’s climate regulation after a disturbance, such as from a meteorite impact or a mass extinction³⁹.

Here, we exposed blue mussels (*Mytilus edulis*) to various degrees of environmental Li enrichment during laboratory experiments. We report Li concentrations and Li isotopes compositions of mussel soft tissues. We aim to define the impact of elevated Li levels in mussel metabolism and, by comparing our results with data collected in the natural environment, to evaluate the mussel ability to biomonitor Li contamination. We also highlight the Li

environmental concentration limit that defines risk for bivalves and assess implications of elevated Li concentrations for coastal organisms and population.

Materials and Methods

Experimental setting and sample preparation

A summary of the experimental setting is sketched in Fig. S1. Forty individual common blue mussels (*Mytilus edulis*) of similar size (43.01 ± 2.18 mm) and wet weight (7.137 ± 1.014 g) were collected in summer 2019 (outside spawning period) *in situ* from La Rochelle, Bay of Biscay, France. The experiment was performed at the Environment Laboratories of the International Atomic Energy Agency (Monaco), based on well-established protocols. Mussels were acclimated at the laboratory environment for one month, at 20.5°C. Batches of five mussels were placed in eight 5-L glass beakers filled with 2 L of filtered (1 µm) seawater pumped from the 30m water depth in Mediterranean Sea (Monaco Bay). Seawater salinity, temperature, and pH_T were 39 ± 1 , 20.5 ± 0.5 °C, and 8.03 ± 0.05 , respectively. Water oxygenation was ensured by constant air bubbling, and a 12 h-12 h light-dark cycle was established.

Two duplicates (A and B) of four experimental conditions were implemented with increasing seawater Li concentrations: CTRL (control group, ~ 0.18 mg Li L⁻¹), Li-0.5 (~ 0.5 mg Li L⁻¹), Li-1.0 (~ 1.0 mg Li L⁻¹), and Li-1.5 (~ 1.5 mg Li L⁻¹). To obtain the desired Li concentrations, dedicated volumes of a concentrated Li reference solution (1000 mg Li L⁻¹, ICP Standard) were evaporated and added as solid salt to the filtered seawater.

The mussels were kept in their experimental aquaria during 4 days, and the culture media (seawater with different Li concentrations) were renewed every day at the same time ($13:40 \pm 40$

min). Due to the paucity of data on Li bioaccumulation process, we decided (1) to investigate the short time process in order to better understand the short term isotope fractionation and uptake processes assuming that Li should act like other alkali elements (i.e., Cs, which quickly reaches a saturation state when accumulated in marine bivalves⁴⁰⁻⁴²) and (2) to use a relatively high exposure concentration based on data available in freshwater studies^{4,43}. Seawater samples were collected before and after each water change to confirm that each aquarium operated as an open system. Between water changes, the mussels were fed for 1 h using non-contaminated seawater containing phytoplankton *Isochrysis galbana* at a concentration of 10^4 cells mL⁻¹. One mussel died after 2 days of the experiment in the duplicate A of the CTRL condition. At the end of the experiment, all individuals were collected and rinsed for 30 min in clean, filtered seawater following the classical protocol for eliminating weakly adsorbed elements from tissue samples⁴⁴. The soft tissues were then extracted, frozen at -28 °C for 72 h, and freeze-dried for further analyses.

Analytical methods

To minimize the possibility of any contamination and procedural blanks, all analytical preparations were performed in a positively pressurized clean laboratory under a fume-hood using only distilled, trace-metal grade reagents and pre-cleaned vessels. Each mussel's soft tissue was weighed in a Teflon reactor, then digested in a mixture of 5 mL HNO₃ and 2 mL H₂O₂ (both trace metal grade, Fisher Scientific, USA) at 90 °C for ~5 h. The obtained solutions were transferred into pre-cleaned polyethylene tubes and gravimetrically diluted up to 20 g with MilliQ water (18 MΩ). Two procedural blanks and two replicates of the Certified Reference Material (CRM) IAEA 407 (Fish Homogenate, IAEA, Vienna) were included in the digestion

120 batch and analyzed with the samples. The recoveries obtained for the CRM were 95 ± 3 %. For
 121 each sample, two solution aliquots were separated for elemental and isotopic analyses.
 122 Lithium concentrations were determined at the IAEA in Monaco by flame atomic absorption
 123 spectroscopy using a ContrAA 700 (Analytic Jena, Germany). The applied analytical procedure
 124 was based on external calibration approach and was preliminary validated, according to the
 125 requirements of international guidelines for method validation⁴⁵. Calibration curves were daily
 126 prepared using proper dilution of commercially available standard solutions. All possible sources
 127 of uncertainty of obtained results were carefully identified. The numerical method of
 128 differentiation described by Kragten⁴⁶ was used to calculate combined standard uncertainties of
 129 obtained measurement results.
 130 Prior to Li isotopic analyses, the Li in the samples must be purified. Lithium extraction and
 131 purification were performed following the procedure of Vigier *et al.*⁴⁷, summarized here.
 132 Sample solution aliquots were dried out and taken up in 0.5 mL of 1 N HCl and loaded on AG
 133 50-X12 cationic resin in 8.5-cm-high Teflon columns. Lithium was purified twice with 1 N HCl.
 134 This fraction was evaporated to dryness before the analysis.
 135 Lithium has two naturally occurring isotopes, one heavy (^7Li , 92.41% abundance) and one light
 136 (^6Li , 7.59% abundance). The isotopic compositions of geological and biological materials are
 137 reported as per-mil variations relative to an isotopic standard (here LSVEC) as $\delta^7\text{Li}_{\text{sample}} [\text{‰}] =$
 138 $((^7\text{Li}/^6\text{Li})_{\text{sample}} / (^7\text{Li}/^6\text{Li})_{\text{LSVEC}} - 1) \times 1,000$.
 139 Lithium isotopic analyses were performed at the CNRS-INSU National Service based at the
 140 École Normale Supérieure de Lyon (France) using a Thermo-Fisher Neptune *Plus* multi-
 141 collector inductively coupled plasma mass spectrometer following the procedure of Balter and
 142 Vigier²⁹ and Bastian *et al.*⁴⁸ developed for low Li concentrations in biological materials. In brief,

samples and standards were analyzed under dry plasma conditions in low-resolution mode. Instrumental mass bias correction was performed externally using the standard bracketing method (using the LSVEC reference material). Analytical blanks, as well as total procedural blanks, were found to be negligible. The internal reproducibility was on average $\pm 0.04\%$. Precision (external reproducibility), estimated from two to three replicate measurements of 14 different samples (mussels and water), ranged from 0.04 to 1.91‰ with an average of $\pm 0.5\%$. In general, the accuracy of reported isotopic compositions are estimated based on analyses of reference materials of known isotopic composition. However, the Li isotopic compositions of biological reference materials corresponding to soft tissue have never been characterized. Thus, this study reports for the first time the Li isotopic composition of a soft-tissue biological reference material IAEA 407 ($\delta^7\text{Li} = 28.3 \pm 1.7\%$, 2SD, $n = 2$). For comparison, the reproducibility of $\delta^7\text{Li}$ obtained for the Li7-N non-biological reference material was 0.4‰ (2SD, $n = 22$)⁴⁸. Note that the IAEA 407 contain $0.68 \pm 0.1 \mu\text{g Li g}^{-1}$ of dry powder, while Li7-N is an isotopically homogeneous solution, analyzed at a Li concentration of 4 ng mL^{-1} .

Statistical treatment

Since the Li data were not normally distributed (i.e., Li concentration and Li isotope ratios do not follow a normal law), we adopted non-parametric statistical tests. The Mann-Whitney-Wilcoxon test was used to compare median values between two observational series, and the Kruskal-Wallis test was used to compare three or more series. Tests were performed using R software⁴⁹ using the respective functions *wilcox.test* and *kruskal.test*. The level of significance for statistical analyses was set at $p < 0.05$.

The relationship between quantitative variables was investigated using principal component analysis (PCA, see section 4.3), a statistical tool that reduces the number of original quantitative variables to fewer dimensions that may explain the majority of the observed variability. Here, PCA was performed using R software⁴⁹ considering the following variables: Al, B, Ca, Fe, K, Li, Mg, Mn, Na, Si, and Sr concentrations in mussels, the Li concentration in seawater (Li_{sw}), and $\Delta^7\text{Li}_{\text{mussel-water}}$.

Results

In the control group ‘CTRL’, the aquarium seawater Li concentration was $0.18 \mu\text{g mL}^{-1}$, which corresponds to the mean global ocean Li concentration⁵⁰. Li concentrations in seawater were set at 0.5, 1.0, and $1.5 \mu\text{g mL}^{-1}$ in the ‘Li-0.5’, ‘Li-1.0’, and ‘Li-1.5’ treatments, respectively. The Li water concentration error is estimated at 10% (2SD). After the experiment, Li concentrations measured in mussels soft tissues were 0.45 ± 0.18 for the CTRL group (2SD, $n = 9$), 1.20 ± 0.53 (2SD, $n = 10$), 1.54 ± 0.96 (2SD, $n = 10$), and $2.19 \pm 1.65 \mu\text{g g}^{-1}$ (2SD, $n = 10$), for the ‘Li-0.5’, ‘Li-1.0’, and ‘Li-1.5’ treatments, respectively (Fig. 1A, Table S2). This corresponds to a fivefold increase of the average mussel Li concentration between CTRL and Li-1.5, in response to an eightfold increase of the seawater Li concentration.

Due to the different isotopic compositions in seawater ($\delta^7\text{Li} = 31.2 \pm 0.3\text{‰}$ in the open ocean^{50,51}) and the pure Li solution ($\delta^7\text{Li} = 10.8\text{‰}$) used to prepare the three exposure conditions, the mussels in each group were exposed to seawater with different Li isotopic compositions ($\delta^7\text{Li}_{\text{sw}}$, which remained constant over the course of the experiment; Table S1): $\delta^7\text{Li}_{\text{sw}}^{\text{CTRL}} = 31.5 \pm 0.7\text{‰}$ (2SD, $n = 5$), $\delta^7\text{Li}_{\text{sw}}^{\text{Li-0.5}} = 18\text{‰}$ (calculated), $\delta^7\text{Li}_{\text{sw}}^{\text{Li-1.0}} = 14.6 \pm 0.01\text{‰}$ (2SD, $n = 2$),

and $\delta^7\text{Li}_{\text{sw}}^{\text{Li-1.5}} = 13.4 \pm 0.1\text{‰}$ (2SD, $n = 5$). These compositions fit along a binary mixing curve (cf. following sections), which demonstrates that, despite a significant Li incorporation by mussels, it remains negligible when compared to the total water Li content of each aquarium. As a consequence, it does not modify neither the water Li content, nor its $\delta^7\text{Li}$ value. The average $\delta^7\text{Li}$ of mussel soft tissues in groups Li-1.0 and Li-1.5 were $21.0 \pm 2.6\text{‰}$ and $21.6 \pm 2.7\text{‰}$, respectively (Table S2, Fig. 1B). The one in group Li-0.5, significantly higher than the latest ($24.4 \pm 3.4\text{‰}$, Wilcoxon test, $p < 0.001$) was significantly lower than that in the control group ($31.7 \pm 2.0\text{‰}$, Wilcoxon test, $p < 0.0001$).

Discussion

Li bioaccumulation by mussels

At the end of the experiment, the Li concentrations measured in mussels of the control group ($0.45 \pm 0.18 \mu\text{g g}^{-1}$) were consistent with that observed in bivalve tissues and organs collected along the French Atlantic coast ($0.43 \pm 0.25 \mu\text{g g}^{-1}$)⁵². This result supports the representative nature of our samples, the reliability of our methods, as well as the lack of bias related to the experimental setup. In both cases (in experiments and in nature), mussel Li concentrations are significantly higher than in seawater ($0.18 \mu\text{g mL}^{-1}$), by a factor of 2.5 on average. This agrees well with a recent study²⁴ that shows that bivalves, and filter feeders in general, accumulate Li the most compared to other organisms from higher trophic groups, such as crustaceans, cephalopods, and fish.

The average Li concentrations displayed by mussels exposed to Li-rich seawater are statistically higher than those of the control group, and increase with seawater Li concentrations, up $2.19 \pm$

1.65 $\mu\text{g g}^{-1}$ when the water Li content is 1.5 $\mu\text{g mL}^{-1}$ (Figs. 1A and 2A). This demonstrates that, on average, mussels accumulate Li proportionally to the concentrations found in their environment, which is a major criterion to validate a biota as a reliable bioindicator of contamination⁵³. These results are consistent with a recent laboratory study that explored key biochemical parameters changed by long-term (28 days) Li exposure on mussels. At 0.25 and 0.75 mg Li L^{-1} in aquarium water, the mussel Li concentrations were found to be $0.9 \pm 0.23 \mu\text{g g}^{-1}$ and $1.4 \pm 0.23 \mu\text{g g}^{-1}$, respectively. These data perfectly fit with our short-term exposure results (Figure 2A), supporting that a bioaccumulation steady-state was reached within the 4-day exposure. The ability of bivalves to bioaccumulate Li can also be evidenced along the coast of North Chile (Table 1). Indeed, this coastal area is characterized by an unusually elevated Li concentration from river waters draining the high altitude Salars (salt flats). When compared to bivalves collected in low Li environments, such as the Atlantic or the Pacific coast, the Chilean bivalves display much higher Li concentrations (by 50 to 270 times higher, see Table 1). Taken altogether (Fig. 2A), all these data are consistent and demonstrate that mussels take up Li in direct proportion to the ambient dissolved concentrations, at least over the range of concentrations tested here.

There is a significant variability among individuals, and a striking observation is that this variability of individual Li concentrations increases with seawater Li concentration (from 44% in the control group to 75% in Li-1.5; Fig. 1A). This suggests that some mussels, when exposed to elevated Li concentrations, can better regulate Li uptake than others. This kind of intra-variability among mussels has already been reported for other trace elements such as Zn, Cd, and Cu¹⁸. Thus, a reliable assessment of environmental Li concentrations requires that several mussels are pooled together to capture the range of individual responses, as this is done for other

contaminants measured in bivalves^{21–23}. Based on the range of seawater Li concentration used in this study, we estimate that accurate Li concentration can be obtained with the analyses of at least five individuals, and likely more when water Li concentration exceeds 1.5 µg mL⁻¹, such as in Northern Chili (since the variability increases with the Li level, Fig. 2). Existing biomonitoring programs usually collect and pool between 80 and 160 individuals per site⁵⁴, so the use of mussels to monitor Li levels along with other contaminants in coastal areas appear largely feasible.

Preferential ⁷Li enrichment in mussels

While the seawater in experimental aquaria have a constant and homogenous $\delta^7\text{Li}$ value for each condition, exposed mussels are ‘isotopically fractionated’ compared to their aquarium water, i.e. with significantly higher $\delta^7\text{Li}$ values (Fig. 2B). This indicates that mussels preferentially accumulate the heavy (⁷Li) isotope in their soft-parts, as compared to the light (⁶Li) isotope. It is possible to calculate the corresponding water-mussel Li isotopic fractionations ($\Delta^7\text{Li}_{\text{mussel-water}}$) using⁵⁵:

$$\Delta^7\text{Li}_{\text{mussel-water}} (\text{‰}) = \delta^7\text{Li}_{\text{mussel}} - \delta^7\text{Li}_{\text{water}} \quad (1)$$

$\Delta^7\text{Li}_{\text{mussel-water}}$ value near 0‰ indicates no Li isotopic fractionation, since mussel $\delta^7\text{Li}$ is equal to water $\delta^7\text{Li}$.

At the end of the exposition, the average $\Delta^7\text{Li}_{\text{mussel-water}}$ ranges from 0.0‰ to +10.3‰ (Fig. 2B). Mussels in the control group exhibit a mean $\delta^7\text{Li}$ value similar to that of the seawater (with $\Delta^7\text{Li}_{\text{mussel-water}} = +0.4 \pm 0.9\text{‰}$, SD, $n = 8$), whereas the average $\Delta^7\text{Li}_{\text{mussel-water}}$ values of the other experimental groups increase from $+5.7 \pm 2.1\text{‰}$ to $+7.5 \pm 3.2\text{‰}$ (from Li-0.5 to Li-1.5 respectively). Li isotopic fractionation is the strongest with the very first - limited - increment in

Li enrichment (5.7‰ in Li-0.5 condition vs. 0.4‰ in CTRL group). Further increases in seawater Li concentration still result in significant (Wilcoxon test, $p < 0.05$), but lower, isotope fractionation (up to 7.5‰ in Li-1.5). This indicates that mussel $\delta^7\text{Li}$ value increases with Li contamination and suggests that even an exposition to a small degree of contamination has a strong and significant effect on mussel Li isotopic compositions. The Li isotopic compositions of mussel soft tissues can therefore reveal even very subtle effects related to environmental Li contamination, which further supports the possibility to use mussel Li isotope ratio to monitor the Li level in waters. Measuring the isotopic composition of mussels has the advantage that there is small Li isotopic variability among mussel individuals and a representative $\delta^7\text{Li}$ value requires 2 individuals only (for a 2–3‰ uncertainty), in contrast to Li concentration. Variabilities exists for isotopic composition, but their magnitude is only of few permill maximum. A recent study shows that the isotopic composition of anthropogenic Li in waters can be distinct from that of natural Li^{12} . Thus, further investigation appears necessary to provide a methodology to trace both Li levels and Li sources (natural or anthropogenic) in the environment.

Li biological control

The biological processing of Li in mussels is affected by the Li concentration in the environment because the mussel-water Li isotope fractionation increases with dissolved Li concentration. The observed ^7Li (heavy) enrichment of mussel soft tissue relative to seawater is intriguing as, in most isotopic systems, biological isotopic fractionation favor light isotopes, mostly because of kinetic effects during ionic transport. Recent work reveals that Li transport in cells, either passive or active, is related to a preferential and significant enrichment in ^6Li ⁵⁶. Li has been considered to be analogous to Na in cells because of the chemical proximity and monovalence of both alkali

elements. Indeed, Li is transported by Na^+/H^+ ubiquitous exchangers (NHEs), which control intracellular pH^{57–60}. Ca/Na exchangers, likely involved in carbonate shell growth, are also known to transport Li^{61,62}. To verify the potential link between Li, Na, and Ca, we measured Li/Na and Li/Ca ratios in every individual mussel. Both Li/Na and Li/Ca ratios in mussels are positively correlated with $\Delta^7\text{Li}_{\text{mussel-water}}$ (Fig. 3). When the Li/Na and Li/Ca ratios are low, the observed Li isotopic fractionations are small or negligible ($\Delta^7\text{Li}_{\text{mussel-water}} \approx 0\text{‰}$), whereas the highest $\Delta^7\text{Li}_{\text{mussel-water}}$ values correspond to the highest Li/Na and Li/Ca ratios. This result confirms the biological coupling between Li and Na⁶³ and suggests that Li transport by NHEs and Ca/Na transporters are at least partly responsible for the measured Li isotopic compositions. As Ca is a major building block of bivalve shells, this link might partly explain the vital effects observed in the Li isotopic signatures of biogenic carbonates^{35–38}. Published Li isotopic compositions of bivalve shells, foraminifera calcite, or corals are scarce, and their interpretation remains disputed^{32,35–38,64}. A physiological control of Li isotopes was suggested by Dellinger *et al.*³⁵ for various species of molluscs, brachiopods, and echinoderms, and by Vigier *et al.*³⁸ and Roberts *et al.*³⁶ for epibenthic foraminifera. All these studies rely on the Li isotope composition of the organism's shells, being either calcitic or aragonitic. As a first approximation, we can compare the mussel soft part $\delta^7\text{Li}$ values of our CTRL group with those reported for *M. edulis* shells by Dellinger *et al.*³⁵. (Fig. 2B). These shells were collected from mussels living in Cable Bay (northwest Wales, UK), and have been cultured at different temperatures⁶⁵. They all show $\delta^7\text{Li}$ values noticeably higher than the $\delta^7\text{Li}$ values obtained for the soft tissues of our CTRL group (this study). It is surprising as the formation of calcite induces a significant isotope fractionation in favor of the ^6Li ⁶⁶. However, both mussel groups do not come from the same location and it is not known if the seawater in which they were cultured had a seawater-like $\delta^7\text{Li}$

value and Li concentration, or if this seawater was contaminated. Although this comparison requires more detailed investigations, all of these results suggest that the temporal variations of ocean Li concentrations may impact both the Li concentrations and the Li isotopic composition of tissues and shells. Since the isotopic investigation of soft tissues is now technically possible with high sensitivity mass spectrometer²⁹, it appears invaluable to a better understanding of the vital effects observed in fossils and potentially for refining paleoclimate reconstructions.

Tracing Li homeostasis and biological thresholds with Li isotopes

In order to bioaccumulate Li as shown in Fig. 2A, the mussel Li excretion flux ($\Phi_{\text{out}}^{\text{Li}}$) must have been lower than its uptake flux ($\Phi_{\text{in}}^{\text{Li}}$), such that:

$$R_f = \frac{f_{\text{out}}^{\text{Li}}}{f_{\text{in}}^{\text{Li}}} < 1 \quad (2)$$

As shown in Fig. 2B, and described above, mussel $\delta^7\text{Li}$ values are systematically higher than seawater $\delta^7\text{Li}$ values. However, due to kinetic effects, Li transport through cell membranes is rather expected to favor the light isotope ^6Li ⁶⁷. It is common to express these isotope variations during transport by the isotope fractionation factor ‘ α ’, which is related to the Δ notation (equation 1) using: $\Delta^7\text{Li} \approx 1,000 \times \ln(\alpha)$. If both, intake (input) and excretion (output) processes favor the light (^6Li) isotope, then both α_{in} (input) and α_{out} (output) are lower than 1 ($\Delta^7\text{Li} < 0$). In that case, mussel ^7Li enrichments are possible only if ^6Li excretion flux is greater than ^6Li uptake flux over the experimental duration (i.e., if $\alpha_{\text{out}} < \alpha_{\text{in}}$, see Fig. 4 for more details). As the degree of ^7Li enrichment is found to increase as a function of the seawater Li concentrations (Fig. 5), the two possible ways to explain these observations are either that (#1) the ratio between the output

and input fractionation factors ($\alpha_{\text{out}} / \alpha_{\text{in}}$) decreases as a function of seawater Li content, or (#2) the ratio of the Li output flux to the Li input flux (R_{ϕ}) increases.

Using a simple box model (described in Fig. 4 and in the Supplementary Material), it is possible to verify more precisely whether the Li regulation in connection to variable Li bioaccumulation by organisms is compatible with their isotopic compositions. Considering that Li transport is likely ensured by ubiquitous ion transporters equally present in all *Mytilus edulis* of similar provenance^{57–59}, we assume that all organisms have a similar value for α_{out} and α_{in} , and therefore favor hypothesis (#2). With this assumption, and using the equations detailed in the Supplementary Material, the average mussel $\delta^7\text{Li}$ value obtained at each condition can be reproduced within uncertainties (Fig. 5). The calculated input and output Li isotope fractionation factors, α_{in} and α_{out} , are 0.999 and 0.989 respectively, and R_{ϕ} is found to increase with increasing seawater Li concentration, as expected (Table S3).

This simple model indicates that, even if mussels bioaccumulate Li, their Li excretion rate increases significantly as Li contamination increases. This balance between input and output depends on the seawater Li level and can be related to Li homeostasis, which varies as a function of Li bioaccumulation, and therefore, of the water contamination. Indeed, the regulation process of a given element can vary according to low or high accumulation scenario, and be caused, for example, by an intense elimination process (excretion), or by storage as an internal inert form of the element (detoxification process).

It is possible to investigate further this aspect by exploring the small but significant $\delta^7\text{Li}_{\text{mussel}}$ variability displayed by individuals from the same experimental group. For each condition, individual $\Delta^7\text{Li}_{\text{mussel-water}}$ values correlate linearly with the Li enrichment factor ($\text{Li}_{\text{m}}/\text{Li}_{\text{sw}}$, the ratio of the Li concentrations in the mussels to that in the water, Fig. 6A). These variations are

significant, of about 2-3‰ (while the external error on the Li isotope ratio is 1.6‰). For all individuals, $\text{Li}_m/\text{Li}_{\text{sw}}$ are higher than 1, which agrees well with the bioaccumulation process described previously. However, the regressions' slopes in Fig. 6A strongly depend on the condition. Mussels exposed to low water Li concentrations (i.e. the CTRL and Li-0.5 groups) display a positive slope, reflecting that $\Delta^7\text{Li}_{\text{mussel-water}}$ increases when the individual enrichment factor increases. On the other hand, mussels exposed to elevated seawater Li concentrations ($> 1\mu\text{g mL}^{-1}$) (Li-1.0 and Li-1.5 groups) display negative slopes (decreasing $\Delta^7\text{Li}_{\text{mussel-water}}$ with the enrichment factor). There is therefore a Li threshold between 0.5 and $1\mu\text{g mL}^{-1}$, above which mussels change their Li biological regulation. Under a significant but limited Li contamination ($<1.0\mu\text{g mL}^{-1}$), the Li excess in the mussel results in a greater Li depuration, which may correspond to a homeostasis mechanism operating to reduce the Li excess. In contrast, the negative correlations observed for mussels exposed to seawater Li concentrations $>0.5\mu\text{g mL}^{-1}$ are best explained by a decrease of R_ϕ (Fig. 6A), indicating reduced Li excretion. Under these conditions, mussels do not - or cannot - entirely eliminate excess Li. This threshold therefore suggests a physiological shift, and we speculate that it may represent the onset of toxicity that is the Li concentration beyond which mussels become “stressed”.

Major element concentrations in the mussels' soft tissues also support a physiological effect induced by Li contamination. Indeed, the principal component analysis (PCA, see Methods section) shows that the essential elements Na, Ca, Fe, and K correlate with each other on the principal component 1 and are clearly distinct from $\Delta^7\text{Li}_{\text{mussel-water}}$, Li_m , and Li_{sw} , which correlate on the principal component 2. Figure 6B shows that the “intergroup” variability is discriminated mostly by $\Delta^7\text{Li}_{\text{mussel-water}}$, Li_m , and Li_{sw} , and therefore depends on the composition and concentration of environmental Li. On the other hand, the “intragroup” variability is mostly

related to variations in essential (major) elements and depends on individual mussel metabolism. This, altogether with the Li threshold highlighted by Li isotopes, strongly suggests a physiological effect induced by Li contamination between 500 and 1000 $\mu\text{g L}^{-1}$ (0.5 and 1 $\mu\text{g mL}^{-1}$). Thus, our results demonstrate that, in addition to being a promising new proxy for Li contamination in coastal ecosystems, mussel Li concentrations and isotopic compositions might also be used to assess Li toxicity since they respond to mussel adaptation and stress due to high environmental Li concentrations. Studies on Li accumulation in organisms being at their infancy and far from being fully understood, further studies would be needed to identify the underlying regulation mechanisms in mussels facing Li contamination.

Implications for coastal environments

The highest dissolved Li concentrations ($>130000 \mu\text{g L}^{-1}$) are found in surface waters that drain the Salars, where Li is mined. Extensive studies of Chilean rivers show that they carry these elevated Li concentrations down to the western Pacific Ocean. Their dissolved Li concentrations are several orders of magnitude greater than those of other large rivers, and local estuaries contain Li concentrations above 1000 $\mu\text{g L}^{-1}$ (100-1000 times more than other estuaries worldwide). A published compendium⁶⁸ shows that marine organisms from the Northern Chilean littoral display Li concentrations considerably higher than those from other parts of the world - unrelated to salt flats - such as the French Atlantic coast or the Kerguelen Islands²⁴. However, as discussed in the previous section, Li isotopes indicate that this Li bioaccumulation has a cost since there is a physiological shift above a threshold between 500 and 1000 $\mu\text{g L}^{-1}$, which is the minimum level found in waters of North Chilean estuaries. Li isotope compositions of marine organisms from natural sites still need to be investigated, but given that the Li transfer to cells is

essentially an active process⁵⁷, passing this threshold may trigger abnormal growth patterns due to chemical stress and damage induced by Li⁶⁹.

Overall, these results highlight the need to monitor the evolution of these ‘Li-rich’ ecosystems, for both their own sustainability, as well as for the local economy and human health. Inhabitants of the Chilean coast consume 8 to 15 times more seafood and shellfish than inland populations, while the proportion of other food types such as meat or vegetables are significantly less variable between the two population groups⁶⁸. This supports the need to quantify and better understand how these coastal marine organisms bioaccumulate Li. The high Li concentrations measured in North Chilean shellfish, fish, and drinking waters explain the high Li concentration measured in the blood (serum), urine, and other tissues collected from the local population⁷⁰. The effects – negative or positive – of these high Li levels in the diet are less known. Figuorea *et al.*⁶⁸ estimated that the local population eats about 20 g day⁻¹ of shellfish and seafood. With a Li concentration of 100 µg g⁻¹, as found in local mussels (Table 1), the Li intake is already 2000 µg day⁻¹ (twice the dose recommended by Schrauzer¹). Given that vegetables, cereals, and waters are also enriched in Li in this area, the Li intake by people living along North Chilean coast is estimated to be close to the medical dose given to patient treated for neurodegenerative disease (Table 1).

In other places in the world, the number of data measured in coastal waters and marine organisms remains scarce, and it is difficult to estimate the impact of Li mining and anthropogenic activities at the global scale. A recent study points that Li is not removed during the waste water treatment plant process¹². As described in the introduction, a significant level of Li pollution has been documented in the Han River crossing Seoul¹². The corresponding Li level is still low compared to what is contained in North Chilean waters, but the predicted considerable

increase of Li production for the next 10 years, along with the development of popular high technology objects, may significantly increase Li levels in coastal environments if its recycling is not massively developed.

Conclusions

This study demonstrates the ability of mussels to bioaccumulate Li, which implies that mussels may be useful as biomonitoring organisms for Li pollution in coastal water. In addition, we identify a preferential enrichment of ^7Li in mussel soft parts. A simple modeling allows us to relate this Li heavy isotope enrichment to an enhanced NHE activity. Experimental data further suggests that Li isotopes, more than Li concentrations, are useful to discriminate an organism's metabolic or physiological threshold when facing high contaminant level in the environment. Combined with ancillary environmental data, our experimental results have profound implications for the fate of coastal ecosystems and shellfish consumption by population living in high environmental Li regions. Finally, they also clarify how biological activity can impact the Li isotope compositions measured in calcitic shells and may bias the record of past ocean variations.

Figure 1: Li concentrations ([Li]) and isotopic compositions ($\delta^7\text{Li}$) of mussel soft tissues as a function of water Li level. Blue mussels were exposed during 4 days to seawater with $[\text{Li}] = 0.18 \mu\text{g mL}^{-1}$ (natural seawater, control group CTRL), $0.5 \mu\text{g mL}^{-1}$ (Li-0.5), $1.0 \mu\text{g mL}^{-1}$ (Li-1.0), or $1.5 \mu\text{g mL}^{-1}$ (Li-1.5). A (pink) and B (green) are the two experimental replicates. Reported errors are $\pm 2\text{SD}$ ($n = 10$ for Li-0.5, Li-1.0, and Li 1.5; $n = 9$ for CTRL). Replicates A and B display the same average values (Wilcoxon test, $p > 0.05$), within uncertainties. The seawater in each experimental group is characterized by a distinct but constant $\delta^7\text{Li}$ (blue lines). **(A)** The variability of mussel Li concentrations increases from 44%(CTRL) to 75% (Li_1.5).

Figure 2: Observed Li bioconcentration and Li isotopic fractionation displayed by blue mussels as a function of seawater Li concentration. **(A)** Mussel Li concentrations: circles and diamonds indicate individual analyses and average values per experimental group, respectively. Black symbols are for bivalves collected in their natural environment (the Bay of Biscay for those at $0.18 \mu g \cdot mL^{-1} [Li]_{water}^{24}$, and Rio Camarones in Chile for those at $6 \mu g \cdot mL^{-1} [Li]_{water}^{68}$). Blue tones symbol color indicates experimental group (i.e., Li concentration in seawater): white, CTRL, $0.18 \mu g \cdot Li \cdot mL^{-1}$; light blue, Li-0.5, $0.5 \mu g \cdot Li \cdot mL^{-1}$; blue, Li-1.0, $1.0 \mu g \cdot Li \cdot mL^{-1}$; and dark blue, Li-1.5, $1.5 \mu g \cdot Li \cdot mL^{-1}$. Pink tones symbol color are from Viana et al.⁷¹ (long-term Li exposure experiment). **(B)** Mussel (symbols as in (A)) and water Li isotopic compositions (orange circles). The dotted line shows the binary mixing curve between natural filtered seawater ($\delta^7 Li = 31.2\text{‰}$) and the Li spike used in the experiment ($\delta^7 Li = 10.8\text{‰}$). For comparison, Li isotopic compositions of *Mytilus eduli* calcite shells collected in Cable Bay, UK, are also reported in green (data from Freitas et al.⁶⁵ and Dellinger et al.³⁵). These mussels were bred at different water temperature from 11 to 20°C, represented by the pale to dark green gradient. The Li concentration and $\delta^7 Li$ value of the seawater in which they grew is not known.

457

458 **Figure 3:** The effect of biological processing on mussel Li isotopic fractionations. $\Delta^7\text{Li}_{\text{mussel-water}}$
 459 values determined in this study (symbol colors as in Fig. 2) as a function of Li/Ca (circles) and
 460 Li/Na (diamonds) ratios in mussels. The red dashed line is the empirical positive correlation
 461 between both parameters (r =correlation coefficients). The Ca/Na ratios measured in mussels
 462 display a restricted range, on average 0.06 ± 0.02 (SD, $n = 38$).

Figure 4: Box model of Li isotope composition in mussel soft tissue. Variables are: $[\text{Li}]$, Li concentration ($\mu\text{g g}^{-1}$ or $\mu\text{g mL}^{-1}$); M_{Li} , Li mass (μg); $\delta^7\text{Li}$, Li isotopic composition (‰); τ_{Li} , Li residence time (d); α , Li isotopic fractionation factor between mussel and water; $\alpha = (^7\text{Li}/^6\text{Li})_{\text{mussel}} / (^7\text{Li}/^6\text{Li})_{\text{water}}$, which relates to $\Delta^7\text{Li}$ as: $\Delta^7\text{Li}_{\text{mussel-water}} = \delta^7\text{Li}_{\text{mussel}} - \delta^7\text{Li}_{\text{water}} \approx 1,000 \times \ln(\alpha)$; α_{in} and α_{out} represent the magnitude of Li isotopic fractionations during uptake and excretion, respectively; and ϕ_{Li} , Li flux ($\mu\text{g d}^{-1}$). Subscripts are: m, mussel; sw, seawater; in, input; and out, output. Using the equations developed in the Supplementary Material, the best average $\delta^7\text{Li}_{\text{m-mod}}$ values (i.e., modeled $\delta^7\text{Li}_{\text{mussel}}$ values) for each experimental condition were determined by minimizing the difference between the modeled and measured $\delta^7\text{Li}$ values (i.e., $\delta^7\text{Li}_{\text{m-mod}} - \delta^7\text{Li}_{\text{m-meas}}$), with α_{out} and α_{in} ranging between 0.9 and 1.

474

475 **Figure 5:** Observed and modeled Li isotopic fractionations in mussels as a function of seawater
 476 Li level. Individual (circles) and average $\Delta^7 Li_{\text{mussel-water}}$ values (diamonds) are reported for the
 477 four experimental conditions. Red circles display the modeled isotope fractionations calculated
 478 by the box model detailed in the text and in the Supplementary Material.

Figure 6: The controls of Li excretion/intake rate and of essential elements on mussel-water Li isotopic fractionation. **(A)** $\Delta^7\text{Li}_{\text{mussel-water}}$ vs. Li enrichment factor (i.e., the ratio of the Li concentration in mussels to that in water, equivalent to the bioaccumulation factor or partition coefficient between mussels and seawater). Symbol colors indicate the experimental conditions as in Fig. 2. Solid lines are intragroup linear regressions. Mussels exposed to relatively Li-poor conditions (CTRL and Li-0.5 groups) show positive slopes, whereas those exposed to Li-rich conditions (Li-1.0 and Li-1.5 groups) define negative slopes. Red lines display model variations and red dots indicate specific R_ϕ values. **(B)** Principal component analysis (PCA) of all mussels (symbols as in (A), shaded ellipses represent intragroup variability). PC1 and PC2 are principal components 1 and 2. Arrow length is proportional to the percentage contribution of these first two principal components to the observed variation of each variable. PC1 and PC2 represent 75% of the variability in the dataset. Variables can be separated into three groups: mussel B, Ca, Fe, K, Mg, Na, and Si concentrations on PC1 and $\Delta^7\text{Li}_{\text{mussel-water}}$, Li_m , and Li_{sw} on PC2.

493 **Table 1:** Compilation of Li concentrations and fluxes estimated for several contexts.

	Sample type	Location and information	Li concentration	Unit	Reference
Environments	Salar draining waters	Chile - Atacama	130000 - 1240000	µg Li/L	BRGM ⁸
		Argentina	600000	µg Li/L	BRGM ⁸
	Geothermal fluids	W USA	200000	µg Li/L	BRGM ⁸
		Italy	480000	µg Li/L	BRGM ⁸
		Mid-oceanic ridges	1000-10000	µg Li/L	Verney-Carron et al., 2015 ⁷²
	Seawater	Pelagic	180	µg Li/L	Broecker, 1982 ⁷³
	Large rivers	Global scale	0.1 - 5	µg Li/L	Huh et al., 1998 ⁷⁴
	Chilean river and tap waters	Azapa, Rio San Jose	90 - 160	µg Li/L	Figueroa et al., 2012 ⁷
		Lluta, Lauca Altiplano	60 - 1830	µg Li/L	Figueroa et al., 2012 ⁷
		Camarones	400 - 5600	µg Li/L	Figueroa et al., 2012 ⁷
		Lauca, Rio Tignamar	20 - 450	µg Li/L	Figueroa et al., 2012 ⁷
		Rio Loa, Salado	1200 - 7700	µg Li/L	Figueroa et al., 2012 ⁷
		Atacama	300 - 2570	µg Li/L	Figueroa et al., 2012 ⁷
	Chilean estuaries	Lluta estuary	1300 - 1580	µg Li/L	Figueroa et al., 2012 ⁷
		Camarones estuary	3500 - 7600	µg Li/L	Figueroa et al., 2012 ⁷
	Urban river	S Korea - Seoul	0.3 - 1.7	µg Li/L	Choi et al., 2019 ¹²
	Wastewaters	S Korea - Seoul	1 - 5	µg Li/L	Choi et al., 2019 ¹²
	Tap water	S Korea - Seoul	0.3 - 0.7	µg Li/L	Choi et al., 2019 ¹²
		Peru - Lima airport	115	µg Li/L	Figueroa et al., 2012 ⁷
		Peru - Pachia	144	µg Li/L	Figueroa et al., 2012 ⁷
		Peru - Pocolla	83	µg Li/L	Figueroa et al., 2012 ⁷
	Mineral waters	Germany	1.7 - 1724	µg Li/L	Seidel et al., 2019 ⁷⁵
	Drinking waters close to Salar	Australia, Chile, Argentina, Bolivia	>1000	µg Li/L	Seidel et al., 2019 ⁷⁵
Humans	Serum	Germany / N Chile	0.03-175	µg Li/L	Seidel et al., 2019 ⁷⁵ , Figueroa et al., 2014 ⁷⁰
	Blood		<60	µg Li/L	BRGM ⁸
	24h Urine	Germany	21 - 1172	µg Li/L	Seidel et al., 2019 ⁷⁵
	Urine	N Chile	140 - 5800	µg Li/L	Figueroa et al., 2014 ⁷⁰
	Toxic threshold in plasma		10000	µg Li/L	Aral and Vecchio-Sadus, 2008 ⁴
	Lethal threshold in plasma		20000	µg Li/L	Aral and Vecchio-Sadus, 2008 ⁴
	Fluxes	Provisory intake recommended by some	1000	µg Li /day	Schrauzer, 2002 ¹
		Li drug (bipolarity)	500000 - 2000000	µg Li/day	Young, 2009 ⁷⁶
		Microdose affecting Alzheimer patients	300	µg Li /day	Andrade Nunes et al., 2013 ⁷⁷
		Li intake by N Chile inhabitants	5000 - 50000	µg Li /day	Figueroa et al., 2013 ⁶⁸
	Tunicates	N Chile - Piure - Coastal Shellfish	33	µg Li/g	Figueroa et al., 2013 ⁶⁸
Marine organisms	Bivalves	Atlantic, Pacific	0.05 - 0.8	µg Li/g	Thibon, Weppe et al., 2020 ²⁴
		N Chile - Oyster - Coastal Shellfish	19	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Clam - Coastal Shellfish	53-83	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Mussel - Coastal Shellfish	111	µg Li/g	Figueroa et al., 2013 ⁶⁸
	Cephalopods	Atlantic, Pacific, Indian ocean	0.05 - 0.2	µg Li/g	Thibon, Weppe et al., 2020 ²⁴
	Gastropods	N Chile - Abalone - Coastal Shellfish	20	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Limpet - Coastal Shellfish	85	µg Li/g	Figueroa et al., 2013 ⁶⁸
	Crustaceans	Atlantic, Pacific	0.1 - 0.3	µg Li/g	Thibon, Weppe et al., 2020 ²⁴
	Fish	Atlantic, Pacific, Indian ocean	0.03 - 0.5	µg Li/g	Thibon, Weppe et al., 2020 ²⁴
		N Chile - Frog fish	18	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Barred Hogfish	20	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Bass	25	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Flounder	51	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - Shark	68	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - H macrophthalmos	86	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - White seabream	99	µg Li/g	Figueroa et al., 2013 ⁶⁸
		N Chile - John Dory	103	µg Li/g	Figueroa et al., 2013 ⁶⁸

495 ASSOCIATED CONTENT

496 **Supporting Information.**

497 Supplementary Material is available and contents supplementary figures, data tables, and box
498 model equations.

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504 **Author Contributions**

505 N. Vigier and M. Metian led the project. F. Oberhänsli, E. Vassileva, and A.M. Orani performed
506 sample preparation and Li concentration measurements. M. Montanes, N. Vigier, and P. Telouk
507 performed the sample Li purification and Li isotopic ratio measurements. F. Thibon modeled the
508 data and wrote the original draft of the manuscript. F. Thibon, N. Vigier, M. Metian, and P.
509 Swarzenski contributed to review and editing the manuscript. All authors have given approval to
510 the final version of the manuscript.

511

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519 TOC GRAPHIC

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