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Behavioural and biochemical responses of the sea snail *Tritia reticulata* to lithium concentration gradient

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A B S T R A C T

Lithium (Li) is present in many modern technologies, most notably in rechargeable batteries. Inefficient recycling strategies for electronic waste containing this element may result in its release into aquatic systems, which may induce harmful effects on wildlife. The present study evaluated the effect of Li contamination on the gastropod *Tritia reticulata* exposed to different concentrations of Li (100, 200, 500 and 1000 μg L^{-1}) for 21 days. Biochemical analyses showed that this species was not significantly affected by this contaminant at the cellular level, as no significant differences were observed in terms of metabolism, oxidative stress, and neurotoxicity. Results further revealed that snails attempted to avoid Li accumulation by burying in the sediment at a faster rate when exposed to the highest concentrations (500 and 1000 μg L^{-1}). More research is needed to fully assess the response of *T. reticulata* to Li contamination, such as investigating longer exposure periods or other endpoints.

1. Introduction

The global demand and consumption of lithium (Li) have increased significantly in recent decades, mostly because of technological development. Lithium has many industrial uses, such as Li-ion batteries (LIB) for electric vehicles and electrical applications, mobile phones, lubricants, renewable energy, as well as in the ceramics and glass industries (Kokkotis et al., 2017; Tabelin et al., 2021; Winslow et al., 2018). However, it is estimated that by 2025 > 80 % of the total Li market will be used to produce LIBs (Harper et al., 2019). Between 2007 and 2017, the production of Li for LIBs witnessed a significant growth, rising from 5160 to 19,780 tons (Jaskula, 2008). Furthermore, it is projected that the production will reach 11 million tons by 2023 (Agusdinata et al., 2018; Bai et al., 2020). With the rise of products containing Li, several concerns have been considered about the environmental impact (Bolan et al., 2021). Incorrect management and the lack of efficient recycling and recovery technologies result in the disposal of Li-rich wastes in landfills. These substances are leaching into aquatic systems, which increases Li concentrations to levels above those found naturally in freshwater and seawater systems. Lithium concentrations in riverine systems are generally low, with studies reporting concentrations below 10 μg L^{-1} in the absence of contamination (Kszos and Stewart, 2003). However, coastal and marine systems can display significantly higher concentrations (≈ 300 μg L^{-1}): as identified by Angino and Billings (1966) at the North Atlantic Ocean Li concentrations range from 174 to 218 μg L^{-1}, while in the Ria de Aveiro (coastal lagoon Norwest Portugal) Li concentrations can reach mean values of 287 μg L^{-1} (see for review: Barbosa et al., 2023). These values are naturally susceptible to hydrological and geological factors; however, contamination from anthropogenic sources has also been reported. For example, Choi et al. (2019) reported that, due to household and industrial pollution, downstream Li concentrations in the Han River in South Korea (1.57 mg L^{-1}) were significantly higher than upstream concentrations (0.28 mg L^{-1}).

The introduction of excessive amounts of metals or other xenobiotics such as Li is a problem in aquatic systems as organisms are forced to respond to these external stressors. Xenobiotic metabolism typically leads to excessive production of reactive oxygen species (ROS), resulting in compensatory responses such as the activation of antioxidant mechanisms (Regoli and Giuliani, 2014). In humans, several studies demonstrated that Li administration activates antioxidant defences (Arraf

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et al., 2012; Khairova et al., 2012; Machado-Vieira et al., 2007), and at very low concentrations it decreased O_2^- production (Marmol et al., 2019). Activation of antioxidant defences and several other defence mechanisms have been shown in marine invertebrates exposed to metals (Bhagat et al., 2016; Ubrihien et al., 2017; Viana et al., 2020). Studies detailing the impacts of Li contamination on marine organisms are still scarce, although this metal is known to have negative effects in high concentrations. Most studies on the impacts of Li on aquatic wildlife focus on invertebrates of the classes Gastropoda and Bivalvia (Rodríguez et al., 2021, 2022; Santos et al., 2023; Thibon et al., 2021; Viana et al., 2020;). In a study by Viana et al. (2020), the mussel *Mytilus galloprovincialis* showed an increase in Li accumulation when exposed to a Li range of concentrations. With an increase in Li, impacts at the physiological and biochemical levels were observed, notably through metabolic depression, oxidative stress, and neurotoxicity, particularly at higher exposure concentrations. In another study by Thibon et al. (2021), the ability to bioaccumulate Li was evidenced in mussels exposed to varying concentrations of Li. Other Li effects are also expected, as Ruocco et al. (2016) reported dose-dependent malformations in embryos of the sea urchin *Paracentrotus lividus*. The available information on Li toxicity indicates that the responses are expected to vary between species (Barbosa et al., 2023; Rodríguez et al., 2022). Reports of varying accumulation, metabolic and antioxidant alterations, and reproductive-level complications justify a species-specific approach to assessing the toxicity of this element (Barbosa et al., 2023; Rodríguez et al., 2022; Thibon et al., 2021). Additionally, studies typically correlate higher concentrations with stronger effects, highlighting a dose-dependent response (Thibon et al., 2021).

To complement the existing literature on Li toxicity to aquatic organisms, the present study aimed to evaluate the response of the gastropod *Tritia reticulata* to different concentrations of this element, ranging from 100 to 1000 μg L^{-1}. *T. reticulata* is a burrowing snail that scavenges the intertidal zone for food. It is widely distributed mostly through the North Atlantic, in European waters (Tallmark, 1980). Although no studies have to date evaluated this species' response to Li contamination, previous work in a species of the same genus, *T. neritea*, has shown that a combination of Li exposure (500 μg L^{-1}) and the increased temperature induced biochemical changes and physiological alterations, which in turn affected organisms' feeding and scavenging capacities (Rodríguez et al., 2021; 2022). Despite this, tolerance for varying concentrations of Li remains unstudied for these gastropods. As an array of responses may be observed in this group of organisms, an extensive evaluation of behavioural, physiological, and biochemical responses was performed in the present study.

2. Material and methods

2.1. Sampling and experimental setup

Tritria reticulata specimens were collected from the Ria de Aveiro coastal lagoon (Portugal), during low tide using a fishing net with a small opening. The selected organisms had a mean length of 26.3 ± 2.3 mm and a mean width of 12.9 ± 1.1 mm. After collection, the organisms were placed in aquaria for acclimation and depuration for 7 days prior to the experiment. During this week, snails were kept in artificial seawater prepared by dissolving a commercially available salt (Tropic Marin®-SEA SALT) in osmosis water (salinity of 30 ± 1). Specimens were kept under a natural photoperiod, at a temperature of 17 ± 1 °C, and fed goldfish food three times a week.

After acclimation, the gastropods were distributed in aquaria and exposed to different Li concentrations for 21 days. Each aquarium contained 5 L of water and clean fine sediments at the bottom (1/4 of the aquarium volume was filled with sediment). The aquaria were spiked with Li concentrations of: A) 100 μg L^{-1}, B) 200 μg L^{-1}, C) 500 μg L^{-1}, D) 1000 μg L^{-1}. Spiking was performed with an aqueous Li stock solution (Inorganic Ventures™). Control aquaria (seawater without adding Li) were also used. Three aquaria were used per treatment, each containing 20 individuals. The mean length and width values of organisms from each treatment were similar to avoid a size-depend response: 27.0 ± 2.4 and 13.5 ± 1.5 mm for CTL; 27.0 ± 2.9 and 13.1 ± 1.3 mm for 100 μg L^{-1}; 24.9 ± 1.3 and 12.4 ± 0.3 mm for 200 μg L^{-1}; 26.0 ± 3.2 and 12.6 ± 1.2 for 500 μg L^{-1}; 26.6 ± 2.6 and 13.1 ± 1.3 mm for 1000 μg L^{-1}. Concentrations were selected based on: i) previous studies in which different Li concentrations were tested with marine invertebrates (Ruocco et al., 2016; Sconzo et al., 1998; Viana et al., 2020); ii) Li concentrations found in marine systems, including the sampling area Ria de Aveiro, reaching values up to ≈ 300 μg L^{-1} (Barbosa et al., 2023); iii) and near-future scenarios considering predicted increase of Li use. The highest concentration used was selected to test the species' response tolerance capacity. The water, prepared as in the acclimation period, was renewed every 7 days and all characteristics (salinity of 30 ± 1, the temperature of 17 ± 1 °C) were re-established, including Li contamination. The water samples were collected immediately after spiking to determine the actual exposure concentrations, which were immediately acidified with 0.25 μL of acid (HNO_3 65 %) for preservation. To assess Li stability over time, in the medium, blank aquaria with 200 μg L^{-1} of Li and no organisms were run, and samples were collected immediately after spiking and after 24 h, 48 h, 72 h and 96 h. The animals were fed during the experimental period every other day (goldfish food, 0.07 g per aquarium) and the food debris that snails did not ingested were removed from the sediment surface to maintain water quality.

At the end of the exposure period (21 days), no mortality was observed and 14 snails from each aquarium were immediately frozen with liquid nitrogen and stored at –80 °C while 3 alive individuals from each aquarium were immediately used to test the burying capacity. For biochemical analyses and Li quantification, a total of 9 frozen organisms was used, corresponding to 3 pools of 3 animals per aquarium. Soft tissues of each pool were homogenised manually with liquid nitrogen, using a mortar and pestle, and 0.3 g of tissue fresh weight (FW) was distributed per aliquot/ microtube.

2.2. Lithium quantification in water and organism samples

The quantification of Li in water samples was performed by inductively coupled plasma ophthalmic emission spectroscopy (ICP-OES) in a Jobin Yvon Activa M. Quality control of the results was ensured using calibration curves with $R^2 > 0.995$. The curves were built based on five calibration standards that ranged in concentration from 10 to 1000 μg L^{-1}. The limit of quantification (LOQ) was considered equal to the lowest calibration standard (10 μg L^{-1}).

Lithium quantification in the tissues required prior solubilization through microwave-assisted acid digestion. After freeze drying and homogenization, 200 mg of each sample were transferred to Teflon tubes, after which 1 mL of HNO_3, 2 mL of H_2O_2 and 1 mL of H_2O were added. The tubes were then placed in a CEM MARS 5 microwave system and subjected to a temperature increase of up to 175 °C for 15 min, which was then held for another 5 min. Finally, samples were collected into polyethylene vials and a final volume of 25 mL was obtained by dilution with ultrapure water. Quality control blanks (reagents without samples) were also run to assess the presence of contamination. After digestion, the samples were analysed by ICP-OES.

2.3. Behavioural parameters

During the experimental period (21 days), mortality was monitored. In addition to mortality, burial capacity was investigated by measuring the capacity of snails to bury into a layer of sediment (approximately 3 cm high). Three animals from each aquarium were used and the time required to be completely buried was recorded.

2.4. Biochemical parameters

To evaluate the biochemical alterations induced in gastropods after exposure, a set of biochemical parameters was performed that included i) electron transport system (ETS) activity, total protein (PROT) and glycogen (GLY) contents, to evaluate the metabolic capacity and energy reserves; ii) superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRed), glutathione peroxidase (GPx) activities, to determine snails antioxidant defence capacity; iii) glutathione S-transferases (GSTs) and carboxylesterases (CbEs) activities, to assess snails biotransformation capacity; iv) lipid peroxidation (LPO), protein carbonylation (PC) and glutathione reduced (GSH) contents, to determine cellular damage and redox balance; v) acetylcholinesterase (AChE) activity, to determine neurotoxic effects. For each biomarker, extraction was performed with specific buffers in a 1:2 (w/v) ratio with the homogenized tissue: phosphate buffer for PROT, GLY, SOD, CAT, GRed, GPx, GSTs, CbEs, PC and AChE; 20% TCA (trichloroacetic acid) was used for LPO; Tris buffer was used for ETS and KPE buffer for GSH. For the extraction procedure samples were sonicated using a TissueLyser II (Qiagen) and centrifuged for 20 min at 10,000 g (or 3,000 g for ETS) at 4 °C. After the extraction was completed, the samples were analyzed in duplicate for the respective parameters using a microplate reader (BioTek). Detailed procedures have already been described in previous works such as Rodríguez et al. (2022), Coppola et al. (2017) and Pinto et al. (2019) and are provided as supplementary material.

2.5. Data analysis

Behavioural (bury capacity) and biochemical results (ETS, PROT, GLY, SOD, CAT, GRed, GPx, GSTs, CbEs, LPO, PC, GSH, and AChE) were submitted to a permutational multivariate analysis of variance using the PERMANOVA add- in PRIMER v6. The results were compared with one factor: Li content. Significance was evaluated by pseudo-F p-values in the PERMANOVA main test. The null hypothesis tested was: Li does not affect *T. reticulata* at the behavioural or cellular level. Comparisons were considered significantly different when $p < 0.05$.

The accumulation of Li in snail tissues was also analysed through the bioconcentration factor (BCF), defined as the ratio between the concentration in the organism and the concentration in the water (measured after spiking) (Arnot and Gobas, 2006).

3. Results

3.1. Lithium concentration in water and organism samples

Lithium concentrations found in water samples collected from the exposure aquaria immediately after spiking are shown in Table 1. The results showed that the control has a baseline Li concentration of 274 μg L^{-1}. Therefore, the contamination in the present study should be interpreted as the addition of 100, 200, 500 and 1000 μg L^{-1} above this baseline value. The final concentrations showed an increase of 40 % compared to the nominal concentrations, regardless of the treatment.

Lithium concentrations in the water samples collected from the blanks immediately after spiking and after 24 h; 48 h; 72 h; 96 h are shown in Table 2. The results demonstrated that the concentrations measured over time were similar, highlighting the stability of Li in the medium.

The average concentrations of Li found in the tissues at the end of the exposure period are presented in Table 3. The results showed that snails tended to bioaccumulate higher concentrations of Li along with the increasing exposure gradient. However, the bioconcentration factor (BCF) decreased with increasing exposure concentration.

Table 1
Lithium concentrations (μg L^{-1}) mean values + standard deviation, in water samples collected immediately after spiking from each treatment.

Treatment	Nominal concentration	Measured concentration	Final concentration*
CTL	0	274±20	274±20
100	100	421±22	147±22
200	200	567±19	293±19
500	500	839±39	565±39
1000	1000	1419±36	1145±36

* Final concentration corresponds to measured concentration in each exposure treatment – concentration measured at CTL.

3.2. Behavioural parameters

The bury capacity did not show statistically significant differences among treatments (Fig. 1). Despite this, a decrease in variability is observed in organisms exposed to higher concentrations of Li, culminating in an overall reduced burying time at the two highest concentrations.

3.3. Biochemical alterations

Metabolic capacity and energy reserves

In terms of metabolic capacity, snails showed no significant differences among treatments, although slightly higher ETS activity was observed in contaminated snails (Fig. 2A). Regarding snail energy reserves (GLY and PROT concentrations), a similar pattern was found, with no significant differences among treatments (Figs. 2B and 2C).

Antioxidant and biotransformation capacity

Regardless of the antioxidant enzyme, snails did not show significant differences in terms of activity among the treatments tested (Fig. 3A – D).

The activity of the GSTs and CbEs enzymes did not show significant differences among treatments (Fig. 4A-C).

Cellular damage and redox balance

Levels of LPO were significantly higher in snails exposed to 200 μg L^{-1} compared to the ones exposed to CTL, 100 and 500 μg L^{-1} (Fig. 5A), while no significant differences among treatments in terms of PC levels were found (Fig. 5B). The GSH content also revealed no significant differences among treatments (Fig. 5C).

Neurotoxicity

The activity of AChE found in snails after the exposure period showed no significant differences among treatments (Fig. 6).

4. Discussion

The increasing use of Li in electronic and medical applications justifies the growing number of studies on the toxicity of this element in aquatic fauna. Although gastropods have been widely used as bioindicators and biomonitors of metal contamination (see reviews Baroudi et al., 2020; Srivastava and Kumar Singh, 2020), few studies have been carried out on the accumulation, physiological and biochemical effects of Li in this group of organisms. In the present study, limited negative effects of this metal were observed in comparison with previous studies in other species of marine snails or in other marine invertebrates. These findings are not entirely surprising, as different behaviour and pre-established defence mechanisms can explain differences in responses between species.

The detection of a toxicant can stimulate organisms to seek refuge to avoid it (Aldaya et al., 2006) and the decrease in population or the

Table 2
Lithium concentrations (μg L^{-1}) mean values + standard deviation, in blank samples collected immediately and periodically for one week after spiking.

Sampling time 0h	24h	48 h	72 h	96 h
278±3	260±23	252±9	247±11	267±10

Table 3
Lithium concentrations (μg g^{-1}) mean values + standard deviation, in snails at the end of the exposure period and BCF values (bioconcentration factor, kg L^{-1}).

	Concentration	BCF
CTL	0.99 ± 0.3	3.64×10^{-3}
100	1.07 ± 0.09	2.54×10^{-3}
200	1.05 ± 0.15	1.79×10^{-3}
500	1.45 ± 0.12	1.73×10^{-3}
1000	2.26 ± 0.32	1.59×10^{-3}

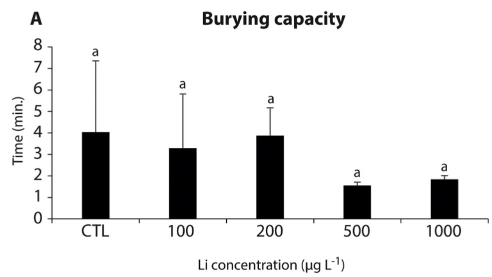

Fig. 1. The time needed by *Tritia reticulata* to bury after exposure to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

absence of a particular species in an environment may be due not only to mortality but also to avoidance behaviour (Novais et al., 2010). This behaviour is quick and easy to detect (Lopes et al., 2004) even when the animals' morphology and physiology seem unaffected (Lefcort et al., 2000). It is also fundamental to determine the effects of stress factors at the population level (Loureiro et al., 2009) and ecosystem as it is related to feeding, predation, reproduction, and migration (Orvain and Sauriau, 2002). Avoidance and preference have been observed in snails under different environmental conditions, such as sediment type and nutritional quality, algal biomass availability and characteristics, intra- and inter-species competition, and environmental contamination (Araújo et al., 2012; 2013; 2016; Barnes and Greenwood, 1978; Berridge et al., 1989; ; Campana et al., 2013; Forbes and Lopez, 1986; Grudemo and Bohlin, 2000; Haubois et al., 2005; Marklevitz et al., 2008a,b). However, avoidance and escape behaviour can be a defence mechanism against chemical stress when the individuals' physical ability to move is not affected (Aldaya et al., 2006; Loureiro et al., 2009; Novais et al., 2010). When this active avoidance behaviour is reduced by the toxic substance, two other possible defence strategies can be adopted: burying in the sediment (sinking) and/or a grouping behaviour (Araújo et al., 2012; Campana et al., 2013; Hampel et al., 2009). In this way, the organisms minimize contact with the chemical, and consequently its effects. Not many burial behaviour studies using gastropods have been carried out with Li (Rodríguez et al., 2022), but some have been carried out with copper (Cu) (Brown, 1982; Cheung and Wong, 1999; MacInnes and Thurberg, 1973). In the study of , different behaviour (floating, burying, and crawling) of *Peringia ulvae* exposed to Cu-enriched sediments were significantly reduced at a concentration higher than 200 μg g^{-1} Cu. The same contaminant also reduced the normal activity of *Babylonia lutosa* (crawling: body extended and movement with foot attached) by increasing the percentage of retracted (body extended, foot not attached, unable to move) or distressed (body retracted in shell) snails as the concentration of Cu increased (Cheung and Wong, 1999). Similarly, *Nassarius obsoletus* started to retract its body at 4.00 ± 5.00 mg L^{-1} Cu (MacInnes and Thurberg, 1973) and *Bullia digitalis* started at 0.2 mg L^{-1} Cu (Brown, 1982). Besides Cu effects, testing the impacts of mercury (Hg) on the freshwater snail *Bellamya bengalensis*, Dhara et al. (2022) demonstrated that the crawling activity and clumping tendency decreased with increasing exposure concentration and the progress of exposure time. Similarly, Campana et al. (2013) demonstrated that the

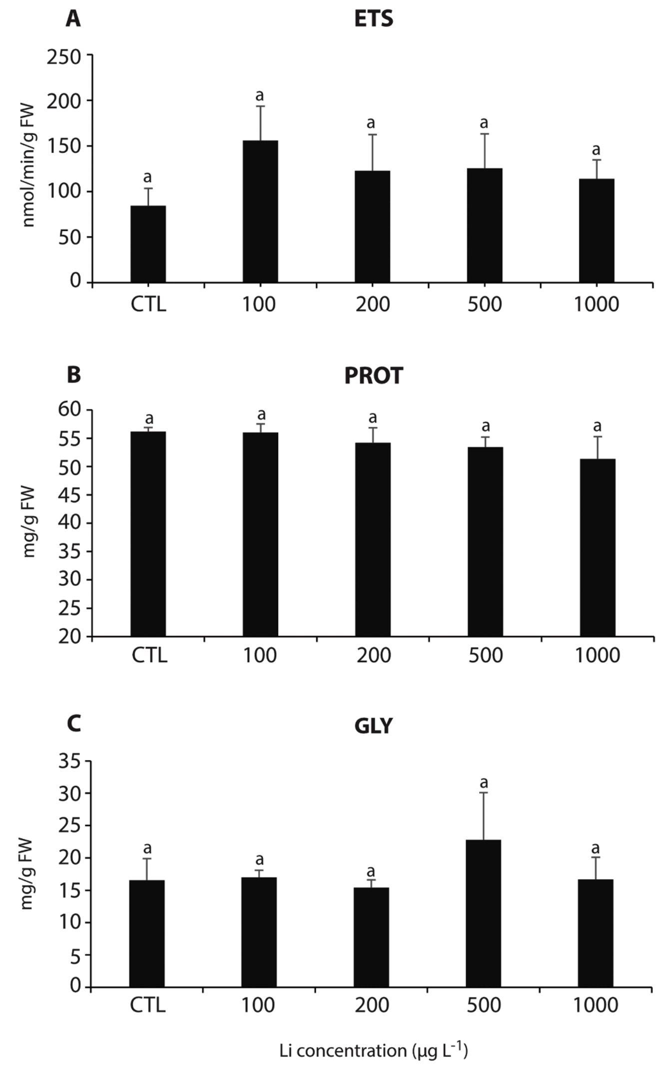

Fig. 2. A: Electron transport system (ETS) activity, B: Protein (PROT) content, C: Glycogen (GLY) content, in *Tritia reticulata* exposed to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

snail *Hydrobia ulvae* continued to bury and crawl at lower cadmium (Cd) and Cu exposures, but at highest Cu exposure they withdrew inside their shell closing the operculum and avoided any contact with the substrate (sinking). In contrast, the reproduction and behavioural responses were generally not affected when organisms were exposed to Cd and zinc treatments. Aloya et al. (2011) further observed that prolonged food searching time and increased self-burial in the sand were highly correlated with increased levels of plastic garbage cover in the gastropod *Nassarius pullus* on garbage-impacted sandy shores of Talim Bay (Philippines). The above-mentioned studies can thus support the hypothesis that, in the present study, *T. reticulata* at the highest Li exposure concentrations showed an attempt to limit their exposure to the metal by reducing time to bury. Avoiding contact with Li-contaminated water would explain the decreased BCF values found along the increasing exposure gradient contributing to limiting Li accumulation in *T. reticulata* tissues, particularly at the highest exposure concentration. In fact, although higher Li concentrations were found in snails exposed to 500 and 1000 μg L^{-1} of Li, accumulation was not proportional to the exposure range which indicates that snails burying capacity could contribute to limiting Li accumulation. However, an excessive tendency to remain buried in the natural environment can also be harmful, since it alters the foraging ability of organisms (Rodríguez et al., 2022).

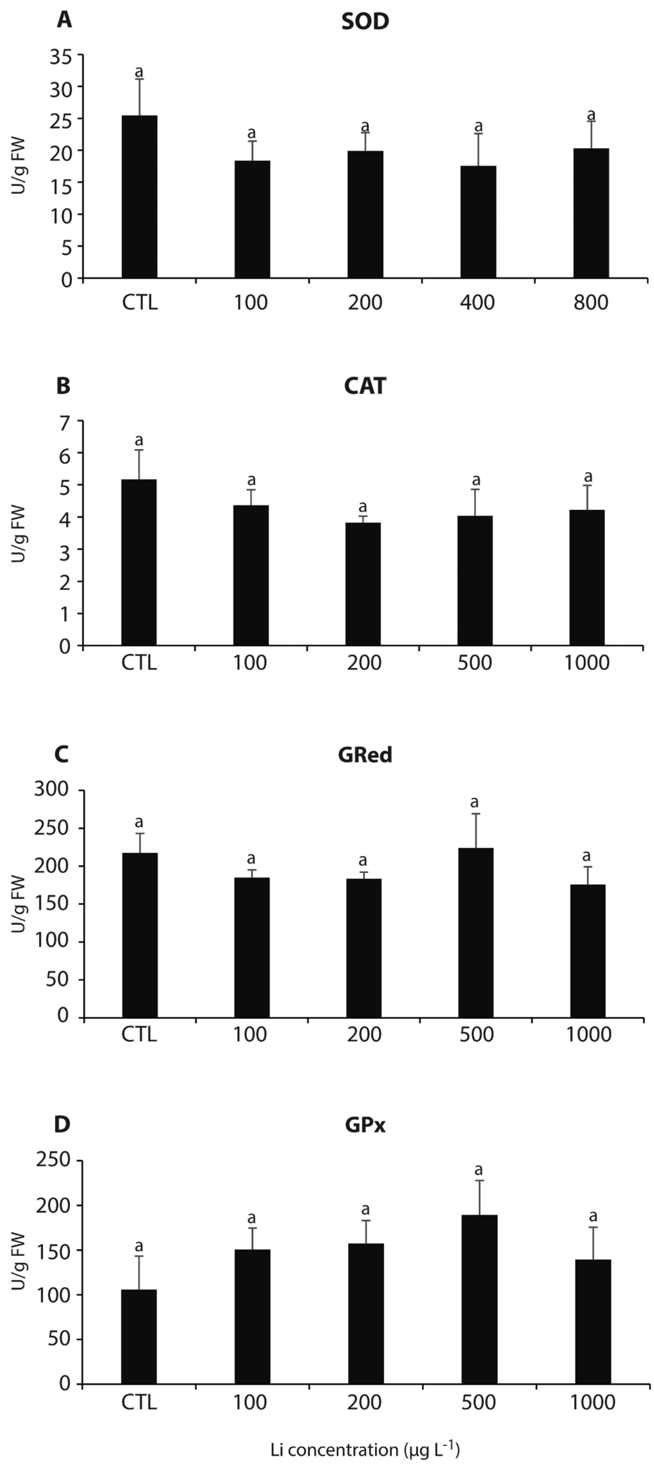

Fig. 3. A: Superoxide dismutase (SOD), B: Catalase (CAT), C: Glutathione reductase (GRed) and D: Glutathione peroxidase (GPx) activities, in *Tritia reticulata* exposed to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

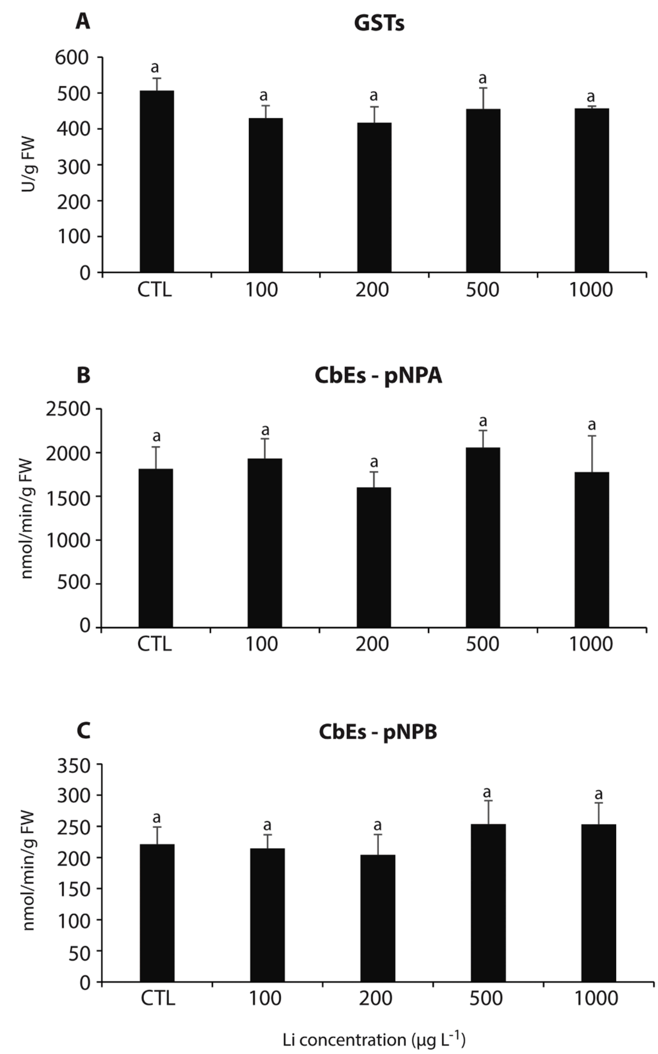

Fig. 4. A: Glutathione S-transferases (GSTs), B: Carboxylesterases (CbEs) with pNPA substrate and C: Carboxylesterases (CbEs) with pNPB substrate activities, in *Tritia reticulata* exposed to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

Organism tolerance to stressors is vital since their survival depends on the ability to balance energy demand and supply (Calow and Forbes, 1998; Sibly and Calow, 1989), with the need to maintain cellular homeostasis and systemic functions (Pörtner, 2001). They also require additional energy reserves because they have to reproduce, grow and move to find food or escape from predators or pollution. As such, metabolism may be a key factor in the effects of stress due to its role in energy balance, adaptation, and tolerance of organisms to stress (Calow and Forbes, 1998; Sibly and Calow, 1989). Electron transport system (ETS) activity has been widely used as a biomarker to determine energy expenditure at the mitochondrial level (Berridge et al., 2005; De Coen and Janssen, 1997; Fanslow et al., 2001). The activity of ETS is associated with the potential metabolic activity of an organism, being a major producer of reactive oxygen species (ROS) (Boveris et al., 1972; Chance et al., 1979; Loschen et al., 1971). Marine invertebrates have been shown to increase their metabolic capacity when under stressful treatments to fuel up their defence mechanisms (Sokolova and Pörtner, 2003). However, under limited stress treatments, marine snails have also been shown to be able to maintain or even decrease their ETS activity by reducing their respiration and filtration capacity to limit the entrance of pollutants (Rodríguez et al., 2022). In a study by Viana et al. (2020) conducted with mussels, significant differences were observed, with control mussels showing significant differences from organisms exposed to Li (100, 250 and 750 µg L^{-1}), with higher values in the control than in Li-exposed mussels. In the present study, no differences in ETS activity were observed regardless of the concentrations tested. Additionally, the snail protein (PROT) and glycogen (GLY) contents were similar under all conditions, which may result from metabolism maintenance. Similarly, Rodríguez et al. (2022) showed no significant changes in ETS activity or energy reserves after exposing the gastropod

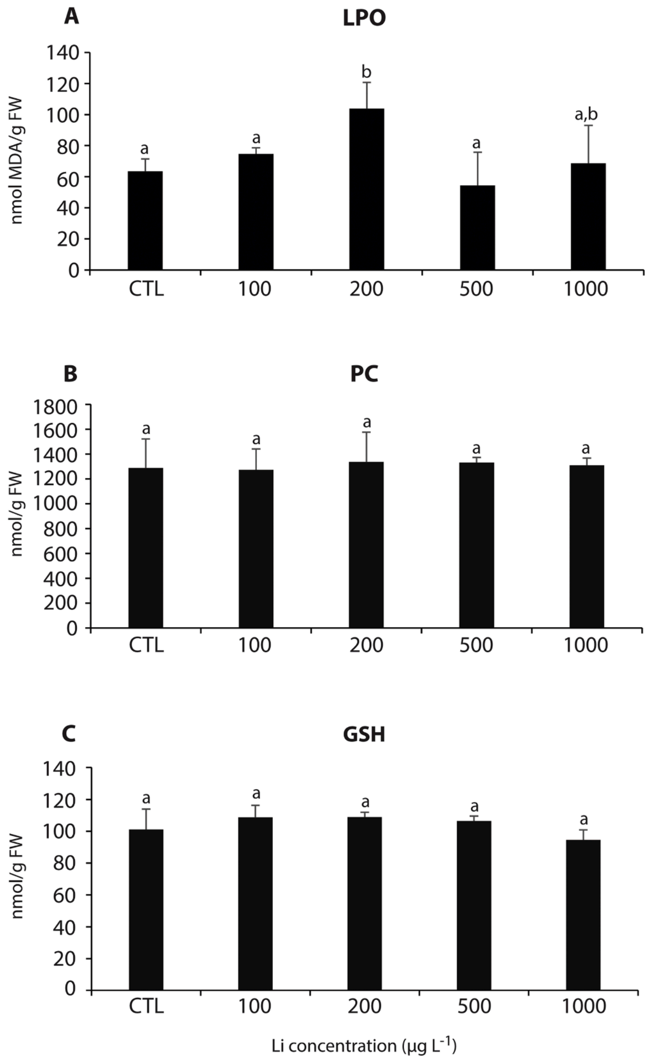

Fig. 5. A: Lipid peroxidation (LPO) levels, B: Protein carbonylation (PC) levels, C: Glutathione reduced (GSH) content, in *Tritia reticulata* exposed to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

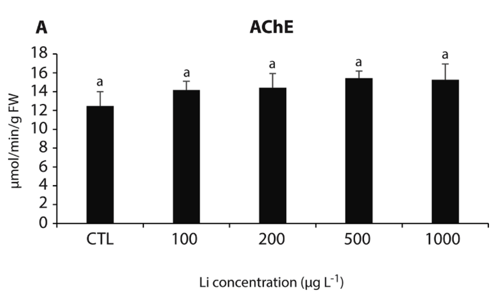

Fig. 6. Acetylcholinesterase (AChE) activity, in *Tritia reticulata* exposed to different Li concentrations. Values are the mean + standard deviation. N = 3 (3 aquaria per treatment).

T. neritea to Li for 28 days, highlighting the similar metabolic responses of organisms of the genus *Tritia* to Li exposure. The lack of alteration of metabolism may result from the adaptation of these organisms to Li in seawater and the capacity to avoid exposure by burying them in the sediment.

Marine invertebrates, including gastropods, have also shown changes at the cellular level when exposed to pollutants. Such changes may include oxidative damage resulting from the overproduction of reactive oxygen species (ROS) (Regoli and Giuliani, 2014). To avoid cellular damage, under stressful conditions, organisms can activate their antioxidant defences to eliminate the excess of ROS produced (Freitas et al., 2016; Regoli and Giuliani, 2014; Velez et al., 2016). Several studies have shown that the antioxidant response in the presence of metals differs between species and the xenobiotic (Berglund et al., 2007; Ji et al., 2006; Martinez-Haro et al., 2011; Mateo and Hoffman, 2001). The first line of antioxidant action is linked to the enzyme superoxide dismutase (SOD), which acts to detoxify products resulting from oxidative stress (Johnson and Giulivi, 2005), by catalysing the dismutation of superoxide radicals (McCord and Fridovich, 1969). Catalase (CAT) is an antioxidant enzyme that catalyses the breakdown of H_2O_2, formed by the action of SOD, into H_2O, and O_2. Another antioxidant enzyme is glutathione peroxidase (GPx), important as a protective system against endogenous and exogenous lipid peroxidation (Wendel, 1981), using reduced glutathione (GSH) as an H donor to catalyse the reduction of H_2O_2 to H_2O reduction, reducing lipid hydroperoxides to alcohols, with simultaneous oxidation of GSH to GSSG. This oxidized glutathione is then reduced to GSH by glutathione reductase (GRed), with NADPH forming NADP. In the present study, all these enzymes (SOD, CAT, GRed, GPx) were analysed, with findings revealing a lack of an antioxidant response in *T. reticulata* exposed to increasing concentrations of Li, although at 500 μg L^{-1} GRed and GPx enzymes showed higher activities than the control snails (but without statistical significance). In a previous study by Rodríguez et al. (2022) with the species *T. neritea*, significant differences in the response of antioxidant enzymes between Li-contaminated and non-contaminated organisms were observed for CAT while GPx activity showed no differences between CTL and Li-exposed snails (500 μg L^{-1}). The divergence between this study and the present one can be attributed to the exposure time which was longer for *T. neritea* (28 days). On the contrary, in mussels exposed to Li for 28 days, a general increase in antioxidant activity (CAT) was observed at a concentration of 100 μg L^{-1}, which began to decrease with increasing Li concentration (Viana et al., 2020). The authors explained that this response corresponds to snails' limited capacity to enhance their antioxidant defences.

Regarding biotransformation enzymes, glutathione S-transferases (GSTs) are Phase II enzymes known to be involved in xenobiotic detoxification by catalysing hydrophobic and electrophilic molecules when conjugated with glutathione, making them less toxic and thus more easily eliminated (Allocati et al., 2018). Carboxylesterases (CEs) are Phase I hydrolytic enzymes well-known for pesticide detoxification and the metabolic transformation of some pharmaceutical drugs (Acena et al., 2017; Solé et al., 2018). In the present study, no significant differences were observable between the activity of biotransformation enzymes of contaminated and non-contaminated organisms, highlighting the absence of a response of *T. reticulata* to Li. Nevertheless, at the highest Li concentrations, CbEs-pNPB activity tended to increase which, probably, contributed to BCF decrease along the increasing exposure gradient. Also, the lack of efficient defence capacity may be related to snails' behaviour of burying in the sediment to avoid accumulation and, consequently, injury.

The oxidative damage of the lipids generating different types of protein carbonyls is evaluated by lipid peroxidation (LPO), and an overview of protein oxidation in the cells is given by the method of protein carbonylation (PC) (Mesquita et al., 2014; Ohkawa et al., 1979). In the present study, it can be observed that there are significant differences only between the concentration of 200 μg L^{-1} and the highest concentration (1000 μg L^{-1}), although between the highest concentration and the remaining concentrations (CTL, 100 and 500 μg L^{-1}) there are no significant differences, suffering from a hormesis effect, with an increase in values at a low concentration (200 μg L^{-1}) failing immediately and rising again at the higher concentration (1000 μg L^{-1}). From this it can be deduced that the oxidative stress increases when organisms are subjected to a concentration of 200 μg L^{-1}, decreasing soon thereafter, but increasing again when the Li concentration increases. In the study by Rodríguez et al. (2022) there are any significant differences when there is Li contamination. In mussels exposed to the same metal as gastropods in this study, an increase of LPO could be observed when we compare non-contaminated with contaminated, with significant differences from the two first concentrations (100 and 250 μg L^{-1}) for the last concentration (750 μg L^{-1}) (Viana et al., 2020). The protein carbonylation (PC) levels in this study do not demonstrate any differences in the results.

Glutathione reduced (GSH) is a crucial molecule in resisting oxidative stress and maintaining the reducing environment of the cell, controls signaling processes, has the function of detoxifying certain xenobiotics, and is considered one of the most important ROS scavengers. This molecule is related to GSSG and its ratio (GSH/GSSG) is used as a biomarker of redox balance (Owen and Butterfield, 2010; Zitka et al., 2012). In the present study, no significant differences were observed between the different concentrations and the control.

The enzyme responsible for the hydrolytic metabolism of the neurotransmitter acetylcholine is acetylcholinesterase (AChE) (English and Webster, 2012). When the activity of acetylcholine is inhibited, there is an accumulation of acetylcholine, causing negative effects on the neurological system (Lionetto et al., 2013). Lithium acts as a calcium channel blocker in psychopathological treatments (Wright and Jarrett, 1991), affecting cell signalling. It also affects membrane potential and ion transport, by interfering with macromolecules at sites specific for sodium (Na) and magnesium (Mg) (Jakobsson et al., 2017). Due to these properties, a neurotoxic effect was to be expected at high concentrations. Viana et al. (2020) had already shown inhibition of AChE in the mussel *Mytilus galloprovincialis* exposed to Li. Despite this, the results presented here indicate that Li does not cause neurotoxicological effects in *T. reticulata* within the concentration range tested and exposure period.

5. Conclusion

The species *Tritia reticulata* is an ecologically important organism that can provide useful information on what marine life is like at the bottom of the ocean, as they are burying scavengers. In the present study, the burial behaviour of the snails indicated that at higher concentrations the organisms take less time to bury themselves as a defence mechanism against Li toxicity. This mechanism turned out to be useful since it limited Li accumulation in *T. reticulata*. Additionally, Li did not induce an antioxidant response in *T. reticulata* within the concentration range tested, nor does it induce neurotoxicity. Overall, the present study showed that *T. reticulata* is tolerant to Li contamination scenarios, which contrasts with previous studies examining different marine invertebrate species. The lack of a response may be related to the already significant baseline concentration of Li in seawater. Lithium exists naturally at varying concentrations in seawater with values often greater than hundreds of μg L^{-1}, which is then susceptible to increase due to contamination. As such, it is not surprising that some organisms would be capable of resisting different degrees of Li contamination. This again shows the importance of a species-specific approach to the determination of Li toxicity, since different organisms respond differently to the same stressor.

Authors credits

Sara Campos, Carla Leite, João Pinto, Bruno Henriques: Laboratory experiments and quantifications; Formal analyses. **Sara Campos:** Writing-original draft; Writing - review & editing. **Amadeu M.V.M. Soares:** Funding. **Mercedes Conradi, Eduarda Pereira, Rosa Freitas:** Conceptualization; Supervision; Writing - review & editing; Funding.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106629.

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