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Highlights

- APs were higher in seawater and mussels at the less industrialized site.
- *Brachidontes rodriguezii* is an effective bioindicator of AP pollution with high bioaccumulation.
- APs <1 mm and fibers dominated all samples; fragments found only in mussel tissue.
- Mussels with high concentration of APs showed digestive gland alterations.
- Oxidative stress was not related to AP bioaccumulation.

Bioaccumulation and effects of anthropogenic microparticles in *Brachidontes rodriguezii* from the Southwestern Atlantic coast

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Abstract

The bivalve *Brachidontes rodriguezii*, a species inhabiting the southwestern Atlantic coast, has emerged as an ideal bioindicator for environmental contamination due to its continuous water filtration, which exposes it to anthropogenic particles (APs), including microplastics (MPs). Although APs such as MPs have been shown to cause biological harm in bivalves, further research is essential to a deeper understanding of these impacts. This study investigates the concentrations and characteristics of APs in the soft tissue of *B. rodriguezii* and the surrounding water column at two contrasting sites in southern Buenos Aires Province, Argentina: Pehuen-Co (PC), a region with lower industrial activity, and Club Náutico (CN), a heavily industrialized and urbanized area. Our results indicate surprisingly higher AP concentrations at PC (22.0 ± 5.7 items/L in seawater; 4.2 ± 2.7 items/g w.w. in soft tissue) compared to CN (4.7 ± 1.2 items/L in seawater; 1.0 ± .3 items/g w.w. in soft tissue), with bioaccumulation factors exceeding 100 for both sites. APs smaller than 1 mm and fibers were predominant across all samples, while fragments were exclusively detected in the soft tissue of mussels. Interestingly, the APs from PC displayed greater color diversity than CN. Health assessments revealed significant site-specific differences: mussels from PC exhibited higher rates of digestive gland alterations (80%) and parasite presence (15%) but no evidence of eosinophilic bodies or hermaphroditism. Conversely, mussels from CN had lower rates of digestive gland alterations (50%), no parasites, but a high incidence of eosinophilic bodies (61%). At the molecular level, no site-specific differences were observed in the expression of genes related to oxidative stress markers such as superoxide dismutase (SOD), reactive oxygen species (ROS), or total thiols. However, protein carbonylation and lipid peroxidation were elevated in mussels from CN. In

conclusion, *B. rodriguezii* bioaccumulates APs in its tissues, correlating with environmental levels, thus confirming its status as an effective bioindicator of AP contamination. The higher levels of APs in PC and the observed histological alterations, along with the lower oxidative stress response, may suggest that APs primarily affect tissue level rather than oxidative stress. These findings highlight the need to consider multiple health indicators in studies of AP contamination and underscore the importance of monitoring and managing pollution even in seemingly less-impacted areas.

Keywords: bioindicator, bivalves, microplastic, biomarkers, histopathology

1. Introduction

Marine aquatic environments are continuously exposed to various pollutants that enter water bodies through multiple pathways, including industrial, domestic, and agricultural effluents, atmospheric deposition, and urban runoff. Many of these contaminants can exert harmful effects on aquatic organisms, compromising their health and survival. Among them, there is a group of contaminants called anthropogenic particles (APs) that refer to a diverse array of organic and inorganic materials either manufactured or generated incidentally often as byproducts of human activity (Mattsson et al., 2021). This category encompasses microplastics (MPs), including synthetic microfibers, and other particulate matter resulting from the degradation of various plastic materials (Collard et al., 2018 and 2021; Gao et al., 2023). They are originated from human activities such as industrial operations, tourism, and inadequate waste management and

recognized as pervasive ecological stressors due to their persistence, widespread distribution, and potential for bioaccumulation in marine organisms (Galloway et al., 2017; Wright & Kelly, 2017;). Marine ecosystems worldwide face growing threats from APs) pollution, with MPs—plastic fragments under 5 mm in diameter— being one of the most studied in recent years (Pourebrahimi & Pirooz, 2023). MPs have drawn scientific attention due to their ability to interact with marine organisms across food webs, from plankton to apex predators, through ingestion, entanglement, and trophic transfer (Benson et al., 2022). However, emerging research highlights that other APs like synthetic textile fibers, tire wear particles, and industrial coatings similarly endanger marine ecosystems (Athey et al., 2022; Forero-López et al., 2024). APs can also disrupt ecological and biogeochemical processes through chemical leaching, habitat alteration, and toxicity, making it essential to understand their sources, behavior, and impacts to develop effective monitoring and management strategies (Mattsson et al., 2021).

Bivalves, particularly mussels, serve as effective bioindicators of anthropogenic pollution due to several key characteristics. First, their filter-feeding behavior allows them to filter large volumes of water, extracting plankton and nutrients while simultaneously accumulating pollutants within their tissues (Strehse & Maser 2020). CResearch indicates that certain bivalve species can filter up to 250,000 MPs per hour, making them ideal organisms for monitoring (Falkenberg et al., 2024). Second, bivalves exhibit a broad geographic distribution, inhabiting various marine environments from coastal waters to estuaries (Prestes et al., 2024) making them effective indicators of APs contamination across diverse ecosystems. Moreover, as sessile organisms, mussels remain fixed in one location, leading to the bioaccumulation of contaminants over time (Chahouri et al., 2023). This characteristic allows them to reflect water quality

consistently within a specific area, providing valuable data on long-term APs pollution trends. Physiological responses of bivalves to contaminants have been widely documented, resulting in genetic and physiological damage. At the molecular level, acute exposure to stressors often triggers oxidative stress responses, measurable through changes in biomarkers such as total thiols, reactive oxygen species (ROS), and the expression of antioxidant enzymes like superoxide dismutase (SOD) (Moreira et al., 2021). These early indicators reflect the organism's attempt to counteract the initial imbalance in redox homeostasis. In contrast, chronic or prolonged exposure can result in cumulative cellular damage, which can be assessed through markers such as protein carbonylation and lipid peroxidation—indicating damage to proteins and cell membranes, respectively (Wang et al., 2022). At the tissue level, contaminants can cause structural alterations, particularly in metabolically active organs like the digestive gland. Histological assessments commonly reveal morphological changes in digestive tubules, the presence of eosinophilic bodies associated with inflammatory responses, and even parasitic infections, all of which reflect impaired physiological status (Ojeda et al., 2021; Otegui et al., 2024). Together, these biomarkers offer a comprehensive view of contaminant-induced effects across multiple levels of biological organization, from molecular alterations to visible tissue damage.

Studies have shown that mussels experience alterations in gene expression and cellular damage even at low concentrations of nanoplastics, indicating their sensitivity to these pollutants (Cole et al., 2020). APs and MPs accumulation in bivalves has been linked to a range of adverse biological effects, including disrupted energy allocation, impaired immune function, and reduced reproductive success (Hussain et al., 2023; Jeon et al., 2021). Bioassays conducted in various mussel species have revealed a range of

physiological and biochemical responses to individual pollutants, highlighting the relevance of monitoring these biological changes to better understand the ongoing environmental stressors (Gudimov and Malavenda, 2022; Dellali et al., 2023). A common objective in these studies is to identify specific responses elicited by a particular pollutant. However, a key limitation of in vitro bioassays lies in their highly controlled experimental conditions which may not fully reflect the complexity of natural ecosystems. In real-world scenarios, pollutants such as APs exist as complex mixtures (Altenburger et al., 2019) that can interact synergistically or antagonistically, potentially altering their combined toxicity to organisms.

The Southwestern Atlantic, particularly along the coast of Buenos Aires Province, Argentina, presents an ideal setting to investigate the ecological impacts of APs due to the varying degrees of human influence along its shoreline. Within his region the Bahía Blanca Estuary (BBE) encompasses heavily industrialized, urbanized zones and relatively undisturbed coastal areas, creating a natural gradient for assessing pollution effects. Varying concentrations of contaminants have been reported throughout the Bahía Blanca Estuary (BBE), with elevated levels consistently observed at sites subjected to greater anthropogenic pressure (Marcovecchio et al., 2021) and compared to that of nearby coastal areas, such as Pehuen Co (Fiori et al., 2024). *Brachidontes rodriguezii*, a dominant species in the mid-intertidal coasts of Buenos Aires Province has been used as a suitable bioindicator of anthropogenic pollution in the BBE due to its wide distribution and ease of collection (Quintas et al., 2017; Buzzi et al., 2017; Quintas et al., 2021).

Although previous studies have reported the presence of APs—including MPs—in water, sediment, and marine organisms from this region (Truchet et al., 2021; Ronda et al., 2023a, 2023b; Fiori et al., 2024), comprehensive investigations into APs bioaccumulation and associated biological effects in *B. rodriguezii* remain scarce. In this study, we examine the accumulation and biological impacts of APs in *B. rodriguezii* collected from two contrasting sites along the Buenos Aires Province: Pehuen-Co (PC), characterized by low anthropogenic impact, and Club Náutico (CN), situated in the heavily industrialized Bahía Blanca estuary. We quantified APs concentrations in both mussel tissues and surrounding waters, and assessed histological and molecular responses in the mussels, focusing on oxidative stress biomarkers and tissue integrity. By comparing responses between these sites, this research aims to deepen our understanding of the ecological risks posed by APs and evaluate the potential of B. *rodriguezii* as a bioindicator for coastal pollution monitoring.

2. Materials and methods

2.1 Study area:

Two coastal sites on the southern coast of Buenos Aires Province, Argentina, were selected based on their differing levels and types of anthropogenic activities: Pehuen-Co (PC) and Club Náutico (CN) within Bahía Blanca Estuary (BBE) (Fig. 1). PC is an exposed sandy beach located in an open bay with an east-west-oriented shoreline. Classified as an intermediate beach, it experiences mesotidal regimes and semidiurnal tides (Isla and Bertola, 2003; Delgado et al., 2012). The permanent population of 681 residents (INDEC, 2010) can increase nearly five-fold during the summer tourism season (Rojas et al.,

2014). Approximately 17 km west of PC lies a Geological, Paleontological, and Archeological Provincial Reserve, which forms part of the Interjurisdictional System of Marine Coastal Protected Areas (Rojas et al., 2014). The BBE is a shallow, turbid estuarine system covering approximately 2,300 km². It features NW-SE-oriented tidal channels, extensive intertidal flats, low marshes, and islands (Perillo and Piccolo, 1991). Geographically, the estuary is situated between 38°30'–39°25' S and 61°15'–63°00' W. The inner part of the estuary is home to ~6,500 residents and features mixed urbanized and rural landscapes, a small tourist area, and an artisanal fishing/recreational port where CN is located. Adjacent to this recreational site is the most industrialized section of the estuary. This area has experienced significant industrial development and population growth, including a city with over 335,000 inhabitants (INDEC, 2023). Industrial activity in this region includes five national harbors and one of South America's largest industrial parks, which comprises refineries, oil terminals, oil product storage tanks, and multiple docks. Effluents from these industrial operations and untreated sewage from industries along the northern estuarine shore are discharged directly into the estuarine waters (Limbozzi and Leitao, 2008).

2.2 Sampling

Sampling was conducted at low tide during winter 2023 to minimize the influence of tourist activity and recreational disturbances at both study sites. The environmental conditions are shown in Table 1.

Table 1: Environmental conditions of sampling at each site, including date, time of sampling, and location.

Site	Sampling date	Time	Latitude	Longitude	Environmental temp. (°C)	Tide height (m)	Water temp. (°C)	pH	Salinity (PSU)
PC	June 6th, 2023	08:39 a.m.	39°00'15"S	61°32'22"W	7.6°C	1.30	7.3	7.9	35.7
CN	July 14th, 2023	09:20 a.m.	38°47'18"S	62°16'43"W	6.3°C	1.05	7.5	8.1	34.6

Mussels of the species *B. rodriguezii* were collected manually. At PC, individuals were gathered from naturally occurring rocks on the beach adjacent to the Provincial Reserve Protected Area, a site chosen to minimize the influence of anthropogenic activity; while at CN, mussels were collected from the pilings supporting a dock. Bivalves measuring between 2 and 3 cm were carefully selected to ensure adult specimens and placed into containers with seawater under cold conditions (4°C) y to minimize metabolic activity. A total of 60 specimens were collected from each site. Of these, 30 specimens were divided into three pools of 10 individuals each and stored at -20°C to analyze APs. The remaining 30 specimens were similarly grouped in 3 pools (10 individuals/each) and stored at -80°C to evaluate reactive oxygen species (ROS), thiols, lipid peroxidation (LPO), and carbonylated proteins (CP). Additionally, 20 extra specimens from each site were collected for individual gene expression of superoxide dismutase (SOD) and histological studies. These mussels were dissected under sterile conditions to extract their gills, which were preserved in RNA*Later*™ (Thermo Fisher Scientific) and stored at

-80°C for subsequent gene expression analysis. The remaining tissue was retained for histological examination. For all collected specimens, weight, length, height, and thickness were recorded, and the condition index (CI) was calculated using the following formula suggested by Zeng et al. (2020):

$$CI = [\text{Total weight (g)} \times 100] / [\text{Body length} * \text{height} * \text{width (cm}^3)]$$

At each site, three replicate 1-liter water samples were collected before the surf zone in pre-treated glass containers and stored at 4°C until laboratory analysis.

Figure 1: Sampling locations on the Southwestern Atlantic Coast: Club Náutico (within Bahía Blanca estuary) and Pehuen-Co, Argentina.

2.2 Anthropogenic particle separation in Brachidontes rodriguezii and seawater:

The entire soft tissue of *B. rodriguezii* was weighed and placed in a pre-muffled beaker and dissolved in 30% H_2O_2 (1:10 v/v) maintaining on a temperature-controlled heating plate set at 45°C until complete digestion of organic matter (Ronda et al., 2023). The resulting supernatants and seawater samples were filtered (pore size = 0.45 µm) and placed in Petri dishes, dried in an electric stove at 50°C, and stored at room temperature. Abundance of APs in the mussels was expressed as items per gram of wet tissue (items/g), considering the soft tissue's total weight and reported per individual (items/ind.) according to the pool (n = 10) The abundances of APs in the seawater samples were expressed as items per liter (items/L).

2.3 Stereomicroscope visualization of anthropogenic particles

Retained particles in the filters were visually inspected under a stereomicroscope (Nikon SMZ1500) with a digital camera attached (Nikon DXM1200F). All sizes of APs were initially considered; however, only particles smaller than 5 mm were found; therefore, all of them will be referred to as "APs". Visual classification of APs was performed using previously established criteria for MPs (Hidalgo-Ruz et al., 2012; Lusher et al., 2013): Photographs of all potential APs and maximum length (mm) were recorded. Shape (fiber, film, fragment, foam, or pellet) and color were also recorded.

2.4 micro-FTIR spectroscopy

A subset of samples (50 % of the total number) were characterized using Fourier-transform infrared spectroscopy in attenuated total reflectance (ATR) mode with a coupled microscope (Nicolet-6700 / Continuum by Thermo Scientific) (micro-FTIR), in the spectral range between 4000 and 600 cm^{-1}. The analyses were performed in reflectance mode on a silver-coated metal plate, with a resolution of 4 cm^{-1}, 128 scans, and an optical velocity of 1.89. Spectra obtained were compared with reference spectra from the database to identify the polymer using a match score greater than 60%that was also confirmed through comparison with the virtual library Open Specy (https://openanalysis.org/openspecy/).

2.5 Controls

Work surfaces were thoroughly cleaned with 70% alcohol, and laboratory coats, cotton clothing, and nitrile gloves were worn. All laboratory equipment, including filter equipment, forks, etc., was also cleaned with 70% alcohol. All solutions, including H_2O_2, ethanol, and distilled water, were filtered. All glassware and glass filters were muffled before use. Petri dishes with control filters were exposed to the air during all procedures to discard contamination with airborne following the same protocol for samples and were processed in parallel during all phases of the analytical method, including the filtering stage, drying, and stereomicroscope observation. The presence of any potential APs in these controls was subtracted from the samples according to its shape, color, and size.

2.6 Bioconcentration factor

The bioconcentration factor (BCF) of APs was calculated following the criteria suggested by Miller et al. (2023) for microplastics. The formula used was: BCF = (items/kg)/(items/L); where items/kg refers to the number of anthropogenic particles found in the soft tissue of the bivalves, and items/L refers to the number of anthropogenic particles found in the water. To ensure consistency with this formula, we standardized the expression of APs concentrations in bivalve tissues to items per kilogram of wet tissue, thereby allowing for accurate BCF calculation according to Miller et al. (2023).

2.7 Histological assessment:

For microscopic analysis, the soft body of 20 mature mussels from each site was fixed in formaldehyde 4% for 24 h at room temperature. Samples were dehydrated through a graded alcohol series and embedded in Paraplast®. Sections of 5 µm thickness were obtained by a rotatory microtome Leica RM 2145 and stained with Masson's trichrome and hematoxylin–eosin, for sex determination and general observation of histological architecture of different organs. Selected sections were photographed using an Olympus BX51 light microscope equipped with an Olympus C-7070 digital camera.

2.8 Analyses of oxidative stress at different levels:

Oxidative stress biomarkers were measured as follows:

2.8.1 Superoxide dismutase expression (SOD)

Gill tissues were homogenized with a Scilogex D160 Handheld Homogenizer (Scilogex, LLC, USA). Total RNA was extracted using TRIzol® RNA Isolation Reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. RNA concentration (~ 500 ng µL-1) and A260/A280 ratio (ratio above 1.8) were assessed using a SPECTROstar Nano Spectrophotometer (BMG Labtech, Ortenberg, Germany). RNA integrity was verified with an agarose bleach gel electrophoresis at 1% (Aranda et al., 2012). After quality control, all samples were chosen and stored at −80 °C for subsequent qPCR gene expression analysis. Primers of *SOD* CDSwere designed using Primer3. (http://biotools.umassmed.edu/bioapps/primer3 www.cgi) and synthesized by IDT (Integrated DNA Technologies). As for housekeeping genes, genes coding for the 18S rRNA and the *elongation factor alpha were* chosen (Bahamonde et al., 2015). Optimal annealing temperatures for primers were between 60 and 62°C. Primer sequences were as follows: *sod* (PF ACTAGTGCAGGATCACATTTC and PR GCATGGACTACTACTGTTCTTC); and two housekeeping 18S (PF CACTGCGAGGATTGACAGATT and PR AACGACACTCGTCCCTCTAA; *efa (*PF CTCCCACTCCAGGATGTTTAC and PR GAGAGACTCGTGGTGCATTT). Primers were checked based on the %E (range 103-110%) and R2 (> 0.99). Real time PCR analysis was performed using SsoFast™ EvaGreen® Supermix (Bio-Rad, Mississauga, ON, Canada). Relative abundance of mRNA levels were assayed using the CFX96TMReal-time PCR System (BioRad). Two NTC (non template control) and two NRT (no reverse transcription controls) were included on all reaction plates. Normalized expression levels for target genes were generated using the △△Cq method (CFX96 Manager Software).

2.8.2 Total reactive oxygen species (ROS) and Total thiols:

The quantification of total reactive oxygen species (ROS) was performed following the protocol described by Pérez-Hernández et al. (2024), using the Fluorometric Intracellular ROS Kit (Green) (Sigma-Aldrich). Relative fluorescence units (RFUs) were recorded using a Cytation 5 fluorometric spectrophotometer (Agilent Biotek Instruments) at an excitation wavelength of 490 nm and an emission wavelength of 520 nm, in accordance with the manufacturer's instructions.

Total thiol content was determined following the protocol described by Pérez-Hernández et al. (2024), with the modification of using 30 mg of frozen biomass for the analysis.

2.8.3 Carbonylated Proteins (CP):

The concentration of protein carbonyl groups was quantified following the method described by Pérez-Hernández et al. (2024), with slight modifications. After lysis and centrifugation 300 µL of the supernatant was incubated with 60 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) for 30 minutes in the dark at room temperature. Following this, 360 µL of cold 20% trichloroacetic acid (TCA) was added, and the mixture was incubated on ice for 15 minutes before centrifugation at 10,000 × g for 5 minutes at 4 °C to remove the supernatant. The resulting pellet was washed multiple times with 300 µL of ethanol:ethyl acetate (1:1), with each wash followed by centrifugation at 10,000 × g for 5 minutes at 4 °C, The pellet was air-dried for 20 minutes at room temperature and dissolved in 300 µL of 6 M guanidine-HCl and incubated at 37 °C for 15 minutes, vortexed every 5 minutes and centrifuged again at 10,000 × g for 5 minutes at

4 °C. InFinally, 150 µL of the resulting supernatant was mixed with 150 µL of 6 M guanidine-HCl per well. Absorbance was measured at 366 nm using a 96-well UV microplate reader. The concentration of carbonyl groups was calculated using the Lambert–Beer law, with a molar extinction coefficient of 22,000 M^{-1} cm^{-1}. Results were normalized to total protein content, determined by the Bradford assay (Bradford, 1976).

2.8.4 Lipid peroxidation (LPO):

Oxidative damage to lipids was assessed by measuring the production of malondialdehyde (MDA), following a slightly modified method from Lushchak et al. (2011). Briefly, an aliquot of the homogenate was diluted in phosphate buffer (1:8) and incubated for 30 minutes at 100 °C with a solution containing trichloroacetic acid, thiobarbituric acid and butylated hydroxytoluene at final concentrations of 0.3%, 4%, and 0.01%, respectively (Pikul and Leszczynski, 1986). Subsequently, the samples were cooled on ice for 5 minutes and then centrifuged at 10,000 g for 15 minutes. The absorbance of the supernatant was measured at 535 nm using a Shimadzu UV/visible spectrophotometer. The concentration of the colored complex was calculated using the molar extinction coefficient of the MDA-TBA complex (156 $mmoles^{-1} \cdot cm^{-1} \cdot L$). Results were expressed in grams of tissue.

2.9 Statistical analyses

The GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, California, USA), the R Software Package 3.6.0 version, and the InfoStat (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) software were used. Data were assessed for normality

and homogeneity of variance using the Shapiro Wilk and Levene tests, respectively. Statistical differences between the biometric data of mussels, anthropogenic particle abundances, sizes, as well as oxidative stress measurements, were analyzed by the t-test or the Wilcoxon signed-rank test for normally and non-normally distributed data. Analyses of correlation were performed using Spearman's coefficient. The statistical significance level accepted for all analyses was $p < 0.05$.

Results and discussion

3.1 Biometric characterization of B. rodriguezii

Although only mussels between 2 and 3 cm in length were collected from both sites, the specimens from CN were shorter in length ($p = 0.0003$, t-student) and exhibited a higher CI ($p = 2.162\ 10^{-8}$, Wilcoxon signed-rank test; Table 2). These findings indicate a notable difference in the morphological and physiological characteristics of mussels collected from the two distinct sites. This observed disparity raises important questions regarding their growth dynamics and environmental adaptations. The CI is a vital metric in bivalve studies, as it reflects the health and nutritional status of these organisms. A higher condition index typically correlates with increased soft tissue mass relative to shell size (Zeng and Yang, 2020), suggesting that the mussels from CN could experience favorable growth conditions. At the population level, *B. rodriguezii* reach significantly higher densities in PC (27444 ± 18975 indiv./m^2) compared to CN (2075 ± 800 indiv./m^2); however, there is evidence of a density-dependent limitation for the development of mollusk biomass in PC (Dos Santos et al., 2018; Dos Santos & Fiori, 2019; Dos Santos, 2022), which could also have contributed to higher values of the condition index in CN.

Since CN was chosen as the "anthropogenically influenced point", this could be attributed mainly to nutrient availability. The inner and middle part of the BBE have been categorized as moderately eutrophic (Carbone et al., 2016). However, the shorter length of the mussels from CN ($p = 3.06 \times 10^{-4}$), despite their higher CI, may imply that these individuals are allocating more energy towards soft tissue development rather than shell growth. This phenomenon can be also indicative of an adaptive strategy where mussels prioritize immediate survival and reproductive fitness over skeletal robustness (Sokolova, 2021). In contrast, the mussels from PC may be experiencing different environmental pressures that limit their growth or affect their CI negatively. Factors such as lower nutrient availability or increased competition could contribute to their comparatively lower robustness (Zeng and Yang, 2020).

Table 2: Biological characteristics of *B. rodriguezii* at both sites. Statistical analyses were performed using the t-test for length (normally distributed data) and the Wilcoxon signed-rank test for condition index (CI), width, and height (non-normally distributed data).*$p < 0.05$; ** $p < 0.01$.

Site	Weight (grams)	Length (mm)	Width (mm)	Height (mm)	Condition Index (CI)
PC	2.11±0.50	26.70±2.71	11.44±0.96	9.87±1.01	70.25±10.63
CN	2.15±0.51	25.20±2.42**	11.15±0.91*	9.58±1.06*	79.24±7.49**

3.2 Environmental concentration of anthropogenic particles and its bioaccumulation in B. rodriguezii

Unexpectedly, the highest concentrations of APs in the seawater were observed at PC (22.0 ± 5.7 items/L), an area characterized by relatively low anthropogenic activity compared to CN (4.7 ± 1.2 items/L, $p = 4.06 \times 10^{-2}$ Fig. 2A). Although no statistically significant differences were detected, the highest concentrations of APs were also observed in the soft tissues of *B. rodriguezii* from PC, both in terms of abundance expressed as items per gram of tissue (4.23 ± 2.74 items/g tissue w.w.) and as items per individual (3.20 ± 1.90 items/individual) (Fig. 2B and C). Concentrations of APs in superficial seawater and the soft tissue of *B. rodriguezii* from PC in this study were found to be one order of magnitude higher than those reported in previous research conducted at a northern beach with similar characteristics (Truchet et al., 2021). Conversely, the concentrations detected in the inner part of the BBE in this study were three orders of magnitude lower than those observed in a prior study (Severini et al., 2019). This counterintuitive finding suggests that the spatial distribution of APs is not exclusively determined by direct human activity. Instead, oceanographic processes, including local currents, tidal patterns, and sediment transport, may drive APs accumulation in less industrialized regions. Previous studies have shown that the BBE plume significantly affects PC by increasing salinity and turbidity (Delgado et al., 2017). This plume transports fine suspended sediments and organic material, but can potentially transport APs from the estuary into the adjacent coastal waters of PC. Given the estuary's documented erosional state and sediment load, it is likely that APs originating from upstream industrial and urban sources are being exported through the plume and deposited along the inner continental shelf, including PC. Furthermore, the

semi-enclosed geomorphology of PC and the presence of slow-moving, recirculating nearshore currents (Delgado et al., 2017) may enhance the retention and local accumulation of these particles in benthic habitats, where *B. rodriguezii* resides.

Our results confirmed that *B. rodriguezii* accumulates APs in its tissues, with bioaccumulation factors exceeding 100, demonstrating its capacity for their significant retention (Fig. 2D). Furthermore, a strong correlation (r^2 = 0.9077) was observed between APs concentrations in water and those accumulated in *B. rodriguezii* tissues (Fig. 2E). These findings underscore the reliability of *B. rodriguezii* as a bioindicator of local APs pollution, supported by several key characteristics that align with established criteria for effective bioindicators (Burger, 2006). First, an ideal bioindicator should exhibit the capacity to bioaccumulate contaminants at levels that are detectable and proportional to environmental concentrations. Our findings reveal that *B. rodriguezii* exhibits a bioaccumulation factor exceeding 100, meaning that it concentrates APs in its tissues at levels significantly higher than those in the surrounding environment. This high bioaccumulation factor enhances its sensitivity as a biological monitor, allowing it to reflect even low levels of environmental APs pollution. Second, bioindicators must be widespread, relatively abundant, and easy to sample in the target environment. *B. rodriguezii* is native to the study area and is commonly found in coastal ecosystems, which makes it accessible and practical for monitoring programs. Its sedentary lifestyle also ensures that the levels of APs detected in its tissues correspond to the local conditions of the sampling area, rather than reflecting contamination from distant sources. Third, bioindicators should demonstrate a clear and quantifiable relationship between contaminant levels in their tissues and environmental concentrations. In this study, *B. rodriguezii* showed a strong correlation between APs concentrations in water

and those in its tissues. This direct relationship confirms its reliability in reflecting local APs pollution levels, providing a robust means of assessing spatial and temporal variations in contamination. Additionally, *B. rodriguezii* is tolerant to a range of environmental conditions, which is an important feature for bioindicators as it ensures they can survive and accumulate pollutants in diverse settings. Its resilience makes it suitable for monitoring APs across different coastal environments, including those with varying degrees of human activity and oceanographic conditions.

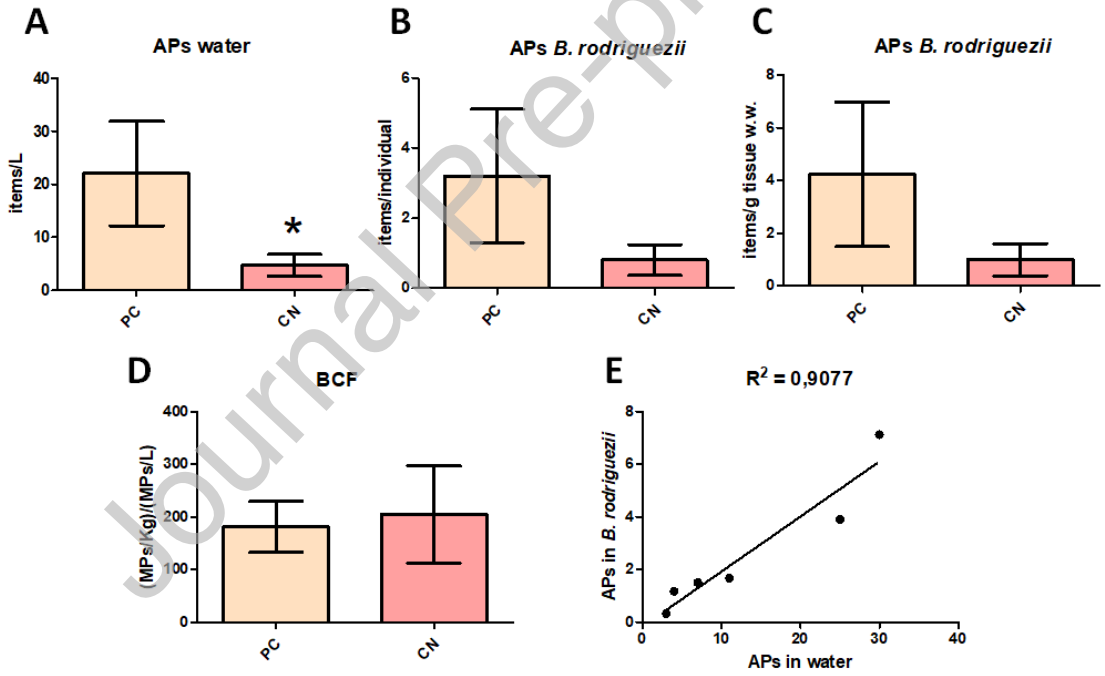

Figure 2: Anthropogenic particles (APs) in the environment and accumulation in *B. rodriguezii.* **A**: APs in the seawater; **B** and **C**: APs in the soft tissues of *B. rodriguezii* expressed as items/individual and g of wet tissue, respectively; **D**: Bioconcentration Factor (BCF); **E**: linear regression analyses of the correlation between APs in *B. rodriguezii* soft tissues and in water. *$p < 0.05$ (Mann-Whitney U test).

3.3 Characterization of anthropogenic particles

In the Fig. 3A the characteristic APs found in seawater and soft tissue of *B. rodriguezii* are shown. Considering particle size, all samples contained particles categorized as "micro" (< 5 mm), with a predominance of those smaller than 1 mm in length. (Fig. 3B, upper panel). In terms of shape, 100% of the particles found in water were microfibers, whereas mussels also contained fragments (Fig. 3B, middle panel). Notably, PC exhibited a greater diversity of colors compared to the CN (Fig. 3B, lower panel). The variety of colors may indicate different sources of contamination for both locations, as the colors present in the water are reflected in those found within the mussels.

Microfibers, often originating from synthetic textiles during washing processes, have been identified as significant contributors to marine pollution (Mishra et al., 2019). Their shape and small size allow them to evade conventional filtration systems, leading to widespread distribution in aquatic environments (Samal et al., 2024). The predominance of microfibers in both PC and CN suggests a pervasive issue that transcends localized pollution sources. Additionally, the presence of fragments in mussels highlights the bioaccumulation potential of these particles within marine organisms, raising concerns about trophic transfer and potential impacts on higher trophic levels.

The observed diversity of colors at PC may further elucidate the complex interplay between various pollution sources. Different colors can be indicative of specific types of materials or dyes used in textiles and plastics, suggesting that monitoring color diversity could serve as a proxy for identifying pollution sources.

Based on the polymeric characterization, all particles were confirmed to be of anthropogenic origin. Most of them were identified as natural and artificial cellulose

particles (NACP) - especially semi-synthetic rayon polymers, accounting for over 80% of the total in most samples. Smaller proportions of polyester (PES) and polyamide (PA) were also identified. Although the overall polymer composition remained consistent across sampling sites, it is noteworthy that polyethylene (PE) particles were detected exclusively in samples from CN. These findings are consistent with previous studies demonstrating that APs vary not only in size but also in polymer composition and origin—factors that can significantly influence their ecological impacts. In the BBE region, where CN is located, microfibers have been shown to primarily consist of cellulose-based materials, polyacrylonitrile (PAN), polyethylene terephthalate (PET), and polypropylene (PP) (Forero-López et al., 2021; Ronda et al., 2023b; Ardusso et al., 2024). Conversely, in the PC area, studies have identified PAN, polypropylene (PP), polyethylene (PE), and polystyrene (PS), alongside microfibers composed of cellulose-PET blends and natural cellulose fibers (Ronda et al., 2023).

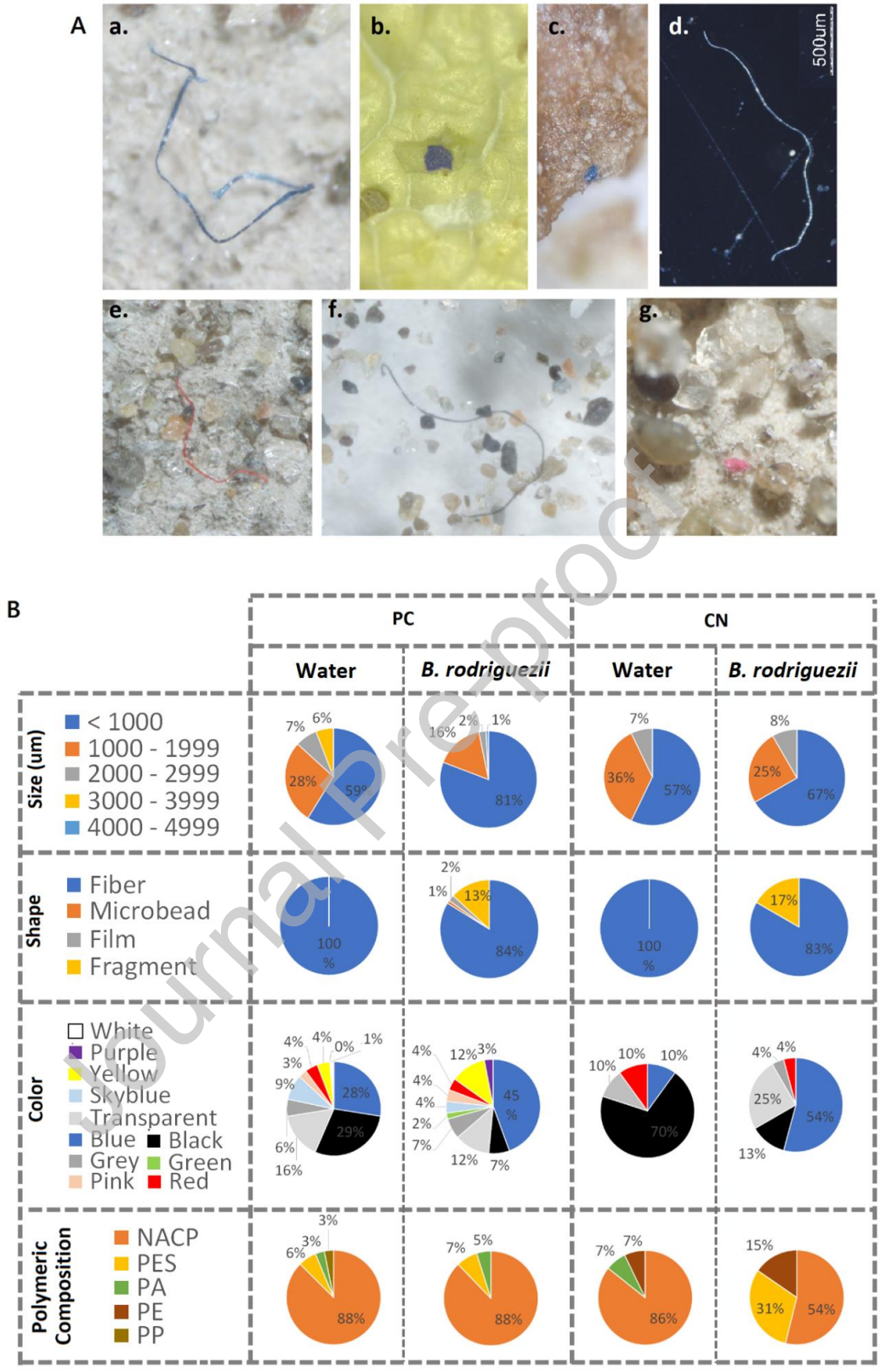

Figure 3: Anthropogenic particles (APs) found in water and soft tissue of *B. rodriguezii.* A: Examples of particles found: a. blue fiber; b. and c. blue fragments; d. transparent fiber; e. red fiber; f. black fiber; g. red fragment. B: Characteristics of APss found in

Pehuen-Co (PC) and Club Náutico (CN) according to their size range, shape, color, and polymeric composition. PP: Polypropylene; PE: Polyethylene; PA: Polyamide (nylon); PES: Polyester; NACP: Natural and artificial cellulose particles.

3.4 Histological assessment

Histological analysis reveals differences in the mussels' tissues from the two sites (Fig. 4). *B. rodriguezii* from PC exhibited higher alterations in their digestive glands (80%), presence of parasites (15%), and no evidence of eosinophilic bodies or hermaphroditism. Mussels from CN site showed less alteration in their digestive glands (50%), no presence of parasites, and a significant percentage of samples with eosinophilic bodies (61%). These findings suggest that environmental factors or stressors at each site may differentially affect the tissue architecture, potentially leading to pathological changes in the mussels. PC is characterized by an exposed sandy beach open to the ocean, where suspended solids are primarily composed of coarse particles, predominantly fine sands (<250 µm). In contrast, CN is a more sheltered environment with finer suspended particles, typically <63 µm (Gelós, 2004). Previous studies have shown that suspended sand particles, especially those <500 µm, can cause mechanical damage to the soft tissues of bivalves, particularly the gills, leading to structural degradation and impaired physiological function (Cheung & Shin, 2005). The tissue alterations observed in mussels from PC may therefore be associated with physical abrasion due to higher sand content in the water column. Simultaneously, previous studies have shown that the ingestion of suspended MPs by bivalves can induce cellular tissue inflammation (Von Moos et al., 2012) and cause gill damage (Cheung & Shin, 2005). Suspended microfibers (MF) have also been linked to negative impacts on the

filtration capacity of coastal mussels, resulting from MF accumulation in the digestive gland (Christoforou et al., 2020). Although the exact cause-effect mechanisms of MF accumulation on mussel filtration and feeding remain unclear, several studies report that very small particles can penetrate cells and the circulatory system, triggering inflammatory responses and a sensation of satiety, which may ultimately lead to mussel starvation (Christoforou et al., 2020). It has been also reported that MPs, a major component of APs, have been shown to induce a pathological condition termed "plasticosis." Charlton-Howard et al. (2023) characterized this disease as macro- and microplastic-associated fibrosis through histological analysis of seabird tissues. Their study demonstrated a linear association between the amount of ingested plastic and the prevalence of collagen deposits in the tubular glands of affected tissues.. By extension, the histopathological alterations in PC mussels could similarly result from chronic exposure to APs, which may compromise their immune defenses and render them more susceptible to parasitic infections. Indeed, similar histological alterations have been observed in specimens of *B. rodriguezii* from the Port of Mar del Plata, a region in the Buenos Aires Province, Argentina, characterized by significant anthropogenic impact (Arrighetti et al., 2019). The histopathological alterations observed in *B. rodriguezii* at PC are likely attributable to mechanical damage induced by suspended sand particles, compounded by the environmental burden of anthropogenic particles (APs). This combination of physical abrasion and pollutant exposure may synergistically exacerbate tissue damage, highlighting the complex impact of both natural and anthropogenic stressors on the species' health.

Conversely, the presence of eosinophilic bodies in most of the specimens from CN may indicate an inflammatory response or chronic oxidative stress (De la Ballina et al., 2022);

moreover, the presence of hermaphroditism in some CN mussels could indicate an adaptive or pathological response (Rapalini et al., 2022). Several studies have demonstrated the adverse effects of sewage on the BBE, impacting benthic (Fiori et al., 2020), planktonic (Dutto et al., 2012), and nektonic (La Colla, 2018) assemblages. At the species level, sewage pollution has affected filter-feeding bivalve mollusks such as the oyster *Crassostrea gigas* (Fiori et al., 2024; Otegui et al., 2024) and even *B. rodriguezii* (Buzzi et al., 2017; Oliva et al., 2017).

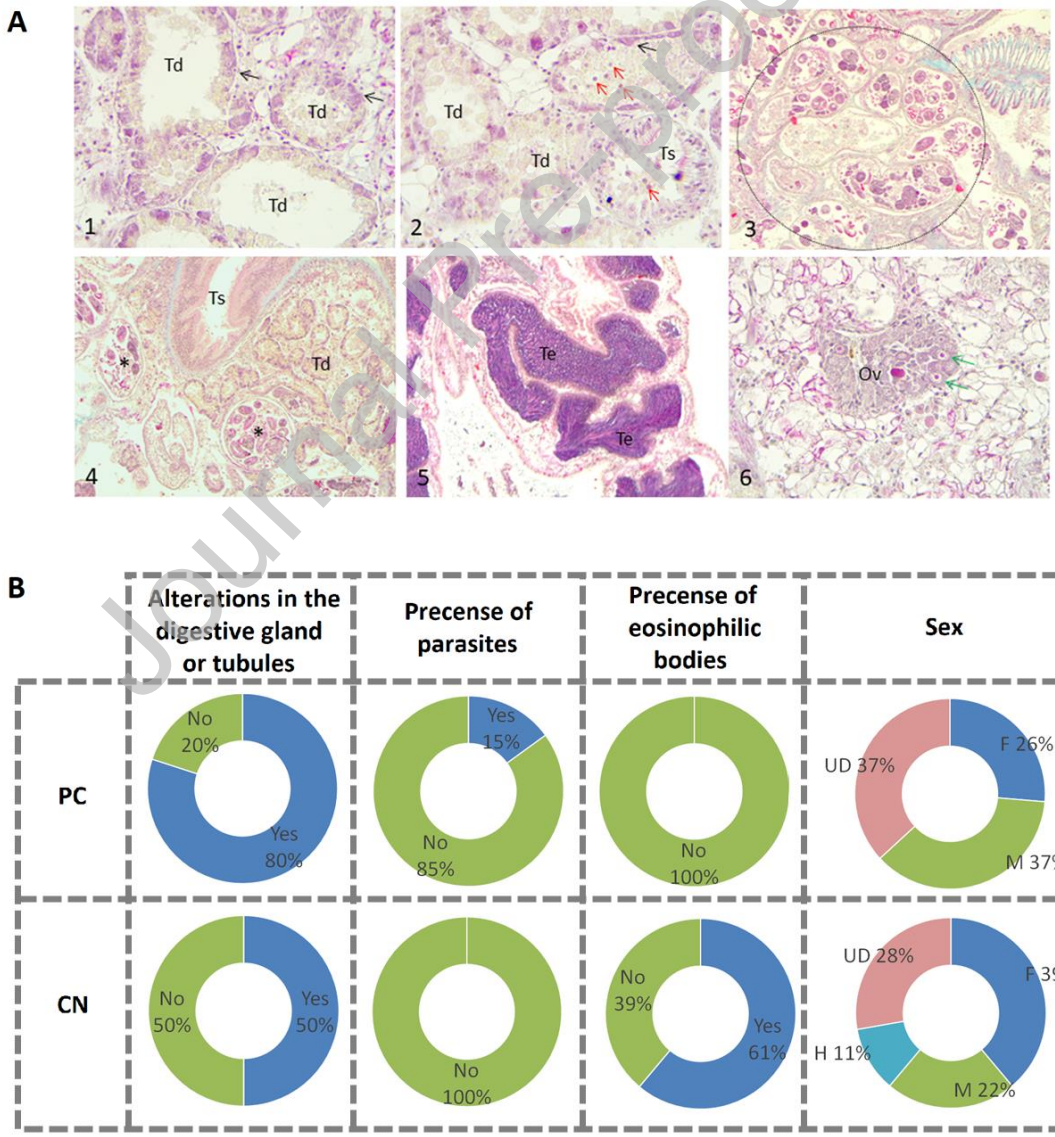

Figure 4: Histological characteristics of *B. rodriguezii.* **A:** Images 1 and 2 show an overview of the digestive gland of *B. rodriguezii* from Pehuen-Co (PC), revealing disorganization in the architecture of the digestive tubules (Td). The basophilic cells (black arrows), typical of the tubules, are disorganized, either clustered or dispersed. The epithelial structures are very low with no active secretion, and eosinophilic bodies can be observed in some tubules (red arrows). Image 2 illustrates the disorganization of columnar cells with microvilli lining the secondary digestive tubules (Ts). Images 3 and 4 show the general view of sporocyst invasion with cercariae in the digestive tubules of the gland (dotted circle and asterisks). Image 5 and 6: sex determination. Image 5 male gonad (Te) containing spermatids, image 6 female gonad (Ov) with green arrows indicating developing oogonia. **B:** Percentage of occurrence of the different observed characteristics.

3.5 Oxidative stress in B. rodriguezii:

Oxidative stress in *B. rodriguezii* was analyzed at several levels of organization. No differences between sites were observed in the gene expression of superoxide dismutase (*SOD*) (Fig. 5 A), suggesting no variations in the regulation of antioxidant defenses at this specific level. Supporting this observation, there were also no significant differences in reactive oxygen species (ROS) levels (Fig. 5B); moreover, the absence of differences in these markers further implies that the capacity of thiol-containing molecules to mitigate oxidative stress is similar at both locations (Fig. 5C). In contrast, differences were evident at oxidative damage, such as protein carbonylation and lipid peroxidation (Fig. 5D and E, respectively), although the latter did not reach statistical significance ($p = 0.07$). The higher levels of protein carbonylation (CPs) and lipid peroxidation in mussels from the CN site indicate greater damage to membrane lipids and proteins suggesting a higher degree of "chronic" or accumulated oxidative stress:

These findings suggest that while the initial antioxidant responses (e.g., SOD expression and ROS levels) appear consistently between the two sites, cumulative oxidative damage is more pronounced in mussels from the CN site. This discrepancy may reflect differences in the intensity or duration of environmental stressors, with the CN site likely experiencing more persistent or severe oxidative challenges. Elevated lipid peroxidation and protein carbonylation levels could have long-term consequences for cellular integrity and functionality, ultimately affecting the overall health and fitness of the mussels. Notably, higher levels of various pollutants associated with finer sediments have been reported in CN, where *B. rodriguezii* can accumulate polycyclic aromatic hydrocarbons (Arias et al., 2010; Oliva et al., 2015), metals (Buzzi et al., 2017), organotin (Quintas et al., 2017), and butylin compounds (Quintas et al., 2021). Further research should focus on identifying the specific stressors driving this chronic oxidative damage at the CN site.

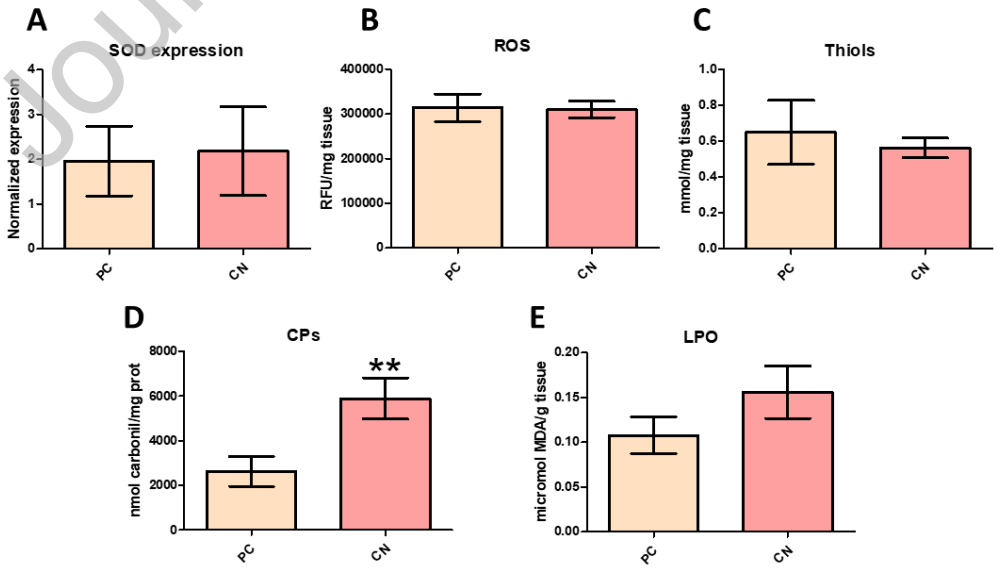

Figure 5: Evaluation of oxidative stress in *B. rodriguezii*. A: Expression of superoxide dismutase (SOD); B: levels of total reactive oxygen species (ROS); C: levels of total thiols; D and E: abundances of carbonylated proteins (CPs) and lipid peroxidation (LPO), respectively. **p < 0.01 (t-student for ROS, Thiols, and LPO; Mann-Whitney U test for SOD).

3. Conclusions

The findings of this study establish *B. rodriguezii* as an effective bioindicator for assessing micro APs contamination along the coasts of Buenos Aires Province, Argentina. This species demonstrates a strong capacity to bioaccumulate APs, with significant correlations observed between bioaccumulated levels and environmental concentrations. The site-specific responses observed in *B. rodriguezii* suggest complex interactions between APs and other environmental stressors along the Southwestern Atlantic coast. In PC, the mechanical damage from suspended sand, the elevated APs concentrations, coupled with histological alterations and subdued oxidative stress responses, suggest that APs may have a greater impact at the tissue than at the biochemical level. Alternatively, mussels from this site might have developed adaptive mechanisms to mitigate oxidative damage. In contrast, organisms from CN exhibed greater lipid and protein damage along with a high prevalence of eosinophilic bodies. These are evidences of chronic stress likely associated with a higher load of fine particles and chemical pollutants such as organic compounds and/or heavy metals. Our findings underscore the importance of considering both physical and chemical stressors when assessing the impact of pollution, as distinct environmental conditions can differentially affect organismal health. A limitation of this study was the use of a single sampling event per site, which restricts the assessment of potential seasonal variations in the

occurrence of APs and mussel responses. Despite this, the consistent detection of APs highlights their pervasive presence in the environment. These findings provide valuable baseline data for a region where information on APs pollution including MPs remains limited and underscore the need for future studies incorporating seasonal and long-term monitoring to better understand temporal dynamics and ecological impacts. Moreover, this study highlights the importance of evaluating multiple health indicators in bioindicator species when assessing the impacts of APs. It also underscores the need for sustained monitoring and effective management of APs contamination, even in areas that may appear less impacted. In this study, PC was selected as a reference site due to its relatively low human activity, particularly during the winter sampling period and its proximity to a protected natural reserve. However, the detection of APs even at this low-impact site highlights the pervasive nature of pollution and suggests that additional environmental factors, such as hydrodynamic transport and atmospheric deposition, may contribute to their distribution. Future studies should aim to incorporate a wider range of sampling sites, including upstream or more remote/pristine locations to better characterize baseline contamination levels and disentangle local versus regional sources and transport mechanisms of APs. Such efforts are crucial for safeguarding coastal ecosystems and mitigating the broader effects of anthropogenic pollution on marine biodiversity.

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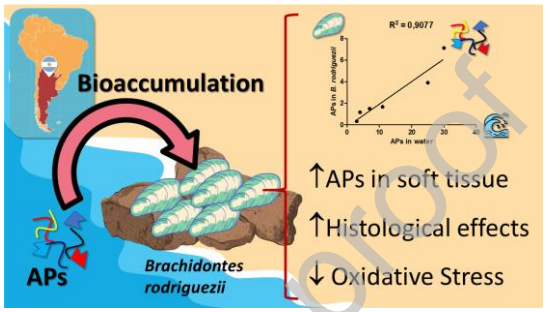
Graphical abstract



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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: