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 ($^{7}Li/^{6}Li$) 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 element[1]. However, as observed for other metals[2,3], Li is toxic and can eventually be lethal beyond a specific threshold [4–6]. Elevated levels of Li in the aquatic environment can be due to natural inputs, mainly from rock erosion[7], 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 2010–2017[8–10], and the demand for Li is expected to follow the greatest growth trajectory among all metals by 2025[9]. Li contamination has already been observed in groundwater and river water with close proximity to mining sites[11], as well as in municipal waters of a modern and densely populated metropolitan area[12]. This is a worrying problem as the various protocols of wastewater depuration appear to be inefficient[12] at removing Li because of the high mobility of Li and its poor ability to adsorb onto particle surfaces[13] (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 metals[18–20]. For instance, mussel-based biomonitoring programs[21–23] 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 levels[24]. 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 (^{6}Li and ^{7}Li, 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 tissues[29]. 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 extinction[39].

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 um) 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 ± 40

97 min). Due to the paucity of data on Li bioaccumulation process, we decided (1) to investigate the
98 short time process in order to better understand the short term isotope fractionation and uptake
99 processes assuming that Li should act like other alkali elements (i.e., Cs, which quickly reaches a
100 saturation state when accumulated in marine bivalves[40–42]) and (2) to use a relatively high
101 exposure concentration based on data available in freshwater studies[4,43]. Seawater samples were
102 collected before and after each water change to confirm that each aquarium operated as an open
103 system. Between water changes, the mussels were fed for 1 h using non-contaminated seawater
104 containing phytoplankton *Isochrysis galbana* at a concentration of 10^4 cells mL^{-1}. One mussel
105 died after 2 days of the experiment in the duplicate A of the CTRL condition. At the end of the
106 experiment, all individuals were collected and rinsed for 30 min in clean, filtered seawater
107 following the classical protocol for eliminating weakly adsorbed elements from tissue samples[44].
108 The soft tissues were then extracted, frozen at –28 °C for 72 h, and freeze-dried for further
109 analyses.
110
111 ***Analytical methods***
112 To minimize the possibility of any contamination and procedural blanks, all analytical
113 preparations were performed in a positively pressurized clean laboratory under a fume-hood
114 using only distilled, trace-metal grade reagents and pre-cleaned vessels. Each mussel's soft tissue
115 was weighed in a Teflon reactor, then digested in a mixture of 5 mL HNO_3 and 2 mL H_2O_2 (both
116 trace metal grade, Fisher Scientific, USA) at 90 °C for ~5 h. The obtained solutions were
117 transferred into pre-cleaned polyethylene tubes and gravimetrically diluted up to 20 g with
118 MilliQ water (18 MΩ). Two procedural blanks and two replicates of the Certified Reference
119 Material (CRM) IAEA 407 (Fish Homogenate, IAEA, Vienna) were included in the digestion

batch and analyzed with the samples. The recoveries obtained for the CRM were 95±3 %. For each sample, two solution aliquots were separated for elemental and isotopic analyses.

Lithium concentrations were determined at the IAEA in Monaco by flame atomic absorption spectroscopy using a ContrAA 700 (Analytic Jena, Germany). The applied analytical procedure was based on external calibration approach and was preliminary validated, according to the requirements of international guidelines for method validation[45]. Calibration curves were daily prepared using proper dilution of commercially available standard solutions. All possible sources of uncertainty of obtained results were carefully identified. The numerical method of differentiation described by Kragten [46] was used to calculate combined standard uncertainties of obtained measurement results.

Prior to Li isotopic analyses, the Li in the samples must be purified. Lithium extraction and purification were performed following the procedure of Vigier *et al.* [47], summarized here. Sample solution aliquots were dried out and taken up in 0.5 mL of 1 N HCl and loaded on AG 50-X12 cationic resin in 8.5-cm-high Teflon columns. Lithium was purified twice with 1 N HCl. This fraction was evaporated to dryness before the analysis.

Lithium has two naturally occurring isotopes, one heavy (^{7}Li, 92.41% abundance) and one light (^{6}Li, 7.59% abundance). The isotopic compositions of geological and biological materials are reported as per-mil variations relative to an isotopic standard (here LSVEC) as $\delta^{7}Li_{sample}$ [‰] = $(((^{7}Li/^{6}Li)_{sample} / (^{7}Li/^{6}Li)_{LSVEC} - 1) \times 1{,}000$.

Lithium isotopic analyses were performed at the CNRS-INSU National Service based at the École Normale Supérieure de Lyon (France) using a Thermo-Fisher Neptune *Plus* multi-collector inductively coupled plasma mass spectrometer following the procedure of Balter and Vigier[29] and Bastian *et al.*[48] developed for low Li concentrations in biological materials. In brief,

143 samples and standards were analyzed under dry plasma conditions in low-resolution mode.
144 Instrumental mass bias correction was performed externally using the standard bracketing
145 method (using the LSVEC reference material). Analytical blanks, as well as total procedural
146 blanks, were found to be negligible. The internal reproducibility was on average ±0.04‰.
147 Precision (external reproducibility), estimated from two to three replicate measurements of 14
148 different samples (mussels and water), ranged from 0.04 to 1.91‰ with an average of ±0.5‰. In
149 general, the accuracy of reported isotopic compositions are estimated based on analyses of
150 reference materials of known isotopic composition. However, the Li isotopic compositions of
151 biological reference materials corresponding to soft tissue have never been characterized. Thus,
152 this study reports for the first time the Li isotopic composition of a soft-tissue biological
153 reference material IAEA 407 (δ^7Li = 28.3 ± 1.7‰, 2SD, n = 2). For comparison, the
154 reproducibility of δ^7Li obtained for the Li7-N non-biological reference material was 0.4‰ (2SD,
155 n = 22)[48]. Note that the IAEA 407 contain 0.68 ± 0.1 µg Li g^{-1} of dry powder, while Li7-N is an
156 isotopically homogeneous solution, analyzed at a Li concentration of 4 ng mL^{-1}.
157
158 ***Statistical treatment***
159 Since the Li data were not normally distributed (i.e., Li concentration and Li isotope ratios do not
160 follow a normal law), we adopted non-parametric statistical tests. The Mann-Whitney-Wilcoxon
161 test was used to compare median values between two observational series, and the Kruskal-
162 Wallis test was used to compare three or more series. Tests were performed using R software[49]
163 using the respective functions *wilcox.test* and *kruskal.test*. The level of significance for statistical
164 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 software [49] 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 $\Delta^7Li_{mussel\text{-}water}$.

Results

In the control group 'CTRL', the aquarium seawater Li concentration was 0.18 µg mL^{-1}, which corresponds to the mean global ocean Li concentration[50]. Li concentrations in seawater were set at 0.5, 1.0, and 1.5 µg 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 ± 1.65 µg 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 (δ^7Li = 31.2 ± 0.3‰ in the open ocean[50,51]) and the pure Li solution (δ^7Li = 10.8‰) used to prepare the three exposure conditions, the mussels in each group were exposed to seawater with different Li isotopic compositions (δ^7Li_{sw}, which remained constant over the course of the experiment; Table S1): $\delta^7Li_{sw}^{CTRL}$ = 31.5 ± 0.7‰ (2SD, *n* = 5), $\delta^7Li_{sw}^{Li\text{-}0.5}$ = 18‰ (calculated), $\delta^7Li_{sw}^{Li\text{-}1.0}$ = 14.6 ± 0.01‰ (2SD, *n* = 2),

and $\delta^7Li_{sw}^{Li\text{-}1.5}$ = 13.4 ± 0.1‰ (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 δ^7Li value.

The average δ^7Li of mussel soft tissues in groups Li-1.0 and Li-1.5 were 21.0 ± 2.6‰ and 21.6 ± 2.7‰, respectively (Table S2, Fig. 1B). The one in group Li-0.5, significantly higher than the latest (24.4 ± 3.4‰, Wilcoxon test, $p < 0.001$) was significantly lower than that in the control group (31.7 ± 2.0‰, 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 ± 0.18 µg g^{-1}) were consistent with that observed in bivalve tissues and organs collected along the French Atlantic coast (0.43 ± 0.25 µg 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 µg mL^{-1}), by a factor of 2.5 on average. This agrees well with a recent study[24] 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 ±

1.65 µg g^{-1} when the water Li content is 1.5 µg 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 contamination[53]. 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 ± 0.23 µg g^{-1} and 1.4 ± 0.23 µg 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 Cu[18]. 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

234 contaminants measured in bivalves[21–23]. Based on the range of seawater Li concentration used in
235 this study, we estimate that accurate Li concentration can be obtained with the analyses of at
236 least five individuals, and likely more when water Li concentration exceeds 1.5 µg mL^{-1}, such as
237 in Northern Chili (since the variability increases with the Li level, Fig. 2). Existing
238 biomonitoring programs usually collect and pool between 80 and 160 individuals per site[54] , so
239 the use of mussels to monitor Li levels along with other contaminants in coastal areas appear
240 largely feasible.
241
242 ***Preferential ^{7}Li enrichment in mussels***
243 While the seawater in experimental aquaria have a constant and homogenous $\delta^{7}Li$ value for each
244 condition, exposed mussels are 'isotopically fractionated' compared to their aquarium water, i.e.
245 with significantly higher $\delta^{7}Li$ values (Fig. 2B). This indicates that mussels preferentially
246 accumulate the heavy (^{7}Li) isotope in their soft-parts, as compared to the light (^{6}Li) isotope. It is
247 possible to calculate the corresponding water-mussel Li isotopic fractionations ($\Delta^{7}Li_{mussel\text{-}water}$)
248 using[55]:
249 $$\Delta^{7}Li_{mussel\text{-}water}\ (‰) = \delta^{7}Li_{mussel} - \delta^{7}Li_{water} \quad (1)$$
250 $\Delta^{7}Li_{mussel\text{-}water}$ value near 0‰ indicates no Li isotopic fractionation, since mussel $\delta^{7}Li$ is equal to
251 water $\delta^{7}Li$.
252 At the end of the exposition, the average $\Delta^{7}Li_{mussel\text{-}water}$ ranges from 0.0‰ to +10.3‰ (Fig. 2B).
253 Mussels in the control group exhibit a mean $\delta^{7}Li$ value similar to that of the seawater (with
254 $\Delta^{7}Li_{mussel\text{-}water}$ = +0.4 ± 0.9‰, SD, n = 8), whereas the average $\Delta^{7}Li_{mussel\text{-}water}$ values of the other
255 experimental groups increase from +5.7 ± 2.1‰ to +7.5 ± 3.2‰ (from Li-0.5 to Li-1.5
256 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 δ^7Li 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 δ^7Li 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 ^{7}Li (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 ^{6}Li [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 $\Delta^7Li_{mussel\text{-}water}$ (Fig. 3). When the Li/Na and Li/Ca ratios are low, the observed Li isotopic fractionations are small or negligible ($\Delta^7Li_{mussel\text{-}water} \approx 0‰$), whereas the highest $\Delta^7Li_{mussel\text{-}water}$ values correspond to the highest Li/Na and Li/Ca ratios. This result confirms the biological coupling between Li and Na [63] 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 δ^7Li 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 temperatures[65]. They all show δ^7Li values noticeably higher than the δ^7Li 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 δ^7Li

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 spectrometer[29], 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 (Φ^{Li}_{out}) must have been lower than its uptake flux (Φ^{Li}_{in}), such that:

$$R_f = \frac{f^{Li}_{out}}{f^{Li}_{in}} < 1 \qquad (2)$$

As shown in Fig. 2B, and described above, mussel δ^7Li values are systematically higher than seawater δ^7Li values. However, due to kinetic effects, Li transport through cell membranes is rather expected to favor the light isotope ^{6}Li[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: $\Delta^7Li \approx 1{,}000 \times \ln(\alpha)$. If both, intake (input) and excretion (output) processes favor the light (^{6}Li) isotope, then both α_{in} (input) and α_{out} (output) are lower than 1 (Δ^7Li <0). In that case, mussel ^{7}Li enrichments are possible only if ^{6}Li excretion flux is greater than ^{6}Li uptake flux over the experimental duration (i.e., if $\alpha_{out} < \alpha_{in,}$ see Fig. 4 for more details). As the degree of ^{7}Li 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 (α_{out} / α_{oin}) 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 δ^7Li 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 δ^7Li_{mussel} variability displayed by individuals from the same experimental group. For each condition, individual $\Delta^7Li_{mussel\text{-}water}$ values correlate linearly with the Li enrichment factor (Li_m/Li_{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, Li_m/Li_{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 $\Delta^7Li_{mussel\text{-}water}$ increases when the individual enrichment factor increases. On the other hand, mussels exposed to elevated seawater Li concentrations (> 1µg mL^{-1}) (Li-1.0 and Li-1.5 groups) display negative slopes (decreasing $\Delta^7Li_{mussel\text{-}water}$ with the enrichment factor). There is therefore a Li threshold between 0.5 and 1 µg mL^{-1}, above which mussels change their Li biological regulation. Under a significant but limited Li contamination (<1.0 µg 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 µg 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 $\Delta^7Li_{mussel\text{-}water}$, Li_m, and Li_{sw}, which correlate on the principal component 2. Figure 6B shows that the "intergroup" variability is discriminated mostly by $\Delta^7Li_{mussel\text{-}water}$, 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 μg L^{-1} (0.5 and 1 μg 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 μg 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 μg L^{-1} (100-1000 times more than other estuaries worldwide). A published compedium[68] 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 Islands[24]. 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 μg 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

393 essentially an active process[57], passing this threshold may trigger abnormal growth patterns due
394 to chemical stress and damage induced by Li [69].
395 Overall, these results highlight the need to monitor the evolution of these 'Li-rich' ecosystems,
396 for both their own sustainability, as well as for the local economy and human health. Inhabitants
397 of the Chilean coast consume 8 to 15 times more seafood and shellfish than inland populations,
398 while the proportion of other food types such as meat or vegetables are significantly less variable
399 between the two population groups [68]. This supports the need to quantify and better understand
400 how these coastal marine organisms bioaccumulate Li. The high Li concentrations measured in
401 North Chilean shellfish, fish, and drinking waters explain the high Li concentration measured in
402 the blood (serum), urine, and other tissues collected from the local population[70]. The effects –
403 negative or positive - of these high Li levels in the diet are less known. Figuorea *et al.*[68]
404 estimated that the local population eats about 20 g day^{-1} of shellfish and seafood. With a Li
405 concentration of 100 μg g^{-1}, as found in local mussels (Table 1), the Li intake is already 2000 μg
406 day^{-1} (twice the dose recommended by Schrauzer[1]). Given that vegetables, cereals, and waters
407 are also enriched in Li in this area, the Li intake by people living along North Chilean coast is
408 estimated to be close to the medical dose given to patient treated for neurodegenerative disease
409 (Table 1).
410 In other places in the world, the number of data measured in coastal waters and marine
411 organisms remains scarce, and it is difficult to estimate the impact of Li mining and
412 anthropogenic activities at the global scale. A recent study points that Li is not removed during
413 the waste water treatment plant process[12]. As described in the introduction, a significant level of
414 Li pollution has been documented in the Han River crossing Seoul[12]. The corresponding Li level
415 is still low compared to what is contained in North Chilean waters, but the predicted considerable

416 increase of Li production for the next 10 years, along with the development of popular high
417 technology objects, may significantly increase Li levels in coastal environments if its recycling is
418 not massively developed.
419

420 **Conclusions**

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

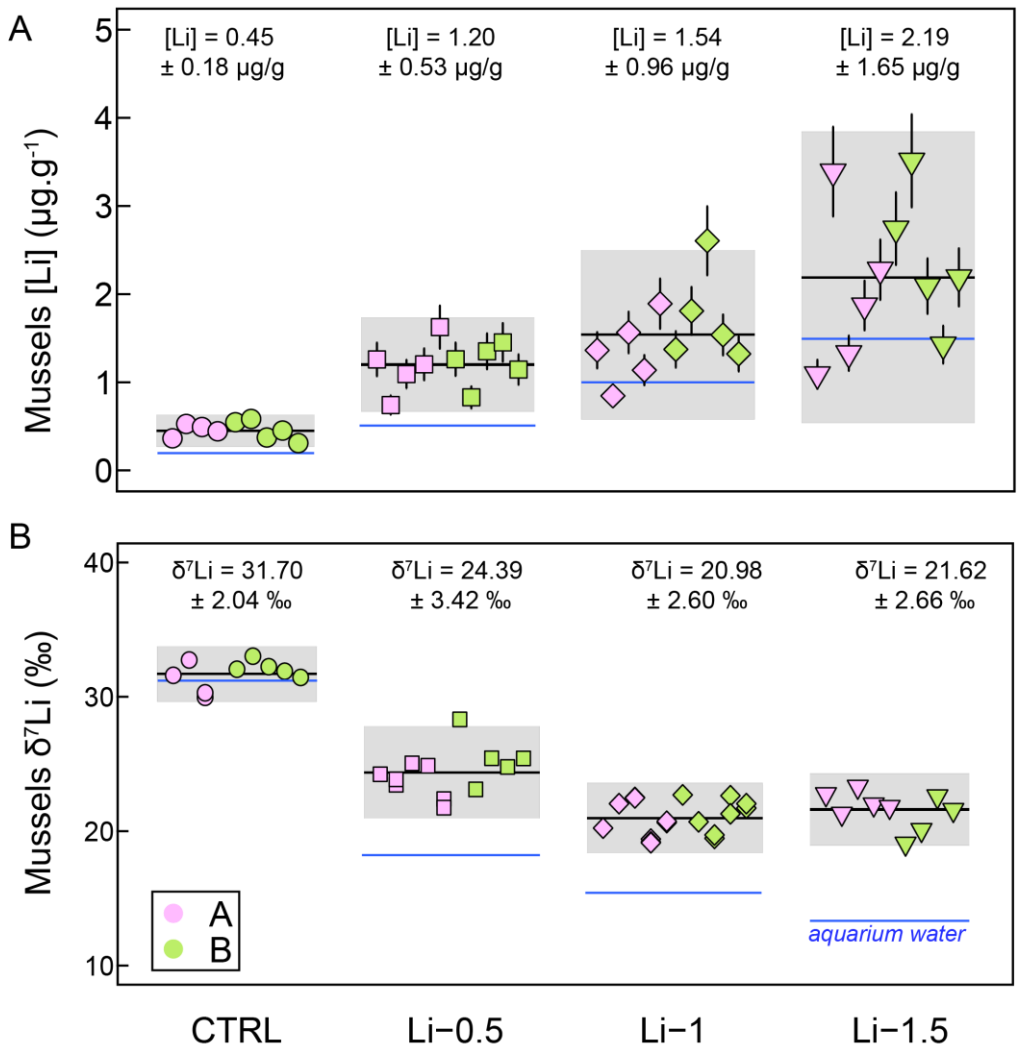
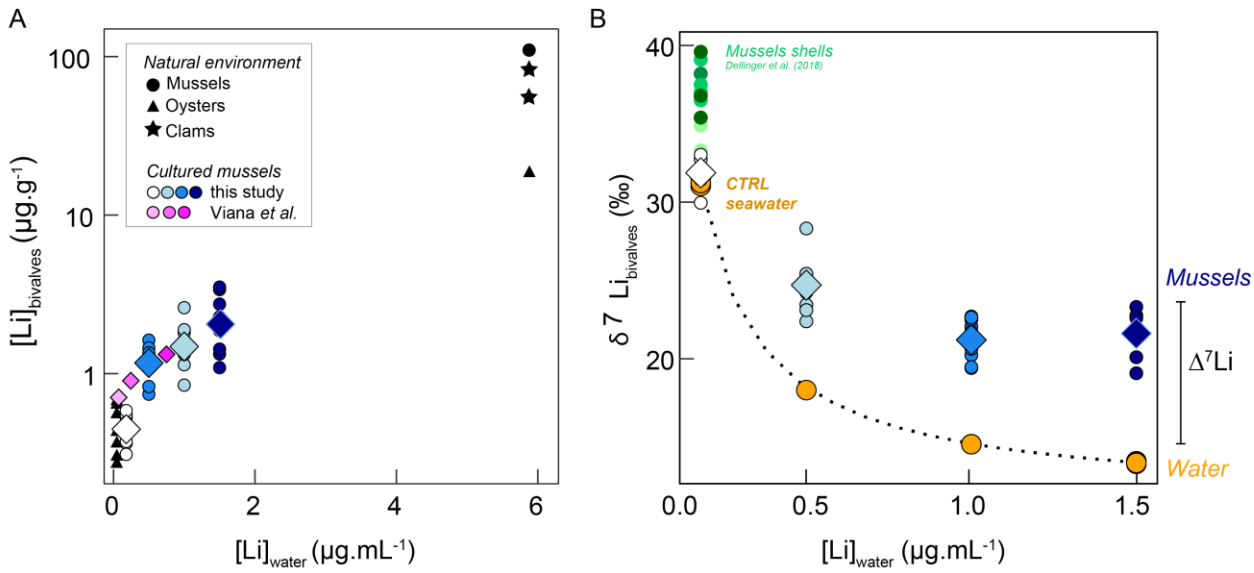
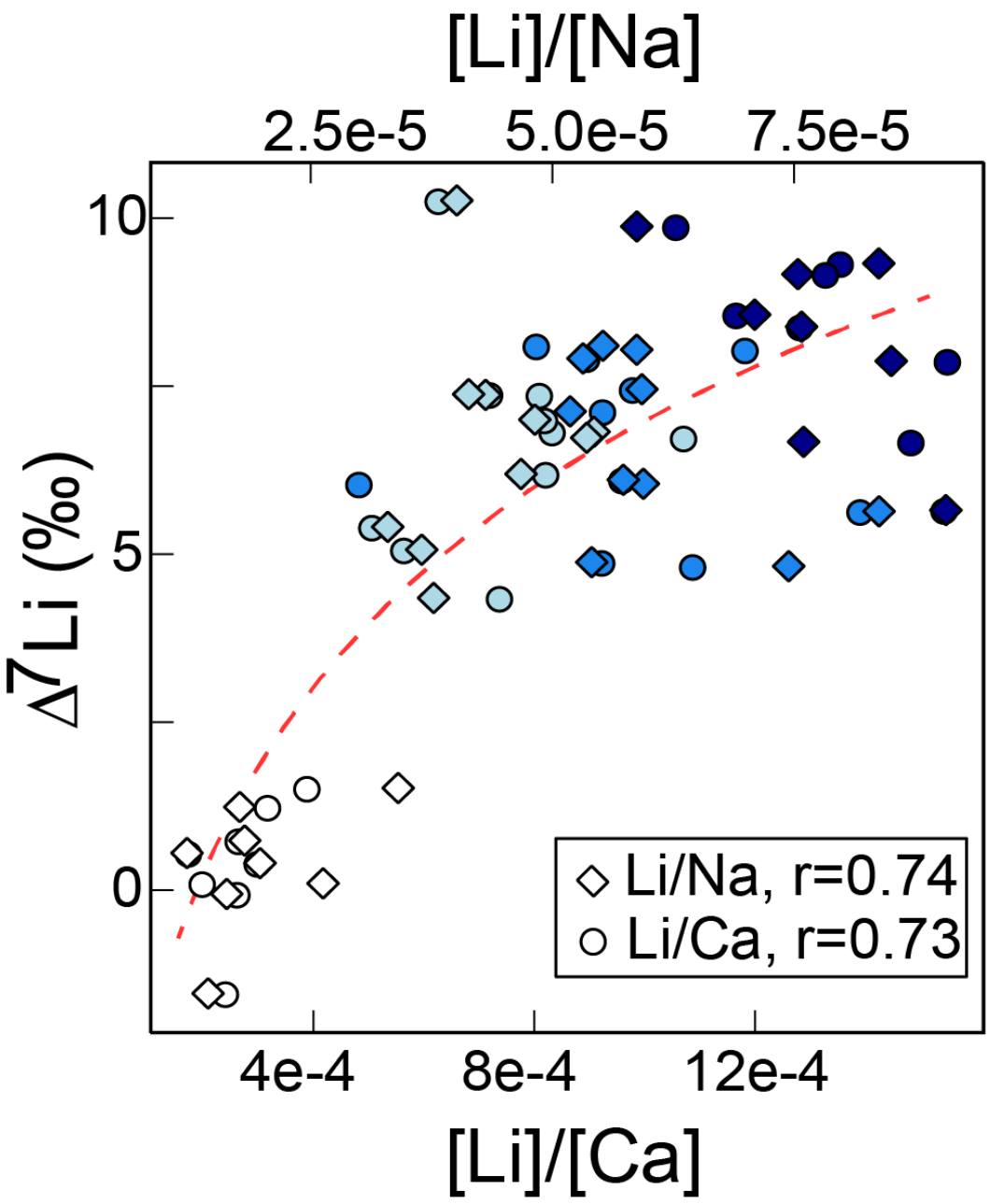


Figure 1: Li concentrations ([Li]) and isotopic compositions (δ^7Li) of mussel soft tissues as a function of water Li level. Blue mussels were exposed during 4 days to seawater with [Li] = 0.18 µg mL^{-1} (natural seawater, control group CTRL), 0.5 µg mL^{-1} (Li-0.5), 1.0 µg mL^{-1} (Li-1.0), or 1.5 µg mL^{-1} (Li-1.5). A (pink) and B (green) are the two experimental replicates. Reported errors are ±2SD (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 δ^7Li (blue lines). **(A)** The variability of mussel Li concentrations increases from 44%(CTRL) to 75% (Li_1.5).



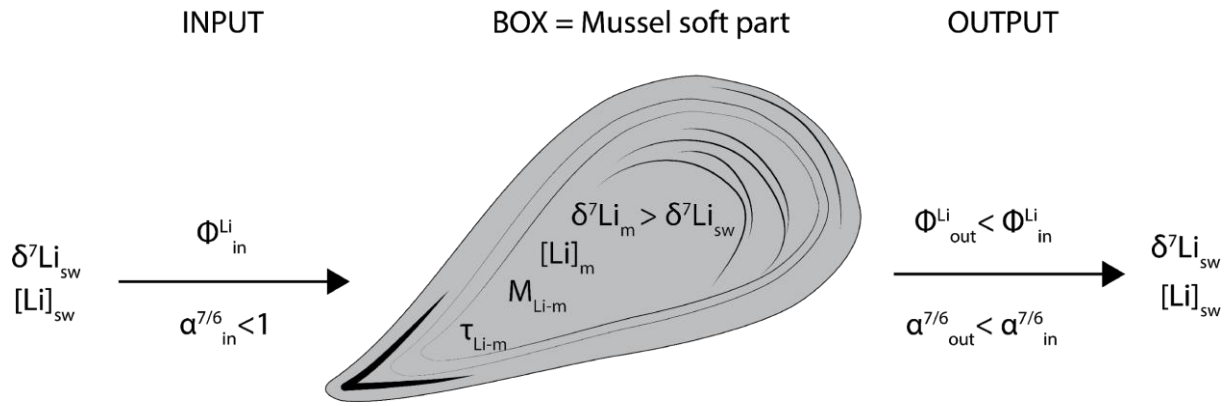
441

442 **Figure 2**: Observed Li bioconcentration and Li isotopic fractionation displayed by blue mussels
443 as a function of seawater Li concentration. **(A)** Mussel Li concentrations: circles and diamonds
444 indicate individual analyses and average values per experimental group, respectively. Black
445 symbols are for bivalves collected in their natural environment (the Bay of Biscay for those at
446 0.18 µg mL^{-1} $[Li]_{water}$[24], and Rio Camarones in Chile for those at 6 µg mL^{-1} $[Li]_{water}$[68]). Blue
447 tones symbol color indicates experimental group (i.e., Li concentration in seawater): white,
448 CTRL, 0.18 µg Li mL^{-1}; light blue, Li-0.5, 0.5 µg Li mL^{-1}; blue, Li-1.0, 1.0 µg Li mL^{-1}; and
449 dark blue, Li-1.5, 1.5 µg Li mL^{-1}. Pink tones symbol color are from Viana et al.[71] (long-term Li
450 exposure experiment). **(B)** Mussel (symbols as in (A)) and water Li isotopic compositions
451 (orange circles). The dotted line shows the binary mixing curve between natural filtered seawater
452 (δ^7Li = 31.2‰) and the Li spike used in the experiment (δ^7Li = 10.8‰). For comparison, Li
453 isotopic compositions of *Mytilus eduli* calcite shells collected in Cable Bay, UK, are also
454 reported in green (data from Freitas *et al.* [65]and Dellinger *et al.*[35]). These mussels were bred at
455 different water temperature from 11 to 20°C, represented by the pale to dark green gradient. The
456 Li concentration and δ^7Li value of the seawater in which they grew is not known.



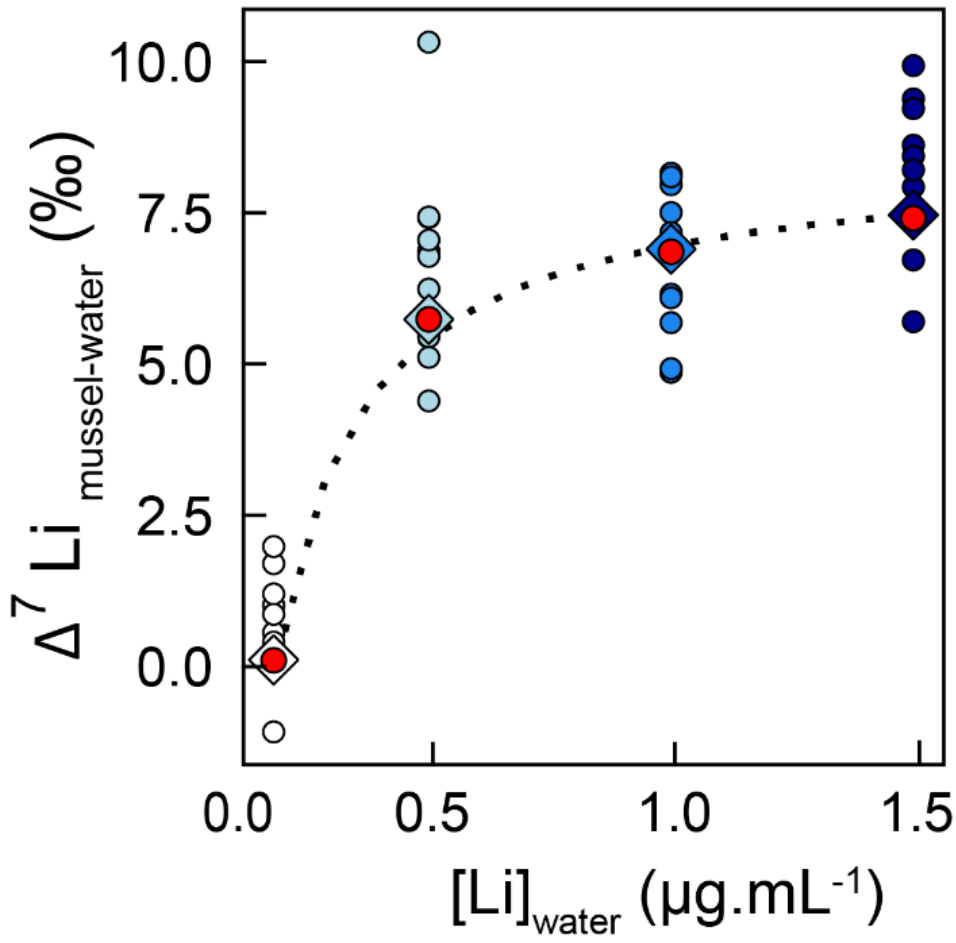
457

458 **Figure 3**: The effect of biological processing on mussel Li isotopic fractionations. $\Delta^7Li_{mussel\text{-}water}$
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).



463

464 **Figure 4**: Box model of Li isotope composition in mussel soft tissue. Variables are: [Li], Li
465 concentration (μg g^{-1} or μg mL^{-1}); M_{Li}, Li mass (μg); δ^7Li, Li isotopic composition (‰); τ_{Li}, Li
466 residence time (d); α, Li isotopic fractionation factor between mussel and water; α =
467 $(^7Li/^6Li)_{mussel}$ / $(^7Li/^6Li)_{water}$, which relates to Δ^7Li as: $\Delta^7Li_{mussel\text{-}water} = \delta^7Li_{mussel} - \delta^7Li_{water} \approx 1{,}000$
468 × ln(α); α_{in} and α_{out} represent the magnitude of Li isotopic fractionations during uptake and
469 excretion, respectively; and ϕ_{Li}, Li flux (μg d^{-1}). Subscripts are: m, mussel; sw, seawater; in,
470 input; and out, output. Using the equations developed in the Supplementary Material, the best
471 average $\delta^7Li_{m\text{-}mod}$ values (i.e., modeled δ^7Li_{mussel} values) for each experimental condition were
472 determined by minimizing the difference between the modeled and measured δ^7Li values (i.e.,
473 $\delta^7Li_{m\text{-}mod} - \delta^7Li_{m\text{-}meas}$), 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 $\Delta^7Li_{mussel\text{-}water}$ 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.

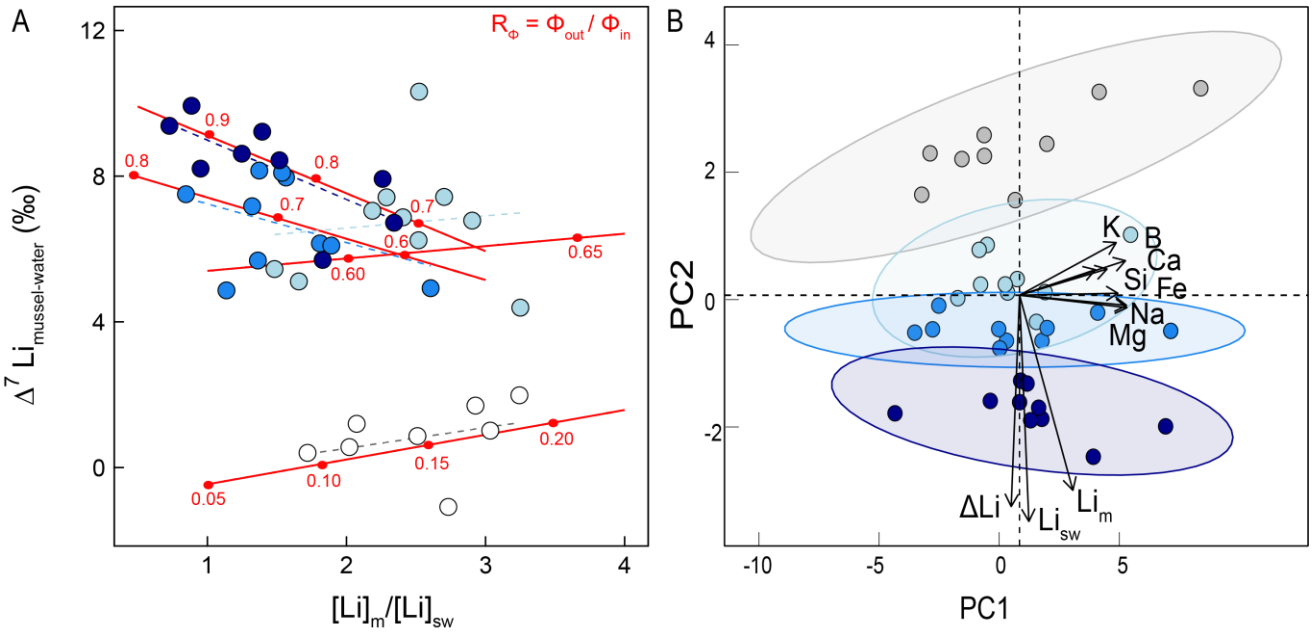


Figure 6: The controls of Li excretion/intake rate and of essential elements on mussel-water Li isotopic fractionation. **(A)** $\Delta^7Li_{mussel-water}$ vs. Li enrichment factor (i.e., the ratio of the Li concentration in mussels to that in water, equivalent to the bioaccumulation factor or partition 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 $\Delta^7Li_{mussel-water}$, Li_m, and Li_{sw} on PC2.

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	Autralia, 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]
Marine organisms	Tunicates	N Chile - Piure - Coastal Shellfish	33	µg Li/g	Figueroa et al., 2013[68]
	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 macrophtalmos	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]

ASSOCIATED CONTENT

Supporting Information.

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

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Author Contributions

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

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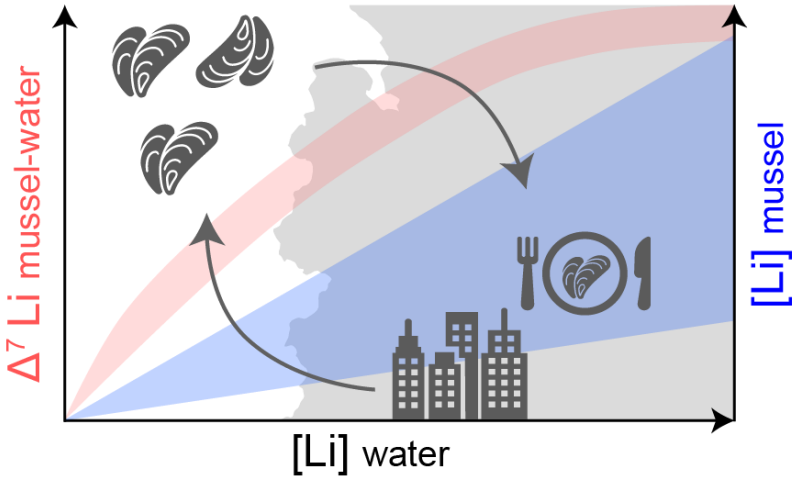
519 TOC GRAPHIC

520 

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