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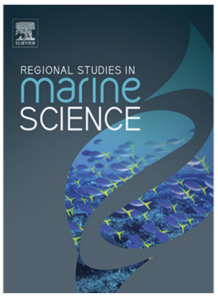

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Oxidative stress response of scallop *Aequipecten tehuelchus* from Patagonia Argentina exposed to inorganic arsenic

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Abstract

Laboratory studies were performed to assess the impact of acute arsenic (As) exposure on the Tehuelche scallop *Aequipecten tehuelchus* from San José gulf (Patagonia, Argentina). The As accumulation in gills, digestive gland and muscle of *A. tehuelchus* was analyzed after 96 hours of exposure at different concentrations (0, 4, 5, 6.3 and 7.9 mg As/L). Accumulation in all tissues increased linearly with the exposure concentration, evidencing no As saturation. A 96 hour median lethal concentration (LC50) value of 7.1 mg As/L was determined, characterizing this species as relatively tolerant to As. The potential effects of As were studied through the production of reactive species, enzymatic activities of catalase and glutathione-S-transferase, metallothioneins, lipid peroxidation, and lipid-soluble antioxidants. In presence of As, the 2',7'-dichlorodihydrofluorescein diacetate oxidation rate and thiobarbituric acid reactive substances content did not show changes in any tissues. Catalase and glutathione S-transferase activities raised in gills and digestive gland and remained unchanged in muscle. Metallothioneins increased in gills and digestive gland and a decreasing pattern of α-tocopherol was observed in gills and muscle. Scallops were slightly affected by As exposure, especially at high levels, being able to counteract its effects by the induction of biotransformation pathways and antioxidant defense mechanisms.

Keywords

Acute toxicity; Antioxidant defence system; Biomarkers; Lethal Media Concentration; Metalloid accumulation; Mollusk.

1. INTRODUCTION

Exposure to contaminants can induce a wide variety of effects in organisms including biochemical, immunological, physiological, and bioenergetics responses to stress, impaired

growth, and reproductive alterations, among others. Depending on nature, temporal and spatial scale of the biological response, impacts on individuals may have implications at the population, community, and ecosystem level (Marsden and Cranford, 2016). Among the most common contaminants in aquatic environments, arsenic (As) is a naturally occurring element released by natural events (for example volcanism, weathering of minerals and ores, leaching and solubilisation of Earth's crust) but principally by human activities such as pesticide application, coal combustion, wood combustion, and waste incineration (Lamela et al., 2019). High levels of this metalloid in water can lead to harm marine life by increasing oxidative damage, interfering with cell events and even causing cell death (Rank et al., 2007). In seawater, the species of dissolved As are limited to arsenate, arsenite, and the organoarsenic compounds, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Kalia and Khambholja, 2015; Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002). The latter three species are derived from biological activity (Azizur Rahman et al., 2012; Caumette et al., 2012; Jenkins et al., 2003; Khokiattiwong et al., 2001). The toxicity of As is related to its oxidation states and its chemical forms (Lai et al., 1999). Marine organisms bioaccumulate As from water, mainly in non-toxic organic forms containing As residues ranging from < 1 to 100 mg/kg, which are found as arsenosugar (in algae) or arsenobetain (in invertebrates and fish) (Francesconi, 2010; Krishnakumar et al., 2016; Mania et al., 2015; Meador et al., 2004; Vieira et al., 2011). Organisms show different sensitivity to different As species, being trivalent As (AsIII) compounds more toxic than pentavalent As (AsV) (Hughes, 2002; Neff, 1997; Sharma and Sohn, 2009). For example, both AsIII and AsV affect the growth of phytoplankton and marine periphyton and some of these impacts appear to occur at concentrations close to those found in seawater, particularly when environmental phosphate levels are low (Kalia and Khambholja, 2015). However, some methylated organic forms are also toxic and may produce adverse effects comparable to those produced by inorganic As (Fattorini et al., 2006).

There are previous studies related to metal accumulation and its effects in bivalves (Giarratano et al., 2014, 2013; Gil et al., 2006; Vázquez et al., 2007), crabs and gastropods (Giarratano et al., 2016, 2015; Marinho et al., 2016; Primost et al., 2017) habiting in Northern Patagonia coastal ecosystems, but just a few studies have focused on metalloids. In that regard, As levels have been reported in several species of bivalves, in gastropods and fishes (Urtubey et al., 2016) and macroalgae *Undaria pinnatifida* (Gil et al., 2015). Sturla Lompré et al. (2019) confirmed the presence of As in different tissues of Tehuelche scallop. The results of the mentioned studies highlight the presence of As in edible tissues of bivalves from San José gulf (Península Valdés) in levels close to or even greater than the limit of total As of 1 µg/g in fresh weight established by the Argentinian Food Code (CAA, 2012) for human consumption. In Australia there is a limitation of the allowed level of inorganic As in seaweed and mollusks of 1 µg/g in fresh weight (Australia New Zealand Food Standards Code, FSANZ, 2012). In sediment from this gulf, Sturla Lompré et al. (2019) reported values between 3.41 ± 0.11 to 4.55 ± 0.22 µg/g dw in winter and summer, respectively. San José gulf is a small, shallow and semi-enclosed basin located on the north coast of Argentine Patagonia and its anthropogenic activity is limited to artisanal and small-scale commercial shellfish fisheries. There are no adjacent cities, industries or ports that allow the existence of an anthropogenic source of As to be assumed. The As in this gulf would have a natural origin related to its biogeochemistry and volcanic activity in the southern Andes Mountains (Lamela et al., 2019). Another source could be the deep water from ascending coastal currents near the San José gulf entering into the gulf (Pisoni et al., 2014; Tonini and Palma, 2017). Due to their high commercial value, scallops have received considerable attention in studies of accumulation, distribution and biological effects of pollutants both in pristine (Peake et al., 2010; Saavedra et al., 2008) as contaminated areas (Guo et al., 2017; Milinkovitch et al., 2015).

The harmful effect of As has been widely investigated in mollusks, particularly in bivalves from different aquatic ecosystems (Diniz et al., 2008; Guo et al., 2017; Marsden and Cranford, 2016; Milinkovitch et al., 2015; Rank et al., 2007; Zhang et al., 2019). Metal(loids)s, such as As, can catalyze the formation of reactive species (RS) that can damage proteins, lipids, and DNA, causing cell injury or cell death. Reactive species may cause lipid peroxidation (LPO), a complex process that destroys lipids in the membrane, destabilizing the structure and function of the cell and its organelles, which can lead to cell death (apoptosis) (Rank et al., 2007). There are different cellular protection mechanisms to avoid or decrease the oxidative damage that RS can cause, including the activity of antioxidant enzymes (such as superoxide dismutase - SOD, catalase - CAT and glutathione-S-transferase - GST), non-enzymatic antioxidants (such as α-Tocopherol - α-T) and induction of metallothionein (MT) expression (Milinkovitch et al., 2015; Viarengo et al., 1999). The balance between free radical damage and antioxidant protection in the lipid phase can be evaluated using the LPO/α-T ratio. This index assumes that higher levels of biomarkers indicate high tissue damage (Lattuca et al., 2009).

However, data provided by a wide battery of biomarkers is difficult to interpret without an integrated overview that globally assesses the potential influence of the pollutant under study (Bertrand et al., 2015). The Integrated Biomarker Response Index (IBR) constitutes a practical and robust tool to assess the susceptibility of exposed organisms integrating the response of multiple biomarkers (Beliaeff and Burgeot, 2002; Devin et al., 2014).

This study was focus on scallop *A. tehuelchus* from Península Valdés, a protected area designated World Heritage Site by UNESCO in 1999 (UNESCO, 1999) due to its remarkable biodiversity and ecosystemic richness, and also for being one of the most important areas for reproduction and breeding of the southern right whale (*Eubalaena australis*). In addition, in San

Jose gulf the scallop has historically represented the main species that supports the artisanal shellfish activity for the small settlement of artisanal fishermen of the gulf (Narvarte, 2001). The present study not only evaluated the mortality of the shellfish resource caused by As, but also analyzed the effects on each tissue of interest separately (not pooled). Studies describing the biochemical effects of As on individual tissues are rare and even less in muscle, which is of particular importance due to human consumption. However, there is evidence of a differential tissues accumulation of this metalloid in bivalves (Chandurvelan et al., 2015; Maher et al., 2014; Saavedra et al., 2008; Xu et al., 2022). Therefore, it is important to analyze toxicological effects and accumulation of As (and its correlation) in tissues separately.

Hence, this study aimed to determine the level of acute As toxicity in Tehuelche scallop *A. tehuelchus* (96 h LC50) collected from San Román (Northern Patagonia Argentina) in August 2016 and to evaluate biomarkers of exposure and damage related to oxidative stress. Finally, to obtain a more holistic view of the biological responses and to evaluate the association between the responses and the exposure concentrations tested, an integrated biomarker response (IBR) was calculated.

2. MATERIALS AND METHODS

2.1 Sampling collection and experimental design

Samples were collected in winter (August 2016) in San Román (42°14′ S–64°13′ W) in the San José gulf located in Northern Patagonia Argentina (42 ° 20'S, 64 ° 20'W). This area has favorable geographical and ecological conditions for the settlement of natural resources of great fishing interest. There, the main artisanal fisheries' product is the Tehuelche scallop (*A. tehuelchus*). Collected Tehuelche scallop (n=135) were transported to the laboratory in cold-thermic containers and individuals with 60-70 mm of shell height were cleaned of epibionts and acclimated for 6 days

in a 30-L aquarium in 1-mm-filtered and UV treated seawater (Biolight Technologies: 2,000 L h–1, 40 W). The salinity was set at 34 ± 1 g/L (refractometer Arcano FG-211 Salinity/ATC 0–100), pH at 8.12 ± 0.05 (pHmeter Consort C931), temperature at 13.0 ± 1.0 °C, continuous aeration (dissolved oxygen > 90%, multiparameter probe YSI model 556) and 12 light:12 dark photoperiod. Seawater was renewed every two days and organisms were not fed either during acclimatization or during exposure.

2.2 Survival test

For exposure tests, sodium arsenite [NaAsO2 solutions, As (III),] was chosen for being the most labile and biotoxic state. Firstly, to define the As exposure range, a tolerance test was assayed using four concentrations (0, 10, 25 and 50 mg As/L). Acute exposures were conducted during 96 h in 10-L plastic aquaria with six organisms in 5 L of test solutions and covered with plastic wrap to prevent evaporation. The mortality was examined at 24 h, 48 h, 72 h, and 96 h. All animals died after 24 h of exposure in 25 and 50 mg As/L treatments, meanwhile, in 10 mg As/L 50% of animals died after 72 h exposure and all died at 96 h.

According to the results of the described experiment mentioned above, an acute toxicity test for a period of 96 h was done with 0; 4; 5; 6.3; 7.9 and 10 mg As/L in triplicate design. Each replicate consisted of 6 organisms per aquarium with 5 L of contaminated seawater, whereas another group of 3 replicates was kept as control (exposed to clean seawater). Physical and chemical conditions were the same as those of the acclimation period, which were checked daily. The mortality was examined every 24 h. The death of scallops was confirmed by their inability to respond to mechanical stimulus. Median lethal concentration (LC50) was calculated according to the EPA (United States Environmental Protection Agency) using Probit 5.1 software.

At the end of the exposure period, three live scallops from each tank (i.e. individuals that actively closed their valves upon mechanical stimulation) were sacrificed to dissect their gills, digestive gland, and adductor muscle resulting in three independent replicates per concentration.

2.3 Quantification of As

For the determination of As, one composite sample of each tissue from three organisms was made per aquarium (n = 3 per condition). Each tissue was carefully excised, thoroughly washed with deionized water, and dried with tissue paper. Scallop's tissues were freeze-dried for 96 h and homogenized with a grinder. Samples of 0.5 g were digested using 10 mL of concentrated nitric acid in a NOVAWAVE SA microwave, using a time-temperature program of 180 °C for 10 min (US EPA 1996). After digestion, samples were filled up to 50 mL with deionized water before analysis with an Agilent 720 inductively coupled plasma optical emission spectrometer (ICP-OES), with axial configuration and multi-element simultaneous detection.

Three replicates of standard reference materials of oyster tissue (NIST-SRM 1566, National Institute of Standards and Technology, Standard Reference Material) were analysed for quality control of data. Precision expressed as coefficient of variation was 5%. Accuracy, expressed as a percentage of recovery, was 90.5%. The detection limit was 0.7 µg/g dry weight (DW). The results were expressed in µg per g of DW.

2.4 Biochemical markers

From each condition, three organisms were taken for the biomarkers determination (n = 3 per condition). The gills, digestive gland, and muscle were carefully excised and individually stored at -80 °C.

Production of RS was evaluated after homogenization with buffer solution (1:5 w/v) at pH 7.75 according to Gallagher et al. (1992). The fluorescent probe 2′,7′ dichlorofluorescein diacetate (DCFH-DA) was added to the homogenate and it was incubated at 40 °C during 30 min before reading. Thereafter, the nonfluorescent compound DCFH was oxidized by RS to the fluorescent compound DCF, which is detected spectrofluorometrically at λexc = 488 nm and λem = 525 nm (Viarengo et al. 1999). Production of RS was expressed as units per minute per milligram of proteins (U/min/mg prot).

For enzyme assays, samples were homogenized in a 1:3 (w/v) ratio of a buffer solution with pH adjusted to 7.6 according to Bainy et al. (1996). CAT activity was evaluated by the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm (Beutler, 1982). One unit of CAT was defined as the amount of enzyme catalyzing the elimination of 1 mmol H_2O_2 per minute. GST activity was determined by incubating reduced glutathione with 1-chloro-2,4-dinitrobenzene as substrate at 25 °C and measuring the increase in absorbance at 340 nm (Habig et al., 1974). One unit of GST was defined as the amount of enzyme catalyzing the formation of 1 mmol of 2,4 dinitrophenyl-S-glutathione per min. Proteins were measured by the method of Lowry et al. (1951), with bovine serum albumin as standard. Results of all enzymes were expressed as units per milligram of proteins (U/mg prot.). All determinations were measured by spectrophotometry using a microplate reader Varikosan LUX except catalase that was measured using a spectrophotometer Jasco UV/Vis 7850.

MT were homogenized in buffer solution at pH 8.6 (1:3 p/v) and analysed by the reaction with 5,5'-dithio-bis-2-nitrobezoic acid (DTNB) according to Viarengo et al. (1997). MT were quantified by the spectrophotometric assay using glutathione (GSH) as standard and expressed as nmol-SH per milligram of proteins (nmol-SH/mg prot).

Thiobarbituric acid reactive substances (TBARS) are formed as a by-product of lipid peroxidation (LPO). The amount of TBARS were measured based on Guerra et al. (2013) at 535 nm, using malondialdehyde (MDA) as standard and were expressed as TBARS pmol equivalents per mg of protein (pmol/mg prot.).

Content of α-T was quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glassy carbon-working electrode at an applied oxidation potential of 0.6 V. Samples were homogenized in deionized water, butylated hydroxytoluene and sodium dodecyl sulfate. Then, α-T was extracted with methanol and hexane, dissolved in methanol:ethanol (1:1, v/v) and injected for HPLC analysis according to Desai (1984). D,L-α-tocopherol was used as standard and the results were expressed as nmol per mg wet weight (nmol/mg WW).

2.5 Data analysis

2.5.1 Integrated Biomarker Response (IBR) index

A general stress index, called "Integrated Biomarker Response" was calculated with biological responses measured in exposed organisms. Variables with strong correlation ($r \geq 0.8$, $p < 0.05$) were excluded to keep non-redundant biomarkers. Then, to select an adequate number of biological responses (no more than eight, Devin et al. 2014) a discriminant analysis was applied to select biomarkers with higher capacity to separate exposure concentrations. Finally, several IBR were calculated changing the order of the biomarkers using R Studio and the median of all the index values was informed as the final index value (Bertrand et al., 2018; Devin et al., 2014).

2.5.2 Statistical treatment

The data are presented as the mean ± standard error (N = 3). Statistical analyses were performed with Statsoft STATISTICA (v. 9.1). Statistical differences in As concentrations and biochemical

parameters in soft tissues were evaluated using two-way ANOVA (Statistica 7.0) considering As concentrations (0, 4, 5 and 6.3 µg As/L) and tissues (gills, digestive gland and muscle) as main factors. Tukey's post hoc test was used to analyze the significant differences among As concentrations for each tissue and among tissues for each As concentration. Correlations between As levels and biomarkers in each tissue were determined through the Pearson's correlation coefficient. A Kruskal-Wallis test was carried out to identify IBR differences between exposure levels. The level of significance for all tests was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Acute toxicity test

No mortality was observed in controls and treatments of 4 and 5 mg As/L during the experiment. LC50 could not be calculated for 24 and 48 h due to the mortality was less than 50%. At 96 h exposure, 18% mortality was registered in 6.3 mg As/L and 78% in 7.9 mg As/L exposure (**Fig. 1**). Mortality reached 100% in the highest dose (10 mg As/L). The 72 and 96 h LC50 was 10.5 and 7.1 mg As/L with 95% confidence intervals ranged between 9.50 and 15.2 and 6.7 y 7.9 mg As/L, respectively.

The results obtained in this study indicated that As presented low acute toxicity for *A. tehuelchus* compared with LC50 value of 3.4 mg As/L reported for *Argopecten irradians* by Nelson et al. (1976). However, those experiments were carried out at 20 °C in contrast with this study at 13 °C. The metabolism of animals is closely related to temperature and that could be the reason for the remarkable difference between the species. Besides, other factors such as size, sex, sexual maturity and inherent differences in defense pathways may explain these variations in LC50 values (Bryant et al. 1985). Since *A. tehuelchus* from San José gulf are exposed to the natural presence of As may

have developed tolerance to As, as reported Azizi et al. (2018). Even though the high LC50 value would indicate that *A. tehuelchus* is less sensitive to As than other bivalve species, this toxic element could affect at the cellular level, threatening the health of the organisms as well as the sustainability of the related shellfish activity.

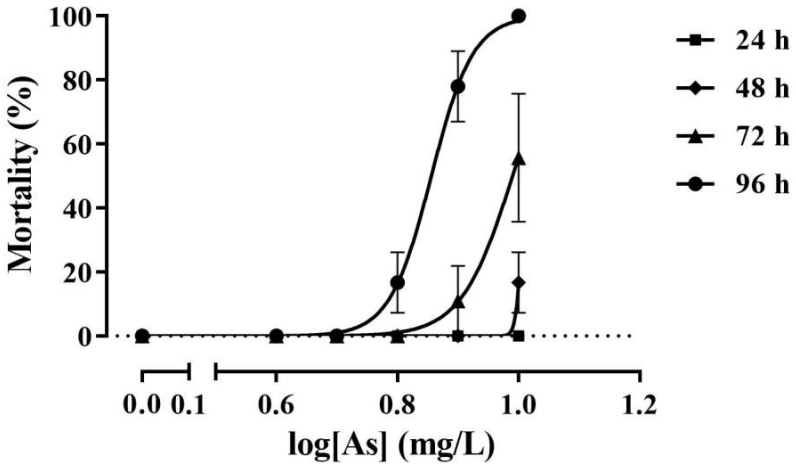


Fig. 1 *Dose-response curves for Aequipecten tehuelchus (n=3; bars correspond to error standard)*

3.2 Arsenic accumulation

At all levels of exposure, the three tissues showed a similar pattern of accumulation, in the following decreasing order: gills > digestive gland > muscle (**Fig. 2**) being the accumulation significantly higher than in control and in all assayed concentrations. Comparing As concentrations, in gills and digestive gland, there were significant differences between 4 and 7.9 mg As/L and no differences were found among 5, 6.3 and 7.9 mg As/L. Similarly, As accumulation in muscle with 4, 5 and 6.3 mg As/L was significantly lower than with 7.9 mg As/L. In controls,

As levels in the three tissues were similar, while in the As traetments, gills and glands had significantly higher levels of As than muscle.

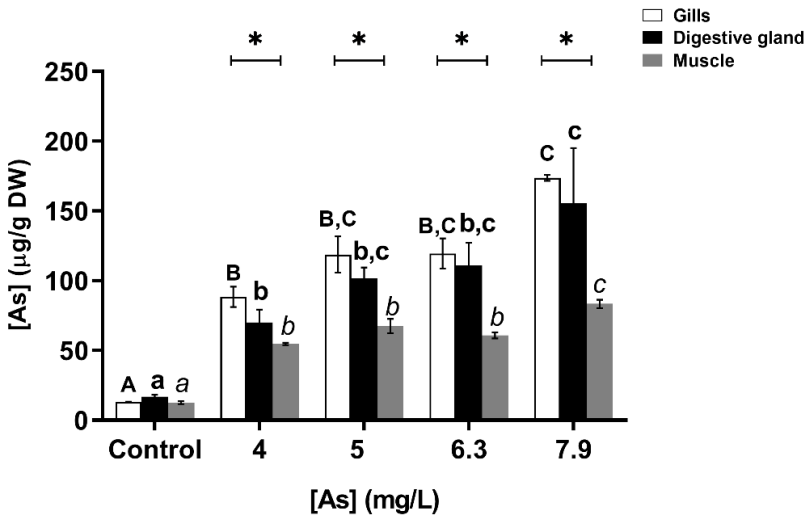


Fig. 2 *As accumulation in gills, digestive gland and muscle of Aequipecten tehuelchus after 96 h exposure. Data are presented as mean ± SEM (N=3). Uppercase, lowercase and italic letters represent significant differences among treatments for gills, digestive gland and muscle, respectively. Asterisks indicate a significant difference among tissues within each treatment (Tukey's test; $p < 0.05$).*

This differential tissue accumulation pattern is consistent with that reported for bivalves from other non-contaminated sites such as the scallop *Pecten maximus* (Saavedra et al., 2008) and the clam *Ruditapes philippinarum* (Chen et al., 2018). However, tissue distribution in *A. tehuelchus* differs from that of scallop *Chlamys farreri* also exposed to As in laboratory where similar bioaccumulation was found among digestive gland, gill and mantle (Zhao et al., 2021).

The higher accumulation showed by gills than digestive gland and muscle may be related to their role as the main route by which metals are incorporated from the dissolved phase in the aquatic organisms, while in the other tissues the exposure is intermittent and indirect via haemolymph and food (Saavedra et al., 2008). A few authors found higher concentrations of total As in the gills than in other tissues of exposed bivalves such as *Pecten maximus*, *Crassostrea virginica* and *Mercenaria mercenaria* (Leatherland and Burton, 1974; Lebordais et al., 2021; Saavedra et al., 2008). The As levels tend to reflect the As content in the diet due to transfer from phytoplankton to filter-feeders. Once inside the organism, this metalloid can be translocated to other excretory organs (kidneys and/or digestive gland) (Hédouin et al., 2010; Marsden and Cranford, 2016; Metian et al., 2008). Furthermore, As appears to be actively secreted into the byssus of mussels, and this may be a significant pathway for the excretion of the element in such species (Ünlü and Fowler, 1979; Yap et al., 2005).

Arsenic acute 96 h exposure resulted in a significant linear increase in the accumulation of As in the three tissues compared to the control, being the correlation coefficients higher than 0.93 for all tissues (Table 1). Similar trend (dose-dependent manner) concerning As absorption from seawater was exhibited by clam *Asaphis violascens* (Zhang et al., 2019). This behavior suggests that *A. tehuelchus* is not able to regulate the internal As concentrations, contrary to other bivalves such *M. galloprovincialis* and *Isognomon isognomon* (Hédouin et al., 2010). Such a trend suggests

that Tehuelche scallop would be a good biomonitor organism of dissolved levels of As. Tehuelche scallop from San José gulf is naturally exposed to As, which enabled them to generate resistance or adaptation to counter As exposure. As occurs in seawater predominantly as the inorganic forms of arsenate and arsenite, being these As species more toxic than organic forms to living organisms (Neff, 2002). Since marine organisms cannot avoid exposure to the potentially toxic inorganic As species, they have evolved biotransformation and detoxification strategies producing less-toxic organo-arsenic compounds which predominate in their tissues (Fattorini et al., 2006; Neff, 2002). The distribution of more than twenty-five As species occurring in marine systems varies markedly among the four marine compartments, namely seawater, sediment/porewater, algae, and animals (Fattorini et al., 2006). Direct comparisons between native populations collected from different areas should be made considering not only the exposure history but also the chemical form in which As is present.

Table 1. *Relationship between acute As exposure level (96 h, 0–7.9 mg As/L) and As accumulation in gills, digestive gland and muscle of scallops A. tehuelchus after acute As exposure (96 h, 0 – 6.3 mg As/L)*

Tissue	Person's correlation coefficient (R^2)	Equation of the line of best fit
Gills	0.958	y = 19.042 x + 13.186
Digestive gland	0.973	y = 17.074 x + 11.743
Muscle	0.932	y = 8.569 x + 16.022

3.3 Biochemical markers

Reactive species, evaluated through the oxidation rate of DCFH-DA, did not register significant differences at any tissues compared to the control neither among exposure treatments (**Fig. 3 a**). These results indicate that, although there was an increase in As levels in tissues, the antioxidant

defense mechanisms would be preventing the generation of RS. Comparing RS levels among tissues, significant differences were recorded in control and treatment 4 mg As/L registering the highest levels in the gill, followed by the digestive gland and muscle. In control, the three tissues differed significantly between them, while in treatment 4 mg As/L, the gills and digestive gland were different from muscle. In gills, the highest CAT activities were registered in the lowest concentration, followed by treatments with 5 and 6.3 mg As/L, while in digestive gland increased significantly in all the treatments compared with control, with no significant differences among As exposures. On the contrary, in the muscle no significant differences were found among As concentrations. Comparing tissues, only catalase activity showed significant differences being digestive gland the tissue with the highest CAT activities followed by gills and muscle (**Fig. 3 b**). Similarly, GST activity in gills, digestive gland and muscle presented the same pattern than CAT activity; but there were no differences among tissues for any treatment (**Fig. 3 c**). A significant increase of MT was detected in digestive gland in 5 and 6.3 mg As/L treatments compared to control but with no differences between control and 4 mg As/L (**Fig. 3 d**). Nevertheless, no significant differences were found between 5 and 6.3 mg As/L, which may suggest that the capacity to sequester the metalloid is diminished as the result of an excess of total As, as also suggested Diniz et al. (2008) in clams. In muscle, MT content decreased significantly in all As exposures, with no differences among them. The mechanisms by which MT act against the presence of toxics have been extensively studied and it is probable that As will initially bind to these sequestering proteins or be incorporated into lysosomes (Viarengo and Nott, 1993). Conversely, the opposite trend was observed in the muscle, being the maximum levels in control and decreasing significantly in all As treatments. However, further tests should be performed to explain the particular effects of As on MT synthesis on this specific tissue. Comparing tissues, in control and the lowest

concentration tested significant differences were observed, being MT levels in muscle significantly higher than in gills and digestive gland.

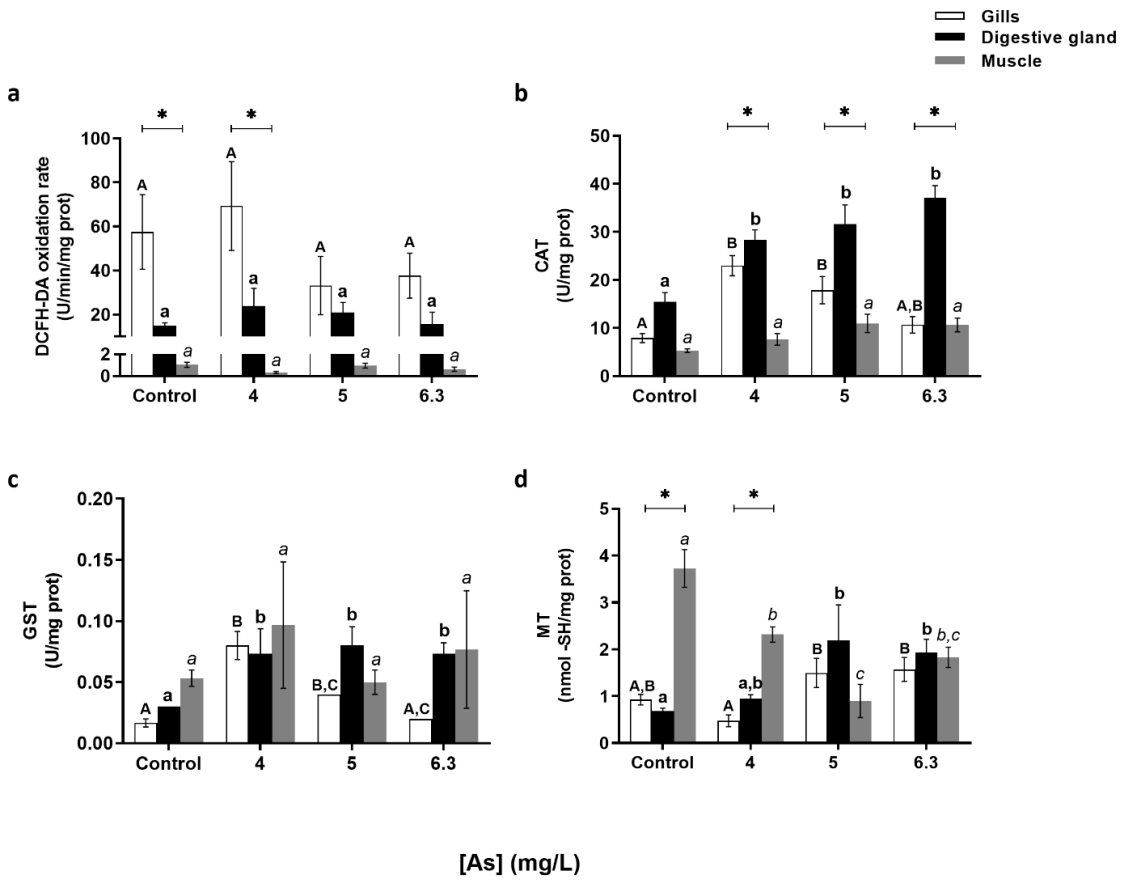


Fig. 3 Oxidation of DCFH-DA (a), catalase (b), glutathione-S-transferase (c) and metallothioneins (d) in gills, digestive gland and muscle of Aequipecten tehuelchus following 96 h exposure. Data are presented as mean ± SEM (N=3). Uppercase, lowercase and italic letters represent significant differences among treatments for gills, digestive gland and muscle, respectively. Asterisks indicate a significant difference among tissues within each treatment (Tukey's test; $p < 0.05$).

The TBARS content did not show significant differences in any tissues among As concentrations. Regarding tissue comparisons, TBARS in digestive gland for all As treatments were significantly higher than in the other tissues with no significant difference between the latter two (**Fig. 4 a**). These results are in line with the finding of no induction of RS, which are known to induce lipid peroxidation. Comparing treatments at each tissue, the content of α-T in gills and muscle of the organisms exposed to the highest concentrations, decreased significantly compared to the control and exposure of 4 mg As/L. In particular, gills showed a significant decrease in treatment 5 and 6.3 mg As/L exposures (significantly different between them) compared to control and treatment 4 mg As/L exposures (with no significant difference between them), but in muscle, no differences were registered among treatments with As. In opposite, no significant changes were observed in the digestive gland (**Fig. 4 b**). The decreasing pattern of α-T is expected due to its consumption in the presence of xenobiotics. Non-photosynthetic organisms such as scallops are not able to synthesize this non-enzymatic antioxidant, which must be incorporated from dietary sources (Fujisawa et al., 2010). In all As treatments, gills were the tissue with the highest α-T values, followed by muscle and then by digestive gland, although without significant differences between the latter two. The balance between oxidative damage and antioxidant protection in the lipid phase can be described through the TBARS/α-T ratio, which assumes that higher levels indicate greater tissue damage (Lattuca et al., 2009). In this case, no significant differences were observed in this index either in the digestive gland or in the muscle, while in gills an increase in

the highest concentration was found compared to the control, probably because the possible damages were not mitigated by the α-T (**Fig. 4 c**).

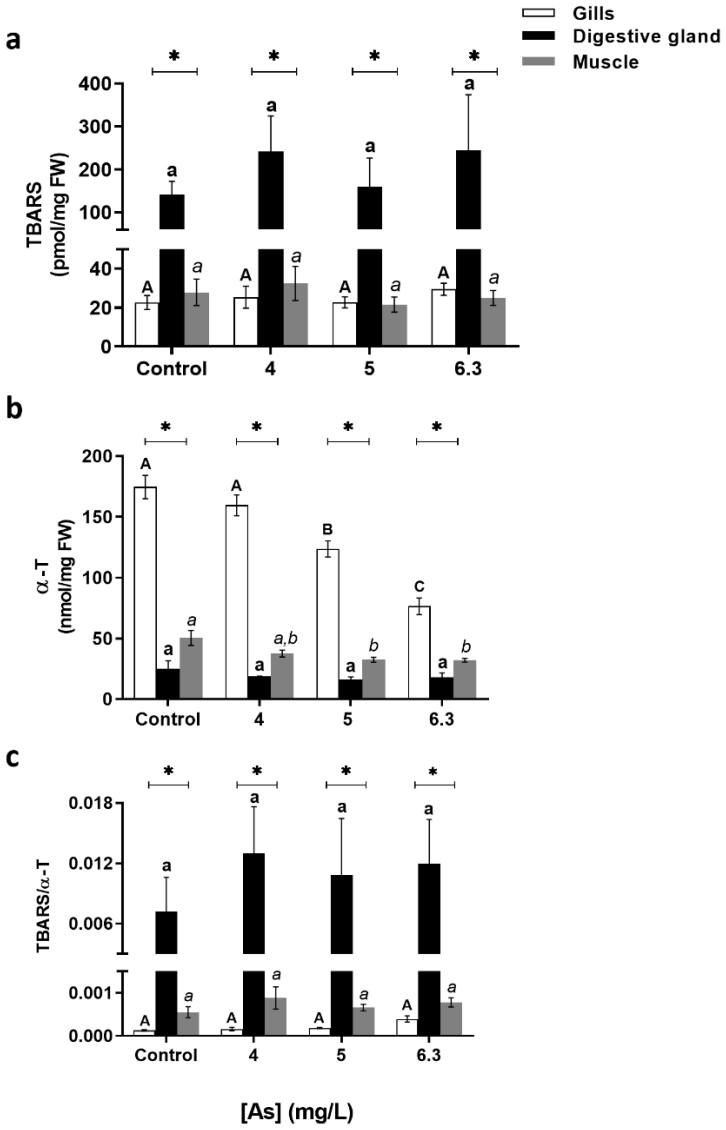


Fig. 4 *Lipid peroxidation (a), α-tocopherol levels (b) and LPO/α-T ratio (c) in gills, digestive gland and muscle of Aequipecten tehuelchus following 96 h exposure. Data are presented as mean ± SEM (N=3). Uppercase, lowercase and italic letters represent significant differences among treatments for gills, digestive gland and muscle, respectively. Asterisks indicate a significant difference among tissues within each treatment (Tukey's test; p < 0.05).*

3.4 Correlation between As tissue accumulation and biomarkers

Table 2 presents the correlation coefficients between biomarkers and As levels in each tissue. As accumulated in gills was significantly positively correlated with α-T. In digestive gland, CAT and GST activities and MT levels correlated positively with As accumulation, while α-T levels showed a negative correlation. In muscle, CAT activity was positively correlated, while MT and α-T levels were negatively correlated. In presence of a toxic element such as As, it was expected induction of antioxidant enzymes to counteract the possible damage caused by RS as well as the consumption of other non-enzymatic antioxidants such as α-T. In that sense and in agreement with these results, Coppola et al. (2018) found an increment of GST in mussel *Mytilus galloprovincialis* exposed to As contributing to prevent higher LPO. Although processes of As transformations or detoxification have not been clearly stated, it is possible that an oxidative response could be avoided by the transformation of inorganic As to less-toxic forms such as organo-arsenic compounds, which have been reported with no ecotoxicological implication (Fattorini et al., 2006). Some authors found that subcellular partitioning of As Regarding the positive correlation between accumulated As and MT in the digestive gland, it can be explained taking into account that the expression of MT is activated as a specific response to metal(loid) toxicity and also as an antioxidant defense to sequester the ions metal (Viarengo et al., 1999). Zhao et al. (2021) studied subcellular partitioning of As in five different tissues of scallop *C. farreri* and found that most of As was storage in the non-toxic form in the metallothionein like protein fraction.

Table 2. *Pearson's correlation coefficient between biomarkers and As accumulation in gills, digestive gland and muscle of A. tehuelchus after acute As exposure (96 h, 0 – 6.3 mg As/L). Significant correlations p < 0.05 with asterisk*

	Biomarkers					
	RS	**CAT**	**GST**	**MT**	**TBARS**	**α-T**
Gills	-0.318	0.423	0.223	0.425	0.231	0.950*
Digestive gland	0.130	0.881*	0.643*	0.667*	0.195	-0.809*
Muscle	-0.304	0.701*	0.096	-0.877*	-0.149	-0.808*

All these correlation analyses were statistically significant (p < 0.001)

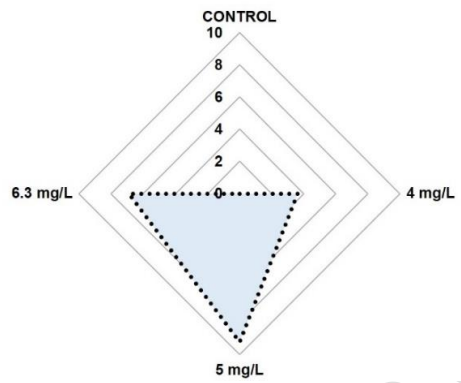
3.5 IBR and correlation with accumulated As

According to discriminant analysis, six biomarkers were selected in exposed organisms: CAT and α-T in gills; MT and α-T in digestive gland and in muscle. In **Fig. 5 a**, obtained values are shown in a star plot, where the grey area integrates the IBR values for each concentration tested. Significant differences in IBR values were observed among all exposure concentrations and control (**Fig. 5 b**).

According to Potet et al. (2018), IBR is able not only to evidence stress levels but also, it could be interpreted as the capacity of organisms to cope with pollutants. In agreement, previous field studies with organisms exposed to complex contaminant mixtures (including metals, metalloids and organic compounds) reported IBR values from 9 to 15 (Bertrand et al., 2018; Bocquené et al., 2004). Furthermore, Brooks et al. (2018) obtained IBR values from 4 to 13 in *Mytilus* sp exposed to mine discharge and As concentrations in soft tissues similar to those recorded in the present work. Even when comparison with studied carried out in field and with other species is not the more suitable option in the present study IBR values of *A. tehuelchus* exposed to As surpassed a value of 9. Moreover, the pattern of IBR values was, in most of the tested conditions, similar with

the accumulation pattern which suggests an association between the measured concentration of the metalloid in tissue and the values of IBR. However, at the higher exposure concentration (6.3 mg/L) the IBR value decreased while the accumulated levels of As were similar than those measured at 5 mg/L. This could be due to a variation in the subcellular distribution of the metalloid in analyzed tissues (Bertrand et al., 2015). Probably, an increase in the precipitated As: soluble As ratio would contribute to a slight decrease in stress levels or need to cope the metalloid exposure.

a



b

IBR ratio					
[As] (mg/L)	Median	Mean	SD	Min	Max
Control	0.00[a]	0.00	0.00	0.00	0.00
4	3.57[b]	3.60	0.43	2.80	4.39
5	9.16[c]	9.22	0.20	8.92	9.57
6.3	6.86[d]	6.88	0.20	6.49	7.24

Fig. 5 *(a) Star plot of Integrated Biomarker Response (IBR) values at different exposure concentrations in Aequipecten tehuelchus exposed to As. (b) Mean, median, standard deviation (SD), minimal (Min), and maximal (Max) values for calculated IBR are shown. Different letter indicates significant differences ($p < 0.05$) among treatments.*

4. CONCLUSION

This study identified that the LC50 of As after 96 h of exposure in scallop *Aequipecten tehuelchus* from San José gulf was 7.1 mg As/L. This species seems to be relatively tolerant to As exposure in comparison with other bivalves' species. The present results indicated that exposure to inorganic As (Ⅲ) caused a proportional dose-dependent and tissue-specific accumulation, tending

to be higher in gills and digestive gland than in muscle. As accumulation induced slight oxidative stress, with no generation of RS nor lipid peroxidation through TBARS, a rise of CAT and GST activities and MT levels and the consumption of the lipid-soluble antioxidant α-T. The integrative analysis of the present results shows that short-term exposure to As concentrations higher than 5 mg As/L produces significant changes in the biochemical metabolism of *A. tehuelchus*. The current study supports the suitability of employing *A. tehuelchus* as a bioindicator of As. However, due to the ubiquitous occurrence of As in the environment and the variable toxicity depending on chemical form, extrapolations of results obtained in laboratory experiments to the natural environment must be avoided. In order to better assess the environmental impact caused by anthropogenic contamination, the authors highlight the importance of measuring chemical speciation of As due to its toxicological relevance.

Ethical Approval

This article does not contain any studies with human participants performed by any of the authors. All procedures performed in studies involving animals were in accordance with national law for the animal welfare and protection in Argentina (Law No. 14.346) and the National Research Council's Guide for the Care and Use of Laboratory Animals (2011).

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Highlights

- Arsenic accumulation in tissues increased linearly with arsenic exposure.
- Arsenic did not induced production of reactive species and TBARS at any tissue.
- Greater antioxidant response in gills and digestive gland than in muscle.
- Tehuelche scallop would be relatively tolerant to As exposure (CL50: 7.1 mg As/L).

Author contribution statement

Julieta Sturla Lompré: Conceptualization, Methodology, Formal analysis, Investigation, Writing original draft, Visualization, Resources. Erica Giarratano: Writing-review-editing, Visualization, Resources, Supervision. Mónica Gil: Writing-review-editing, Visualization, Resources, Supervision. Lidwina Bertrand: Formal analysis, Writing-review-editing. Gabriela Malanga: Writing-review-editing, Visualization, Resources, Supervision

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: