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Evaluation of tissue-specific Bisphenol A accumulation in mussels upon acute exposure: A pilot study

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ABSTRACT

Tissue-specific accumulation of Bisphenol A is evaluated for the first time in the mussel *Mytilus galloprovincialis* to trace the contaminant path within the selected tissues. Evaluation occurred in Mantle, Gills and Digestive gland after acute exposure to concentrations of the contaminant ranging from 6,25 to 125 ppb. Different uptakes were detected depending on the Bisphenol A exposure concentration: specifically, at low Bisphenol A doses (from 0 to 12.5 ppb) no substantial differences were observed among Mantle, Gills and Digestive gland. Starting from 31.25 ppb of Bisphenol A exposure, higher uptake in the Mantle was detected (up to 116.0 μg/g dry weight) and morphological alteration of reproductive organs was observed. The results obtained, supported by histological analysis, provided novel insights into the pathways of Bisphenol A bioaccumulation and the variability of tissue-level responses. Overall, this integrated strategy offers a powerful tool to investigate contaminant dynamics, paving the way to a deeper understanding of long-term effects and supporting more effective risk assessment and environmental regulation.

1. Introduction

The monitoring of environmental pollution is generally based on chemical analysis of abiotic compartments such as water and sediments as well as on the determination of contaminant levels in biota. The accumulation of contaminants in living organisms, widely studied in the last thirty years, occurs either through the aqueous phase or through the ingestion of sediment particles (Kukkonen and Landrum, 1995). The relative significance of the two accumulation pathways depends both on the feeding behavior of the organism, and on the contaminants' speciation (Pontoni et al., 2022; Paolella et al., 2024). In this context, bivalve mollusks have often been used as model organism in monitoring programs and research studies addressing the environmental pollution in coastal marine ecosystems (Goldberg, 1986; Dimitriadis and Koutsoubas, 2008; Carroll et al., 2009; Gupta and Singh, 2011; Whaley-Martin et al., 2012; Zuykov et al., 2013; Winnberg et al., 2014; Moraitis et al., 2018; Zhao et al., 2018; Calisi et al., 2022; Gonçalves and Bebianno, 2023; Daniel et al., 2024; Chen et al., 2025; Falfushynska et al., 2025; Iuffrida and Franzellitti, 2025, García-Pimentel et al., 2025, Marinaro et al., 2025, Romano et al., 2025, Rosati et al., 2024, Soegianto et al., 2025). Use of bivalves is due to several reasons: i) they are sessile filter feeder organisms and accumulate contaminants quickly; ii) they have a wide distribution and are easy to collect; iii) they respond to contaminants with measurable physiological and biochemical changes; iv) they are a widely consumed commercial product.

Nowadays, attention is focusing on emerging contaminants which, although found in seawater at very low concentrations (from μg/L to ng/L), represent a serious concern for environmental quality and human health (Mille et al., 2021; Goeury et al., 2022). Among them, Bisphenol A (BPA), a commonly used plasticizer (Thomaidis et al., 2013), recognized as an endocrine disruptor (Beausoleil et al., 2018), plays a special role because of its relative abundance (Paolella et al., 2025). Despite its high toxicity even at low concentrations (Tarafdar et al., 2022), it continues to be widely produced, utilized and discarded. It enters the

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environment not only through direct discharge into water bodies from industrial wastewater (Ragavan et al., 2013) but also from hazardous waste landfill leachate (Rezaei Adaryani and Keen, 2022), illegal dumping, and the degradation of plastic products (Halden, 2010).

BPA bioaccumulation by *Mytilus galloprovincialis* has been already investigated by evaluating the BPA content in the whole organism (Gatidou et al., 2010; Salgueiro-González et al., 2016; Fabrello and Matozzo, 2022; Castellani et al., 2025; Paolella et al., 2025). The sole evaluation in the whole mussels only provides information about the safety of the bivalve as food. No information is obtained concerning the biological routes and related specific effects on animal physiology. For instance, no systematic investigation has been conducted exposing mussels to known concentration of emerging contaminants measuring then the concrete uptake in each tissue to be directly correlated to observed biological responses. Such experimental gap, together with the recent development of an accurate method to detect BPA in small samples (Paolella et al., 2025) compelled us to perform this novel experimental approach aimed to: i) evaluate BPA distribution into selected mussel tissues (i.e. Mantle, Gills and Digestive gland) dissected from exposed animals; ii) clarify how BPA concentration in water may affect the uptake route; iii) correlate BPA tissue-specific concentration to precise physiological responses.

2. Materials and methods

2.1. Chemicals and standards

Analytical standards of BPA, chromatography grade ultrapure water, acetic anhydride and pyridine were sourced from Sigma-Aldrich (Germany). BPA d_{16}, used as internal standard, was obtained from Dr. Ehrenstorfer (Switzerland). Polypropylene (PP) filters and Florisil 30–60 U.S. mesh were supplied by VWR Chemicals (Italy). Acetone was purchased from Carlo Erba (Italy), and dichloromethane was provided by Honeywell Riedel-de Haën (France).

The standard stock solution for analytical calibration was obtained dissolving the solid standard in dichloromethane. Working solutions were obtained dissolving solid BPA in ultrapure water, produced by a PURELAB Option-Q ultrapure water system (ELGA, France). To prevent contamination, the use of plastic containers was minimized, and glassware was cleaned with chromic mixture.

2.2. Exposure design and sampling

To extract BPA from mussel tissues, organisms were purchased from Eurofish (Italy) between November and December 2022. Worthy, the mussels we used for the experimental setup were from non-polluted class A water. Mussels were not subjected to any treatment for human consumption which could affect mussels' wellness and survival. Special samples for experiments grown in wild conditions and not processed for food purposes were used. After collecting, mussels were kept in a holding tank for 7 days to acclimatize. After this period, they were distributed equally among tanks (10 individuals per tank) in the ratio of 1 individual/L of mechanically filtered and sterilized seawater. Tanks were continuously aerated and kept under ambient conditions. The exposure lasted 120 h. Shell feeding was added every 24 h, and seawater was changed every 48 h, reintroducing the contaminant at the same concentrations to ensure steady levels of the contaminant. This experimental protocol and the stability of BPA concentrations in seawater were previously validated and described in Paolella et al. (2025). A further tank, containing only the organisms, with no BPA, was used as control test. To evaluate the accumulation in tissues, animals were then exposed to BPA in five different tanks at seawater BPA concentrations of 6.25, 12.50, 31.25, 62.50, and 125.00 ppb, respectively and again compared with the control. The exposure design was supported by previously reported concentrations of BPA into the seawater (Xie et al., 2022; Soltani Nejad et al., 2023). From each tank, 6 individuals were taken to assess BPA bioaccumulation in the whole organism and the remaining 4 were used to determine the BPA fate in the various tissues. Hence for each BPA concentration tested, $n = 4$ tissues were processed for tissue specific BPA detection.

After the treatment, mussel tissues (Mantles, Gills and Digestive glands) were dissected, freezed at −20 °C, and homogenized using an Ultra Turrax T25 instrument (IKA-Werke GmbH & Co. KG, Germany) adding 1 mL of distilled and filtered water to each tissue. The samples were then freeze-dried using an Alpha 2–4 LSC plus (Martin-Christ, Germany) before undergoing chemical extraction, derivatization and GC–MS analysis.

2.3. Histological analyses

For histological analysis two further experiments were set: a control tank and 125 ppb one again with 10 individuals for each condition. Mantle tissues were divided into two aliquots. One fixed in Bouin for 24 h, dehydrated through an increasing series of alcohols and embedded in paraffin for histological study. To show the general morphology, 5 μm sections were stained with haematoxylin-eosin (Di Lorenzo et al., 2021; Marinaro et al., 2024). All morphological observations were carried out using a Zeiss Axioskop System (Axiocam 305 color and Zen imaging software-blue edition) (Spampinato et al., 2024). The remaining aliquot of male samples was processed for the BPA detection described below.

2.4. Extraction and derivatization

Mussel tissue samples were processed following these steps that were also utilized in previous research (Paolella et al., 2025):

- **Step 1 (Extraction):** The tube containing the sample, BPA d_{16}, and dichloromethane was placed into a Sonorex RK52 ultrasonic bath (Bandelin, Germany) for 45 min. The sample was then filtered through polypropylene organic filters (0.45 μm pore size, 13 mm diameter) and transferred to a new Pyrex tube;
- **Step 2 (Acetylation):** The filtered solvent was dried under gentle airflow. After evaporation, 50 μL of pyridine and 50 μL of acetic anhydride were added to the cuvettes, and the sample was heated by means of SBH200D thermostatic block (Stuart Scientific, England) at 85 °C for 2 h. Following this, a liquid-liquid extraction was performed with chloroform and ultrapure water. The organic phase, containing the acetylated BPA and $BPAd_{16}$ was washed three times with water to remove polar impurities.
- **Step 3 (Clean-up):** The sample underwent clean-up first through Florisil resin and then through a polypropylene filter (0.45 μm pore size, 13 mm diameter). Finally, the organic phase was again air-dried under gentle flow.

2.5. Determination of acetylated BPA by GC–MS

The gas chromatography–mass spectrometry (GC–MS) system consisted of an Agilent 6850 gas chromatograph coupled with a 5973 mass selective detector. It was equipped with an HP-5MS capillary column (Agilent). The injector operated in splitless mode, and the liner

temperature was set to 250 °C. The column oven was programmed with the following thermal settings: start at 60 °C, hold for 2 min, ramp up to 200 °C at 10 °C per minute, hold for 1 min, then increase to 290 °C at 15 °C per minute, and hold for 15 min.

2.6. Statistical analysis

All data are expressed as mean ± standard deviation (SD). To evaluate differences among exposure groups and different tissues, one-way Analysis of Variance (ANOVA) was performed, followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using GraphPad Prism (version 6.0.1, GraphPad Software, Inc., La Jolla, CA, USA).

2.7. Calculation of Bioconcentration factors (BCF)

To evaluate the bioaccumulation kinetics of BPA across distinct tissue compartments, the Bioconcentration Factor (BCF):

$$BCF = \frac{C_{tissue}}{C_{water}}$$

was determined, for each tissue, by applying a linear regression model. In this context, the slope of the regression line serves as a direct indicator of uptake efficiency relative to environmental concentrations. Since the initial measurements were recorded in μg/g d.w. and μg/L, we normalized the BCF values to the conventional unit of L/kg w.w. to align with international standards for bioconcentration assessment, (e.g. REACH, OECD guidelines). The reliability of this linear approach was confirmed using the coefficient of determination (R^2) and *P*-value analysis, with significance thresholds set at significance level of $P < 0.05$. Error margins for the BCF values were estimated through the propagation of the standard error associated with the slope.

3. Results and discussions

As shown in Fig. 1, Mantles, Gills and Digestive glands are very variable in each individual, both in terms of size and wet (Fig. 1A) / dry (Fig. 1B) weight. Averagely, the dry tissues had a weight 88% less than the wet tissues. Mantles, Gills and Digestive glands overall contribute to 60% of the total mass.

BPA accumulation was measured in the Mantle, Gills, and Digestive gland of each mussel, after dissection. BPA accumulation in the remaining part of the mussels (from here named "Other tissues") was obtained by closing the BPA mass balance and calculating the differences from the whole mussels and the examined tissues (Mantles, Gills, and Digestive gland).

Fig. 2 shows the different uptake depending on the BPA exposure concentration: specifically, for the Fig. 2A-C (starting from 0 to 12.5 ppb) no substantial differences were observed among Mantle, Gills and Digestive gland. Accumulation was not showing any specific trend or hierarchy in tissues. On the contrary, starting from 31.25 ppb of BPA in water (Fig. 2D), higher uptake in the Mantle (10.8 ± 2.0 μg/g, d.w.) and Digestive gland (11.3 ± 3.5 μg/g, d.w.) was detected if compared to the Gills (7.4 ± 0.8 μg/g, d.w.) and Other tissues (2.5 ± 1 μg/g, d.w.). While BPA levels remained relatively basal and consistent across the initial exposure range (0–31.25 ppb, Fig. 2A-D), a significant shift in accumulation dynamics was observed at 62.5 ppb (Fig. 2E). At this concentration, the Mantle began to emerge as the primary sequestration site, showing not only higher mean values but also a marked increase in inter-individual variance, with one replicate reaching 54.4 μg/g, d.w. This heterogenicity became even more pronounced at 125 ppb (Fig. 2F), where Mantle concentrations spanned a wide range (44.4 to 108.8 μg/g, d.w.), likely reflecting individual differences in the saturation of detoxification or excretion pathways. Notably, the hierarchy of tissue accumulation also shifted at the highest dose; branchial levels (48.1 ± 15.0 μg/g, d.w.) eventually surpassed those of the digestive gland (33.7 ± 9.7 μg/g, d.w.). This reversal from the low-dose trend suggests that under high-exposure scenarios, direct branchial uptake mediated by constant water filtration becomes the dominant pathway, potentially bypassing or overwhelming the digestive route.

As expected, BPA in tissues was strictly related to the BPA concentration in water. From 0 to 12.5 ppb exposure there was an uptake rate of about 6% in all the tissues. By contrast, from 31.25 ppb, this value increases to 15% (of which, about 40% is found in the Mantle). To justify such a result, we propose two possible non-except alternative hypotheses: (*i*) there is a concentration threshold in water and BPA bioavailability may be limited by interactions with natural organic matter (Paolella et al., 2024; Yao et al., 2024), explaining the basal uptake observed below 31.25 ppb; (*ii*) mussels are capable to detoxicate themselves over time as reported in our previous study (Paolella et al., 2025).

Finally, BPA concentrations detected in the tissues were correlated with BPA concentrations in the water (Fig. 3) and by linear regression the bioconcentration Factor (BCF) was calculated and reported in Table 1. BCF data confirms a distinct, tissue-specific partitioning of BPA within the organism. Again, we observed a clear gradient of affinity, with the Mantle emerging as the primary site of sequestration. The calculated BCF for the Mantle was 556.8 ± 160.5 L/kg d.w., a value significantly higher than those recorded for other tissues. An interesting contrast appears when comparing the Gills (BCF: 361.6 ± 57.4 L/kg d.w.) and the Digestive gland (BCF: 241.3 ± 71.2 L/kg d.w.). The higher accumulation in the branchial tissue suggests that direct uptake from the water column—likely through filtration and respiration is a more efficient pathway for BPA entry than the dietary route under these

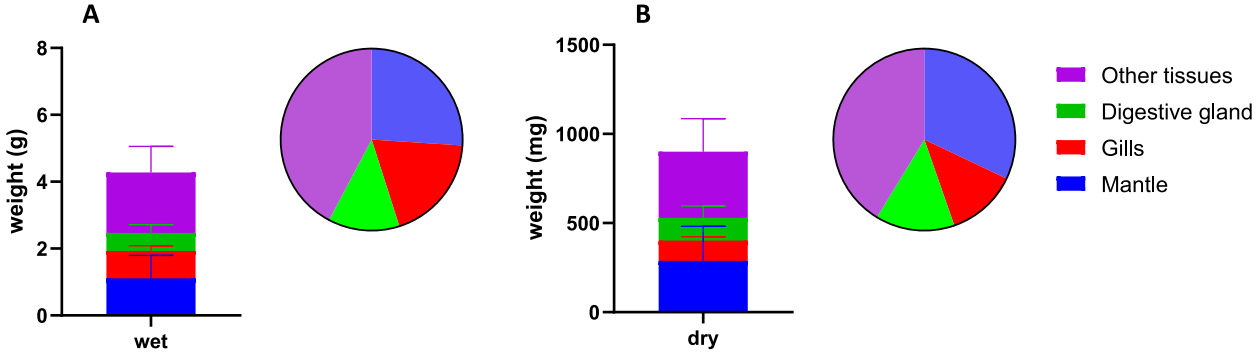

Fig. 1. Mass distribution in *M. galloprovincialis*: Stacked column chart and pie chart representing mass distribution among tissues considering both wet weight (A panel) and dry weight (B panel), $N = 8$.

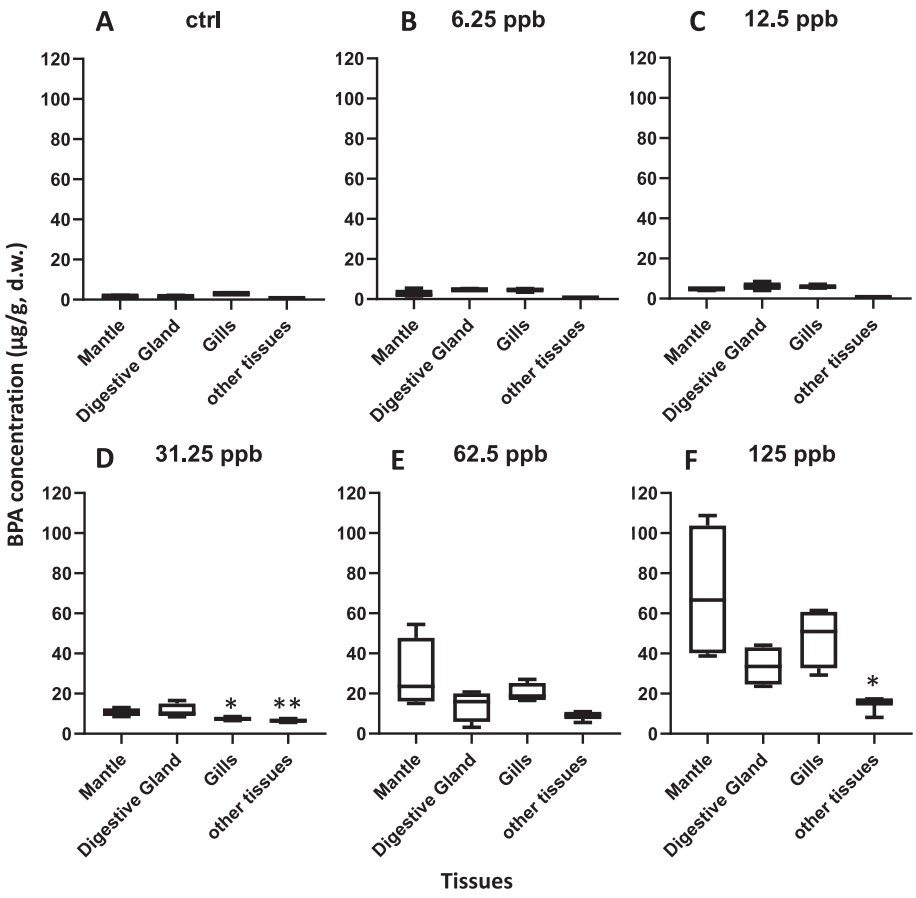

Fig. 2. Quantification of the different BPA concentration. Box plot represent the BPA uptake (y axis) in Mantle, Digestive gland, Gills and Other tissues (x axis) relatively to the different concentration of exposure. d.w. = dry weight. $p < 0.05$, $^{**}p < 0.01$ vs Mantle (One-way ANOVA followed by Tukey's post-hoc test).

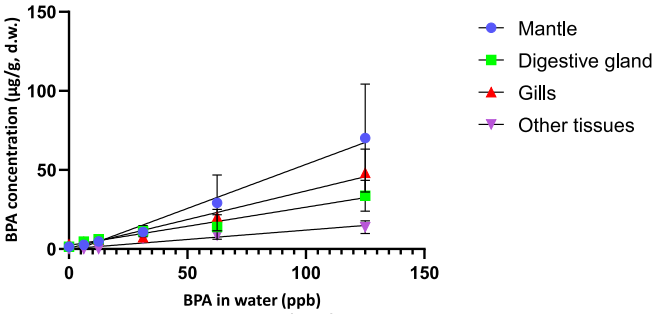

Fig. 3. Linear correlation of BPA concentration in water (x) and accumulation (y) in Mantles (blue circles), Digestive gland (green squares), Gills (red triangles) Other tissues (purple triangles). BCF factors were calculated from the slopes and reported in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

experimental conditions. Meanwhile, the "Other tissues" showed a much lower BCF of 118.3 ± 25.2 L/kg d.w., indicating that BPA tends to concentrate in specific target organs rather than diffusing evenly across the whole body (Table 1).

The BCF values obtained (ranging from ~120 to ~560 L/kg, d.w.) are consistent with findings in other bivalve species (Staniszewska et al., 2014; Liu et al., 2023). The preferential accumulation in the Mantle confirms its role as the primary metabolic sink for lipophilic contaminants in mussels, consistent with its high lipid content. This is consistent with the physiological role of the Mantle in mollusks, which is often involved in both storage and shell formation processes. Indeed, *M. galloprovincialis* is a sessil filter-feeding organism able to capture food (Burge et al., 2016) as well as pollutants which get in touch with the Gills, move to the labial palp and are finally moved into the Digestive gland from which BPA can be adsorbed (thus move to other tissues or circulate) or expelled. Within this context, the higher and sensibly variable concentration of BPA detected in the Mantle could be the result of its internalization or expulsion processes. Therefore, individual biological variability becomes more pronounced as the organism's metabolic, or storage capacity is challenged.

By considering the tissue-specific moisture, even more nuanced understanding of BPA partitioning within the organism is provided. Indeed, our analysis of wet and dry weights revealed significant differences in hydration levels, with moisture content ranging from 73.9% in

Table 1
Linear regression parameters and calculation of BCF for the investigated tissues.

	Slope	R^2	Equation	*P* value	BCF (L/Kg, d.w.)	BCF (L/Kg, w.w.)
Mantle	0.5568	0.7501	y = 0.5568*x – 2.178	<0,0001	556.8 ± 160.5	144.49 ± 41.65
Digestive gland	0.2413	0.8179	y = 0.2413*x + 2.329	0,0488	241.3 ± 71.2	56.4 ± 16.7
Gills	0.3616	0.8651	y = 0.3616*x + 0.5465	0.3572	361.6 ± 57.4	50.44 ± 8
Other tissues	0.1183	0.8456	y = 0.1183*x + 0.1282	0.0002	118.3 ± 25.2	24.36 ± 5.2

the Mantle to 86.2% in the Gills (Fig. 1). By normalizing BCF values to wet weight (w.w.), the Mantle remained the primary accumulation site with a BCF of 144.49 ± 41.65 L/kg w.w. Interestingly, although the Gills showed a higher accumulation rate on a dry weight basis, their high water content resulted in a lower wet-weight BCF (50.44 ± 8 L/kg w.w.) compared to the Digestive Gland (56.4 ± 16.7 L/kg w.w.).

The tissue-specific approach of this work highlights how the Mantle concentrates BPA nearly three times more effectively than the respiratory apparatus when considering the entire living tissue. The Mantle possesses both the highest affinity for BPA and a substantial share of the total biomass making it the definitive reservoir for the contaminant. Conversely, the 'Other tissues', despite their lower bioconcentration potential, play a crucial role in systemic distribution. Because they represent the largest mass fraction, they function as a primary dilution sink, sequestering a significant portion of the total body burden without reaching the high, potentially toxic concentrations seen in the specialized organs (i.e. Mantles, Gills). The non-uniform distribution of BPA among different tissues has profound implications for trophic transfer and marine food web stability. In a real-world ecological scenario, predators such as fish or crustaceans often consume specific parts of the mussel or are exposed to concentrated 'hotspots' of contaminants. Since the mantle in *M. galloprovincialis* acts as a primary reservoir—reaching concentrations significantly higher than the average whole-body burden—it represents a concentrated source of BPA for higher trophic levels. A predator feeding on these specific tissues would receive a 'burst' of contaminant far exceeding the levels predicted by standard monitoring models based on whole-organism homogenization. This suggests that current environmental risk assessments, by averaging the concentration over the entire body mass, likely underestimate the actual dietary exposure and toxicological risk to predators. Such a discrepancy highlights a critical gap in marine pollution monitoring, where tissue-specific accumulation may drive the impact of endocrine

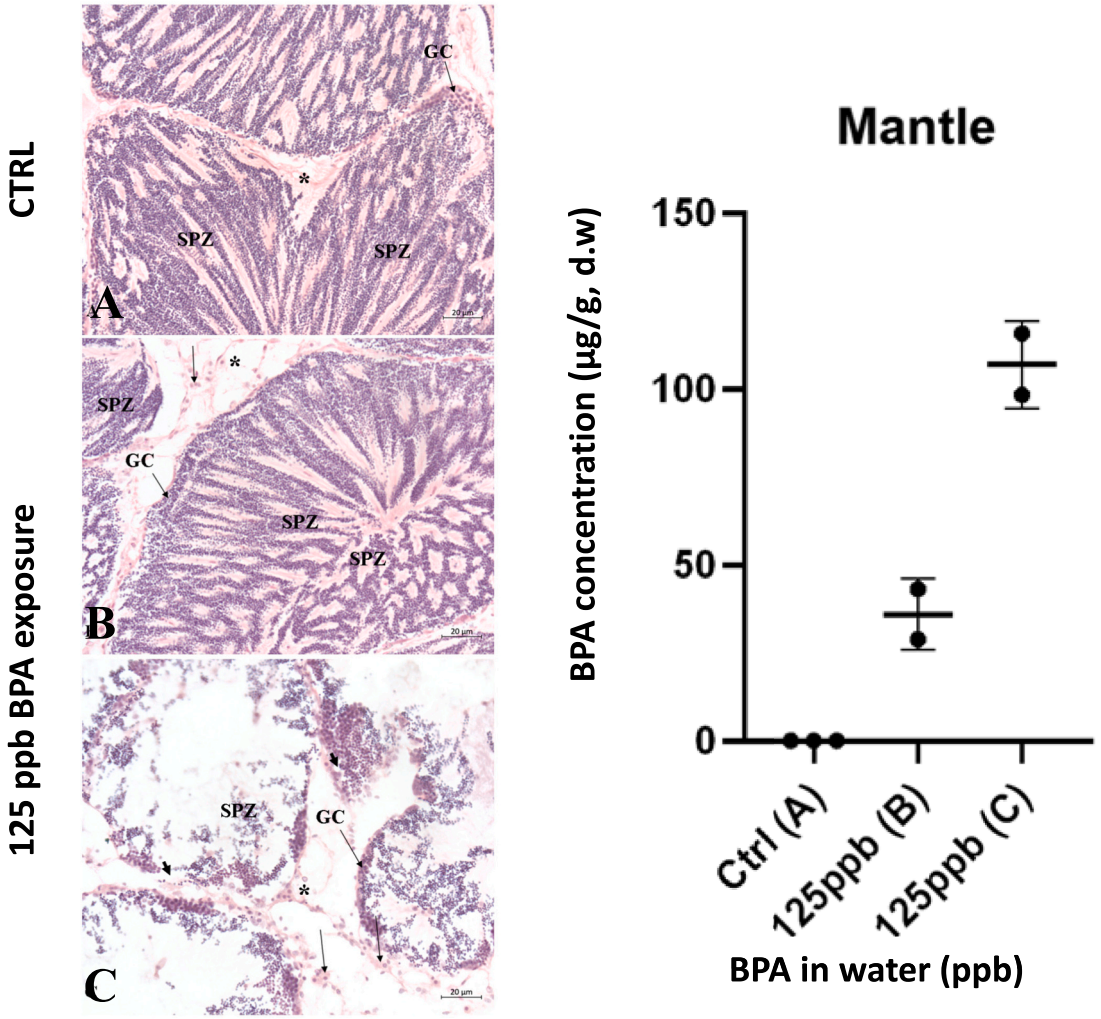

Fig. 4. Haematoxylin-eosin staining of *M. galloprovincialis* testis sections according to BPA concentration. A) Control: intact spermatic follicles, rich in germ cells (GC) and spermatozoa (SPZ). B) Animals exposed to BPA with low levels of tissue accumulation: preserved spermatic follicles architecture with the presence of germ cells (GC) and spermatozoa (SPZ). C) Animals treated with BPA with high accumulation in tissues: a greater increase in disorganized spermatic follicles is observed, indicating significant alterations in cross-communication between germ cells (arrowhead). Arrow, haemolymph cells; asterisk, interstitial connective tissue. Scale bars = 20 µm.

disruptors on the ecosystem more severely than previously estimated. In other words, from a toxicological standpoint, these BCF values were well below the REACH Regulation (annex XIII) 'Bioaccumulative (B)' threshold of 2.000 L/kg w.w. suggesting a moderate risk profile regarding long-term sequestration. Nevertheless, the sequestration of BPA in the mantle—the reproductive hub of the mussel—represents a high-risk scenario for population stability. Unlike the digestive gland, which processes and eliminates toxins, the mantle acts as a long-term reservoir that exposes developing gametes to endocrine disruption during the entire pre-spawning period. (Aarab et al., 2006; Prisco et al., 2017).

For these reasons, morphological alterations in the Mantles exposed to the highest concentration of BPA, were finally evaluated (Fig. 4). It is important to note that mussels' Mantle contains the gonads (i.e. testis or ovaries). Hereby, testicular morphology in response to BPA exposure was reported. The control testis showed a typical organization with sperm follicles surrounded by connective tissue (Chianese et al., 2025) rich in immature germ cells along the periphery of the wall follicles and mature spermatozoa toward the central lumen ready for release (Fig. 4A). In two out of four male mussels, where lower BPA accumulation was detected in the tissue (36.1 ± 10.0 μg/g, d.w.) exposed to BPA (125 ppb), it was possible to observe the same gonadal organization of the control (Fig. 4B). Other testis from the remaining 2 animals showed sensibly higher BPA accumulation (107.18 ± 12.44 μg/g, d.w.). Here, highly disrupted morphology (Fig. 4C) was evident. In fact, in Fig. 4C many sperm follicles were characterized by significant cellular disorganization in the testis. Emptying of sperm follicles was evident, which themselves were characterized by germ cells disconnected from each other and from the basement membrane. Finally, the presence of hemolymph cells in the interstitial zone of the connective tissue was recorded.

4. Conclusions

While the use of nominal concentrations may represent a limitation of this pilot study, the consistency of the observed accumulation patterns with previous validated models (Paolella et al., 2025) supports the reliability of the findings regarding tissue-specific BPA distribution. This study provides a novel perspective on BPA accumulation in *M. galloprovincialis*, demonstrating that the contaminant does not distribute uniformly but concentrates in specific 'hotspots', primarily the mantle. Our findings challenge the reliability of current marine monitoring protocols based on whole-body analysis, as this traditional approach tends to dilute and mask critical concentrations in sensitive organs. Finally, our results strongly suggested that the altered physiology induced by the presence of BPA may be related not just to its concentration in the water but is affected by the uptake process of the contaminant by the organisms. This highlights the need for a paradigm shift in environmental monitoring, moving toward tissue-specific investigations to better evaluate the impact of emerging contaminants.

Next steps will be to verify the possibility to correlate both the time and the amount of BPA exposure to the tissues specific bioconcentration and quantify their effects. Further studies need to be carried out in such direction to define a dependency between the presence of BPA in specific tissue, the exposure time and mussels' specific responses to contaminants. This approach should be also used to improve the investigations on the reversibility of the contamination, to assess the capability of the different mussels' tissue to bioconcentrate or depurate themselves from BPA uptake.

CRediT authorship contribution statement

Giulia Paolella: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sicong Yao:** Writing – review & editing, Investigation, Formal analysis. **Massimiliano Fabbricino:** Writing – review & editing, Supervision, Funding acquisition. **Annamaria Locascio:** Writing – review & editing, Supervision, Funding acquisition. **Luigi Rosati:** Writing – original draft, Investigation, Formal analysis, Data curation. **Ludovico Pontoni:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Maria Sirakov:** Writing – original draft, Visualization, Validation, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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