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Large-scale survey of lithium concentrations in marine organisms

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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Li is bio-reduced along the marine trophic webs.
- Organs involved in osmoregulation processes display the highest Li concentrations.
- The studied parameters are trophic group, habitat, location, and taxonomical group.
- All investigated ecological parameters are necessary to explain Li variability.

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ABSTRACT

Trace metals such as Cu, Hg, and Zn have been widely investigated in marine ecotoxicological studies considering their bioaccumulation, transfer along trophic webs, and the risks they pose to ecosystems and human health. Comparatively, Li has received little attention, although this element is increasingly used in the high-tech, ceramics/glass, and medication industries. Here, we report Li concentrations in more than 400 samples, including whole organisms and different organs of bivalves, cephalopods, crustaceans, and fish. We investigated species from three contrasting biogeographic areas, i.e. temperate (Bay of Biscay, northeast Atlantic Ocean), tropical (New Caledonia, Pacific Ocean), and subpolar climates (Kerguelen Islands, southern Indian Ocean), among diverse trophic groups (filter-feeders to meso-predators) and habitats (benthic, demersal, and pelagic). Although Li is homogeneously distributed in the ocean (at 0.18 µg/mL), Li concentrations in soft tissues vary greatly, from 0.01 to 1.20 µg/g dry weight. Multiple correspondence analyses reveal two clusters of high and low Li concentrations. Li distributions in marine organisms appear to be mostly geographically independent, though our results highlight a temperature dependency in fish muscles. Li is consistently bio-reduced through the trophic webs, with filter-feeders showing the highest concentrations and predatory fish the lowest. Strong variations are observed among organs, consistent with the biochemical similarity between Na and Li during transport in the brain and in osmoregulatory organs. Fish gills and kidneys show relatively high Li concentrations (0.26 and 0.15 μ g/g, respectively) and fish brains show a large range of Li contents (up to 0.34 μ g/g), whereas fish liver and muscles are Li depleted (0.07 ± 0.03 and $0.06 \pm 0.08 \mu g/g$, respectively). Altogether, these results provide the first exhaustive baseline for future Li ecotoxicology studies in marine coastal environments.

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1. Introduction

Numerous trace metals are released to rivers, coastal areas, and ultimately the oceans by human activities, and their behaviors in many marine taxa such as bivalves, cephalopods, crustaceans, and fish have been thoroughly investigated in past decades (e.g. Bustamante et al., 2003; Eisler, 2010; Metian et al., 2013). However, lithium (Li) concentrations in marine organisms have received little attention, despite the exponentially increasing use of this element in high-tech industries due to its unique physicochemical properties. Li in the oceans is dominantly derived from two natural sources, i.e. high-temperature hydrothermal fluxes at mid-ocean ridges and river inputs. It exits the ocean mainly via the formation of marine authigenic aluminosilicate clays on the seafloor (Chan et al., 2006). Due to its long oceanic residence time (~1.2 million years) and its weak capacity to adsorb onto marine particles (Decarreau et al., 2012), Li is homogeneously distributed throughout the water column (Misra and Froelich, 2012). Thus, the oceanic concentration of dissolved Li is constant at $0.183 \pm 0.003 \,\mu\text{g/mL}$, irrespective of latitude and depth (Aral and Vecchio-Sadus, 2011; Misra and Froelich, 2012; Riley and Tongudai, 1964).

Worldwide anthropogenic Li production has strongly increased in the past decade (from 28 to 43 thousand tons between 2010 and 2017), with its growing use in several industries (Labbé and Daw, 2012; USGS, 2017). Thus, Li has the highest predicted annual growth rate among metals during 2017–2025 (+18%), far surpassing cobalt (+8.8%; Leguérinel et al., 2018). Indeed, Li-ion batteries are expected to account for more than 80% of the total Li market in 2025, although Li in batteries has the worst recycling recovery rate compared to Al, Co, Cu, Mn, and Ni (Harper et al., 2019). Li from waste-disposal practices in the USA contaminates groundwater at levels toxic to some freshwater organisms (50–170 µg/L) and far above Li concentrations in most rivers in the USA and worldwide (\sim 1.5 µg/L) (e.g. Huh et al., 1998; Kszos and Stewart, 2003). Li contamination also results from the use of agricultural amendments (Millot et al., 2010; Négrel et al., 2010). Recently, a positive correlation was reported between population density and Li content in the Han River and tap waters of the megalopolis of Seoul (Korea), highlighting the first known large-scale Li pollution in an urban area (Choi et al., 2019). Consequently, considerable further anthropogenic contributions of Li to the environment are expected in the coming decades, raising the necessity to understand the role and impact of Li in coastal and marine ecosystems.

It remains unclear how Li affects ecosystems. Li may be somewhat beneficial to organism health, as is the case for humans (Giotakos et al., 2013; Liaugaudaite et al., 2017; Ohgami et al., 2009; Schrauzer and Shrestha, 1990): some authors consider Li to be an essential trace element, recommending a daily Li intake of 14.3 µg/kg (Schrauzer, 2002). Indeed, since 1949, various forms of Li (mostly Li₂CO₃) have been used as the major treatment for bipolar disorder because Li presents a neuroprotective effect (Machado-Vieira, 2018; Szklarska and Rzymski, 2018; Won and Kim, 2017). However, Li₂CO₃ is poisonous beyond the maximum therapeutic dose (10.4 mg/L in blood; Timmer and Sands, 1999) and may lead to death at concentrations above 20 mg/L (Aral and Vecchio-Sadus, 2008; Schou et al., 1968). Above a given critical concentration, Li is also toxic to aquatic organisms, affecting some of their metabolic functions. For instance, Li-enriched seawater disrupts embryogenesis in urchins, zebrafish, and amphibians (Hall, 1942; Kao et al., 1986; Kiyomoto et al., 2010; Stachel et al., 1993).

The bioconcentration of trace elements is a well-known process (Bryan, 1979) that depends on their bioavailability in a given media and their bioaccessibility in trophic sources. Thus, the chemical form of an element (dissolved, particulate, adsorbed, or complexed) influences its capacity for absorption and assimilation by living organisms. Furthermore, the concentrations of some contaminants increase along trophic levels (Atwell et al., 1998). Such biomagnification is well documented in various food webs around the world for Hg and organic contaminants (Kelly et al., 2007). In the ocean and coastal areas, abiotic

(temperature, salinity, pH) and biotic factors (physiological characteristics, habitats, trophic ecology) can also affect organism metal accumulation capacities. To date, Li concentrations in marine organisms and their controlling factors have not been intensively investigated (Ansari et al., 2004; Luoma, 1983). Although significant differences in Li concentrations have been observed between selected marine species and organs (English et al., 2015; Guérin et al., 2011; Noël et al., 2012), changes of Li concentrations along the trophic webs remains unknown.

The aims of the present study are (1) to establish a biogeochemical baseline for future ecotoxicological studies in marine and coastal environments, and (2) to provide a preliminary assessment of the behavior of Li in food webs. Thus, we provide an extensive dataset of Li concentrations across different marine groups (bivalves, cephalopods, crustaceans, and fish) and assess their variations in terms of ecological and geographical factors. We analyzed organs (brain, digestive gland, gills, gonads, kidneys, liver, and muscles) and whole organisms of 33 species from three climatically contrasted zones (tropical: New Caledonia; temperate: Bay of Biscay; subantarctic: Kerguelen Islands).

2. Materials and methods

2.1. Sampling

For our analyses, we selected 33 species from three distinct biogeographic areas: the Bay of Biscay (northeast Atlantic Ocean), New Caledonia (western tropical Pacific Ocean), and the Kerguelen Islands (southern Indian Ocean; see Supplementary Fig. S1). These areas were chosen to explore the effect of climate on Li bioaccumulation in marine organisms: the Bay of Biscay represents a temperate system, New Caledonia a tropical system, and the Kerguelen Islands a subpolar environment. Species were carefully selected to be comparable across the biogeographic areas in terms of taxonomy, position in the water column (habitat), and trophic ecology.

Samples from the Bay of Biscay were bought from fishermen at the local seafood market in La Rochelle (France) between October 2018 and March 2019. Pelagic organisms from the Kerguelen Islands were collected on cruises of the RV "La Curieuse" in February 1998 (austral summer) with a IYGPT trawl (International Young Gadoid Pelagic Trawl, opening $12 \times 7 \text{ m}^2$) with 10 mm mesh size in the cone, while benthic fish were collected from net fishing at the same period. Samples from New Caledonia were collected in March and October 2007. The samples from Kerguelen and New Caledonia were gathered from a former laboratory collection and have been the subject of several previous studies focusing on other trace elements (Bustamante et al., 2003; Chouvelon et al., 2008; Cipro et al., 2018; Metian et al., 2013). The analyzed organisms included molluscs (bivalves and cephalopods), crustaceans (Malacostraca), and fish (Chondrichthyes and Actinopterygii). The organisms were divided into trophic groups to assess the influence of food and feeding ecology characteristics on Li bioaccumulation (Metian et al., 2013; Cipro et al., 2018). We defined the trophic groups according to feeding mode (filter-feeders, grazers/scavengers, predators) and preferred predator diet (i.e., invertebrates, small fish, or both). In addition, the trophic level of each fish species (Froese and Pauly, 2020) was used to better define any relationship between an organism's Li content and its position in the food webs. These trophic levels are based on the organism diet and the trophic level of their food items. They are estimated using nitrogen isotopes ($\delta^{15}N)$ to quantitatively position species in their respective food webs (Kline and Pauly, 1998).

Immediately after sampling, all organisms were weighed (wet weight, ww) and measured (total length, i.e., from the head to the base of the caudal fork for fish, mantle length for squids, shell width for bivalves, and carapace length for some crustaceans). The collected species and their weights (wet and dry) and lengths are reported in Supplementary Table S1. Fish were dissected in a clean laboratory to sample the dorsal muscle, liver, kidneys, gills, and brain. Dissected organs of the king scallop Pecten maximus were the adductor muscle, gonads, digestive gland, and gills, whereas only the muscles and gonads of the glory scallop Mimachlamys gloriosa were collected. Whole Pacific oysters Crassostrea gigas from two brands, "Fines de Claires" and "Spéciales", from the Bay of Biscay were analyzed. Muscles from the blue shrimp Litopenaeus stylirostris and the European spider crab Maja brachydactyla were sorted. Cephalopod digestive glands were analyzed too (except for S. officinalis and L. vulgaris). In addition to the digestive gland, only the mantle muscle was collected for the broadclub cuttlefish Sepia latimanus and the oval squid Sepioteuthis lessoniana, whereas remaining tissues (mantle muscle, gut, head, arms, and skin) were merged for the flying squid Todarodes cf. angolensis and the greater hooked squid Moroteuthopsis ingens. Tissue samples were stored in clean individual plastic bags, fresh weighed (ww), and put at -20 °C until being freeze-dried during 48 h. After drying, samples were again weighed (dry weight, dw), ground to a fine powder with a porcelain mortar and pestle, and stored in clean individual plastic vials.

2.2. Sample dissolution and Li concentration analyses

To minimize procedural blanks, the following procedures were performed in a pressurized clean laboratory, under a fume hood, and using distilled reagents and pre-cleaned vessels. Between 200 and 250 mg aliquots of each freeze-dried tissue sample were weighed and poured into a Corning® 50 mL centrifuge tube. Sample dissolution was performed overnight at room temperature in a mixture of 6 mL of 65% ultrapure HNO₃ and 2 mL of 37% ultrapure HCl (reverse *aqua regia*). For mineralization, the dissolved samples were transferred to a microwave and heated to 120 °C over 30 min, then held at 120 °C for 15 min. After mineralization, all samples were diluted to 50 mL with Milli-Q water. Procedural blanks and the reference material IAEA-436 (tuna fish flesh; International Atomic Energy Agency, Austria) were prepared via the same protocol to evaluate contamination and data precision and accuracy.

Li concentrations were analyzed using a Thermo Fisher X-Series II Inductively Coupled Plasma Mass Spectrometer, Li concentrations are reported in µg/g dw. Calibration and spike solutions were prepared using a 1000 mg/L Li monoelemental commercial solution. The biological reference material IAEA-436 was analyzed several times during each analytical session to monitor the accuracy and reproducibility of the measured Li concentrations; the Li concentration of this biological reference material is not certified, but was estimated by Azemard et al., 2006 ($0.040 \pm 0.009 \,\mu\text{g/g}$ dw, confidence interval from 0.018 to 0.062 μ g/g dw). Due to the uncertainties on the Li concentration of this reference material, we developed a spike-addition method to verify (1) the accuracy of our Li measurements and (2) the completeness of the mineralization process. For each of the twelve mineralization sessions, four aliquots of ~250 mg of IAEA-436 were digested. In three of these tubes, spikes of different Li concentrations (0.5, 1.0, or 5.0 μ g/L) were added. We achieved good recovery rates for the spike concentrations (98.2 \pm 3.4%, n = 12), demonstrating the near-100% yield of our entire chemical procedure. The calibration solutions were run several times during each analytical session to monitor instrumental drift and correct for the memory effect. Blank contributions were about 5%, and internal errors on measurements were 2%. Non-spiked IAEA-436 reference material provided good reproducibility (4%) between the twelve analytical sessions, and a mean Li concentration of $0.020 \pm 0.001 \,\mu\text{g/g} \,\text{dw} (n = 12)$, within the confidence interval published by Azemard et al., 2006. During our analytical sessions, the detection limit was $0.0125 \,\mu g/g \, dw$ for 250 mg of sample taken up in 50 mL of diluted reverse aqua regia; only two samples had concentrations below this value and were sidelined: the liver of Scyliorhinus canicula and the brain of Spondyliosoma cantharus.

2.3. Statistical data processing

As the Li concentrations in our samples are not normally distributed, even transformed, differences among data groups (i.e., between biogeographic areas, taxonomic groups, trophic groups and levels, habitat, and organs) were tested statistically using the R software package (R Core Team, 2017) following two non-parametric tests: the Mann-Whitney-Wilcoxon test for comparisons between two data series, and the Kruskal-Wallis test for comparisons between three or more series. The level of significance for statistical analyses was set at $\alpha = 0.05$. The detailed statistical treatment is presented in the Supplementary Material.

The relationships between Li concentrations and biological parameters were investigated using multiple correspondence analyses (MCA, see Section 3.1), a statistical tool that reduces the number of original qualitative variables to a smaller number of dimensions that explain the majority of the observed variability. In this study, MCA was performed in R considering the following variables: habitat, location, trophic group, taxonomical group, and organ type (predictor categorical variables); Li concentration was then added as a single dependent quantitative variable. We used the "ACM" function of "FactoMineR" R package (quanti.sup subfunction was filled to take Li into account).

3. Results and discussion

Li concentrations ranged from the detection limit ($0.01 \ \mu g/g \ dw$) to 1.20 $\mu g/g \ dw$ (digestive gland of *Pecten maximus*) (Supplementary Table S2). As the oceanic Li concentration is homogeneous at 0.18 $\mu g/mL$ (Aral and Vecchio-Sadus, 2011; Misra and Froelich, 2012; Riley and Tongudai, 1964), the large range of Li concentrations observed in marine organisms must result from different bioaccumulation dynamics related to biotic and abiotic processes. To investigate this, we first examine the entire dataset to identify which factors are related to high - or low - Li concentrations, and then investigate these factors in more details.

3.1. Multifactorial control of Li bioaccumulation by marine species

The relationship between Li and biological parameters (modalities of the predicted categorical variables) was investigated using MCA, as described in Section 2.3. In Fig. 1, the calculated coordinates of all variable modalities are plotted in a two-dimensions space. Li concentration was added as a quantitative variable, and is represented by the grey arrow. The proximity between different modalities in Fig. 1 indicates a connection between them, such as for "bivalves" and "filter-feeders", or "fish" and "predators of invertebrates".

It is notable that the modalities "fish", "predators of invertebrates", "predators of small fish", and "demersal" are associated (group 1, Fig. 1), which was expected considering that most fish are demersal. "Bivalves", "benthic", and "filter-feeders" form a second group (group 2), which is consistent with the fact that collected filter-feeders are benthic bivalves. "Cephalopods" are associated with "pelagic" and "predators of invertebrates and small fish" in group 3, as this group is represented by four pelagic squid vs. only two species of benthic cuttlefish. "Crustaceans" and "grazers/scavengers" constitute group 4. The association of these two last modalities is also related to sampling: the only collected grazer is a crustacean. Each group includes several parameters (biogeographic area, trophic group, taxonomy, habitat, or tissue), indicating that most of the modalities are confounded. There is clearly an imbalance across potential predictors that is related to the sampled organisms. However, sampling location is reasonably well crossed with other predictors and it is possible to evaluate independent effects of this variable (see Section 3.2).

The Li concentration trend (grey arrow) points almost directly towards group 2 and away from group 1, indicating that samples whose modalities belong to those groups 2 and 1 correspond to high and low Li concentrations, respectively. Whether feeding mode (filter-feeding)



Fig. 1. Results of the multiple correspondence analyses (MCA) performed over the entire dataset (Supplementary Table S2). Modality coordinates are reported in the space of dimensions 1 and 2 (Dim1 and Dim2), the first two dimensions resulting from MCA, which are ordered according to the percentage of the data variability that they explain. Qualitative variables considered here are trophic group, taxonomic group, habitat, organ type, and location. The grey arrow shows Li concentration as a quantitative variable. Although this model cannot discriminate which factor is the major control on Li distribution, it highlights that some modalities of different variables (e.g. filter-feeders, bivalves, benthic, and gonads) are grouped and have higher Li concentrations than others (demersal, liver, fish, and predator of small fish, in particular). Abbreviations: FF, filter-feeder; GS, grazer; PI, predator of invertebrates; PISM, predator of invertebrates and small fish; BB, Bay of Biscay; NC, New Caledonia; Kerg, Kerguelen Islands; Dig. GL, digestive gland.

or taxonomy (bivalve) is related to high Li values cannot be discerned with this data set, however, the MCA shows that filter-feeding bivalves display higher Li values. The impact of each variable on Li concentration is further discussed in the following sections.

3.2. Impact of biogeographic area

To determine whether Li concentration differs according to biogeographic areas, we chose three locations characterized by contrasted climates and environments, here represented by the annual mean sea surface temperature (SST): New Caledonia (SST = 21 °C), the Bay of Biscay (SST = 16 °C), and the Kerguelen Islands (SST = 1 °C) (https:// www.watertemperature.org/).

For comparing Li concentrations of similar tissues, we choose muscle, the only ubiquitous organ among the various studied organisms (note that gonads from Pectinidae are also considered, since gonads and muscle were pooled together in New Caledonian individuals). At first glance, the observed differences in Li concentrations between biogeographic areas are not drastic, as shown in Fig. 2 and Supplementary Table S3. The largest difference (of 0.17 μ g/g dw, p < 0.01) is observed between Li concentrations in crustacean muscles from the Bay of Biscay and New Caledonia. For fish and cephalopod muscles, differences between biogeographic areas are less than 0.06 µg/g dw (on average). Li concentrations in muscles and gonads of Pectinidae from the Bay of Biscay are similar to or slightly lower than those of Pectinidae from New Caledonia. In the MCA, the predicted variable "location" is not highly confounded with other variables (cf. Fig. 1 Section 3.1), allowing discussion on its direct effect on Li concentrations. The modalities "Bay of Biscay", "Kerguelen Islands", and "New Caledonia" are not aligned with observed variations in Li concentrations (Fig. 1), indicating that, considering all species, there is no clear and simple relationship between tissue Li concentrations and a biogeographic / climatic gradient. The similar Li concentrations in Pectinidae are also consistent with the results of Chassard-Bouchaud et al. (1984), which showed that Li retention by *Mytilus edulis* (common mussel) is geographically independent.

Nevertheless, in fish, Li concentrations in muscles (n > 20 per location) increase significantly (p < 0.0001) from 0.03 \pm 0.01 µg/g dw (New Caledonia) to 0.14 \pm 0.17 μ g/g dw (Kerguelen Islands) with decreasing SST, similar to crustaceans (although we have fewer data on crustaceans) (Fig. 2). The fish, as poikilotherms, are metabolically influenced by seawater temperature: typically, lower temperatures mean slower metabolism (Mehta, 2017). Therefore, the higher Li concentrations in muscles of fish from the Kerguelen Islands are consistent with a slower Li turnover (and therefore a higher Li retention time), compared to fish from the Bay of Biscay and New Caledonia. This trend is not observed for cephalopods, but we only had a few samples from New Caledonia (n = 8). It is consistent with the temperature effect on Hg and Pb retention in the black-lip oyster Saccostrea echinata (Denton and Burdon-Jones, 1981), with increasing metal half-life in visceral mass when temperature decreases. Accumulation and depuration experiments of Cu and Pb conducted on the girdled horn sea snail Cerithidea cingulata and the Starry flounder Platichthys stellatus, respectively, also reported higher tissue concentrations after depuration at low temperature compared to higher temperatures (Prabhakara Rao et al., 1988; Varanasi, 1978). A more detailed investigation of Li bioaccumulation processes is however necessary to explore the relationship between Li concentrations and metabolic rate among different species.

3.3. Impact of trophic group

Collected organisms allow exploring Li accumulation in different trophic groups (detailed in Section 2.1). Considering all tissues, Li concentration among all filter-feeders is $0.42 \pm 0.25 \,\mu$ g/g dw, a value significantly higher than for any other trophic groups (p < 0.0001; Fig. 3). In



Fig. 2. Li concentrations (µg/g dw) in different marine organisms grouped by biogeographic area and indicating mean annual sea surface temperature (SST): NC, New Caledonia, SST = 21 °C (red); BB, Bay of Biscay, SST = 16 °C (orange); Kerg., Kerguelen Islands, SST = 1 °C (blue). Abbreviations: Biv., bivalves; Crust., crustaceans; Ceph., cephalopods. Numbers at the right of each plot indicate the number of analyses (n) for each location. We note that n = 5 for NC and BB crustaceans and NC Pectinidae, n = 8 for NC cephalopods, and n > 20 for other groups. Asterisks indicate p-values from the Mann-Whitney-Wilcoxon test (see Supplementary Material for details) and highlight the significance of the observed differences between pairs of groups: ns (not significant) for p > 0.05, * for p < 0.01, *** for p < 0.001 (highly significant difference). Outliers are plotted as grey circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contrast, Li concentrations in grazers/scavengers and in predators of invertebrates, of invertebrates and small fish, and of small fish are low $(0.10 \pm 0.02, 0.10 \pm 0.11, 0.09 \pm 0.07, and 0.07 \pm 0.00 \,\mu\text{g/g}$ dw, respectively) and not statistically different (p > 0.05). These differences are in agreement with our MCA results (Section 3.1) indicating that Li concentrations align from the modality "predators of small fish" towards the modality "filter-feeders".

Considering muscle tissues alone avoids potential bias due to the incorporation of Li-enriched sedimentary particles in the gut, for instance in the digestive gland of filter-feeders (see Section 3.4). Muscle tissues display the same trend between filter-feeders and other trophic groups (Fig. 3B), although not significant due to the paucity of muscles sampled among filter-feeders (n = 10).

The same Li decrease among trophic groups and among taxonomic groups is observed in the three biogeographic areas (Fig. S3). All areas combined, Li concentrations decrease from bivalves to fish (p < 0.05) as: bivalves ($0.42 \pm 0.50 \mu g/g dw$) > crustaceans ($0.19 \pm 0.19 \mu g/g$)



Fig. 3. Box plots showing Li concentrations (µg/g dw) of (A) all organs/tissues and (B) muscles vs. trophic group. The numbers at the top of each plot indicate the number of analyses for each trophic group (n). Trophic groups are: FF, filter-feeders; GS, grazers/ scavengers; PI, predator of invertebrates; PISM, predator of invertebrates and small fish; PSM, predator of small fish. Asterisks and 'ns' indicate statistical significance as in Fig. 2. Outliers are plotted as grey circles. The high variability of Li concentrations observed at the PI level is mainly *G. piabilis* and *G. nicholsi*, which are mostly planktivores.

dw) > cephalopods $(0.12 \pm 0.10 \ \mu g/g \ dw)$ > fish $(0.08 \pm 0.17 \ \mu g/g \ dw)$ dw; Fig. 4). The variability among Li concentrations was limited, except for fish $(0.01-0.77 \ \mu g/g \ dw)$ and, to a lesser extent, cephalopods $(0.02-0.23 \mu g/g dw)$, as previously reported for other trace elements (e.g., Rainbow, 2002). This variability may partly be explained by the various habitats (benthic, demersal, and pelagic) occupied by species within these two groups. However, sampled organisms did not show a significant effect of habitats on Li concentrations. In contrast, the MCA (Fig. 1) indicates that the benthic habitat is close to the high Li concentration group (group 2). This is strongly influenced by the dominance of Li-rich filter-feeders among benthic species, whose Li enrichment in the digestive gland is discussed in more details in the following section. The Li concentration variability within groups can also be related to the presence of various tissues (see Section 3.4), and result from species differences in ecological need, swimming behavior, or metabolic activities (Canli and Atli, 2003).

Since filters-feeders are generally at lower trophic levels than predatory fish, our results strongly suggest that trophic ecology influences Li concentrations in marine organisms, but a bio-reduction, not biomagnification, of Li is observed along the considered trophic webs (Fig. 3A). Both comparisons including all organs (Figs. 3A, 4) and muscles only (Fig. 3B) demonstrate the consistent bio-reduction of Li along marine food webs, similar to that previously described for the



Fig. 4. The median and range of Li concentrations (μ g/g dw – all tissue considered) among different taxonomic groups. The numbers at the top of the plot indicate the number of analyses (n) per taxonomic group. The dashed blue line indicates the seawater Li concentration (0.18 μ g/mL). Subdivisions are made by the habitat: benthic species (brown) live on the seafloor, demersal species (or benthopelagic, orange) move in the water column just above the seafloor, and pelagic species (yellow) live in the water column. Abbreviations: Biv, bivalves; Crust, crustaceans; Ceph, cephalopods. Asterisks and 'ns' indicate statistical significance as in Fig. 2. Outliers are plotted as grey circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two essential trace metals, Cu and Zn. In contrast, Hg is known to significantly bioamplify towards the top of the food webs (Atwell et al., 1998). Consensus has not been reached on other elements such as As, Cd, and Se, although several studies argue for their bioamplification in specific cases (Barwick and Maher, 2003; Rahman et al., 2012; see review in Suedel et al., 1994). These observations suggest that the risk assessment on human health is limited to Li intake from shellfish consumption and not from Li-poor mesopredators (e.g. fish and cephalopods), which is in marked contrast to Hg (Atwell et al., 1998; Chouvelon et al., 2012; Lavoie et al., 2013).

3.4. Li distribution in the tissues of marine organisms

Our dataset allows us to further explore the internal distribution and accumulation of Li among various organs. Comparison of the different tissues within each taxonomic group (Fig. 5, Table 1; excluding crustaceans, for which only muscles were analyzed) shows that cephalopods are the only group for which there is no significant difference (p > 0.05) between Li concentrations in muscles and digestive gland. For the other groups, Li is unequally distributed among organs (Fig. 5, Table 1). In fish, Li is most concentrated in the gills, moderately concentrated in the kidneys and brain, and least concentrated in the liver and muscles. In bivalves (*P. maximus*), both gills and digestive gland display high Li concentrations compared to muscles and gonads. These observations are supported by the MCA results (Section 3.1): the Li trend extends from the modalities "liver", "brain", "muscles", and "kidneys" towards "gills" and "gonads" in the high Li concentration group (group 2; Fig. 1).

In fish, the liver is usually reported to be a storage organ for many trace elements (Cd, Cu, Fe, Hg, Pb, and Zn; Metian et al., 2013). However, our data show that the liver is depleted in Li compared to the gills and kidneys (Figs. 5, 6), regardless of geographic origin (Table S2). Nonetheless, liver Li concentrations are higher than those in muscles, except for *S. canicula* and *C. gunnari* (Fig. 6, Table S2). The relatively low Li concentrations in fish liver (average $0.07 \pm 0.03 \ \mu\text{g/g}$ dw) demonstrate that Li is not stored in this organ; our results instead show that the brain, gills, and kidneys play a key role in Li bioaccumulation. The kidneys and gills are involved in ionoregulation, and brain functions mostly depend on Na



Fig. 5. Li concentrations (μ g/g dw) measured in the whole body ('whole') and different tissues of the analyzed marine organisms. The dashed blue line indicates the seawater Li concentration (0.18 μ g/mL). Fish show the greatest variability in Li concentrations, both within each organ type and among different organs. Abbreviations: Biv., bivalves; Crust., crustaceans; Ceph., cephalopods. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

channels that are able to transport Li as well. Gills are in direct contact with seawater and, together with the kidneys, play a critical role in osmoregulation of marine organisms and the maintenance of their internal Na balance (Greenwell et al., 2003). Li uptake in fish most likely occurs through Na channels (Tkatcheva et al., 2007) and ubiquitous Na-H Exchangers (NHEs) located in cell membranes (Counillon et al., 2016). Li⁺ ions can penetrate gill cells via NHEs by following Na⁺ pathways (Milosavljevic et al., 2014; Tkatcheva et al., 2007). The high Li concentrations measured in gills and kidneys is thus consistent with Li accumulation primarily controlled by NHEs during water filtration, explaining why these two organs appear crucial to Li distribution in marine organisms. This implies that seawater is likely a major pathway of Li incorporation, although additional particulate pathways, such as predation, might participate to Li incorporation. Future studies should now further investigate the relative importance of different Li incorporation pathways in fish.

Table 1

Average Li concentrations (μ g/g dw \pm SD) in various organs of bivalves, cephalopods, crustaceans, and fish from the entire dataset (Supplementary Tables S1 and S2). n: number of individuals.

	п	Bivalves	п	Cephalopods	п	Crustaceans	n	Fish
Brain							11	0.10 ± 0.10
Digestive gland	10	0.80 ± 0.27	31	0.10 ± 0.04				
Gills	10	0.58 ± 0.04					12	0.26 ± 0.13
Gonads	10	0.30 ± 0.07						
Kidneys							11	0.15 ± 0.06
Liver							57	0.07 ± 0.03
Muscles	10	0.08 ± 0.01	28	0.10 ± 0.03	10	0.19 ± 0.10	163	0.06 ± 0.08
Whole individuals	25	0.42 ± 0.14	20	0.15 ± 0.05				

Gills functions may also partly explain the observed Li bioreduction between filter-feeders and other trophic groups (cf. Section 3.3). Indeed, Li becomes more concentrated in the muscles of organisms that use gills both for respiration and feeding (e.g., filter-feeders) compared to organisms that use gills for respiration only (crustaceans, cephalopods, and fish). Thus, organ function may play a key role in how Li is distributed and internally regulated. Unfortunately, gills from filter-feeders could not be further analyzed to investigate this relationship. Among fish, aside from muscles, we observe no trend according to trophic groups for Li concentrations in liver, kidneys, or gills (Fig. S4). However, we note that Li concentrations in fish brain increase with trophic level (from 3 to 5, p <0.01, Fig. 7), a trend deserving further investigation.

In the king scallop *Pecten maximus*, the highest Li concentration was observed in the digestive gland ($0.80 \pm 0.27 \,\mu$ g/g dw, Fig. 6), apparently consistent with its major role in detoxification and the storage of several trace elements, such as Ag, As, Cd, Cu, and Zn, which reach high concentrations in this tissue (Bustamante and Miramand, 2005; Metian et al., 2008a, 2008b; Saavedra et al., 2008). Nevertheless, the considered Pectinidae live in benthic sediments and are likely to ingest fine particles from the muddy sand, such as silts and clays (Mikulich and Tsikhon-Lukamina, 1981; Shumway et al., 1987), which can contain Li at concentrations >100 μ g/g (Tardy et al., 1972), several orders of magnitude above that dissolved in seawater. Therefore, the high digestive gland Li concentrations may be due to the presence of sediment particles

that remained in the gut portions sampled with the digestive gland tissue, as the *Pecten* were not depurated before dissection. This interpretation is consistent with the presence of Li-rich particles observed by Chassard-Bouchaud et al. (1984) in the intestine lumen of the pearl oyster *Pinctada fucata martensi*. Similarly, the Li concentrations in gonads may be overestimated as this organ is crossed by the intestine, which serves also for gamete emission (Fig. 6). Although not evidenced yet, Li⁺ ions from the ingested food and sediment particles may pass through the intestine to be stored in the digestive gland and gonads, as suggested for the blue mussel (*Mytilus edulis*) and the transparent razor shell (*Cultellus pellucidus*) (Chassard-Bouchaud et al., 1984).

4. Conclusions

This work provides the first extensive dataset of Li concentrations and distributions among marine organisms and their tissues in contrasted biogeographic areas (the Bay of Biscay, New Caledonia, and the Kerguelen Islands). Within trophic webs, Li is consistently bio-reduced in the three regions. Li heterogeneities among organs are consistent with the biological incorporation of Li by Na transporters. Although further study is required at high trophic levels, the observed Li bio-reduction towards predators suggests a weak impact of their consumption on human health. However, this investigation also highlights the need to assess Li bioaccumulation in



Fig. 6. Average Li concentrations (µg/g dw) in the organs of selected fish and bivalve species from the Bay of Biscay. Colors indicate organ types, small grey dots indicate individual analyses, and vertical lines indicate the range of observed values. Abbreviations: PI, predator of invertebrates; PISM, predator of small fish and invertebrates; FF, filter-feeders. In most studied species, gills display higher Li concentrations than other organs, whereas muscles and liver show lower Li concentrations.



Fig. 7. Average Li concentrations (μ g/g dw) in fish brain vs. (a) trophic groups and (b) trophic levels. Fish trophic levels are from Froese and Pauly (2020). Small grey circles are individual measurements. Vertical lines reflect the range of values per trophic group or level. Numbers at the top indicate the number of analyses (n) per trophic group or level. Asterisks and 'ns' indicate statistical significance as in Fig. 2.

bivalves and shellfish and their potential impact on human health in case of Li contamination.

CRediT authorship contribution statement

F. Thibon: Formal analysis, Writing - original draft, Visualization, Writing - review & editing. L. Weppe: Methodology, Validation, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. N. Vigier: Conceptualization, Supervision, Project administration, Writing - review & editing. C. Churlaud: Methodology, Validation, Investigation, Writing - review & editing. T. Lacoue-Labarthe: Resources, Writing - review & editing. M. Metian: Writing - review & editing. Y. Cherel: Resources, Writing - review & editing. P. Bustamante: Conceptualization, Supervision, Project administration, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2020.141453.

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