Chemosphere 343 (2023) 140260

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Oxidative stress and histopathological effects by microplastic beads, in the crayfish *Procambarus clarkii*, and fiddler crab *Leptuca pugilator*

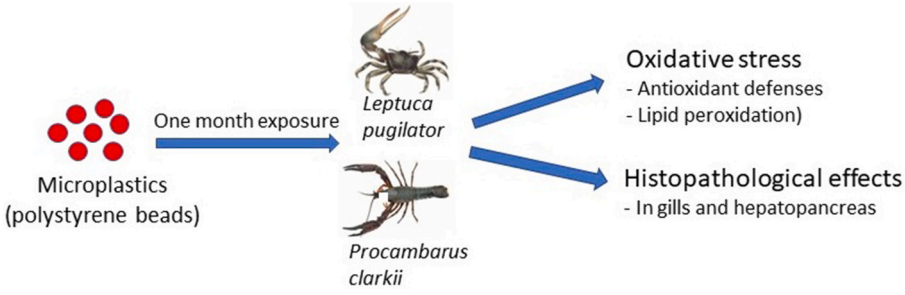
G.R. Silveyra [a], P. Silveyra [b], M. Brown [c], S. Poole [c], I. Vatnick [c], D.A. Medesani [a], E.M. Rodríguez [a,*]

[a] Dept. of Biodiversity and Experimental Biology, FCEN, University of Buenos Aires, Institute of Biodiversity, Experimental and Applied Biology (IBBEA), CONICET-UBA, Ciudad Universitaria, Pab. II, C1428EGA, Buenos Aires, Argentina
[b] Dept of Environmental and Occupational Health, Indiana University Bloomington, School of Public Health, Bloomington, IN, 47401, USA
[c] Dept. of Biology, Widener University, Chester, PA, 19809, USA

HIGHLIGHTS

- Crabs and crayfish exposed to polystyrene beads accumulate them and increased their antioxidant defenses.
- Lipid peroxidation was observed in both species.
- Histopathological effects in both hepatopancreas and gills were also observed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: James Lazorchak

Keywords:
Crustaceans
Polystyrene
Antioxidant defenses
Hepatopancreas
Gills

ABSTRACT

The present study was aimed at evaluating the *in vivo* effects of microplastics (MP), in terms of oxidative stress and histopathological effects, in two crustacean species: *Procambarus clarkii* and *Leptuca pugilator*. In addition, MP accumulation in the hepatopancreas (HP) of both species was also determined. Adults of both crayfish and crabs were exposed for one month to fluorescent polystyrene beads (size: 1 μm) at nominal concentrations of 1000 or 5000 particles/mL. During the exposure, animals were maintained under controlled feeding, aeration, temperature, and photoperiod conditions. At the end of the exposure, HP and hemolymph (HL) samples were harvested for analysis of oxidative damage and total antioxidant levels. Additionally, the presence of MPs in both tissues was confirmed. Significant differences with the control groups were observed in lipid peroxidation levels in HP in animals exposed to the lowest concentration in *P. clarkii* and to the highest concentration in *L. pugilator*. A marked increase in antioxidant levels was also observed in the HL at both concentrations in *P. clarkii*, and at the highest MPs concentration in *L. pugilator*. Moreover, several histopathological changes were detected in both gills and HP, including hypertrophied lamellae, lifting or collapse of gill epithelia, loss of normal shape of hepatopancreatic tubules, and epithelial atrophy in the HP tissue. We conclude that exposure to MP beads at selected concentrations results in oxidative damage, induces histopathological changes in gills and HP, and triggers an antioxidant response in two crustacean species.

* Corresponding author.
E-mail address: enrique@bg.fcen.uba.ar (E.M. Rodríguez).

https://doi.org/10.1016/j.chemosphere.2023.140260
Received 17 August 2023; Received in revised form 21 September 2023; Accepted 22 September 2023
Available online 22 September 2023

1. Introduction

With the increasing reliance on plastics as an everyday item, the environmental implications of these materials are a growing concern (Crawford and Quinn, 2017). Over time, UV radiation and mechanical abrasion generate the fragmentation of plastics into very small pieces called microplastics (MPs), plastic particles <5 mm, which eventually could be degraded to smaller particles, called nanoplastics <1 μm (Crawford and Quinn, 2017; Issac and Kandasubramanian, 2021). Near 300 million tonnes of plastics (mainly polyethylene, polypropylene, and polystyrene) are released annually into aquatic ecosystems, and it is predicted, to reach 1800 tonnes in 2050 (Rai et al., 2021).

Once entering the marine environment, and due to their size and abundance, these MPs are ingested by marine organisms, potentially causing multiple harmful effects at several levels by themselves and/or by other pollutants adsorbed on them (Vieira de Araujo et al., 2021; Rai et al., 2021; Issac and Kandasubramanian, 2021; Crawford and Quinn, 2017). Bioaccumulation of all kinds of MPs in several tissues of aquatic fauna, including crustaceans, has been reported extensively (Crawford and Quinn, 2017; González Pisani et al., 2022; Yin et al., 2022). Moreover, the experimental exposure to MPs of either invertebrate or fish species trophically related, has shown that these pollutants can be transferred along trophic webs (Farrell and Nelson, 2013; Mattsson et al., 2015; Wang et al., 2019, 2021).

Accumulation of MPs in tissues of crustaceans is correlated with several harmful effects, oxidative stress among them (Yu et al., 2018; González Pisani et al., 2022). The hepatopancreas especially shows a high MP bioaccumulation, together with significant activation of the antioxidant defenses (Yu et al., 2018; Wang et al., 2021). Oxidative stress is the root cause of several other deleterious processes, leading to cell death, histopathological effects, and ultimately, systemic failure and death (Lushchak, 2011). Other effects, such as a reduction in the reproductive output of crabs, have been reported after chronic exposure to polypropylene microfibers (Horn et al., 2020).

This study was aimed at evaluating the oxidative stress and histopathological effects caused by exposure to MP, in both the freshwater crayfish *Procamabrus clakii* and the estuarine crab *Leptuca pugilator*, a species widely distributed in rivers and estuaries of the U.S., and therefore potentially subject to the negative impact of MPs. Based on previous studies made in crustaceans, we hypothesized a triggering of antioxidant defenses in the hepatopancreas of both studied species. Despite this, we expect to observe several histopathological lesions in both hepatopancreas and gills, this latter tissue being the first barrier facing exposure to MPs.

2. Materials and methods

2.1. Animals

Adult *P. clarkii* crayfish (N = 64) were obtained from Carolina Biological Supply in September 2021. About 100 adult crabs from both sexes of *Leptuca pugilator* were collected at low tide off the Nature Trail at the Wetlands Institute in Stone Harbor, NJ, also in September 2021.

Upon arrival, crayfish were immediately sexed, held into large aquaria filled with aged tap water, and allowed to acclimate for two weeks. Following this acclimation period, the crayfish were transferred to individual containers (4 ¾“ × 4 ¼“) filled with 400 ml of dechlorinated aged tap water (pH = 7.6 ± 0.2; hardness = 125 mg/L, as CaCO3 equivalents), and continuously aerated. Once in the laboratory, collected crabs were also held in large aquaria for two weeks of acclimation. The bottoms of the aquaria were covered with about 5 cm of mud (from the marsh) in a slope, and filled with enough 12 g/L saline water made with Instant Ocean ™ salt so the crabs could move in or out of the water; short segments of PVC pipes of different diameters provided refugia. At the end of the acclimation, crabs were transferred to individual containers (4 ¾“ × 4 ¼“) filled with 400 ml of 12 g/L saline water and aerated continuously. All animals were held in a temperature-controlled room (23 °C ± 2 °C) with a 12:12 light-dark cycle.

2.2. Bioassays

The toxicological bioassay was conducted in semi-static conditions according to the standard procedures recommended by the American Public Health Association et al. (2005). The code of ethics for animal experiments stated in the Declaration of Helsinki was always followed.

Polystyrene fluorescent beads, 1 μm in diameter (Fluoresbrite Microparticles – Poly science®) were used to prepare a stock solution with 4×10^5 particles per mL. Small aliquots were added to the individual containers, to achieve three concentrations: 0 (control), 1000, and 5000 particles/mL. To validate the nominal concentrations, water samples from all treatments were measured by spectrometry at 470 nm and emission at 526 nm. Baseline values of the controls were used to correct the treatment values.

Ten to fifteen animals were assigned to every treatment, housed individually in aquaria containing 400 mL that were completely changed twice a week. At the end of the 30-d exposure, samples of hemolymph (HL, 200–300 μL each) were withdrawn from the prebranchial sinus at the base of the fifth pereiopod of each animal, with a tuberculin syringe fitted with a 29G needle, and stored at −80 °C until analysis. Animals from both species were first anesthetized by submersion in ice water and then sacrificed. The HP was then quickly dissected and weighed at 0.0001 g precision. The hepatosomatic index (HI) was calculated as the weight of HP/final body weight x 100. After separating a portion for histological analysis, HPs were stored at −80 °C until they were processed. The gills of *L. pugilator* were also dissected for histological purposes. All animals were weighed at the beginning and end of the assays, in order to estimate their weight gain (WG), as ((FW-IW)/IW) x100, IW and FW being the initial and final body weight, respectively.

2.3. Bioaccumulation

Since only *P. clarkii* had enough biomass to analyze MPs bioaccumulation, in addition to oxidative stress and histological analysis, estimation of the number of particles in both HP and HL was only made in this species. The stored HP was digested in glass tubes with 2 mL KOH 10% at 100 °C, until the tissue was digested entirely. Tubes were covered with aluminum foil to avoid water evaporation; 200 μL of each sample were placed by duplicate in a black 96 microplate reader, and fluorescence was measured with excitation at 470 nm and emission at 526 nm. Hemolymph was analyzed without previous digestion. Standard curves were prepared with serial dilutions of the stock solution to estimate the number of particles in the samples, by interpolation. Baseline control values were used to correct the treatment values. The bioconcentration factor (BCF) was calculated as the concentration of MP in the HP (number of particles/mg), divided by the concentration of particles in the tested solution (number of particles/mL).

2.4. Antioxidant and TBARS determination

Hemolymph samples of both species were homogenized and centrifuged at 13,000 g for 20 min, at 4 °C. Then, each sample was diluted 1/100 and aliquoted for further determination. Levels of antioxidants, as total antioxidant capacity (TAC) were measured in the HL using a colorimetric assay kit (Antioxidant Assay (Cayman®), measuring the absorbance in an Infinite M Plex (Tecan®) spectrophotometer, at a wavelength of 750 nm.

Levels of TBARS (thiobarbituric acid reactive substances, indicative of lipid peroxidation) were measured in the hepatopancreas using a colorimetric assay kit (TCA method, Cayman®), at 530 nm; HP samples weighing 25 mg were homogenized in 250 μl of RIPA buffer and centrifuged at 1,600 g for 10 min, at 4 °C. A standard curve ranging from 0 to 0.4 mg/mL was prepared, according to the kit's instructions; 100 μL

Table 1
Validation of the concentration used for assayed species.

Species	Nominal concentration (number of particles/mL)	Measured concentration (N = 5)
Procambarus clarkii	1000	1450 ± 32
	5000	7242 ± 332
Leptuca pugilator	1000	1569 ± 111
	5000	7417 ± 267

of each sample or standard were added to a 96-well plate, and absorbance was measured in an Infinite M Plex (Tecan®) spectrophotometer, at 530 nm.

2.5. Histopathology

Both HP and gill samples were fixed in Bouin's 2000™ (American MasterTech Scientific) for 6 h at 22 °C and then transferred to 70% alcohol for storage until further processing. Tissues were processed by the Translational Research/Pathology Shared Resource Lab at the Kimmel Cancer Research at Jefferson University. This lab embedded the tissues in paraffin, cut them to 5 μm thickness, stained them with an E&O stain, scanned them at 40x magnification, and uploaded the images to a shared drive. We examined those images, and the incidence of each pathological change observed in either HP or gills was estimated semi-quantitatively.

2.6. Statistical analysis

Results were analyzed by means of a one-way ANOVA, followed by Tukey multiple comparisons (Sokal and Rohlf, 1981) once the assumptions for this test were confirmed. A minimum confidence level of 5% was considered for statistical significance.

3. Results

Validation of the nominal concentrations by spectroscopy yielded values ranging between 45 and 57% over the nominal values (Table 1). No mortality was registered during the assays. Weight gain and the HI, measured at the end of the assay, are shown in Table 2. Only L. *pugilator* showed a significant ($p < 0.05$) decrease in weight gain in the highest MP concentration and control. No significant ($p > 0.05$) decrease in the HI was noted between the highest MP concentration and control, for either species.

Bioaccumulation of particles in both hepatopancreas and hemolymph of *P. clarkii* is shown in Table 3. A bioconcentration factor higher than 1 was observed for both concentrations. The number of particles detected in hemolymph represented 26.6% and 31.3% of particles measured in water (per mL in both cases), for the low and high concentration assayed, respectively (Table 3).

Table 2
Weight gain and hepatosomatic index of both species, at the end of the 30-d assay. The asterisk indicates significant ($p < 0.05$) differences with respect to control.

Species	Treatment	Weight gain	Hepatosomatic index
P. clarkii	Control	6.68 ± 2.80	5.06 ± 0.35
	Low concentration	7.67 ± 2.19	4.47 ± 0.32
	High concentration	10.12 ± 2.40	4.73 ± 0.34
L pugilator	Control	14.49 ± 3,59	4.52 ± 1.70
	Low concentration	7.78 ± 3,59	4.39 ± 0.41
	High concentration	5.92 ± 3,00 *	3.13 ± 0.46

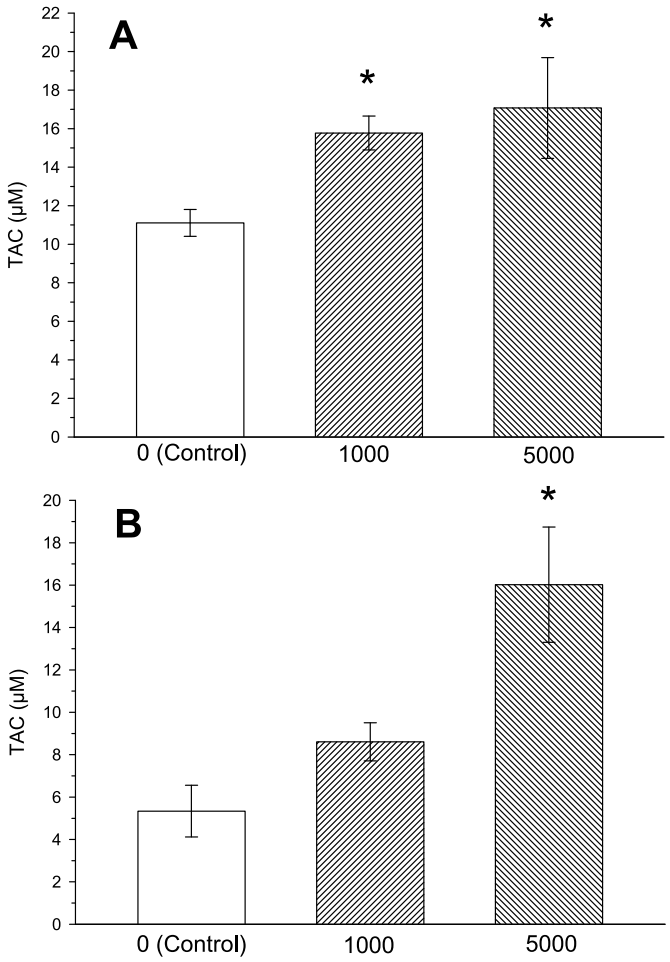


Fig. 1. Levels of total antioxidant capacity (TAC) in the hemolymph of *P. clarkii* (A), and *L. pugilator* (B), at the end of the assay. Values are means ± standard errors. Asterisks indicate significant differences with respect to control ($p < 0.05$).

A marked increase in TAC was observed in the HL, at both concentrations in *P. clarkii* (Fig. 1A), and the high MPs concentration in *L. pugilator* (Fig. 1B). Significant differences in lipid peroxidation were observed in the HP of *P. clarkii* exposed to MPs at the lowest concentration, and in that of *L. pugilator* exposed to the highest concentration, compared to control groups (Fig. 2).

Table 4 shows the results of the semi-quantitative histopathological analysis made on *L. pugilator* gills and the HP of both species. The main pathologies observed in gills were hypertrophy, i.e., augmented size of epithelial cells, leading to a reduced space for hemolymph circulation (Fig. 3B), and some degree of hyperplasia. The rupture of pillar cells, which mediate the normal separation between both epithelia in secondary lamellae, produced two different lesions: telangiectasia, i.e., increased separation of the epithelia, and consequently bagging of hemolymph (Fig. 3C), or eventually a collapse of the lamellae epithelia; in

Table 3
Bioaccumulation of particles in the hemolymph (per mL) and hepatopancreas (per mg) of *P. clarkii*. Bioaccumulation factors are also expressed.

Measured concentration of particles in water, per mL (Nw)	Number of particles in hemolymph	Number of particles in hepatopancreas (Nh)	Bioaccumulation factor (Nh/Nw)
1450 ± 32	385 ± 548	5596 ± 4704	3.86
7242 ± 332	2266 ± 1348	8283 ± 4750	1.14

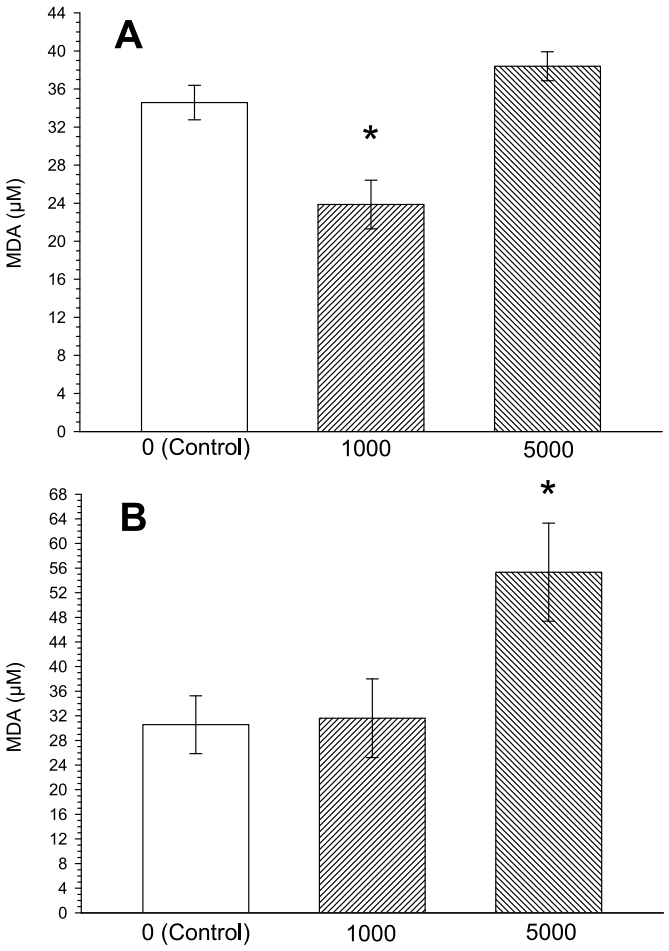


Fig. 2. MDA levels in the hepatopancreas of *P. clarkii* (A), and *L. pugilator* (B), at the end of the assay. Values are means ± standard errors. Asterisks indicate significant differences with respect to control ($p < 0.05$).

Table 4
Histopathological effects observed in the gills and hepatopancreas of *Leptuca pugilator*, and the hepatopancreas of *Procambarus clarkii*, at the end of the assay. The relative incidence of each pathology is indicated (+ = low; ++ = intermediate; +++ = severe).

Histopathology	Relative incidence		
	Control	1000	5000
Gills – *L. pugilator*			
Hypertrophy		++	+++
Hyperplasia		++	+++
Telangiectasia		+	+
Collapsed lamellae		++	+++
Hepatopancreas – *L. pugilator*			
Enlarged lumen		++	+++
Vacuolization		+	+
Atrophied epithelium		+	++
Necrosis		+	++
Hepatopancreas – *P. clarkii*			
Enlarged lumen		+	+++
Vacuolization		+	+++
Atrophied epithelium		+	++
Necrosis		+	++

both cases, hemolymph circulation is restricted (Fig. 3D).

The HP of both species showed similar histopathologies, such as an enlarged lumen of hepatopancreatic tubules, i.e., a loss of the normal star-like shape of tubules transversally cut (Fig. 4B, D). Vacuolization was also observed, in terms of a higher number and size of vacuoles (Fig. 4D). Finally, atrophy, commonly associated with necrosis, was also seen in the HP of animals exposed to MP. All the referred histopathologies had a higher incidence as MP concentration increased (Table 4).

4. Discussion

As a general rule, the smaller the size of plastic particles, the higher the probability of being incorporated into animal tissues (Crawford and Quinn, 2017). In this sense, nanoplastics (e.g., particles < 1 μm) have the highest chance to accumulate inside cells. For instance, Jeong et al. (2017) reported the uptake of 0.05 μm polystyrene microbeads by the digestive system of copepods, affecting growth, and causing oxidative stress. However, *C. maenas* crabs exposed for 2–3 wk to 10 μm fluorescently labeled microspheres, significantly accumulated these particles, but no apparent intake to tissue cells or hemolymph seems to occur; instead, microspheres were retained in the external surface of gills lamellae, and inside the lumen of the digestive system (Watts et al., 2014). Therefore, the particle size seems to be a major factor for intake into the tissues. Farrell and Nelson (2013) showed the uptake of 0.5 μm fluorescent polystyrene particles into the hemolymph of the crab *Carcinus maenas*. Yu et al. (2018) have reported a progressive accumulation of 0.5 μm fluorescent polystyrene particles in the gills, liver, and gut of *Eriocheir sinensis* exposed during 7 d. Moreover, Wang et al. (2021) observed the bioaccumulation of 5 μm fluorescent polystyrene microspheres in the crab *Charybdis japonica*, not only in gills and hepatopancreas but also in muscles, indicating that MPs could be translocated to the hemolymph to be distributed to several tissues, yielding a bioconcentration factor of 5.20×10^3. In our study, 1 μm polystyrene beads were shown to accumulate in the HP, although the bioconcentration factors found in the current study were modest. However, the fact that the assayed MPs were detected in HL indicated that they are able to reach, and eventually accumulate, in other tissues.

The production of oxidative stress due to microplastic pollution has been well-documented in several invertebrate species. Many studies on mollusk species reported the activation of both enzymatic and non-enzymatic antioxidant defenses, against a wide variety of MPs (Sendra et al., 2021). In other aquatic fauna, including crustaceans, oxidative stress caused by MPs has been reported, associated with metabolic disorders and inhibition of growth, among other processes affected (Wang et al., 2019). In the current study, lipid peroxidation was enhanced in the hepatopancreas of both species, at the low concentration in *P. clarkii* and the high concentration in *L. pugilator*. On the other hand, the antioxidant defenses were increased in the hemolymph at both concentrations in *P. clarkii*, and at the high MPs concentration in *L. pugilator*.

Compared to other crustacean species, the exposure to 0.5 μm polystyrene particles induced a higher expression of several antioxidant enzymes in the hepatopancreas of the crab *E. sinensis*, such as superoxide dismutase and glutathione peroxidase, together with glutathione; this effect was only observed at the lower concentrations; at higher ones, the activity of antioxidant enzymes was probably inhibited by the assayed MPs (Yu et al., 2018). Similarly, the exposure of *C. japonica* crabs to 1000 particles/mL of 5 μm polystyrene microspheres caused an upregulation of antioxidant defenses; however, as the hepatopancreatic concentration of MPs increased, such defenses collapsed due to a severe hepatic injury (Wang et al., 2021). As mentioned above, the inhibition of antioxidant defenses was not observed at the highest concentration assayed in our study, at least in the HL. However, the high lipid peroxidation observed in the hepatopancreas of *L. pugilator*, indicates that the antioxidant defenses triggered by the exposure to MPs were not enough to suppress the oxidative stress in that tissue, whose role in detoxification is crucial.

The loss of the normal shape, as well as the epithelial atrophy including necrosis, have been previously reported in the hepatopancreas of crustaceans exposed to other pollutants, such as herbicides (Silveyra de Melo et al., 2019), and insecticides (Lavarías et al., 2022). As shown by these latter authors, necrosis and atrophied epithelium of

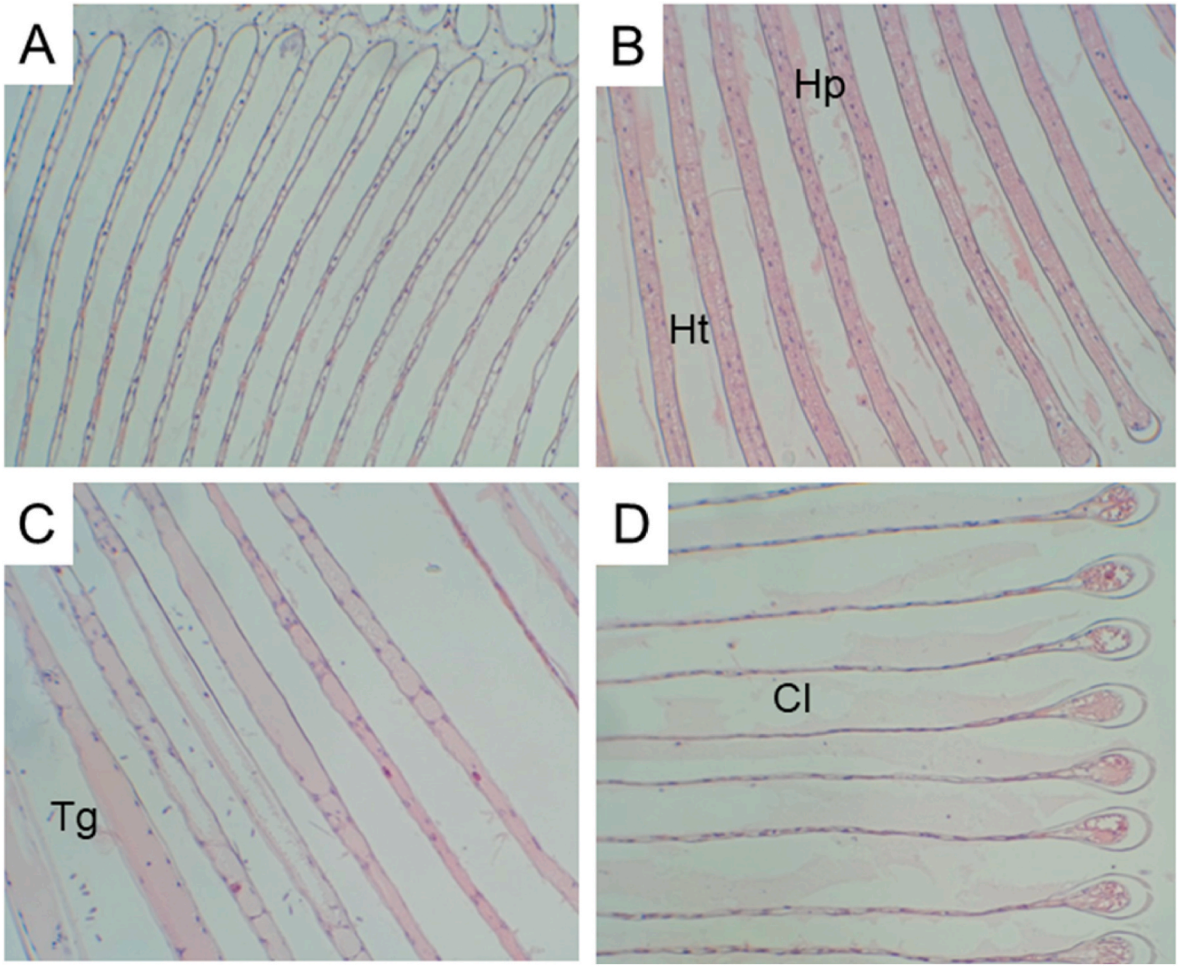


Fig. 3. Histopathologies observed in gills of *Leptuca pugilator* exposed to microplastics. A: control; B–D: exposed to 5000 particles/mL Ht: hypertrophy; Hp: hyperplasia; Tg: telangiectasia; Cl: collapsed lamellae. Magnification: 80x

hepatopancreatic cells correlate with other biomarkers of oxidative stress, such as lipid peroxidation, suggesting that the excess of ROS (reactive oxygen species) generated by MPs and not compensated by the antioxidant defenses, would be the responsible for the pathologies observed in the hepatopancreas of *P. clarkii* and *L. pugilator*. As observed in the current study, the previous studies above mentioned also found an intense vacuolization in several hepatopancreatic cell types, probably related to an enhanced detoxification process, to sequester pollutants. Since the HP is the organ not only responsible for the digestion and absorption of nutrients but also for the synthesis of vitellogenin, both somatic growth and reproduction can be seriously affected by the damage to this organ.

It is well known that excess ROS could produce a series of cellular changes in several tissues, triggering several pathological lesions. The inflammatory response has been a typical effect described for MPs in tissues of aquatic animals (Vieira de Araujo et al., 2021), and such response has been directly associated with oxidative stress (Wang et al., 2021). For instance, significant alterations have been reported in the digestive gland and gills of mollusks exposed to MPs, such as thickening and disorganization of the gill epithelium (Sendra et al., 2021). In our study, marked hypertrophy, and even hyperplasia of gill lamellae, was seen in the crab *L. pugilator*, which implies a reduced efficiency in the gas and ion exchange carried out by gills. On the other hand, the death of pillar cells, which maintain a normal separation between the opposite epithelia in a single lamella, would lead to the other pathologies observed in the current study, i.e., the collapse of lamellar epithelia or, instead, separation of such epithelia (telangiectasia). In any case, the normal circulation of hemolymph is impaired, again reducing the efficiency of gills for gas and ion exchange with the internal medium.

5. Conclusions

We conclude that even when both decapod crustacean species studied displayed an antioxidant response after 30 days of exposure to MP beads, exposure to the beads at two concentrations resulted in oxidative damage, together with several histopathologies in gills and hepatopancreas. Our current results make evident the need for further studies aimed at deepening the effects of MPs at longer times of exposure and lower concentrations. The study of other critical variables able to be affected, such as reproductive potential, is also highly desirable.

Author contributions statement

Gabriela R. Silveyra: Investigation, Methodology, Conceptualization. Patricia Silveyra: Methodology, Resources. Megan Brown: Investigation. Shirley Pool: Investigation. Itzick Vatnick: Investigation, Methodology, Supervision. Daniel A. Medesani: Methodology. Enrique M. Rodríguez: Supervision, Conceptualization, Writing- Original draft presentation, Resources.

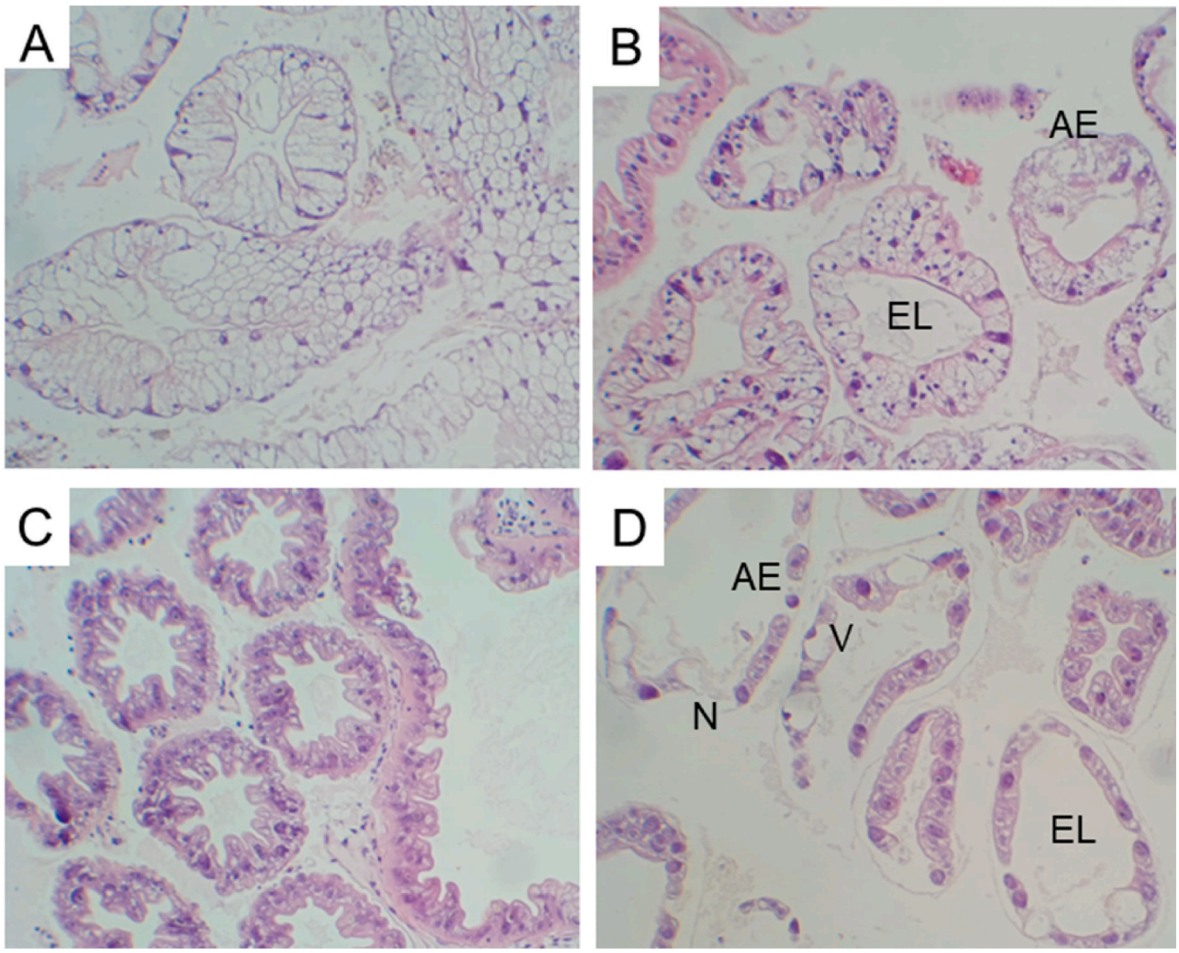


Fig. 4. Histopathologies observed in hepatopancreas of *Leptuca pugilator* and *Procambarus clarkii* exposed to microplastics. A and C: control; B and D: exposed to 5000 particles/mL. EL: enlarged lumen; AE: atrophied epithelium; N: necrosis; V: vacuolization. Magnification: 80x

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.

Acknowledgements

This work was supported by a grant from ANPCYT, Argentina (PICT 2016–0040, PICT2020-2020-SERIEA-036 69), and UBACYT (code 20020190100014BA).

References

American Public Health Association, American Water Works Association, Water Pollution Control Federation, 2005. Standard Methods for the Examination of Water and Wastewaters. American Public Health Association, Washington, DC.

Crawford, B.C., Quinn, B., 2017. Microplastic Pollutants. Elsevier, Netherlands.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.

González Pisani, X., Sturla Lompré, J., Pires, A., López Greco, L., 2022. Plastics in scene: a review of the effect of plastics in aquatic crustaceans. Environ. Res. 212, 113484 https://doi.org/10.1016/j.envres.2022.113484.

Horn, D.A., Granek, E.F., Steele, C.L., 2020. Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (*Emerita analoga*) mortality and reproduction. Limnol. Ocean. Lett. 5, 74–83. https://doi.org/10.1002/lol2.10137.

Issac, M.N., Kandasubramanian, B., 2021. Effect of microplastics in water and aquatic systems. Environ. Sci. Pollut. Res. 28, 19544–19562. https://doi.org/10.1007/s11356-021-13184-2.

Jeong, C.B., Kang, H.M., Lee, M.C., Kim, D.H., Han, J., Hwang, D.S., Souissi, S., Lee, S.J., Shin, K.H., Park, H.G., Lee, J.S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci. Rep. 7, 41323 https://doi.org/10.1038/srep41323.

Lavarías, S.M.L., Colpo, K.D., Landro, S.M., Ambrosio, E.S., Rodrigues Capítulo, A., Arrighetti, F., 2022. Deleterious effects of two pesticide formulations with different toxicological mechanisms in the hepatopancreas of a freshwater prawn. Chemosphere 286, 131920. https://doi.org/10.1016/j.chemosphere.2021.131920.

Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006.

Mattsson, K., Ekvall, M.T., Hansson, L.A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ. Sci. Technol. 49, 553–561. https://doi.org/10.1021/es5053655.

Rai, P.K., Lee, I., Brown, R.J.C., Kim, K.H., 2021. Environmental fate, ecotoxicity biomarkers, and potential health effects of micro- and nano-scale plastic contamination. J. Hazard Mater. 403, 123910 https://doi.org/10.1016/j.jhazmat.2020.123910.

Sendra, M., Sparaventi, E., Novoa, B., Figueras, A., 2021. An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci. Total Environ. 753, 142024 https://doi.org/10.1016/j.scitotenv.2020.142024.

Silveira de Melo, M., Goncalves dos Santos, T.P., Jaramillo, M., Nezzi, L., Rauh Muller, Y. M., Nazari, E.M., 2019. Histopathological and ultrastructural indices for the assessment of glyphosate-based herbicide cytotoxicity in decapod crustacean hepatopancreas. Aquat. Toxicol. 210, 207–214. https://doi.org/10.1016/j.aquatox.2019.03.007.

Sokal, R.R., Rohlf, F.J., 1981. Biometry. Freeman, New York.
Vieira de Araujo, F., Oliveira Castro, R., Lopes da Silva, M., Muniz Silva, M., 2021. Ecotoxicological effects of microplastic and associated pollutants. In: Kibenge, S.B., Baldisserotto, B., Chong, R.S.M. (Eds.), Aquaculture Toxicology. Elsevier, Netherlands, pp. 189–225. https://doi.org/10.1016/B978-0-12-821337-7-00009-8.
Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: a review. Ecotoxicol. Environ. Saf. 173, 110–117. https://doi.org/10.1016/j.ecoenv.2019.01.113.
Wang, T., Hu, M., Xu, G., Shi, H., Leung, J., Wang, Y., 2021. Microplastic accumulation via trophic transfer: can a predatory crab counter the adverse effects of microplastics by body defence? Sci. Total Environ. 754, 142099 https://doi.org/10.1016/j.scitotenv.2020.142099.
Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T.S., 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ. Sci. Technol. 48, 8823–8830. https://doi.org/10.1021/es501090e.
Yin, J., Li, J.Y., Craig, N.J., Su, L., 2022. Microplastic pollution in wild populations of decapod crustaceans: a review. Chemosphere 291, 132985. https://doi.org/10.1016/j.chemosphere.2021.132985.
Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.