Effect of acute cadmium exposure on oxidative stress and antioxidant system of the scallop *Aequipecten tehuelchus*

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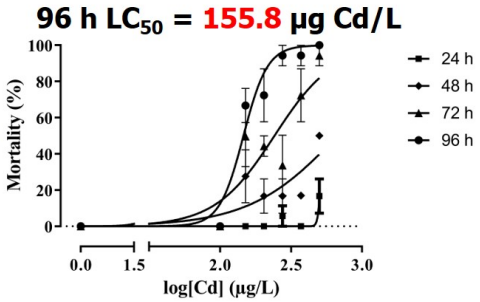
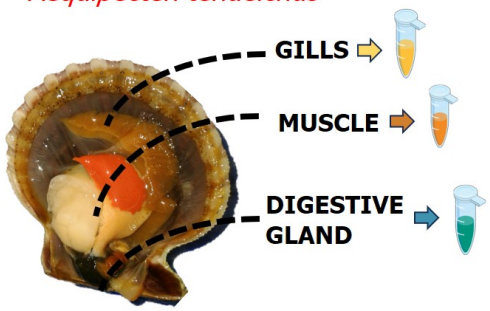
Scallops exposed to: 0, 25, 50, 100, 150, 204, 275, 371, 500 µg Cd/L for 96 h.



Sublethal effects with: 0, 25, 50, 100, 150 and 204 µg Cd/L for 96 h.

Aequipecten tehuelchus



- RONS
- GST
- LPO

→ NO CONCENTRATION RESPONSE

- CAT
- α-T
- β-C

→ CONCENTRATION RESPONSE (GILLS, DIGESTIVE GLAND)

- MT

→ CONCENTRATION RESPONSE (GILLS)

Effect of acute cadmium exposure on oxidative stress and antioxidant system of the scallop *Aequipecten tehuelchus*

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Abstract

This study aimed to assess the impact of acute exposure (96 h) to Cd in gills, digestive gland and muscle of the Tehuelche scallop *Aequipecten tehuelchus* from San José gulf in Patagonia, Argentina. Scallops were exposed to Cd concentrations of 0, 25, 50, 100, 150, 204, 275, 371, and 500 µg/L, and mortality rates were recorded after 96 hours of exposure. Surviving organisms were analyzed for the biochemical response through reactive oxygen and nitrogen species (RONS), activities of catalase (CAT) and glutathione-S-transferase (GST), metallothioneins (MT), lipid peroxidation (LPO) and liposoluble antioxidants α-tocopherol (α-T) and β-carotene (β-C). The mean lethal concentration (LC_{50}) was 155.8 µg Cd/L, a lower value than other scallops' species, showing that *A. tehuelchus* has a particular sensitivity to Cd. In the three tissues, at all exposure concentrations, there was no significant response in RONS levels, GST activity or LPO. Nevertheless, CAT activity and α-T levels decreased in the gills but increased in the digestive gland, with no significant response in the muscle. Two-way ANOVA revealed a significant interaction between Cd concentration and tissue on MT, which increased significantly in gills, decreased in digestive gland with 100 compared to 50 µg Cd/L; whereas in muscle a significant increase was observed with 25 µg Cd/L compared to control. The results show a significant effect of Cd in scallop's gills on CAT activity and α-T levels, highlighting this tissue as the primary target against relevant concentrations of metal in seawater. The effect on digestive gland and muscle was minimal. The overall results suggest that Cd toxicity is tissue-specific. This study will help reduce the existence knowledge gap regarding potential impacts of acute exposure to Cd in a bivalve species with high ecological and commercial importance, as well as identifying the most responsive biomarkers associated with Cd stress for monitoring assessment.

Keywords

Metal exposure; Biochemical response; Scallop tissues; Metallothioneins; Argentine Patagonia; Marine bivalves

1. Introduction

It has been estimated that most of the cadmium (Cd) (99%) present in surface waters (at a depth of 1 meter) is due to physical processes such as upwelling and advection (Delgadillo-Hinojosa et al., 2015; Tanahara et al., 2021). In recent years, Cd emissions from anthropogenic sources into the marine environment have increased, due to its high commercial production for diverse applications (McGeer et al., 2011; Smail et al., 2012). The dominant and most bioavailable form of Cd in the sea is a free ion (Cd^{2+}). Organic Cd complexes constitute a significant part of the dissolved Cd, although less bioavailable than their inorganic forms (Neff, 2002). In addition, there is a strong association between the vertical distribution of Cd with that of inorganic nutrients, mainly phosphate and nitrate, suggesting that the oceanic biogeochemistry of this metal is controlled by the cycle of organic matter (Baars et al., 2014).

Bivalves are widely used worldwide to monitor the pollution of coastal environments (Brenner et al., 2014; Marsden and Cranford, 2016; Zuykov et al., 2013). Pectinid bivalves appear to accumulate Cd to a greater degree than other organisms (Liu et al., 2012; Metian et al., 2007). Cadmium is mainly incorporated through the diet, by the consumption of phytoplankton (O'Mara et al., 2019; Schmitz et al., 2015). It tends to accumulate preferentially in the liver, digestive gland and kidney, probably sequestered in insoluble granules or bound to tissue proteins, such as metallothioneins (MT) (Jebali et al., 2014; Moncaleano-Niño et al., 2017; Neff, 2002; Zhao et al., 2023). Trace metal concentrations in

organisms can be influenced by many intrinsic factors such as species, age, sex and reproductive stage, and extrinsic factors such as temperature, salinity, food availability, among others (Azizi et al., 2018; Geng et al., 2015; Jebali et al., 2014; Marsden and Cranford, 2016).

Meta-analysis reveals the species-, dose- and duration-dependent effects of Cd toxicities in marine bivalves. When Cd accumulation exceeds the capacity of detoxification, marine bivalves may adapt to the Cd stress or may activate other biological responses like antioxidation system and apoptosis to maintain cellular homeostasis (Fang et al., 2010; Gao et al., 2022; Sokolova et al., 2012). Antioxidant enzymes play a key role in mitigating the damaging effects of free radicals. At the cellular level, Cd binds to intracellular MT (Le et al., 2016) or to other macromolecules, leading to DNA mutations, affecting protein structure and function, and initiating lipid peroxidation (LPO) (Benedetti et al., 2015). Researches on scallops have highlighted the effects of Cd exposure on antioxidant enzymes and LPO (Giarratano et al., 2023; Milinkovitch et al., 2015; Nardi et al., 2018), as well as the induction of MT (Gao et al., 2016; Giarratano et al., 2023; Nardi et al., 2018; Zapata et al., 2012).

A previous ecotoxicology study carried out by Giarratano et al. (2023) with the commercial scallop *Aequipecten tehuelchus* exposed for 7 and 14 days to sublethal Cd concentrations revealed significant effects in gills, digestive gland and muscle. Those effects included generation of reactive species, modulation of enzymatic activities and MT production, with no evidence of lipid peroxidation. In order to deepen knowledge and to generate toxicity data for a priority metal such as Cd, this study seeks to evaluate how short-term exposure at high Cd concentrations affect the most important bivalve species supporting small inshore fisheries that operate within the northern Patagonian gulfs (Soria et al., 2016).

The main objective of this study was to measure the oxidative response of the Tehuelche scallop (*A. tehuelchus*) from San José gulf (SJG) (Northern Patagonia) after acute exposure to Cd. In particular, the aims were (1) to establish the lethal concentration of Cd that causes the death of 50% of a group of test animals after 96 h of exposure (96 h LC_{50}), (2) to evaluate the effects of sublethal Cd concentrations in gills, digestive gland and muscle of *A. tehuelchus* through several biomarkers and (3) to use a multi-biomarker integrated approach (IBRv2) for the evaluation of the effect induced by Cd on organismal health.

2. Methodology

2.1 Experimental conditions

About 400 organisms were collected by scuba diving in San Román in SJG in the winter of 2017, selected due to the availability of the resource and the high activity of artisanal fishermen observed during previous work. The organisms were acclimatized for one week in 10 aquaria of 30 L of capacity each (n = 40 *per* aquarium) with constant aeration, filtered seawater (10, 5 and 1 µm filters with UV disinfection), temperature of 13.0 ± 1.0 °C, salinity of 35 ± 1 g/L and photoperiod 12:12 similar to natural conditions. Solid wastes were siphoned every 24 hours and 50% of the water was replaced every 48 hours. During the whole acclimatization period, the physicochemical parameters of water were daily controlled, using a multiparameter probe YSI 556 for temperature and dissolved oxygen, a pH meter Consort C931 for pH and a refractometer Alla-France 0-100 for salinity.

2.2 Determination of 96 h LC_{50}

A preliminary dose range-finding study was performed to establish the concentrations to be tested in LC_{50} assay. For this purpose, 20 organisms (n = 4 *per* condition) were exposed

112 to 0, 100, 500, 2500 and 5000 mg Cd/L and mortality was recorded every 24 h. The dose
113 range-finding study consisted of establishing the highest and lowest concentration to be
114 tested in LC_{50} assay, the former being the concentration that causes at least 50% mortality at
115 24 h and the latter causing less than 50% mortality at 96 h. Logarithmic transformation
116 (Log10) was then used to derive intermediate concentrations for the LC_{50} assay (Stephenson,
117 1984). Then, the exposure concentrations were 0, 25, 50, 100, 150, 204, 275, 371, and 500
118 µg Cd/L, which were prepared using cadmium chloride ($CdCl_2$) with filtered seawater as
119 indicated for the acclimatization stage. For LC_{50} assay, 6 organisms were placed in an
120 aquarium with 5 L of each concentration. Two controls without Cd were also carried out.
121 The experimental conditions were the same as those in the acclimation period. Cumulative
122 mortality was recorded every 24 h for 96 h of exposure to determine the LC_{50} of Cd. It was
123 considered dead the organism with a retracted and lax mantle, with the shells completely
124 open and/or without response to mechanical stimuli. No water replacements were made
125 during the exposure period and the scallops were not fed to prevent interaction between Cd
126 and food during the experiment (Zhang et al., 2015).

127 Biomarkers were analyzed in organisms from those concentrations where 100% survival
128 occurred after 96 h of exposure, and at those where mortality occurred but it was possible to
129 sample 3 organisms in good health (rapid response of valve opening and closing to
130 perturbations, swimming by propulsion, and mantle not retracted or discolored). One
131 organism per aquarium (n=3) was taken for biomarkers determinations. Bivalves were
132 dissected into gills, digestive gland and muscle on ice and stored at -80 °C. Water
133 contaminated with Cd was discarded according to the guidelines of the Laboratory Safety
134 and Biosecurity Committee of CCT CONICET-CENPAT.

2.3 Biochemical markers

2.3.1 Reactive oxygen and nitrogen species (RONS)

Reactive species determination was performed through the oxidation of 2,7 dichlorodihydrofluorescein diacetate (DCFH-DA) according to González et al. (2015) and Viarengo et al. (1999). The samples were homogenized (1:5 p/v) in buffer solution 100 mM Tris-HCl, pH 7.75, with 2 mM EDTA and 5 mM $MgCl_2$. The homogenate was centrifuged at 10,000 g for 20 minutes at 4 ºC and the supernatant was used for RONS determination. The reaction was performed in a buffer solution of 30 mM HEPES, pH 7.2, 200 mM KCl and 1 mM $MgCl_2$ with the addition of the fluorescent compound DCFH-DA at a final concentration of 40 µM. The resulting non-fluorescent DCFH is oxidized by reactive species to the fluorescent compound DCF, which is fluorometrically detected using λex = 488 nm and λem = 525 nm. The reaction mixture was incubated at 37 ºC and a Varikosan LUX plate reader was used for the measurement. Production of RONS was expressed as units *per* minute *per* milligram of proteins (U/min/mg prot). Total protein concentrations were measured at 750 nm using bovine serum albumin (BSA) as a reference standard (Lowry et al., 1951).

2.3.2 Antioxidant enzymes

Samples were homogenized in a 1:3 (w/v) ratio of buffer solution containing 20 mM Tris-Base, 1 mM EDTA, 1 mM DL-dithiothreitol, 0.5 M sucrose, 0.15 M KCl and 0.1 mM PMSF, with pH adjusted to 7.6 according to Bainy et al. (1996). Catalase (CAT) activity was evaluated by the rate of decomposition of H_2O_2 at 240 nm (Beutler, 1982). One CAT unit was defined as the amount of enzyme that catalyzes the elimination of 1 mmol of H_2O_2 *per* minute. Glutathione S-Transferases (GST) activity was determined by incubating GSH 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 GST unit was defined as the amount of enzyme that catalyzes the formation of 1 mmol of 2,4 dinitrophenyl-S-glutathione *per* minute. Determinations of both enzymes were made using a Varikosan Lux plate reader and were expressed in units *per* milligram of proteins (U/mg prot.).

2.3.3 Metallothioneins (MT)

The content of MT was analyzed according to Viarengo et al. (1997). The tissues were homogenized (1:3 p/v) in a solution of 20 mM Tris-HCl, pH 8.6, with 0.5 M sucrose, 0.006 mM leupeptin, 0.5 mM PMSF and 0.01% β-mercaptoethanol. The homogenate was centrifuged at 14,000 g for 40 minutes at 4°C and the resulting supernatant was used for subsequent precipitation of ethanol/chloroform in two stages (6,000 g for 10 minutes at 4 °C). After ethanol/chloroform acid fractionation of the tissue homogenization, the resulting precipitate containing MT was resuspended in 5 mM Tris-HCl buffer with 1 mM EDTA pH 7 followed by a reaction with (5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). Metallothioneins were quantified using spectrophotometric assay using GSH as standard and the supernatant absorbance was read at 412 nm. The determinations were made using a Jasco UV/Vis 7850 spectrophotometer and a Varikosan LUX plate reader and expressed as nanomol of thiol groups *per* milligram of protein (nmol-SH/mg prot).

2.3.4 Liposoluble antioxidants

Contents of α-tocopherol and β-carotene (α-T and β-C) were measured in 40 mg of samples homogenized in 100 µL of deionised water, 15 µL of 4% butylated hydroxytoluene (w/v) and 100 µL of 3% sodium dodecyl sulphate (w/v). The samples were extracted with

200 µL of methanol and 900 µL of hexane. After centrifugation at 5,000 g for 5 minutes, 800 µL of the supernatant was taken and the solvent was removed by evaporation under a stream of nitrogen. The extracts were dissolved in methanol:ethanol (1:1). After filtering through nylon membranes (0.22 µm pore size), the samples were injected for analysis by high-performance liquid chromatography (HPLC) reverse phase with electrochemical detection. A Bioanalytical System LC-4C amperometric detector was used applying an oxidation potential of +0.6 V (Desai, 1984). The determinations were made using HPLC equipment, Waters 510 pump with UV-Vis Water 486 detector and ESA Coulochem II electrochemical detector, with computerized data acquisition system, column: Supelcosil LC-8 (3.3 cm x 4.6 mm; 3 µm). Flow was established at 0.9 mL/min and the mobile phase of 20 mM lithium perchlorate at 90:10 of methanol:water. D, L-α-tocopherol and β-carotene (Sigma, St. Louis, MO) were used as standards and the results were expressed as nmol/mg wet weight (ww).

2.3.5 Lipid peroxidation (LPO)

Lipid peroxidation was measured by the quantification of thiobarbituric acid reactive substances (TBARS) according to Guerra et al. (2013). The samples were homogenized in a 1:10 (w/v) saline solution (0.9%) at pH 7.0. Then, 0.8 M HCl was added in 12.5% trichloroacetic acid (TCA) to 250 µL of homogenate before the addition of 1% thiobarbituric acid (TBA). The samples were incubated for 10 minutes at 100 ºC in a water bath, cooled to room temperature and centrifuged at 1,500 g for 10 minutes at 4 ºC. TBARS levels were measured at 535 nm, using malondialdehyde (MDA) as standard. Results were expressed as TBARS picomol equivalents *per* milligram of tissue in wet weight (pmol/mg WW). The determinations were made using a Varikosan LUX plate reader.

2.4 Integrated biomarker response (IBRv2) analysis

IBRv2 (version 2) described by Sanchez et al. (2013) was used for assessing the effects of Cd exposure on scallop *A. tehuelchus*. Briefly, log transformation of the data of biochemical indicators was compared between the treatment groups (Y_i) and the control group (Y_0). Then, the standard deviation (s) and the general mean (m) of the data previously calculated were standardized according to the equation ($Z_i = Y_i$-m)/s. Finally, the biomarker deviation index (A) was calculated ($A = Z_i$-Z_0, where Z_0 is the standardized biomarker in the control group) to obtain the IBRv2 values (IBRv2= $\Sigma|A|$) for each concentration and tissue. Besides, the biomarker deviation index (A) of a single biomarker was also used to draw the star plot. The IBRv2 values were obtained through the IBRtools package (Resende et al., 2022).

2.5 Data and statistical analyses

The Probit method (US EPA program v.1.5) was used to determine the 96 h LC_{50} and the corresponding 95% confidence intervals. Statistical analyses were performed with STATISTICA (v. 9.1 Statsoft), with a significance level of $p < 0.05$. Natural logarithm transformations of the data were performed when necessary, in order to comply with the conditions of normality and homogeneity of variance. A two-way ANOVA analysis using concentrations and tissues as factors was performed to identify significant differences. Tukey's *post hoc* test was then used to compare concentrations within each tissue and to assess the differences among tissues at each concentration. The data is presented as mean and standard error (n = 3).

3. Results

3.1 Determination of 96 h LC_{50}

Figure 1 displays the dose-response curves for Cd at different exposure times. During the 96-hour exposure period, no mortality was observed at the lowest tested concentrations (0, 25, 50 and 100 µg Cd/L), while approximately 70% of mortality occurred at the intermediate concentrations (150 and 204 µg Cd/L). At the highest concentrations (275, 371 and 500 µg Cd/L), the mortality rate reached 100 % at 96 hours. The 96 h LC_{50} was 155.8 µg Cd/L with 95% confidence intervals of 136.5 and 174.8 µg Cd/L.

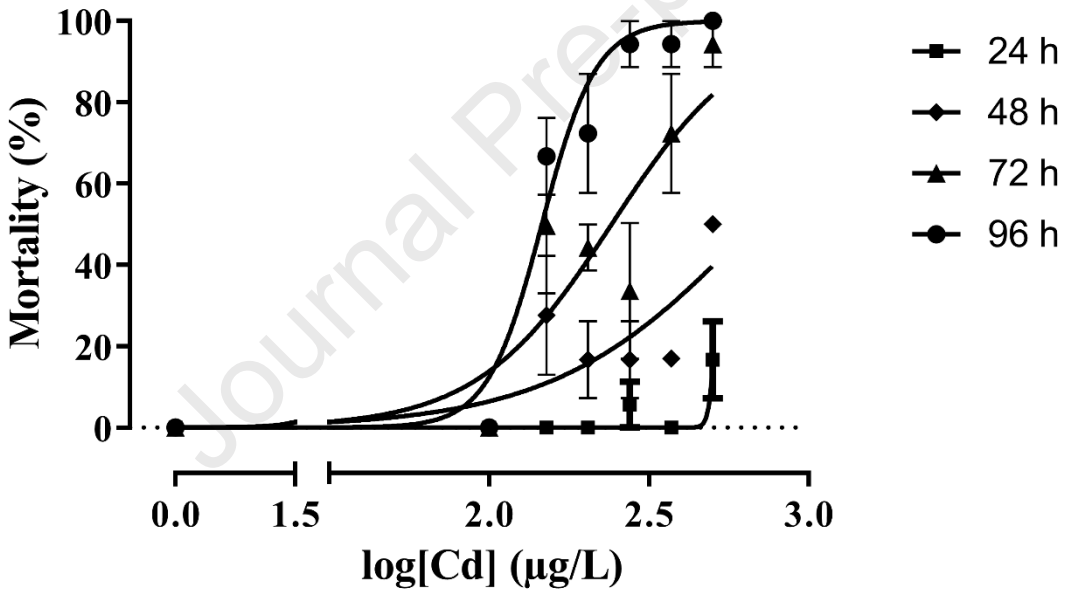


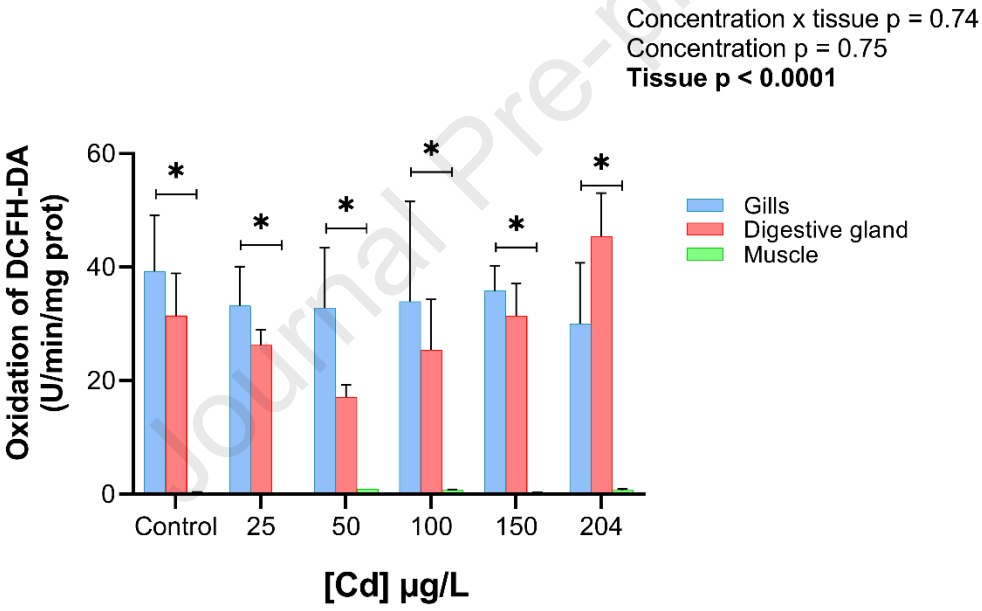
Fig. 1 Dose-response curves for *A. tehuelchus* exposed to Cd at 24, 48, 72 and 96 h.

232

233 *3.2 Biochemical markers*

234 *3.2.1 Reactive oxygen and nitrogen species*

235 Reactive species levels did not vary significantly with Cd concentrations within each
236 tissue ($p > 0.05$), but showed significant variation among tissues ($p < 0.05$) (Fig. 2). For all
237 treatments, gills and digestive gland showed higher values (up to two orders of magnitude)
238 than muscle ($p < 0.05$), where no induction of RONS was measured (Fig. 2).

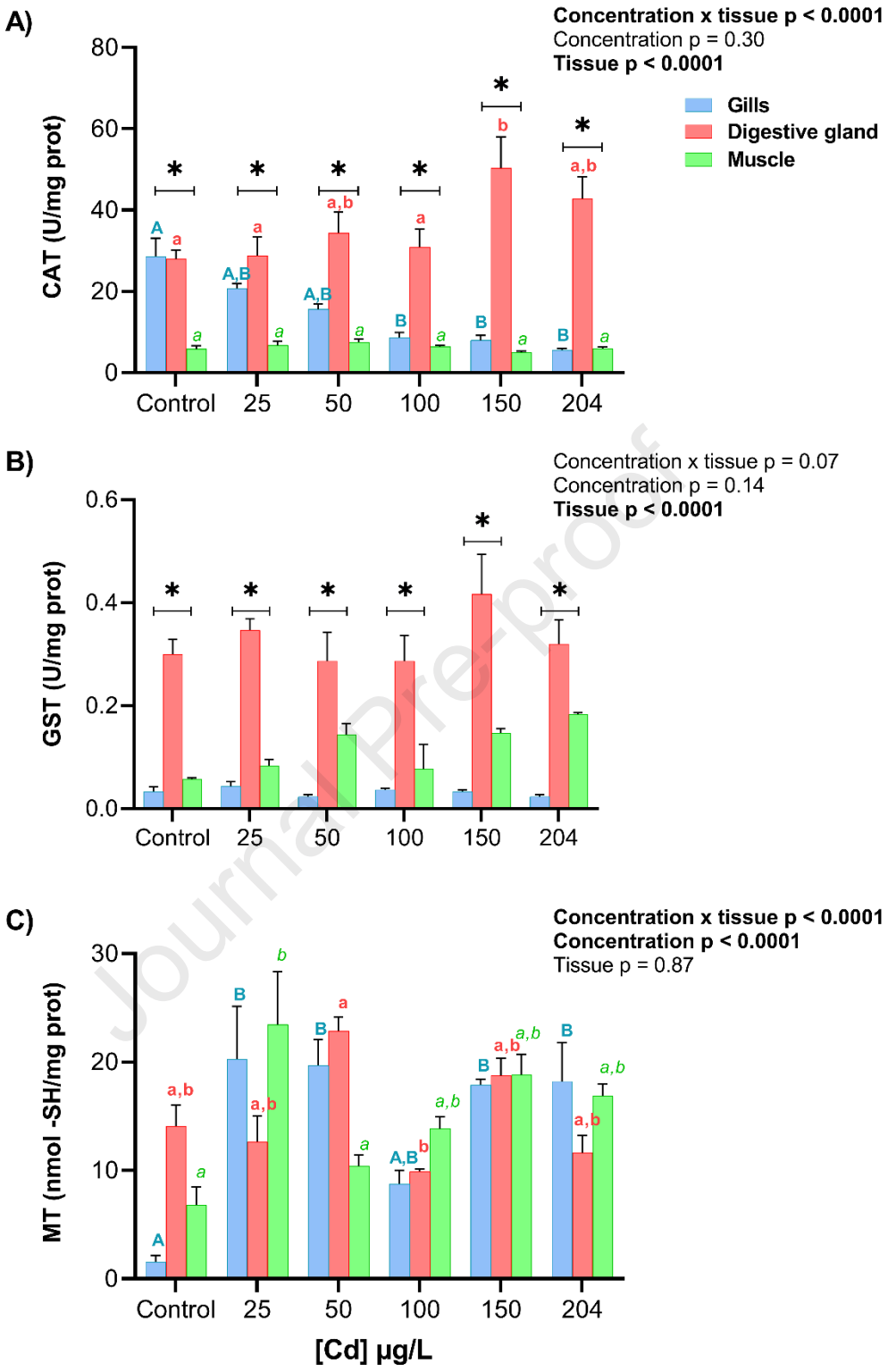


239 Fig 2. Oxidation of DCFH-DA in gills, digestive gland and muscle of *A. tehuelchus* after
240 96 h of Cd exposure (mean ± SE). Asterisks indicate significant differences among tissues
241 within each Cd concentration.

242

3.2.2 Antioxidant enzyme activities and metallothioneins

Catalase activity showed a significant effect on the interaction of Cd concentrations and tissue (Fig. 3A). Compared to control, a significant decrease (81%) was found in gills with 100, 150 and 204 µg Cd/L, meanwhile, an increment of 80% was measured in digestive gland with 150 µg Cd/L ($p < 0.05$). CAT activity did not vary significantly in muscle ($p > 0.05$). In control, CAT activity was significantly lower in muscle than in gills and digestive gland ($p < 0.05$). In all Cd exposures, CAT activity was similar in gills and muscle, although significantly lower than in digestive gland ($p < 0.05$). The GST activity in the three tissues showed no significant differences among Cd concentrations ($p > 0.05$). When comparing tissues, the activity of GST in digestive gland was significantly greater than in gills and muscle ($p < 0.05$), while no differences were found between the last two tissues (Fig. 3B) ($p > 0.05$). Regarding MT, their levels showed a significant interaction between Cd concentration and tissue (Fig. 3C). In gills, MT showed a significant increase compared to the control at all concentrations ($p < 0.05$), except with 100 µg Cd/L. In digestive gland, a significant decrease was measured with 100 µg Cd/L compared to 50 µg Cd/L ($p < 0.05$), whereas in muscle a significant increase was observed with 25 µg Cd/L compared to control ($p < 0.05$).

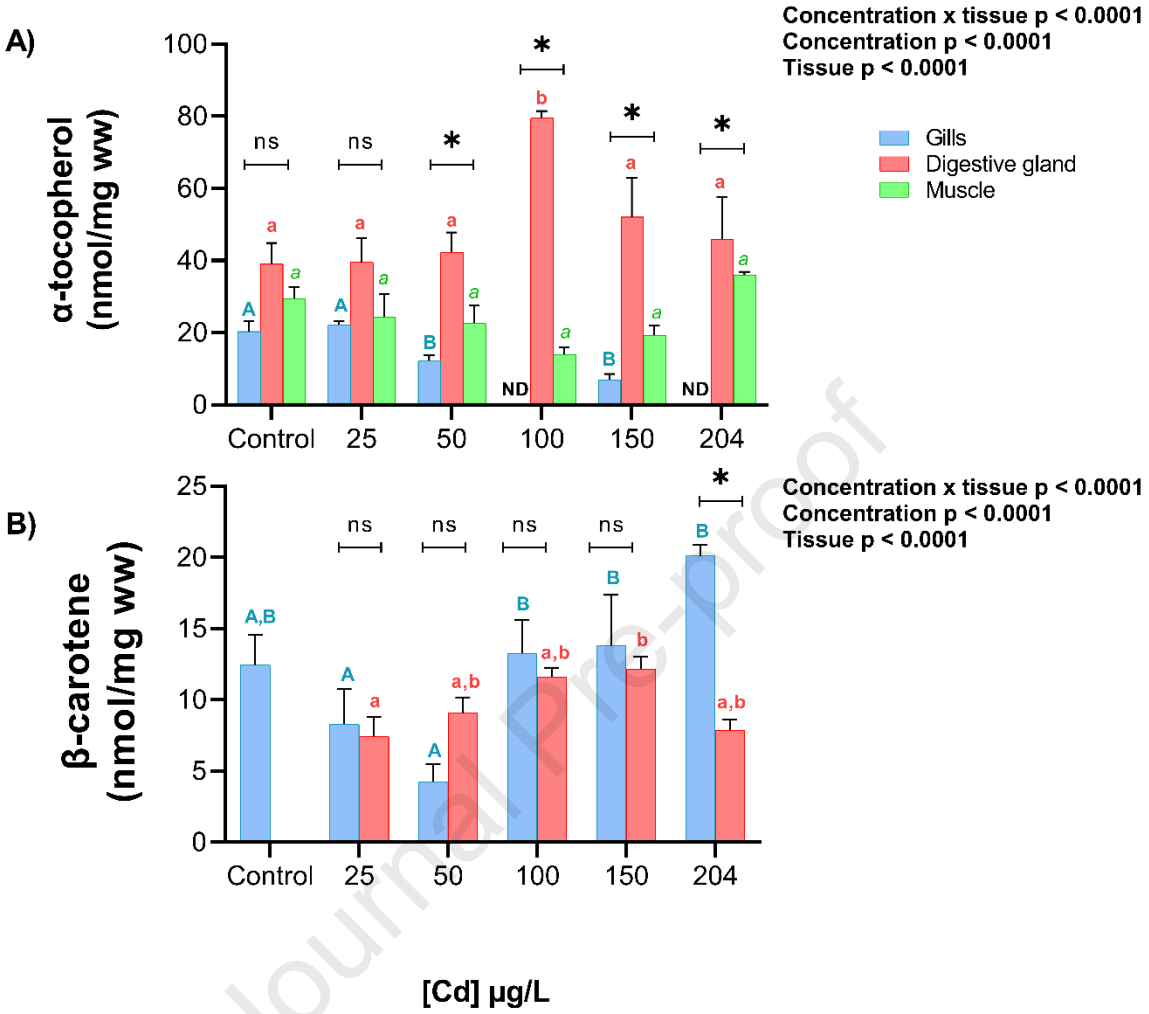


262 Fig. 3 Catalase (A), glutathione-S-transferase (B) and metallothioneins (C) in gills, digestive
263 gland and muscle of *A. tehuelchus* after 96 h of Cd exposure (mean ± SE). Asterisks indicate
264 significant differences among tissues within each Cd concentration. Uppercase (blue),

lowercase (red), and italic letters (green) represent significant differences among Cd concentrations for gills, digestive gland and muscle, respectively.

3.2.3 Liposoluble antioxidants

Levels of α-tocopherol in gill showed significant decreases with 50 and 150 µg Cd/L ($p < 0.05$) and were not detectable with 100 and 204 µg Cd/L (Fig. 4A). In digestive gland, α-T level with 100 µg Cd/L showed a significant increase compared with all treatments ($p < 0.05$), being the double of control. In muscle, there were no significant variations in α-T levels among treatments ($p > 0.05$). In the case of β-C levels, no significant variation was observed in gills among treatments ($p > 0.05$). However, in digestive gland, there was a significant increase with 150 µg Cd/L compared with the lowest Cd concentration treatment ($p < 0.05$). In muscle, β-C levels remained below the detection limit in all cases (Fig. 4B).



277 Fig. 4 Content of (A) α-tocopherol and (B) β-carotene in gills, digestive gland and muscle of
278 *A. tehuelchus* after 96 h of Cd exposure (mean ± SE). ND: non-detectable. Asterisks indicate
279 significant differences among tissues within each Cd concentration. Uppercase (blue),
280 lowercase (red), and italic letters (green) represent significant differences among Cd
281 concentrations for gills, digestive gland and muscle, respectively.

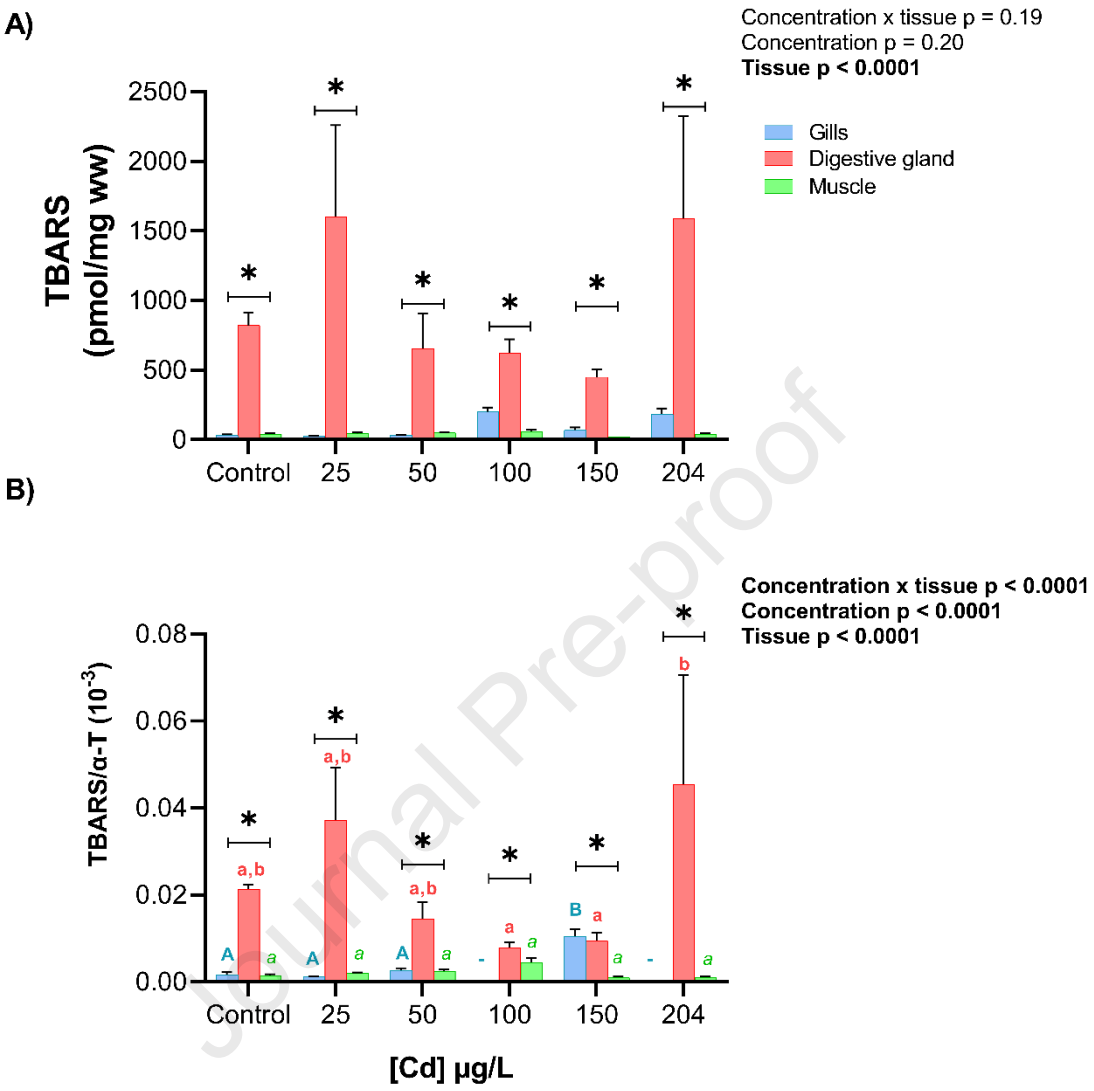
282

283 *3.2.4 Lipid peroxidation*

284 TBARS levels showed a significant tissue-specific effect (Fig. 5A), with the highest levels
285 recorded in digestive gland, up to 60 and 80 times higher than in gills and muscle,
286 respectively. In both the control group and with 25 and 204 µg Cd/L, TBARS levels were
287 significantly higher in digestive gland compared with gills and muscle ($p < 0.05$), with no
288 significant difference between the latter two tissues ($p > 0.05$).

289 The TBARS/α-T index describes the relationship between lipid damage and liposoluble
290 antioxidant defense (Fig. 5B). The highest values were recorded in digestive gland with 204
291 µg Cd/L, which was similar to that measured with 25 µg Cd/L and control. Those values
292 were an order of magnitude higher than in gills and muscle ($p < 0.05$).

293



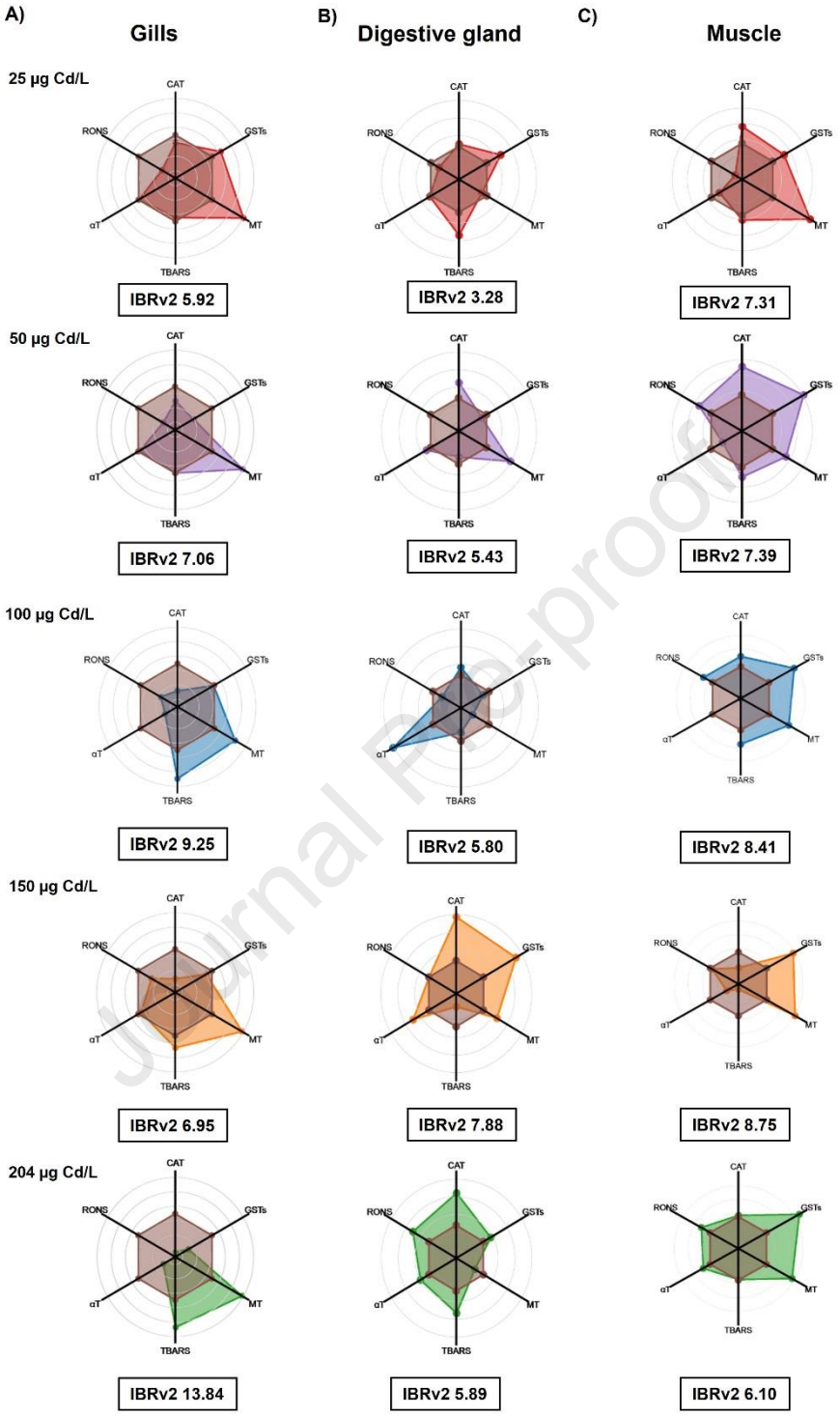
294 Fig. 5 Content of (A) TBARS and (B) TBARS/α-T index in gills, digestive gland and
295 muscle of *A. tehuelchus* after 96 h of Cd exposure (mean ± SE). Asterisks indicate significant
296 differences among tissues within each Cd concentration. Uppercase (blue), lowercase (red),
297 and italic letters (green) represent significant differences among Cd concentrations for gills,
298 digestive gland and muscle, respectively.

299

300 3.3 IBRv2 analysis

The above biomarker responses for all Cd concentrations tested and their respective IBRv2 values are shown in Fig. 6. β-carotene was not included in this analysis because it was not detected in the digestive gland control or at any concentration in muscle. The IBRv2 value is associated with a star plot where each arm of the graph represents a different biomarker, and the brown shaded area represents the zero-value established by the control for each tissue. Biomarker induction is indicated by the area above zero and biomarker inhibition is indicated by the area below zero. In gills (Fig 6A), MT showed the highest scores at all Cd concentrations indicating an induction compared to control, meanwhile the same induction was observed for TBARS with 100, 150 and 204 µg Cd/L. On the other hand, CAT, GST, α-T and RONS kept close to zero at all Cd concentrations. In digestive gland (Fig. 6B), inductions were observed for most biomarkers at the highest concentrations (150 and 204 µg Cd/L), while TBARS and MT showed inhibition. Conversely, at lower concentrations (25, 50, and 100 µg Cd/L), most biomarkers remained unchanged or were inhibited. Induction was measured for TBARS and GST (25 µg Cd/L), CAT and MT (50 µg Cd/L), and α-T (50 µg Cd/L). In muscle (Fig. 6C), most biomarkers showed an induction compared to the control at all concentrations, except with 150 µg Cd/L where only GST and MT showed an induction.

The highest IBRv2 levels were recorded in gills at the highest concentration, while the lowest levels were observed in digestive gland at the lowest exposure concentration. In both digestive gland and muscle, IBRv2 levels showed minimal variation among exposure concentrations. The gills exhibited a rising IBR pattern with increasing exposure concentration (except at the 150 µg Cd/L treatment).



324 Fig. 6. Calculated IBRv2 index utilizing the biochemical parameters after 96 hours of exposure to
325 Cd. Star plots for each tissue and concentration; and IBRv2 values for tissue: (A) gills, (B) digestive
326 gland and (C) muscle. Abbreviations: RONS (reactive oxygen and nitrogen species), CAT (catalase),

GST (glutathione-S-transferase), MT (metallothioneins), TBARS (lipid peroxidation) and α-T (α-tocopherol).

4. Discussion

The 96h CL_{50} value of 155.8 µg Cd/L obtained for *A. tehuelchus* was considerably lower than 1480 µg Cd/L reported for the scallops *Argopecten irradians* (Nelson et al., 1976), 396 µg Cd/L for *Argopecten ventricosus* (Sobrino-Figueroa et al., 2007) and than 960 µg Cd/L reported for the mussel *Mytilus edulis* (Nelson et al., 1988). The low CL_{50} value shows that *A. tehuelchus* is a very susceptible species to Cd exposure compared to other bivalves.

Cadmium is a non-redox active element that cannot directly trigger ROS production (Cuypers et al., 2010). This explains the absence of RONS induction in the three tissues over the range of studied Cd concentrations. However, Cd is able to produce oxidative challenges to organisms. Different responses to CAT and GST activities in the presence of Cd have been reported. Figueira et al. (2012) reported both significant increases and decreases in clams exposed to Cd, which could be due to an interaction between Cd and the catalytic subunit of CAT, as suggested by Wrónska-Nofer et al. (1999). The decrease in CAT activity observed mainly in gills may be related to some degree of inhibition at high concentrations of Cd. No clear pattern was found between metal concentration and GST activity in the three tissues. Wang et al. (2011) showed that Cd contamination increased the activity of GST in both gills and digestive gland of clam *Ruditapes philippinarum,* whereas Ramos-Gómez et al. (2011) found for the same species exposed to contaminated sediment no significant response of this enzyme. Regarding the synthesis of MT, gills showed an increase in response to Cd, while the other tissues did not react. Previous studies have reported MT induction in gills, digestive gland and other soft tissues of marine bivalves exposed to Cd (Liu and Wang, 2011;

Pytharopoulou et al., 2011; Serafim and Bebianno, 2007). Particularly, Serafim and Bebianno (2010) and Wang et al. (2011) showed an increase in MT in both gills and digestive gland of clams in the presence of Cd. The observed lack of response in digestive gland concerning to MT could be attributed to the relatively short exposure time. Indeed, in experiments with *A. tehuelchus* exposed to Cd for 7 and 14 days, an increase in digestive gland was observed compared to the control (Giarratano et al., 2023). On the other hand, the increase in MT levels in gills is expected since they are the first organs exposed to entry of Cd present in water, as well as, in phytoplankton (Fernández Galindo et al., 2010).

In heterotrophic organisms, α-T and β-C are not synthesized internally but rely exclusively on dietary sources. The decrease in α-T levels observed in gills can be explained by its consumption, possibly to mitigate cellular damage in response to xenobiotics or stress factors. In contrast, increased levels of lipid-soluble antioxidants were observed, even though the scallops were not fed. The increase in α-T in digestive gland and β-C levels in both digestive gland and gills may be due to a redistribution of these antioxidants between different tissues within the organism. This hypothesis is based on proposed explanations for similar results in other species. In that sense, Bertrand et al. (2016) reported higher levels of α-T levels in shrimp *Palaemonetes argentinus* in cephalothorax exposed to chlorpyrifos than in control conditions and proposed a possible mobilisation of α-T from the abdomen to the cephalothorax. Previous studies have also described α-T transport from muscle to liver in fish (Lie et al., 1994; Parazo et al., 1998). Cahu et al. (1995) proposed a similar redistribution mechanism in shrimp *Penaeus indicus*, involving transport of this molecule from muscle to the hepatopancreas. Similarly, the movement of carotenoids has been documented in various sea urchin tissues (Pérez et al., 2015). Liu et al. (2020) identified a novel carotenoid-

producing bacterium, *Brevundimonas scallop*, isolated from *Chlamys nobilis*, as a new source of carotenoids in marine bivalves. They therefore hypothesised that the carotenoids present in the tissues were not exclusively dietary but were synthesised by this bacterium. Furthermore, the presence and increase of β-carotene in scallop tissues found in our study can be attributed to *in situ* synthesis within the organism by bacteria.

In this study, the highest TBARS levels were found in digestive gland, but values were similar in exposed and control scallops. In this experiment, no tissue damage through TBARS was found, with similar results to those found by Figueira et al. (2012) in experiments with clams in the whole tissue. An increment of TBARS indicates tissue damage, meanwhile, levels similar to control would indicate no significant effect of the contaminant or that the antioxidant system was efficient in avoiding damage.

The IBRv2 index was analyzed to assess comprehensively the toxicological effect of Cd exposure stress on *A. tehuelchus*. The elevated IBRv2 value in gills indicates that this tissue was more affected by Cd than the digestive gland and muscle.

The pronounced activation of gill defence system is due to direct contact with waterborne toxics. Conversely, the digestive gland with almost no response, suggests insufficient Cd concentrations and time exposure to induce oxidative stress. The muscle showed minimal reactivity to contaminants, highlighting the need to consider tissue specificity and exposure duration when assessing oxidative stress in marine organisms exposed to contaminants.

5. Conclusion

This study investigated the effects of different Cd concentrations on the antioxidant defence system of the marine scallop *A. tehuelchus*. The LC_{50} was found to be lower than for other

bivalve molluscs and scallops, indicating a higher sensitivity to Cd. Responses to different concentrations showed a low effect at lower exposure concentrations and a higher effect at higher concentrations, although this relationship varied among tissues. This reveals distinct tissue-specific patterns that may be related to their specific functions. Cadmium exposure did not induce production of RONS and consequently, no tissue damage was measured through TBARS. Gills were the most responsive tissue, and no clear patterns were observed in digestive gland and muscle. This finding could be related to assayed Cd concentrations and/or exposure time that could be not enough to induce oxidative stress on this scallop.

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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.

Ethical Approval

All procedures performed in present study 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). Sampling of scallops in the Natural Protected Area Valdés Peninsula was authorised by the Ministry of Tourism of Chubut Province (Disp. 36/17-SsCyAP). All efforts were made in order to minimize the suffering of the organisms and reduce the number of used individuals.

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Highlights

- Tehuelche scallop is more sensitive to Cd than other species (LC50: 155.8 µg Cd/L).
- CAT and α-T decrease in gills, increase in digestive gland, no response in muscle.
- MT significantly rises in gills.
- No Cd effect on RONS, GST, or LPO in any tissue.
- Gill is the most affected tissue, digestive gland and muscle show no clear response.

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:

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