Effects of microplastics on head-kidney gene expression and biochemical biomarkers in adult zebrafish

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Introduction & objective

Environmental contamination by microplastics (MPs) has been recorded worldwide, with highest reported concentration above 100 mg/m^3 within oceanic gyres (Goldstein et al., 2012). MPs have been isolated from various fish tissues, such as gastro-intestinal tract, liver, and gills, the reported toxicological effects suggest that MPs could lead cause tissue damage, inflammation, oxidative stress, metabolic alterations and affect acetylcholinesterase (AChE) activity (Fackelmann and Sommer, 2019).

The objective of this study was to test a set of molecular and enzymatic biomarkers. Eight genes (gene set 1) were selected from the results of a previous study conducted in our lab, which found differently expressed genes in zebrafsh liver after a similar MPs exposure. Four genes (Gene set 2) were selected for their known role in the immune system, for which head kidney is the primary organ in teleost. Moreover, acetylchlinesterase (AChE) and lactate dehydrogenase (LDH) activity were measured in zebrafish tissues.

Materials & methods

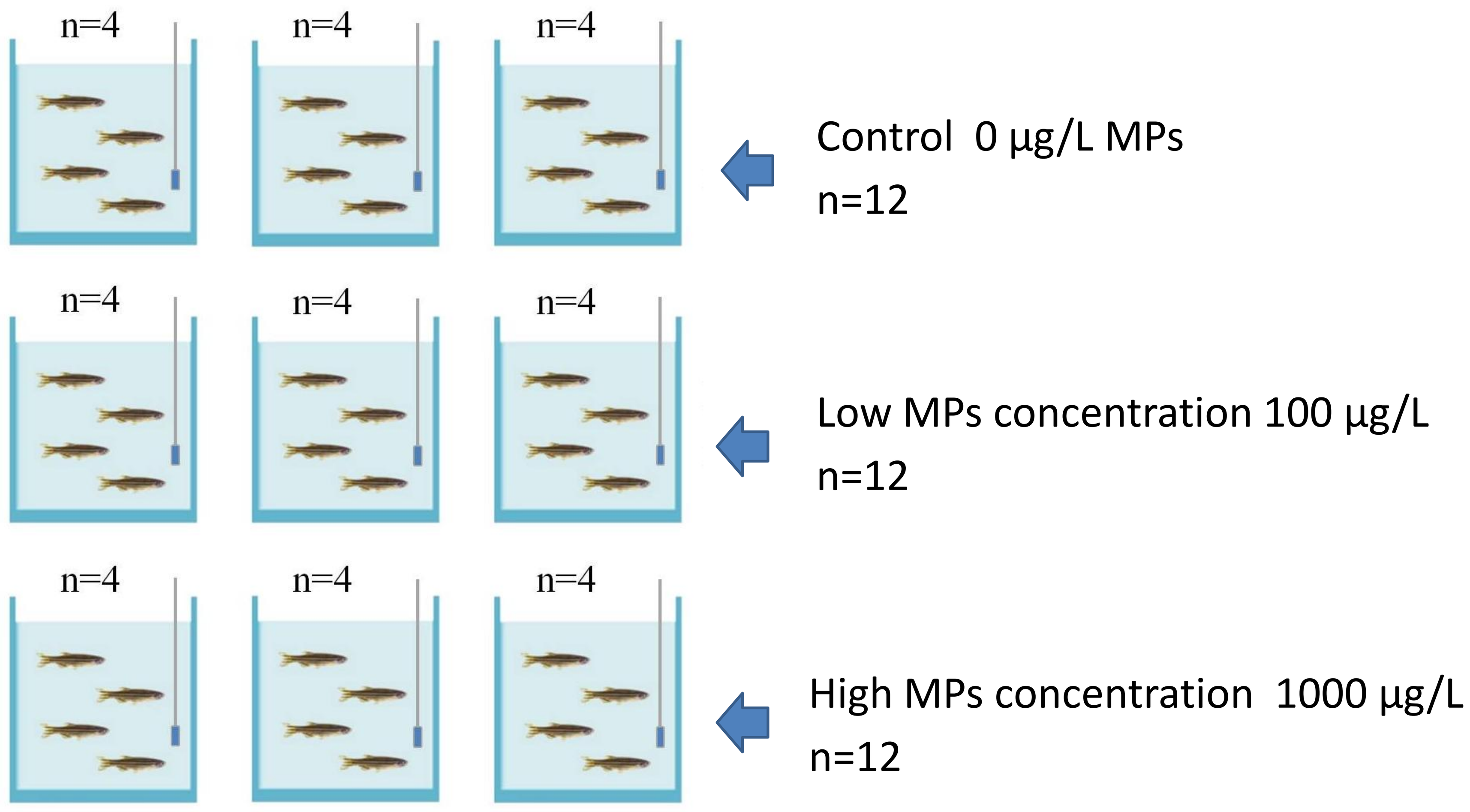
Experimental design: MP particles were administered daily for 21 days in association with food. The control group was given regular dry fish food, the two other groups were fed with control food spiked with a MPs mix (50% HD-PE and 50% PS). The water was changed daily before the feeding, and the tanks accurately rinsed to avoid MP accumulation over time.

Microplastics: PS and HD-PE irregularly shaped fragments were purchased from Toxemerge Pty Ltd as virgin microplastic powders (dimensional distribution: 90% < 90 µm; 50% < 50 µm; 10% < 25 µm).

Tissues sampling: All fish were euthanized through anesthetic overdose (MS-222 - 200 mg/L). The head-kidney was dissected and immediately submerged in RNAlater (10 µl/mg of tissue).

RNA extraction, cDNA synthesis and RT-qPCR: Head-kidneys from three fish from the same biological replicate were pooled together. Total RNA was extracted from 30 mg of tissue using the kit RNeasy plus mini (Qiagen). Reverse transcription and RT-qPCR were performed according to the method described in Limonta et al. (2019). The results were normalized based on the house-keeping genes: beta actin, ribosomal protein L8, and eukaryotic translation elongation factor 1.

Acetylcholinesterase and lactate dehydrogenae activity: AChE activity was quantified in the whole zebrafish head, according to the spectrofluorimetric method described in Casini et al. (2006). The LDH activity was measured in the whole body except the head, according to the method described by Menezes et al. (2006).

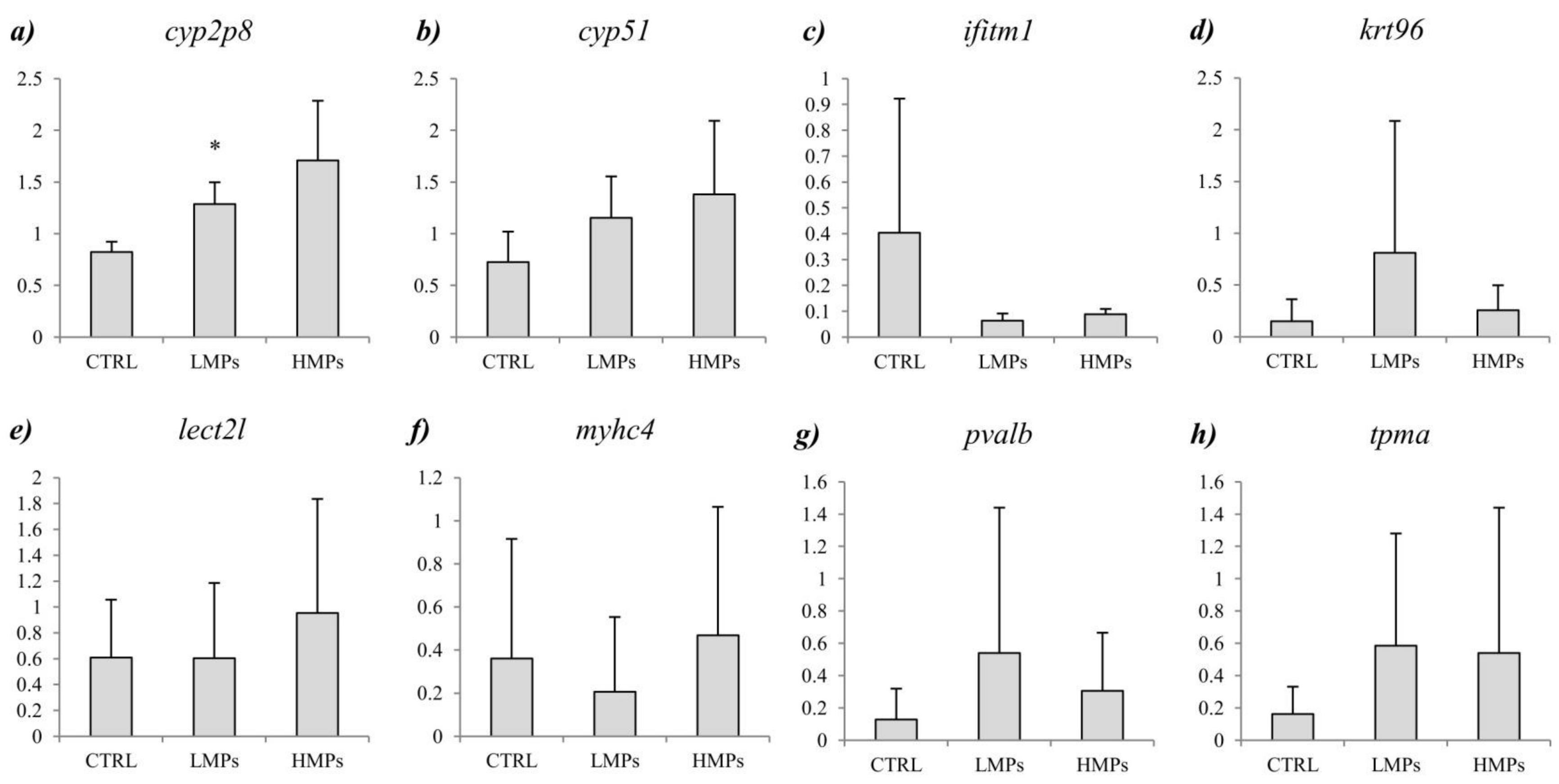


Experimental setup. Adult zebrafish (n=36) were kept in filtered and dechlorinated water. Photoperiod:12h light:12h dark. Water temperature: 28 °C.

Results & discussions

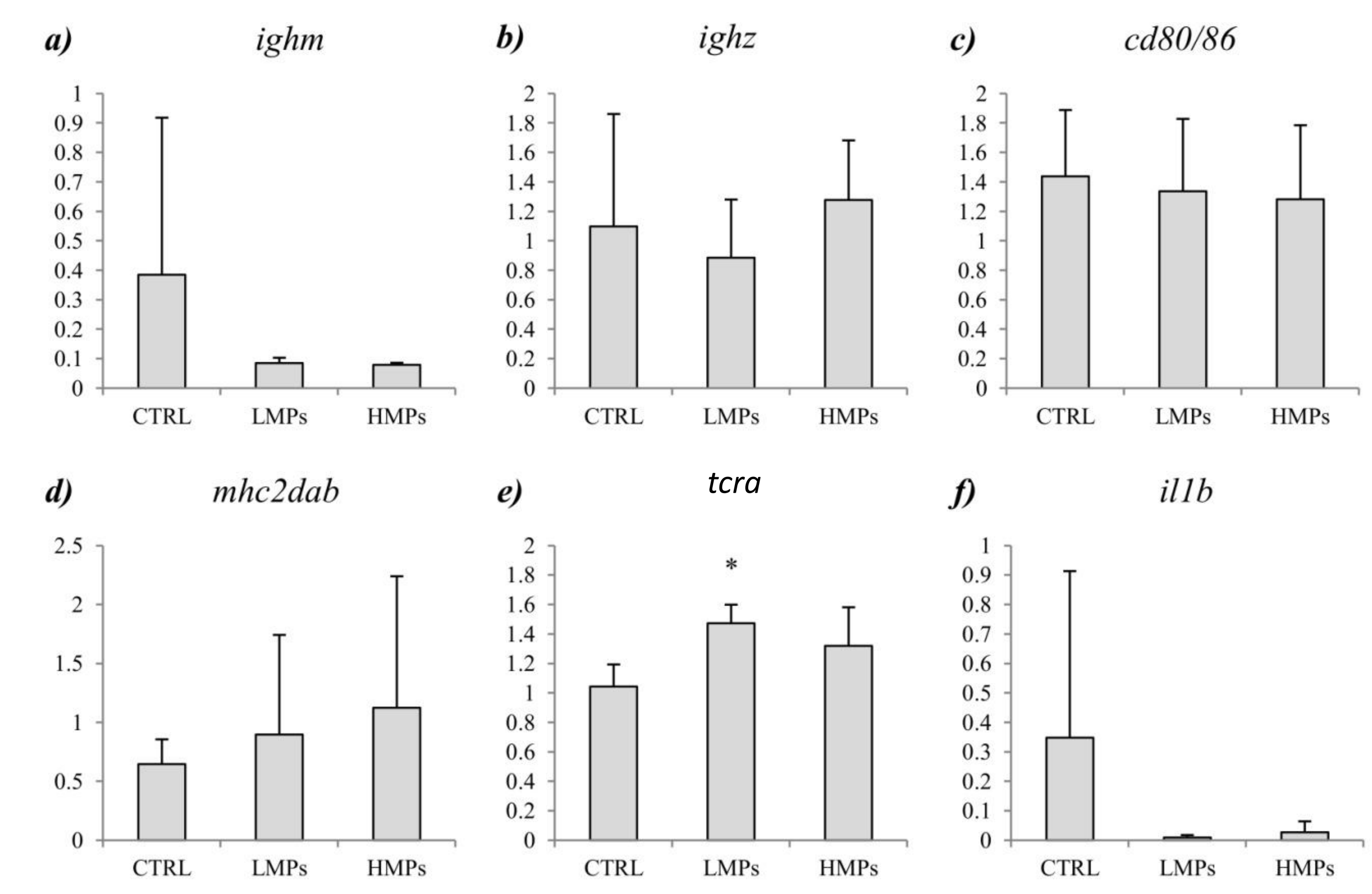
Molecular biomarkers (Gene set 1)

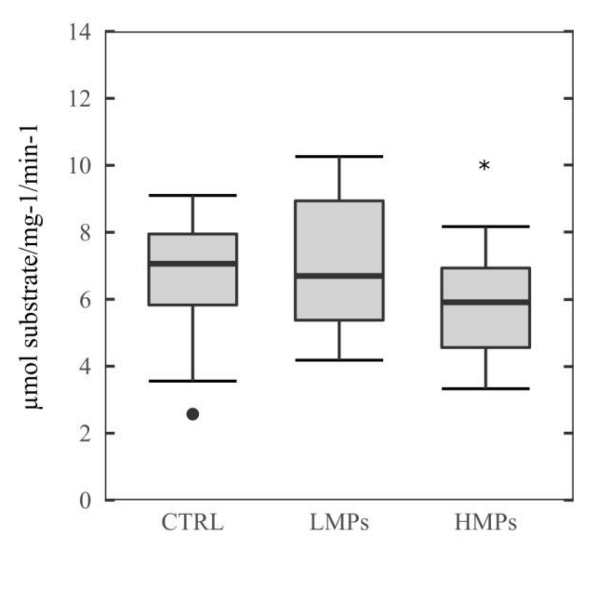
- The mRNA level for this gene was significantly higher in fish treated with 100 µg/L of MPs in respect to the control (p=0.01, *t*-test).
- C*yp2p8* is a gene involved in the detoxification metabolism which activates in response to xenobiotics such as PAHs (Curtis et al. 2017). It's expression in relation to MPs contamination should be further investigated, to confirm whether it may be responsive to MPs exposure



Molecular biomarkers (Gene set 2)

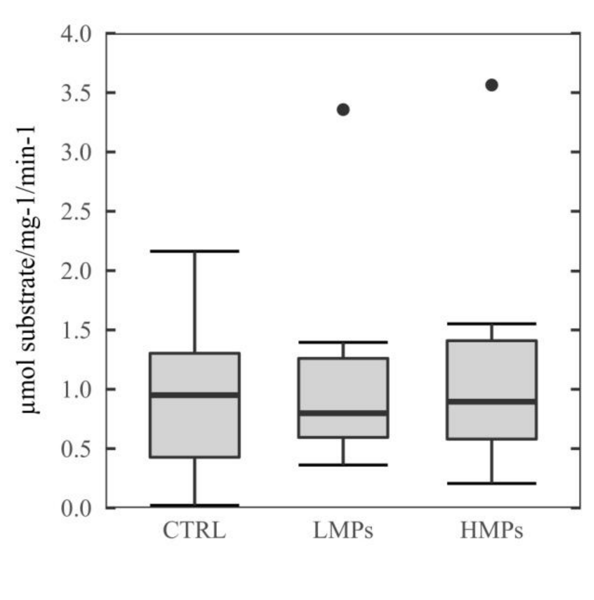
- Among the set of immune genes, treatment with 100 µg/L of MPs was able to up-regulate the t-cell receptor (*tcra*), compared to the control (p=0.02, *t*-test).
- T-cells receptors are responsible for recognizing antigens bound to either MHCI and MHCII molecules. It has been reported that MPs could cause gut dysbiosis (Qiao et al., 2019), and if pathogens are involved they may cause an activation of the adaptive immune response.





Acetylcholinesterase

- A significant 18 % inhibition was found after exposure to 1000 µg/L of MPs, compared to the control (p<0.05 *t*-test).
- Low concentration of MPs may not be able to affect AChE activity, as reported in a study by Barboza et al. (2019) as well.



Lactate dehydrogenase

- The LDH activity results show no significant differences across the three groups (CTRL, LMPs, HMPs).
- Neverthless, some studies reported increase in LDH activity following MPs exposure, (Banaee et al., 2019).

Conclusions

Overall, these findings contribute to the available data regarding the biological effects of MPs on fish, and in particular on the head-kidney, an organ in which the effects of MPs are still rarely studied. The results indicate that MPs could affect genes involved in detoxification metabolism and immune system. In light of this we believe that the nature of the relationship between MPs and the immune system should be further investigated.

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