Toxicopathic effects of lithium in mussels

Nadezhna Fraga [a,b], Denis Benito [a,b], Tifanie Briaudeau [a,b], Urtzi Izagirre [a,b], Pamela Ruiz [a,b,*]

[a] CBET+ Research Group, Department of Zoology and Animal Cell Biology + One Health, Faculty of Science and Technology, University of the Basque Country (UPV-EHU), Sarriena Auzoa z/g, E-48940, Leioa-Bizkaia, Basque Country, Spain
[b] Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza z/g, E-48620, Plentzia-Bizkaia, Basque Country, Spain

HIGHLIGHTS

- There is scarce information concerning lithium toxicity in marine organisms.
- LC50 for mussels was established at 153.78 mg/L Li after 9 days of exposure.
- Li was bioaccumulated in mussel tissue after 21 days of exposure.
- Environmentally relevant Li concentrations caused histopathological effects.
- The prevalence and intensity of the histopathological alterations increased over time.

GRAPHICAL ABSTRACT
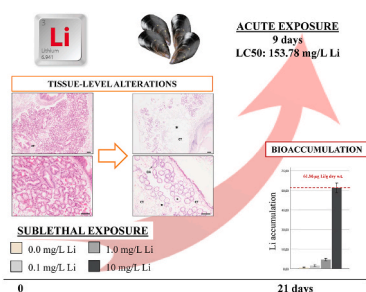
ARTICLE INFO

ABSTRACT

The rising use of lithium (Li) in industrial processes, modern technology and medicine has generated concerns in the scientific community, in particular its potential impact on the environment. Unfortunately, there is only scarce information concerning the toxicity of lithium in marine organisms. The objective of this study is to determine the toxicity of Li using *Mytilus galloprovincialis* as model organism, based on acute and sublethal toxicity tests. In the first experiment, mussels were exposed for 9 days to a range of acute concentrations of Li (0, 2, 5, 13, 34, 89, 233 and 610 mg/L Li) in order to find the median lethal concentration. In the sublethal experiment, mussels were exposed to environmentally relevant concentrations of Li (0, 0.1, 1, 10 mg/L Li) for 21 days. Digestive gland and gonad samples were taken at day 0, 1, 7 and 21 for histopathological analysis. Samples of the whole mussels were taken for chemical analysis at day 0 and after 21 days. Results showed that *M. galloprovincialis* had a LC50 value of 153 mg/L Li after 9 days of exposure. Lower concentrations (environmentally relevant), led to Li bioaccumulation in a dose-dependent manner and histopathological effects in a time-dependent manner. Atrophy of the digestive alveoli epithelium and degeneration of the digestive gland were observed after 21 days of exposure. These findings open new perspectives for the understanding of the toxic effects of Li on marine organisms and evidence the need for further long-term research at different levels of biological organizations.

* Corresponding author. CBET+ Research Group, Department of Zoology and Animal Cell Biology + One Health, Faculty of Science and Technology, University of the Basque Country (UPV-EHU), Sarriena Auzoa z/g, E-48940, Leioa-Bizkaia, Basque Country, Spain.
E-mail address: pamela.ruiz@ehu.eus (P. Ruiz).

1. Introduction

New ways of transport (electric vehicles, e-bikes, drones), communicative technologies (smartphones, tablets, laptops, small home appliances) and energy storage systems have caused an increased usage of batteries (Comission, 2006/66/EC), in particular Li-ion batteries. Lithium converts chemical energy into electric energy very efficiently; reducing costs and increasing manufacturing capacities (IEA, 2017). In addition, Li is commonly used in medicine for therapeutic treatment of psychiatric diseases such as manic depression (bipolarity) and other affective disorders (Moore, 1995).

Although the use of Li has clearly increased (Comission, 2006/66/EC), Li waste collection rate is still very low (Bolan et al., 2021; Comission, 2006/66/EC; Swarnakar and Choubey, 2014; Zeng et al., 2014). In the case of Li-ion batteries for instance, the European Parliament do not set strong collection targets or reporting obligations to promote the recovery of their chemicals; instead, Li-ion batteries are classified as "other batteries'' and tend to be placed in landfills with municipal solid waste (Bolan et al., 2021; Thibon et al., 2021a). Protocols of wastewater depuration appear to be inefficient at removing Li due to its high mobility (Choi et al., 2019). Consequently, Li can be transported long distances from its source, increasing its presence in the environment.

The increasing use of Li, associated with mining and extracting activities, as well as inefficient depuration protocol have led to an increase in Li concentrations in the environment (Thibon et al., 2021a). In the aquatic environment, Li has been found at concentrations exceeding 0.18 mg/L (Aral and Vecchio-Sadus, 2011; Reiman and Caritat, 1998). High levels of the metal have been documented in water sources from places with major Li extraction showing concentrations between 1 and 5 mg/L Li in Chile (Zaldívar, 1980), Austria (Kapusta et al., 2011) and Argentina (Concha et al., 2010). Extremely high concentrations of Li (14 mg/L) have also been measured in lakes such as the Dead Sea (Aral and Vecchio-Sadus, 2008, 2011). The increasing use of Li and its resulting presence in the environment have brought up new concerns in the scientific community and its potential impact on the environment and human health (Aral and Vecchio-Sadus, 2008, 2011; Bolan et al., 2021; Bradley et al., 2017). However, studies assessing the potential toxicity of Li in the aquatic environment are still very scarce (Thibon et al., 2021a, 2021b). In seawater environment, embryogenic disruption has been reported in different organisms such as striped bass, squid and sea urchin (Crawford, 2003; Dwyer et al., 1992; Ruocco et al., 2016). In squid embryos, LiCl inhibited development along the animal-vegetal axis and anterior midline (Crawford, 2003), whereas it induced malformations in sea urchin embryos in a dose-dependent manner when added to the eggs before fertilization (Ruocco et al., 2016). The lethal concentration was reported for freshwater organisms such as *Dreissena polymorpha* (US EPA, 2008), and was established in a range of 185–232 mg/L. Nevertheless, lethal concentrations for marine organisms remain unknown, which are essential for toxicological assessment. *Mytilus* sp. is the most used sentinel species in the world (Goldberg, 1975; ICES, 2012). In the same way, mussels may be useful as biomonitoring organisms to assess Li pollution in coastal waters as recently described by Thibon et al. (2021b) who reported that mussel tissues showed the highest concentrations of Li across the trophic web.

Regarding sublethal effects in the seawater environment, Rodríguez et al. (2021) studied the effects of Li contamination together with temperature changes on gastropods' feeding behavior. Viana et al. (2020) provided information on toxic effects of Li at biochemical level in *Mytilus galloprovincialis*, characterized by a decrease in metabolic activity and induction of oxidative stress and neurotoxicity. Those works showed the importance of analyzing the biological sublethal effects of Li in mussels. To complete the knowledge of the toxicopathological effects of Li in mussels, different biological organization levels such as histopathological alterations must be analyzed in different tissues at different concentrations and time exposure.

The increasing use of Li is evident and concerns about this metal are increasing. This use may have significant effects on the environment and thus, on the health and well-being of the aquatic living organisms. Therefore, the importance of researching the toxicity of this metal as an emerging contaminant in marine ecosystems is crucial (Aral and Vecchio-Sadus, 2008, 2011; Bolan et al., 2021; Comission, 2006/66/EC; Thibon et al., 2021b). The aim of this work is to assess the acute and sublethal effects of Li in mussels *Mytilus galloprovincialis* by histopathological analysis.

2. Material and methods

2.1. Sampling and acclimatization

Intertidal mussels (*Mytilus galloprovincialis*) of 2.5–3.5 cm shell length were collected from the low tide-mark level in Plentzia (Basque Coast; 43°24′N, 2°55′W) in October 2019 for the acute toxicity test and in November 2020 for the sublethal toxicity test.

Upon arrival, mussels were transported to the laboratory and maintained in 20 L polyethylene containers with running seawater to acclimatize for 7 days. They were kept under the following laboratory conditions: water temperature – 18-19 °C; pH – 7.8; conductivity – 48.000–51.000 Ω; salinity – 33 PSU; ammonium/ammonia concentration – <0.5 mg/L; photoperiod – 12/12 h. Mussels were not fed during the first 5 days of the acclimatization period, so that depuration may occur. From day 6, they were fed daily with a commercial marine microalgae mixture (1:10; 3.5 mL; Acuinuga, A Coruña, Spain). After acclimatization, the animals were used for the exposure experiments (acute toxicity test and sublethal toxicity test).

2.2. Experimental design

2.2.1. Acute toxicity test

Lithium was applied as LiCl (>99%, ACROS, USA). A mother solution (5 g Li/L) was prepared diluting 31.25 g LiCl in 1 L seawater. Fibbonacci scale numbers were used to select the eight exposure concentrations for the acute experiment: 0 (control), 2, 5, 13, 34, 89, 233, 610 mg/L Li.

In total, 8 experimental groups were set with 3 replicas. Each concentration was tested in a total of 15 mussels (5 per replica) which were kept in 0.5 L polyethylene bottles under continuous aeration for 9 days. The contaminant was provided daily after water changes. Mussels were fed a commercial marine microalgae mixture every day, after water changes. Mortality was checked daily and dead specimens were removed. Mortality data was used to calculate the LC50 and the LT50.

2.2.2. Sublethal toxicity test

A range of environmentally relevant concentrations of Li was selected for the sublethal test (Aral and Vecchio-Sadus, 2008, 2011; Reiman and Caritat, 1998): 0 (Control), 0.1 mg/L Li (L; low concentration), 1 mg/L (M; medium concentration) and 10 mg/L Li (H; high concentration).

Mussels were distributed in 4 experimental groups (n = 57) with 3 replicas per exposure concentration. They were set in 4 L polyethylene tanks under continuous aeration. A set of mussels (n = 15) was sacrificed at the starting point of the experiment (T0). Mussels were fed a commercial marine microalgae mixture every day. The contaminant was provided every two days, after water changes.

2.2.2.1. Sampling and sample processing.
Biological samples (digestive glands and gonads) were collected at day 0 (n = 10) and at days 1, 7 and 21 (n = 12; 4 per replica) for histopathological analysis. Besides, 5 mussels of each experimental group were dissected out at days 0 and 21 and stored at −80 °C for chemical analysis in order to determine the bioaccumulation.

2.2.2.2. Chemical analysis in mussels. Samples from 5 individuals at day 0 and day 21 per experimental group were pooled to obtain a minimum of 1 g dry weight (d.w.). After lyophilization, tissue samples were digested in acid (HNO3) at 180 °C for 15 min, using a microwave system (MARS 5 Xpress CEM Corporation Instrument). Lithium content was determined by inductively coupled plasma with mass detector (ICP-MS) (7700x, Agilent Technologies, Palo Alto, USA) using a MicroMist micro-uptake glass concentric nebulizer (Glass Expansion, West Melbourne, Victoria, Australia). Detection limit for Li (in µg metal/g flesh d.w.) was 0.001 for Li. ICP-MS analysis was carried out for Li content in mussel soft tissue by the Analytical Chemistry Service of the University of the Basque Country (SGiKER).

2.2.2.3. Mantle and digestive gland histopathological analysis. Digestive gland and gonad samples collected for histopathological analysis were fixed in 4% neutral buffered formaldehyde (24 h, 4 °C), dehydrated in graded solutions of ethanol and cleared in xylene, prior to paraffin embedding. Paraffin blocks were cut using a rotary microtome to obtain 5 µm thick sections. Histological sections were stained with haematoxylin-eosin.

Sex and gamete developmental stages were identified in gonad histological samples as described by Ortiz-Zarragoitia et al. (2011) adapted from Kim et al. (2006): undifferentiated stage (inactive), early gametogenesis (gamete development has begun but no mature gametes are visible), advanced gametogenesis (equal proportion of developing and ripe gametes are observed in follicles), mature (follicles are full of mature gametes), spawning (active emission of gametes, follicles may appear empty depending on degree of spawning) and post-spawning (empty follicles with occasional residual gametes remaining).

Histopathological analysis in mussel digestive gland was carried out following guidelines from Bignell et al., (2008). Amongst the histopathological lesions analyzed, the following alterations were identified: epithelial thinning of the digestive alveoli, integrity of the digestive gland and digestive tissue degeneration. The prevalence (percentage of occurrence of an alteration within an experimental group) was calculated for the most common observed parasites (*Nematopsis* spp. and *Mytilicola* spp.) and histopathological alterations (granulocytoma, brown cell and haemocytic infiltration) identified in the digestive gland of mussels.

Epithelial thinning of the digestive alveoli of mussels measured as atrophy index has been reported to be indicative of general stress in several studies (Benito et al., 2019; Garmendia et al., 2011; Kim et al., 2006). This parameter was rated using a numerical grading from 0 to 4 as described by Kim et al. (2006) and has been commonly used for mussels (Benito et al., 2019; Cuevas et al., 2015).

Loss of digestive gland histological integrity occurs in response to pollutant exposure (Benito et al., 2017; Garmendia et al., 2011; Marigómez et al., 2006). The integrity of the digestive gland was determined semi-quantitatively assessing the density of the digestive alveoli in relation to the interstitial connective tissue using scores from 1 to 4 as follows: (1) the majority of the digestive gland tissue presents a high density of digestive alveoli almost without visible interstitial connective tissue; (2) the majority of the digestive gland tissue presents a high density of digestive alveoli, the interstitial connective tissue is visible, but the distance between digestive alveoli remains shorter than the mean alveolar radius; (3) parts of the digestive gland tissue present lower digestive alveoli density and the distance between alveoli is similar or superior to the mean alveolar radius; (4) the majority of the digestive gland tissue present low digestive alveoli density and the distance between alveoli is similar or superior to mean alveolar radius.

2.3. Statistics

Statistical analyses were performed using IBM SPSS 25.0 for Microsoft. Each data set was tested for normal distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). LC10 and LC50 were determined using Probit analysis at the corresponding time intervals. Differences between survival curves were estimated using the non-parametric Kaplan-Meier test. The non-parametric *U* test was applied on chemical data to determine the effect of exposure time in different experimental groups. For the semiquantitative histopathological results, the non-parametric Kruskal-Wallis test was carried out to identify differences between concentrations and between exposure times (p < 0.05). For post hoc comparisons, Dunn's test was applied. To compare prevalence of histopathological alterations a Z-score test was performed (p < 0.05). A 95% significance level (p < 0.05) was established for all statistical analyses carried out.

3. Results

3.1. Acute toxicity test

No significant changes in mortality rate were observed in control and lower exposure concentrations (from 0 to 89 mg/L Li) throughout the testing period (<10%; Fig. 1). Survival rate decreased significantly in the two highest concentrations (233 mg/L Li and 610 mg/L Li), with a 0% survival rate detected upon exposure to 610 mg/L Li at day 8. LT50 for mussels exposed to the highest Li concentration (610 mg/L) was earlier (4 days) than in mussels exposed to 233 mg/L (6 days).

The LC50 and LC10 for *M. galloprovincialis* were 153.78 and 15.13 mg/L Li, respectively, after 9 days of exposure (Table 1).

3.2. Sublethal toxicity test

No significant mortality was recorded throughout the experiment.

3.2.1. Chemical analysis in mussels

Li concentration in soft tissues was similar in mussels at day 0 and in control mussels after 21 days, ranging from 1.03 to 1.17 µg Li/g d.w. with no significant differences among times. After 21 days of exposure, Li exposed mussels showed significantly higher Li levels than the control group, with the highest Li concentration (61.86 µg Li/g d.w.; Fig. 2) detected in soft tissues of mussels exposed to 10 mg/L Li.

3.2.2. Mantle and digestive gland histopathological analysis

The highest level of atrophy index was measured in mussels exposed to 10 mg/L Li for 21 days and was significantly different to levels detected in T0 mussels and 10 mg/L Li exposed mussels for 1 day
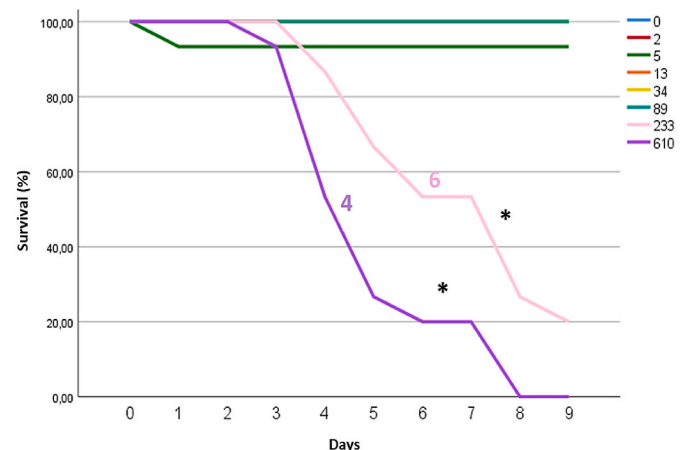

Fig. 1. Cumulative survival curves of *Mytilus galloprovincialis* (n = 15) when exposed to different Li concentrations (0–610 mg/L). Asterisks indicate significant differences among mussel populations exposed to different contaminant concentrations. Numbers indicate median lethal time (LT50). Survival curves which cannot be seen mean 100% of survival.

Table 1
Lethal concentration (mg/L) values (LC10 and LC50) for *Mytilus galloprovincialis* (n = 15) exposed to different Li concentrations (2, 5, 13, 34, 89, 233, 610 mg/L).

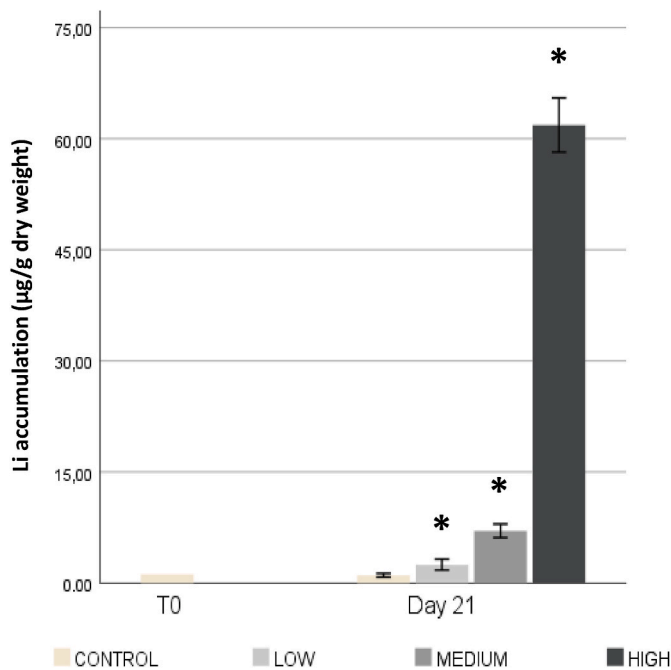| Time (Days)	LC10 (mg/L)	LC50 (mg/L)
4	9.42	1665.42
5	11.92	430.91
6	12.17	310.53
7	12.17	310.53
8	15.18	165.21
9	15.13	153.78

Fig. 2. Concentrations of Li in μg/g tissue d.w. in mussel soft body of mussels (n = 5). The asterisk indicates significant differences between experimental groups and the control of the same sampling time (p < 0.05).

(Fig. 3). The level of affection and the reduction in the height of the digestive epithelium of the alveoli was evident as observed in Fig. 4.

Overall, the connective tissue index increased in all Li exposed groups throughout time (Fig. 5). Mussels exposed to 1 mg/L Li, showed significantly higher connective tissue index at day 21 than day 1. In 10 mg/L Li exposed mussels, index levels were significantly higher at days 7 and 21 in contrast to day 1. Significant differences were also detected in experimental groups at the same sampling time. Mussels exposed to 10 mg/L Li had higher connective tissue index than control mussels at day 7 and 21 (see Fig. 6). Moreover, connective tissue index of mussels exposed to 10 mg/L Li was significantly higher than the one observed in 0.1 mg/L Li exposed mussels at day 21.

Parasites found by histopathological analysis were *Mytilicola intestinalis* copepod and the gregarine *Nematopsis* sp. The prevalence of *Mytilicola intestinalis* ranged from 25 to 83% in the experimental groups, while the gregarine parasite showed a prevalence of 100% in all the groups.

Within inflammatory responses, granulocytomas, haemocytic and brown cell infiltrations were found (see Fig. 7) (Fig. 8). Granulocytomas were observed in the majority of the groups at different exposure time, with a slightly higher prevalence detected in the control (17%) than in the rest of the experimental groups at day 7.

The highest prevalence of haemocytic infiltration in mussels exposed to 0.1 and 1 mg/L Li concentrations was found at days 1 and 7 respectively. In the group exposed to 10 mg/L Li, the infiltration prevalence
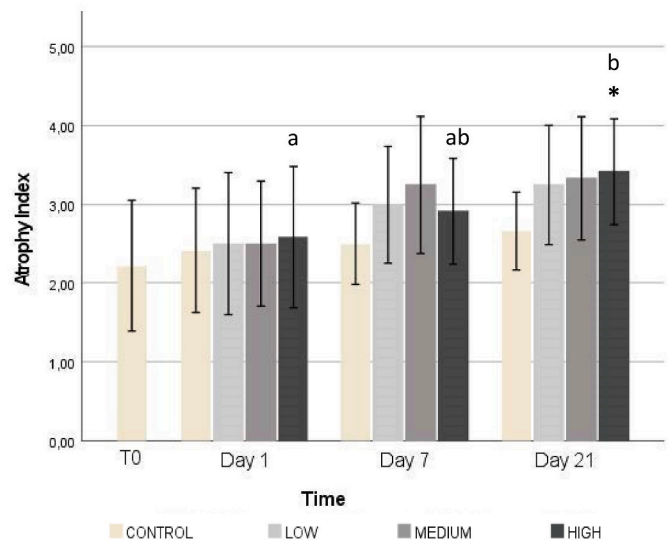

Fig. 3. Atrophy index in digestive gland of *Mytilus galloprovincialis* (n = 12) exposed to different concentrations of Li (0, 0.1, 1, 10 mg/L) at different exposure times (0, 1, 7 and 21 days). Intervals indicate standard deviation. The asterisk indicates significant differences between experimental groups and the control of the same sampling time (p < 0.05). The letters indicate significant differences for each treatment throughout time (p < 0.05).

remained high (92–100%) until the end of the experiment (Table 2).The highest prevalence was found at days 7 and 21. In addition to the infiltration of the vesicular interstitial connective tissue, a characteristic haemocytic infiltration in the lower part of the epithelium of the digestive tract upwards to the lumen could be easily distinguished (Fig. 7).

Brown cell infiltration prevalence in the digestive tissue was highest (83%, 92%) by days 7 and day 21 in mussels exposed to 1 mg/L Li (Table 2). These brown cells were located at the base of the digestive tract epithelium and through the epithelial cells. Moreover, some brown cells were also located around the digestive alveoli forming aggregates (see Fig. 8). The prevalence of brown cell infiltration in the gonad tissue was significantly higher in mussels exposed to the lowest concentration of Li at day 21 (75%) than at the beginning of the experiment (22%).

Digestive gland degeneration around the stomach was more frequently observed (83%) in individuals exposed to the highest Li concentration at day 21 (Table 2) (see Fig. 9). Overall, degeneration prevalence was higher at all times in the medium (1 mg/L) and high Li concentrations (10 mg/L) than in the lower one (0.1 mg/L) and the control.

Gamete developmental stages exhibited no significant changes in relation to the exposure to Li (data not shown). Most of mussels were in post spawning stages as expected for the season experiment was performed.

4. Discussion

The use of Li has increased due to the high demand of batteries for modern technology (mobile phones, laptops, tablets, photo cameras, cars) and its therapeutic use. However, few information is available in relation to the toxicity of this emergent pollutant in the marine environment (Tkatcheva et al., 2015). In order to increase the knowledge of the effects of Li in marine organisms, the present work describes the acute and sublethal effects of this pollutant on ecologically relevant *Mytilus galloprovincialis* sentinel species.

An acute toxicity test has been headed to evaluate Li lethality for mussels during 9 days of exposure. Acute toxicity testing has been previously used to establish the impact of Li in aquatic organisms (Khangarot and Das, 2009; Milam et al., 2005). The lethal concentrations
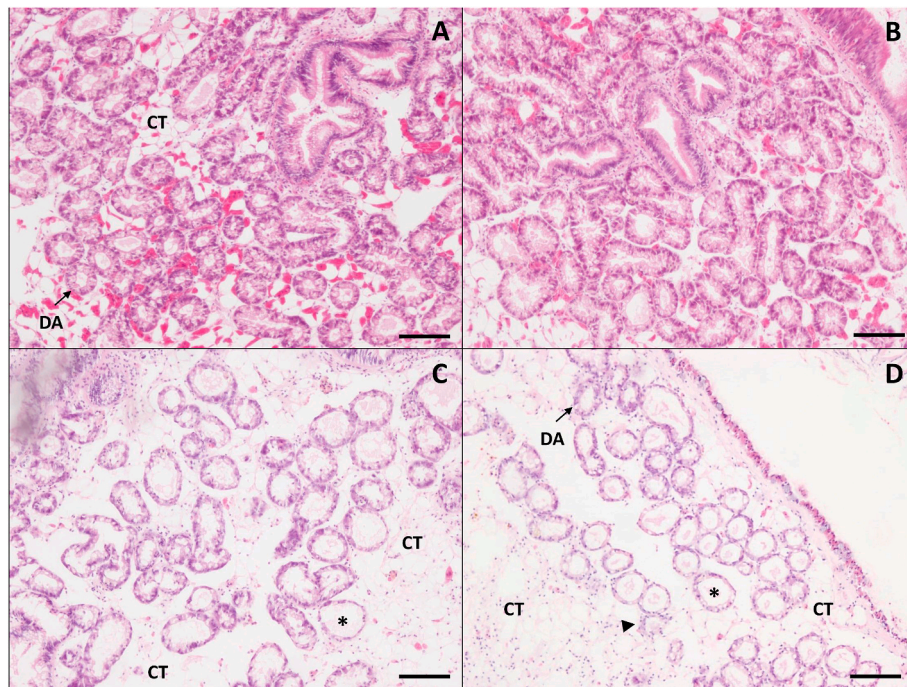
Fig. 4. Sections of the digestive gland stained with haematoxylin-eosin in (A, B) control mussel, (C, D) mussel exposed to 10 mg/L Li for 21 days. Note the high atrophy levels (*) in the digestive alveoli. Scale bars: 100 μm, CT: connective tissue, DA: digestive alveoli; arrow head: degenerative alveoli.
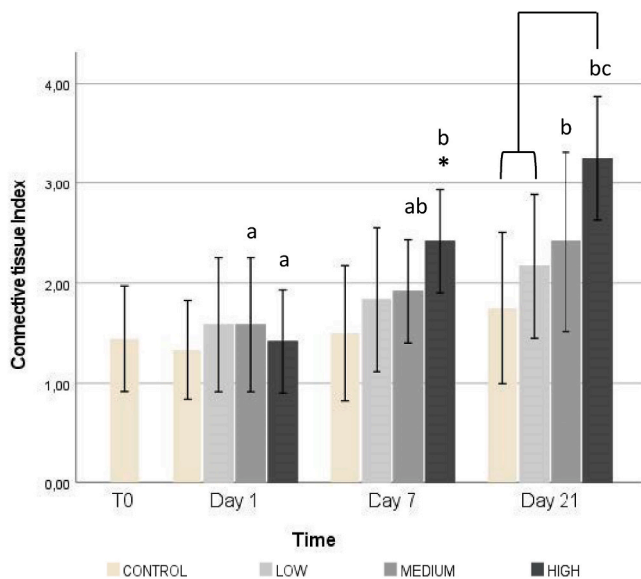

Fig. 5. Connective tissue index in digestive gland of *Mytilus galloprovincialis* (n = 12) exposed to different concentrations of Li (0, 0.1, 1, 10 mg/L) at different exposure periods. Intervals indicate standard deviation. The asterisk and the bars indicate significant differences between experimental group and the control of the same sampling time (p < 0.05). The letters indicate significant differences for each treatment throughout time(p < 0.05).

reported for freshwater fish such as *Pimephales promelas* and *Tanichthys albonubes* were in the range of 1.2–62 mg/L Li, which implies a relatively low acute toxicity compared to concentrations found in the environment with the exception of Li mining areas (Aral and Vecchio-Sadus, 2008). However, there is not enough information to evaluate the relative importance of Li toxicity in marine ecosystems. The LC50 for *M. galloprovincialis* is 153.78 mg/L Li after 9 days of exposure, which is comparable to the LC50 of freshwater mussel *Dreissena polymorpha* established at 185–232 mg/L Li after only 24 h of exposure with LiCl (US

EPA, 2008). Nevertheless, it seems that the toxicity for freshwater bivalves is higher than for the marine bivalve, which could indicate a different sensibility of organisms to Li or the influence of salinity (Kszos et al., 2003).

The LC50 found for mussels is still far from Li concentrations reported in seawater (0.18 mg/L; Reiman and Caritat, 1998) and in mining sites (1 mg/L; Concha et al., 2010; Kapusta et al., 2011; Zaldívar, 1980), or from the highest concentration found in the environment which is in the Dead Lake in Israel (14 mg/L; Aral and Vecchio-Sadus, 2008, 2011). At first, it seems that there is no extreme risk for the environment, however, Li is not a target component for most of the routine chemical analysis in water and more information is required to measure Li levels in different environments and assess its evolution in different ecosystems.

In order to get a complete toxicopathological characterization for this metal, analysis of the effect that Li can exert on biota at sublethal levels must be achieved. To determine an accurate realistic and effective sublethal exposure concentration, the LC10 value was determined in 15.13 mg/L Li for mussels of the present study. Moreover, realistic concentrations found in different environmental scenarios 0.1 mg/L Li, (Reiman and Caritat, 1998), 1 mg/L Li (Concha et al., 2010; Kapusta et al., 2011; Zaldívar, 1980) and 10 mg/L Li (Aral and Vecchio-Sadus, 2008, 2011) were used in a mid-term experiment (21 days). Concentrations were kept below 15 mg/L Li (LC10) to ensure a sublethal exposure.

In the present study, an evident bioaccumulation of Li was observed in a dose-dependent manner with highest bioaccumulation recorded in mussels exposed to 10 mg/L Li for 21 days (61.86 μg Li/g d.w.). In mussels soft tissue, Li levels were similar to those recorded in a previous study by Viana et al. (2020), where mussels exposed to 0.1 and 0.75 mg/L of Li for 28 days, bioaccumulated the metal at 0.7 and 1.4 μg/g d. w., respectively. The accumulation of Li in mussels exposed to 0.1 mg/L and 1 mg/L in the present study is slightly higher (2.47 and 7.03 μg Li/g d.w., respectively) but comparable with this data, showing a reproducibility and a stable accumulation pattern of Li in mussels. Although these levels of Li are not expected to produce acute toxicity, potential sublethal effects of the metal should be assessed, as suggested by Aral and
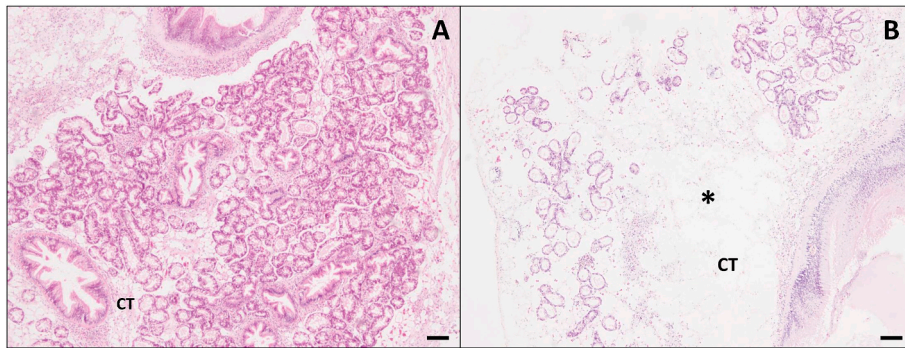
Fig. 6. Sections of the digestive gland stained with haematoxylin-eosin in a mussel exposed to 10 mg/L Li at (A) day 1 of exposure and (B) day 21 of exposure. Note the high proportion of connective tissue (*) in the digestive gland as a result of Li-exposure. Scale bars: 100 μm, CT: connective tissue.
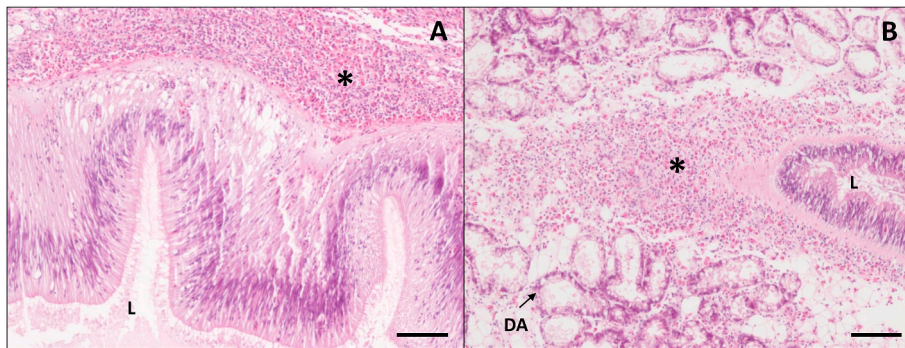

Fig. 7. Sections of the digestive gland stained with haematoxylin-eosin in mussels exposed to 10 mg/L Li at day 7 of exposure (A. B). Note the haemocytic infiltration (*) under the stomach epithelium as a result of Li-exposure. Scale bars: 100 μm, DA: digestive alveoli, L: lumen of the stomach.
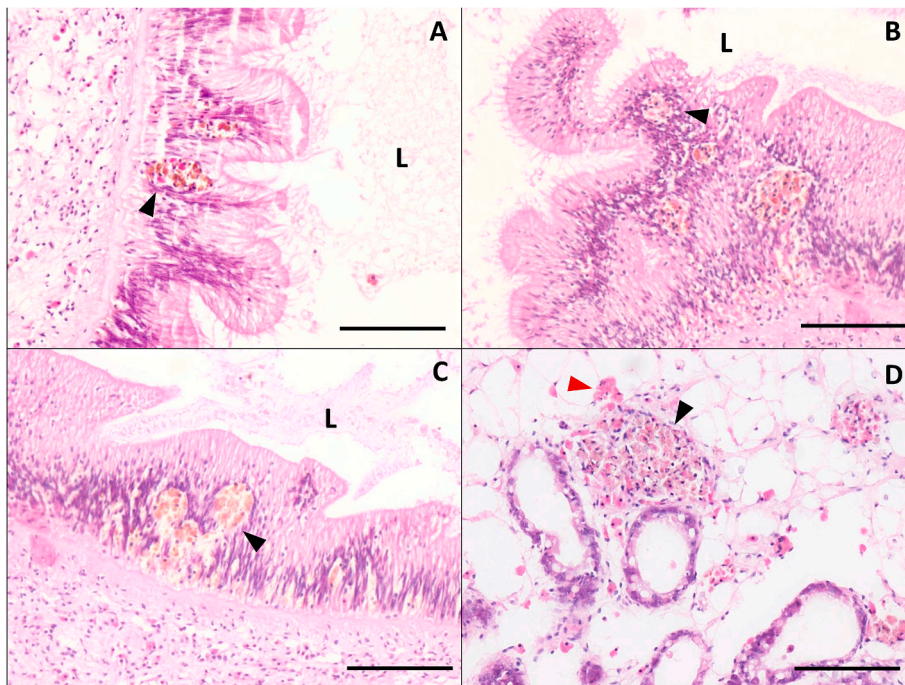

Fig. 8. Sections of the digestive gland stained with haematoxylin-eosin in a mussel exposed to 1 mg/L Li at day 21 of exposure. Note the brown cell infiltration in the stomach epithelium (A, B, C) and around the digestive alveoli (D) as a result of Li-exposure. Scale bars: 100 μm, L: lumen of the stomach, black arrow head: brown cells, red arrow head: haemocytes.
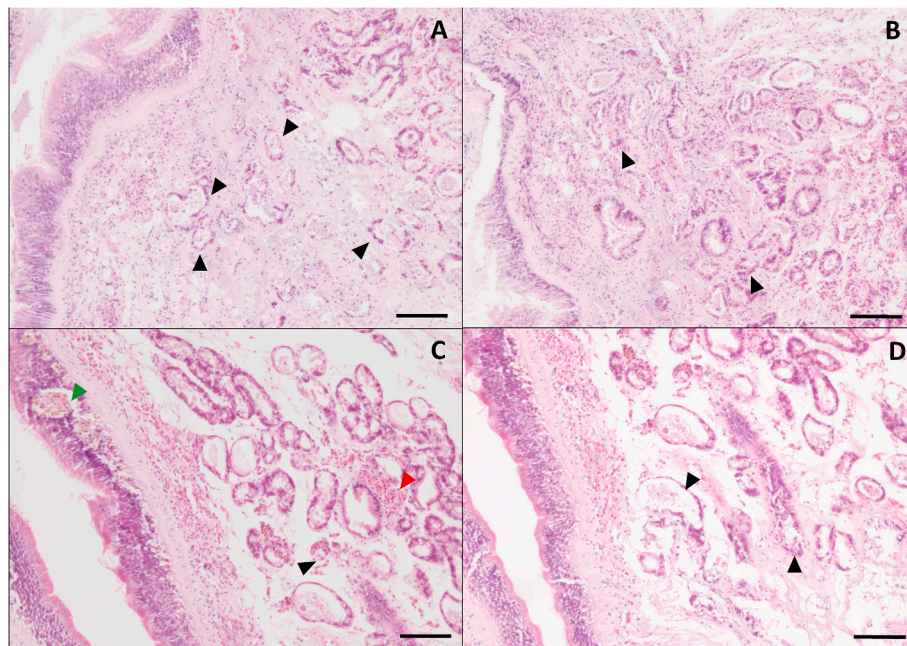
Fig. 9. Sections of the digestive gland stained with haematoxylin-eosin in a mussel exposed to 10 mg/L Li at day 21 of exposure. Note the increase in degenerative digestive alveoli (A, B, C, D) as a result of Li-exposure. Scale bars: 100 μm, *: degeneration area, black arrow head: degenerative digestive alveoli, red arrow head: haemocytes, green arrow: brown cells.

Table 2
Prevalence (%) of Nematopsis sp., Mytilicola sp., granulocytomas, haemocytic infiltrations, brown cell infiltrations in gonad (GO) and digestive gland (DG) and degeneration of the digestive gland which is next to the stomach found after histopathological analysis of *Mytilus galloprovincialis* samples (n = 12) in sublethal experiment. C: control; L: low, 0.1 mg/L Li; M: medium, 1 mg/L Li; H: high, 10 mg/L Li.

	T0	Day 1				Day 7				Day 21			
		C	L	M	H	C	L	M	H	C	L	M	H
Nematopsis sp. (DG)	100	100	100	100	100	100	100	100	100	100	100	100	100
Mytilicola sp. (DG)	56	83	50	33	50	42	25	25	42	50	50	25	67
Granulocytoma (DG)	11	0	8	8	8	17	8	8	0	9	0	8	8
Haemocytic inf. (DG)	33	25	83	75	75	42	67	83	100	25	58	58	92
Brown Cells (GO)	22	50	67	58	67	33	50	33	42	42	75	42	50
Brown Cells (DG)	22	25	50	42	58	42	25	83	50	33	67	92	67
Degeneration (DG)	11	17	17	25	25	25	33	67	75	33	17	50	83

Vecchio-Sadus (2008).

In mollusks, the organ which plays the main role in the metabolism, detoxification and in biological responses to pollutants is the digestive gland (Garmendia et al., 2011; Kim et al., 2006; Marigómez et al., 2006). Other researchers such as Garmendia et al. (2010) and Rocha et al. (2016) have demonstrated that mollusks exposed to pollutants exhibit a significant loss in the digestive gland epithelium, which leads to atrophy of the digestive epithelium or less digestive diverticula in relation to connective tissue. This may be associated with a degenerative process indicating pathological conditions in bivalves (Benito et al., 2017; Carella et al., 2015). In the present study, significant differences in atrophy of the digestive alveoli epithelium were found upon exposure to Li and throughout time. This means that Li not only affects in a dose-dependent manner but also in a time-dependent manner when referring to digestive gland atrophy. Similar results were obtained by Pinto et al. (2019), who described a progressive increase in digestive system atrophy in a dose-dependent manner in mussels exposed to 0.1, 1 and 10 mg/L of other emerging pollutants such as lanthanum.

Together with atrophy, the proportion of connective tissue in the digestive gland may increase due to the loss of the digestive alveoli. In this way, a reduction of integrity of the digestive gland may happen. High proportion of connective tissue compared to the proportion of digestive tissue has been reported to indicate a loss of integrity of the digestive gland caused by adverse environmental conditions or as a response to pollutant exposure (Benito et al., 2017; Garmendia et al., 2011; Marigómez et al., 2006). In agreement with these studies, high values of connective tissue index were also found in the digestive gland of mussels exposed to Li. Moreover, connective tissue proportion changes significantly after 21 days of exposure both in mussels exposed to the medium and the high concentration of Li. As this alteration is indicative of potential tissue-level effects that would compromise the detoxification and digestive functions of the digestive gland, these results are of critical importance when considering the potential impact of sublethal doses of Li in mussels. Moreover, considering the trends observed in the present study, potential effects are still to be expected at low concentrations in the mussel digestive system if the exposure is maintained for longer.

Brown cell infiltration in the gonad has been directly linked to resorption processes during the gametogenesis and can be induced when environmental conditions are unfavorable for spawning (Newell, 1989; Suárez et al., 2005). These statements appear to be in line with the present work, since the energy demand could be higher in Li exposed mussels as a consequence of the ongoing detoxification processes (that could lead to epithelial thinning, among other effects). Under this demanding condition, gonad reabsorption would be intensified, as described by Soto et al. (2000). However, this brown cell prevalence

could also be explained by the slow and less intense process of gametogenesis that mussels perform in winter, which ends up in no effective spawning (Newell, 1989). Since our mussels were collected in November, further research is needed at different seasons, in order to demonstrate the effects of Li at different natural condition that is known to modulate the responses to pollutants (Blanco-Rayón et al., 2020; Benito et al., 2019).

Several authors stated that brown cells and haemocytes are included in response mechanisms of mollusks to metals (Lowe and Moore, 1979; Thomson et al., 1985), and that metal accumulation is suggested to happen in brown cells (Janssen and Scholz, 1979). These cells play an important role in metal detoxification, they might transport metals across the epitheliums to the organs (Zaroogian and Yevich, 1993). The higher haemocytic infiltration values in mussels exposed to the medium concentration (1 mg/L Li) compared to the ones exposed to the highest concentration (10 mg/L Li) suggest that the latter group of mussels presents a lack of haemocytic response capability, which would indicate that these animals are highly affected by the contaminant. Moreover, brown cell migration through the epithelial cells of the stomach has been reported previously in mussels exposed to metals (Benito et al., 2019; Calabrese et al., 1984; Soto et al., 1996), which coincides with present observations in mussels exposed to the different concentrations of Li.

The prevalence of the degeneration of the digestive alveoli near the stomach also confirms a stress situation in mussels exposed to the highest concentration of Li. Degenerative alterations such as alveoli dilation or breakdown, have been reported in mussels exposed to metals such as copper and mercury (Krishnakumar et al., 1990) and complex mixture of contaminants (Bignell et al., 2011). Almost all the mussels exposed to higher concentrations of Li presented degenerative alveoli in regions near the stomach epithelium together with the previous described histopathological alterations. This suggests that the digestive gland was degenerating from the first part of the duct-system of the digestive gland and then would probably extent to the secondary ducts and alveoli. Several authors explained that this degenerative process may be due to the destabilization of the lysosomes, which may lead to autolytic processes and finally to atrophy and degeneration of the digestive alveoli as has been reported in different mollusks (Benito et al., 2017 Brooks et al., 2011; Calabrese et al., 1984; Lowe, 1988; Múgica et al., 2015). Thus, in future experiments, it would be interesting to assess the responses to Li at different biological organization levels, specially focusing in lysosomal alterations.

Haemocytic infiltration is considered an important biomarker of inflammatory response in bivalves and it is often linked to neoplasia or xenobiotics (Sheir and Handy, 2010; Villalba et al., 2001). In the present work, the haemocytic infiltration prevalence increased until day 7 but decreased at day 21 in mussels exposed to the medium concentration, which may indicate that mussels were not able to sustain their response and detoxification of Li. The inhibition of this immune response was also described in other studies with mussels exposed to diverse contaminants (Grundy et al., 1996; Pipe et al., 1999). In the present study, the high prevalence of haemocytic infiltration in mussels exposed to the highest concentration of Li remain high during the whole experiment related to the degeneration of the digestive alveoli. Higher haemocytic activity could reflect tissue damage along the degeneration of the digestive alveoli (Gosling, 2015).

It has been described that Li has adverse effects at reproduction level in freshwater organisms, such as *Daphnia magna*, decreasing brood number after 21 days to Li exposure (Martins et al., 2022). In the present study, mussels exposed to Li did no exhibit significant changes in gamete development. Mussels of this experiment were mainly in post spawning phase, which is characterized by empty follicles only presenting a few residual reabsorbing gametes, thus, the detection of histopathological alterations in gonad was not expected. Those stages are the common ones for the season the experiment was performed (Ortiz-Zarragoitia et al., 2011). Nevertheless, Li effects in reproduction of mussels cannot be discarded and should be checked in further studies that should be performed in different seasons.

The present study revealed that LC50 value for *Mytilus galloprovincialis* is low to provoke extreme effects within the environmental concentrations observed nowadays. However, the results obtained clearly demonstrated that Li is accumulated in soft tissues, implying that long-term exposure could still generate toxicopathic effects. Furthermore, exposure to environmentally relevant concentrations caused histopathological effects such as increased atrophy of the digestive epithelium, haemocytic and brown cell infiltration and loss of digestive gland integrity.

Based on the present acute toxicity results, Li concentrations currently found in the environment do not represent a threat for the ecosystem. However, the possible sublethal effects of Li should not be underestimated as demonstrated by histopathological alterations observed; instead, Li biological effects should be assessed based on a complete battery of biomarkers. Overall, the present findings open new perspectives for the understanding of the toxic effects of Li on marine organisms and evidence the need for further long-term research at different biological organization levels.

Credit author statement

Nadezhna Fraga: Conceptualization, Methodology, Investigation, Writing - Original Draft, Denis Benito: Methodology, Investigation, Validation, Investigation, Writing - Review & Editing Tifanie Briaudeau: Methodology, Investigation, Validation, Writing - Review & Editing, Urtzi Izagirre: Conceptualization, Methodology, Investigation, Validation, Writing - Review & Editing, Supervision, Pamela Ruiz: Conceptualization, Methodology, Investigation, Validation, Writing - Review & Editing, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgements

References

Aral, H., Vecchio-Sadus, A., 2008. Toxicity of lithium to humans and the environment-a literature review. Ecotoxicol. Environ. Saf. 70, 349–356. https://doi.org/10.1016/j.ecoenv.2008.02.026.

Aral, H., Vecchio-Sadus, A., 2011. Lithium: environmental pollution and health effects. In: Encyclopedia of Environmental Health. Elsevier, pp. 116–125. https://doi.org/10.1016/B978-0-444-52272-6.00531-6.

Benito, D., Niederwanger, M., Izagirre, U., Dallinger, R., Soto, M., 2017. Successive onset of molecular, cellular and tissue-specific responses in midgut gland of *Littorina littorea* exposed to sub-lethal cadmium concentrations. Int. J. Mol. Sci. 18, 1–26. https://doi.org/10.3390/ijms18081815.

Benito, D., Ahvo, A., Nuutinen, J., Bilbao, D., Saenz, J., Etxebarria, N., Lekube, X., Izagirre, U., Lehtonen, K.K., Marig omez, I., Zaldibar, B., Soto, M., 2019. Influence of season-depending ecological variables on biomarker baseline levels in mussels (*Mytilus trossulus*) from two Baltic Sea subregions. Sci. Total Environ. 689, 1087–1103. https://doi.org/10.1016/j.scitotenv.2019.06.412.

Bignell, J.P., Dodge, M.J., Feist, S.W., Lyons, B., Martin, P.D., Taylor, N.G.H., Stone, D., Travalent, L., Stentiford, G.D., 2008. Mussel histopathology: effects of season, disease and species. Aquat. Biol. 2, 1–15. https://doi.org/10.3354/ab00031.

Bignell, J.P., Stentiford, G.D., Taylor, N.G.H., Lyons, B.P., 2011. Histopathology of mussels (*Mytilus sp.*) from the Tamar estuary, UK. Mar. Environ. Res. 72, 25–32. https://doi.org/10.1016/j.marenvres.2011.05.004.

Blanco-Rayón, E., Ivanina, A.V., Sokolova, I.M., Marigómez, I., Izagirre, U., 2020. Sex and sex-related differences in gamete development progression impinge on biomarker responsiveness in sentinel mussels. Sci. Total Environ. 740, 140–178. https://doi.org/10.1016/j.scitotenv.2020.140178.

Bolan, N., Hoang, S.A., Tanveer, M., Wang, L., Bolan, S., Sooriyakumar, P., Robinson, B., Wijesekara, H., Wijesooriya, M., Keerthanan, S., Vithanage, M., Markert, B., Fränzle, S., Wünschmann, S., Sarkar, B., Vinu, A., Kirkham, M.B., Siddique, K.H.M., Rinklebe, J., 2021. From mine to mind and mobiles–Lithium contamination and its risk management. Environ. Pollut. 290, 118067 https://doi.org/10.1016/j.envpol.2021.118067.

Bradley, D.C., Stillings, L.L., Jaskula, B.W., Munk, LeeAnn, McCauley, A.D., 2017. Lithium, chap. K. U.S. Geological Survey Professional Paper 1802, p. K1–K21. In: Schulz, K.J., DeYoung Jr., J.H., Seal, R.R., Bradley, D.C. (Eds.), Critical Mineral Resources of the United States—Economic and Environmental Geology and Prospects for Future Supply. https://doi.org/10.3133/pp1802K.

Brooks, S., Harman, C., Zaldibar, B., Izagirre, U., Glette, T., Marigómez, I., 2011. Integrated biomarker assessment of the effects exerted by treated produced water from an onshore natural gas processing plant in the North Sea on the mussel *Mytilus edulis*. Mar. Pollut. Bull. 62, 327–339. https://doi.org/10.1016/j.marpolbul.2010.10.007.

Calabrese, A., MacInnes, J.R., Nelson, D.A., Greig, R.A., Yevich, P.P., 1984. Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel *Mytilus edulis*. Mar. Environ. Res. 11 (4), 253–274.

Carella, F., Feist, S.W., Bignell, J.P., De Vico, G., 2015. Comparative pathology in bivalves: aetiological agents and disease processes. J. Invertebr. Pathol. 131, 107–120. https://doi.org/10.1016/j.jip.2015.07.012.

Choi, H.B., Ryu, J.S., Shin, W.J., Vigier, N., 2019. The impact of anthropogenic inputs on lithium content in river and tap water. Nat. Commun. 10, 1–7. https://doi.org/10.1038/s41467-019-13376-y.

Concha, G., Broberg, K., Grandér, M., Cardozo, A., Palm, B., Vahter, M., 2010. High-level exposure to lithium, boron, cesium, and arsenic via drinking water in the Andes of northern Argentina. Environ. Sci. Technol. 44 (17), 6875–6880. https://doi.org/10.1021/es1010384.

Crawford, K., 2003. Lithium chloride inhibits development along the animal vegetal axis and anterior midline of the squid embryo. Biol. Bull. 205 (2), 181–182. https://doi.org/10.2307/1543241.

Cuevas, N., Zorita, I., Costa, P.M., Franco, J., Larreta, J., 2015. Development of histopathological indices in the digestive gland and gonad of mussels: integration with contamination levels and effects of confounding factors. Aquat. Toxicol. 162, 152–164. https://doi.org/10.1016/j.aquatox.2015.03.011.

Dwyer, F.J., Burch, S.A., Ingersoll, C.G., Hunn, J.B., 1992. Toxicity of trace element and salinity mixtures to striped bass (*Morone saxatilis*) and *Daphnia magna*. Environ. Toxicol. Chem. 11, 513–520. https://doi.org/10.1002/etc.5620110409.

Garmendia, L., Soto, M., Cajaraville, M.P., Marigómez, I., 2010. Seasonality in cell and tissue-level biomarkers in *Mytilus galloprovincialis*: relevance for long-term pollution monitoring. Aquat. Biol. 9 (3), 203–219. https://doi.org/10.3354/ab00245.

Garmendia, L., Soto, M., Vicario, U., Kim, Y., Cajaraville, M.P., Marigómez, I., 2011. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. J. Environ. Monit. 13, 915–932. https://doi.org/10.1039/c0em00410c.

Goldberg, E.D., 1975. The mussel watch-a first step in global marine monitoring, 10.1016/0025-326X(75)90271-4 Mar. Pollut. Bull. 6, 111–114.

Gosling, E, 2015. Marine bivalve molluscs. John Wiley & Sons.

Grundy, M.M., Ratcliffe, N.A., Moore, M.N., 1996. Immune inhibition in marine mussels by polycyclic aromatic hydrocarbons. Mar. Environ. Res. 42 (1–4), 187–190. https://doi.org/10.1016/0141-1136(95)00033-X.

ICES, 2012. Integrated Marine Environmental Monitoring of Chemicals and Their Effects. By Davies IM and Vethaak AD. ICES Cooperative Research Report No. 315.

International Energy Agency (IEA), 2017. Tracking Clean Energy Progress 2017.

Janssen, H.H., Scholz, N., 1979. Uptake and cellular distribution of cadmium in. Mytilus edulis. Mar. Biol. 55 (2), 133–141.

Kapusta, N.D., Mossaheb, N., Etzersdorfer, E., Hlavin, G., Thau, K., Willeit, M., Praschak-Rieder, N., Sonneck, G., Leithner-Dziubas, K., 2011. Lithium in drinking water and suicide mortality. Br. J. Psychiatry 198 (5), 346–350. https://doi.org/10.1192/bjp.bp.110.091041.

Khangarot, B.S., Das, S., 2009. Acute toxicity of metals and reference toxicants to a freshwater ostracod, *Cypris subglobosa* Sowerby, 1840 and correlation to EC50 values of other test models. J. Hazard Mater. 172 (2–3), 641–649. https://doi.org/10.1016/j.jhazmat.2009.07.038.

Kim, Y., Ashton-Alcox, K.A., Powell, E.N., . Gonadal Analysis. in: Histological Techniques for Marine Bivalve Molluscs. NOAA Tech. Mem. NOS NCCOS 27. https://hdl.handle.net/1834/30810. Silver Spring, pp. 1–10.

Krishnakumar, P.K., Asokan, P.K., Pillai, V.K., 1990. Physiological and cellular responses to copper and mercury in the green mussel *Perna viridis* (Linnaeus). Aquat. Toxicol. 18 (3), 163–173. https://doi.org/10.1016/0166-445X(90)90024-J.

Kszos, L.A., Beauchamp, J.J., Stewart, A.J., 2003. Toxicity of lithium to three freshwater organisms and the antagonistic effect of sodium. Ecotoxicology 12 (5), 427–437. https://doi.org/10.1023/a:1026160323594.

Lowe, D.M., 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Mar. Ecol. Prog. Ser. 46 (1/3), 91–100. http://www.jstor.org/stable/24827568.

Lowe, D.M., Moore, M.N., 1979. The cytology and occurrence of granulocytomas in mussels. Mar. Pollut. Bull. 10 (5), 137–141. https://doi.org/10.1016/0025-326X(79)90081-X.

Marigómez, I., Soto, M., Cancio, I., Orbea, A., Garmendia, L., Cajaraville, M.P., 2006. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the prestige oil spill (Monitoring Campaign 2003). Mar. Pollut. Bull. 53, 287–304. https://doi.org/10.1016/j.marpolbul.2005.09.026.

Martins, A., Dias da Silva, D., Silva, R., Carvalho, F., Guilhermino, L., 2022. Long-term effects of lithium and lithium-microplastic mixtures on the model species *Daphnia magna*: toxicological interactions and implications to 'One Health. Sci. Total Environ. 838, 155934 https://doi.org/10.1016/j.scitotenv.2022.155934.

Milam, C.D., Farris, J.L., Dwyer, F.J., Hardesty, D.K., 2005. Acute toxicity of six freshwater mussel species (glochidia) to six chemicals: implications for daphnids and Utterbackia imbecillis as surrogates for protection of freshwater mussels (Unionidae). Arch. Environ. Contam. Toxicol. 48 (2), 166–173. https://doi.org/10.1007/s00244-003-3125-3.

Moore, J.A., 1995. An assessment of lithium using the IEHR evaluative process for assessing human developmental and reproductive toxicity of agents. Reprod. Toxicol. 9 (2), 175–210. https://doi.org/10.1016/0890-6238(94)00069-7.

Múgica, M., Sokolova, I.M., Izagirre, U., Marigomez, I., 2015. Season-dependent effects of elevated temperature on stress biomarkers, energy metabolism and gamete development in mussels. Mar. Environ. Res. 103, 1–10. https://doi.org/10.1016/j.marenvres.2014.10.005.

Newell, R.I.E., 1989. Species profiles: life histories and environmental requirements of coastal fish and invertebrates (north and mid-atlantic) blue mussel. US Fish Wildlife Serv. Biol. Reproduct. 82 (11), 102. US Army Corps of Engineers (TREL-82-4). https://apps.dtic.mil/sti/citations/ADA212654.

Ortiz-Zarragoitia, M., Garmendia, L., Barbero, M.C., Serrano, T., Marigómez, I., Cajaraville, M.P., 2011. Effects of the fuel oil spilled by the Prestige tanker on reproduction-related parameters in wild mussel populations. J. Environ. Monit. 13, 84–94. https://doi.org/10.1039/C0EM00102C.

Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Di Cosmo, A., Soares, A.M.V.M., Polese, G., Pereira, E., Freitas, R., 2019. Ecotoxicological effects of lanthanum in *Mytilus galloprovincialis*: biochemical and histopathological impacts. Aquat. Toxicol. 211, 181–192. https://doi.org/10.1016/j.aquatox.2019.03.017.

Pipe, R.K., Coles, J.A., Carissan, F.M.M., Ramanathan, K., 1999. Copper induced immunomodulation in the marine mussel *Mytilus edulis*. Aquat. Toxicol. 46 (1), 43–54.

Reiman, C., Caritat, P., 1998. Chemical Elements in the Environment: Factsheets for the Geochemist and Environmental Scientist. Springer Science & Business Media, Berlin, p. 398.

Rocha, T.L., Sabóia-Morais, S.M.T., Bebianno, M.J., 2016. Histopathological assessment and inflammatory response in the digestive gland of marine mussel *Mytilus galloprovincialis* exposed to cadmium-based quantum dots. Aquat. Toxicol. 177, 306–315. https://doi.org/10.1016/j.aquatox.2016.06.003.

Rodríguez, B.M., Bhuiyan, M.K.A., Freitas, R., Conradi, M., 2021. Mission impossible: reach the carrion in a lithium pollution and marine warming scenario. Environ. Res. 199, 111332 https://doi.org/10.1016/j.envres.2021.111332.

Ruocco, N., Costantini, M., Santella, L., 2016. New insights into negative effects of lithium on sea urchin *Paracentrotus lividus* embryos. Sci. Rep. 6, 32157 https://doi.org/10.1038/srep32157.

Sheir, S.K., Handy, R.D., 2010. Tissue injury and cellular immune responses to cadmium chloride exposure in the common mussel *Mytilus edulis*: modulation by lipopolysaccharide. Arch. Environ. Contam. Toxicol. 59 (4), 602–613. https://doi.org/10.1007/s00244-010-9502-9.

Soto, M., Cajaraville, M.P., Marigómez, I., 1996. Tissue and cell distribution of copper, zinc and cadmium in the mussel, *Mytilus galloprovincialis*, determined by autometallography. Tissue Cell 28 (5), 557–568. https://doi.org/10.1016/s0040-8166(96)80058-9.

Soto, M., Ireland, M.P., Marigómez, I., 2000. Changes in mussel biometry on exposure to metals: implications in estimation of metal bioavailability in 'Mussel-Watch' programmes. Sci. Total Environ. 247 (2–3), 175–187. https://doi.org/10.1016/s0048-9697(99)00489-1.

Comission Staff Working Document on the Evaluation of the Directive 2006/66/EC on Batteries and Accumulators and Waste Batteries and Accumulators and Repealing Directive 91/157/EEC.

Suárez, M.P., Alvarez, C., Molist, P., San Juan, F., 2005. Particular aspects of gonadal cycle and seasonal distribution of gametogenic stages of *Mytilus galloprovincialis* cultured in the estuary of Vigo. J. Shellfish Res. 24 (2), 531–540.

Swarnakar, A., Choubey, S., 2014. Hazardous Chemical Present in Batteries and Their Impact on Environment and Humans. Retrieved from. https://www.researchgate.net/publication/274636169.

Thibon, F., Metian, M., Oberhansli, F., Montanes, M., Vassileva, E., Orani, A.M., Telouk, P., Swarzenski, P., Vigier, N., 2021a. Bioaccumulation of lithium isotopes in mussel soft tissues and implications for coastal environments. ACS Earth Space Chem 5 (6), 1407–1417. https://doi.org/10.1021/acsearthspacechem.1c00045.

Thibon, F., Weppe, L., Vigier, N., Churlaud, C., Lacoue-Labarthe, T., Metian, M., Cherel, Y., Bustamante, P., 2021b. Large-scale survey of lithium concentrations in marine organisms. Sci. Total Environ. 751, 141453 https://doi.org/10.1016/j.scitotenv.2020.141453.

Thomson, J.D., Pirie, B.J., George, S.G., 1985. Cellular metal distribution in the Pacific oyster, *Crassostrea gigas* (Thun.) determined by quantitative X-ray microprobe analysis. J. Exp. Mar. Biol. Ecol. 85 (1), 37–45. https://doi.org/10.1016/0022-0981(85)90012-7.

Tkatcheva, V., Poirier, D., Chong-Kit, R., Furdui, V.I., Burr, C., Leger, R., Parmar, J., Switzer, T., Maedler, S., Reiner, E.J., Sherry, J.P., Simmons, D.B., 2015. Lithium an emerging contaminant: bioavailability, effects on protein expression, and

homeostasis disruption in short-term exposure of rainbow trout. Aquat. Toxicol. 161, 85–93. https://doi.org/10.1016/j.aquatox.2015.01.030.

US EPA, 2008. ECOTOX Retrieval Database.

Viana, T., Ferreira, N., Henriques, B., Leite, C., De Marchi, L., Amaral, J., Freitas, R., Pereira, E., 2020. How safe are the new green energy resources for marine wildlife? The case of lithium. Environ. Pollut. 267, 115458 https://doi.org/10.1016/j.envpol.2020.115458.

Villalba, A., Carballal, M.J., López, C., 2001. Disseminated neoplasia and large foci indicating heavy haemocytic infiltration in cockles *Cerastoderma edule* from Galicia (NW Spain). Dis. Aquat. Org. 46 (3), 213–216. https://doi.org/10.3354/dao046213.

Zaldívar, R., 1980. High lithium concentrations in drinking water and plasma of exposed subjects. Arch. Toxicol. 46, 319–320. https://doi.org/10.1007/BF00310450.

Zaroogian, G., Yevich, P., 1993. Cytology and biochemistry of brown cells in *Crassostrea virginica* collected at clean and contaminated stations. Environ. Pollut. 79 (2), 191–197. https://doi.org/10.1016/0269-7491(93)90069-z.

Zeng, X., Li, J., Singh, N., 2014. Recycling of spent lithium-ion battery: a critical review. Crit. Rev. Environ. Sci. Technol. 44 (10), 1129–1165. https://doi.org/10.1080/10643389.2013.763578.