Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Microplastics in aquaculture: accumulation and physiological effects in experimental models and species of commercial interest.

PhD student: Nico Cattaneo – Tutor: Prof. Ike Olivotto
Reproductive and Developmental Biology Lab, DiSVA

Introduction

Microplastics (MPs; size < 5mm) contamination is a worldwide problem, and studies have demonstrated their presence also in fish feed posing serious issues for the aquaculture sector.

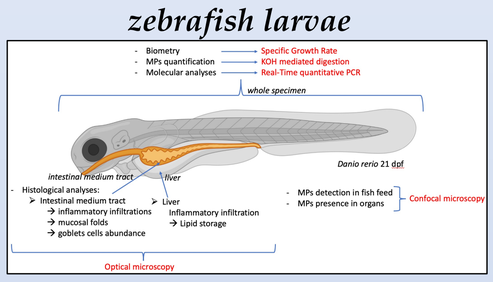

Zebrafish (Danio rerio)

AIM:
The present study investigated for the first time through a comparative approach, the effects of different sized fluorescent MPs included in a diet intended for zebrafish (*Danio rerio*). A comparison based on fish different life cycle stages (larval, juvenile, and adult) and exposure time, dietary MPs size and concentration was performed applying a set of laboratory analysis able to elucidate their possible effects on fish growth and welfare, MPs translocation among tissues and organs and the presence of biological barriers able to trap MPs.

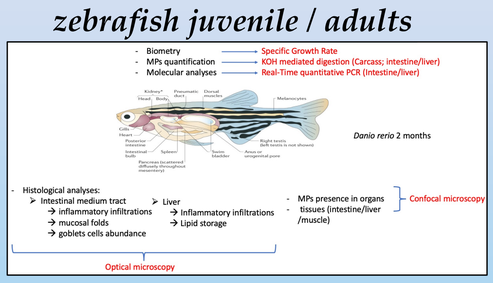
Materials and Methods

Analyses

zebrafish larvae



zebrafish juvenile / adults



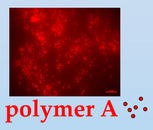
Diets formulation

Zebrafish feed*

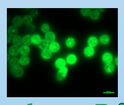
Ingredients	g/kg
Fish meal	490
CPSP 90	123
Wheat gluten meal	120
Pea protein concentrate	120
Wheat starch	55
Fish oil	60
Soya lecithin	8
Mineral and vitamin	14
Binder	10

Control: Zebrafish feed
A50: Zebrafish feed + 1-5µm [0.05g/kg]
A500: Zebrafish feed + 1-5µm [0.5g/kg]
B50: Zebrafish feed + 40-47µm [0.05g/kg]
B500: Zebrafish feed + 40-47µm [0.5g/kg]

Amino formaldehyde polymer - FMV-1.3**
size: 1-5µm
Peak emission of 636nm
when excited at 584nm

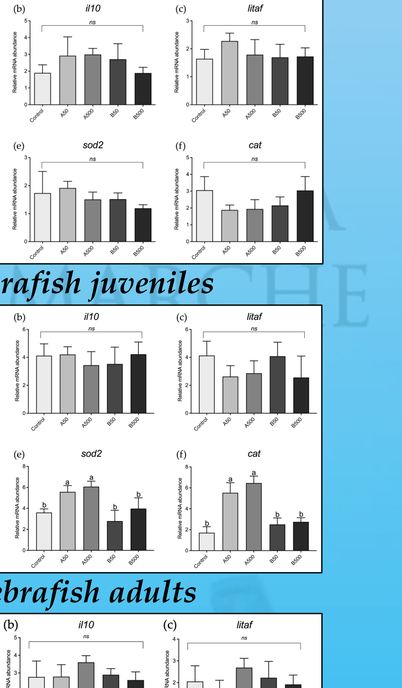

polymer A

Polyethylene - UVPMS-BR-0.995***
size: 40-47µm
Peak emission of 607nm
when excited at 575nm

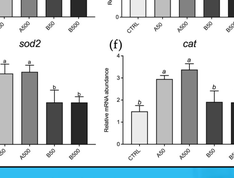

polymer B

*Diets were prepared in the pilot feed mill facility available at the Department of Agriculture, Food, Environmental and Animal science of University of Udine (Italy). A control diet MPs-free (named Control) was formulated to resemble the proximate composition of a commercial standard diet available for zebrafish (Zebrafeed; Sparos LDA, Olhão, Portugal) Cospheric LLC (Goleta, CA, USA):
**https://www.cospheric.com/fluorescent_violet_tracer_microparticles_2um.htm
***https://www.cospheric.com/UVPMSBR_fluorescent_red_spheres_density0995.htm

Experimental design



Sampling schedule

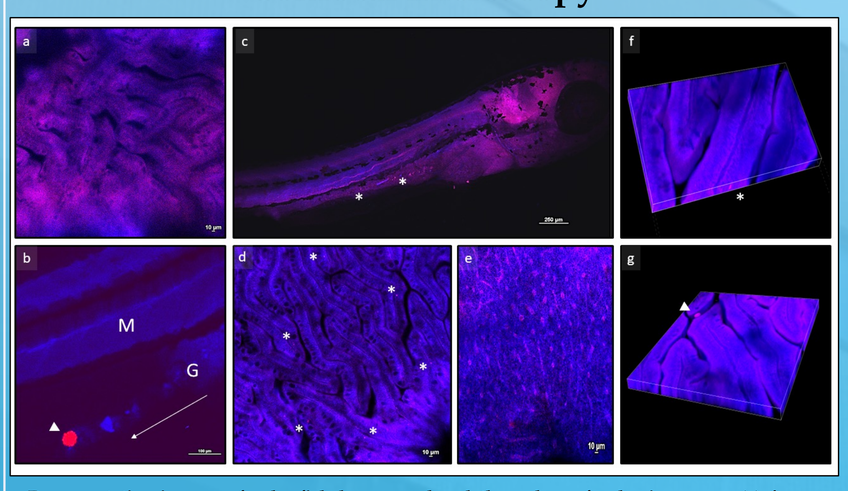


Results

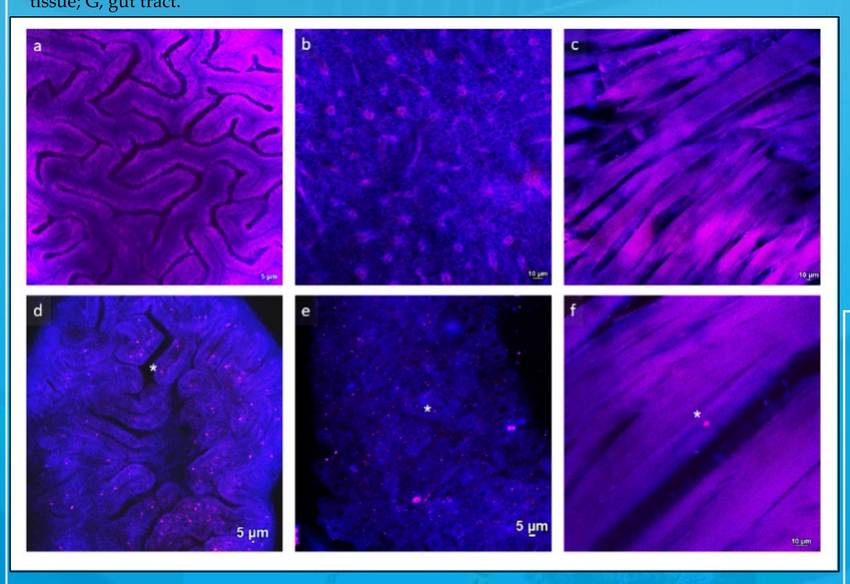
Growth and survival

No differences in survival and specific growth rate were found in zebrafish larvae, juveniles, and adults (*data not shown*) fed on the different diets.

Confocal microscopy

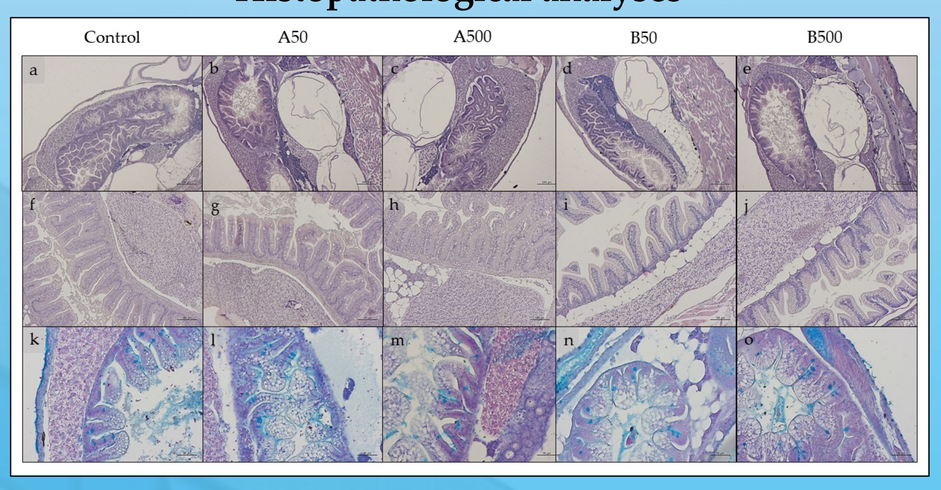


Representative images of zebrafish larvae analysed through confocal microscopy. (**a**) focus on intestine from zebrafish larva fed control diet; (**b**) polymer B fluorescent microbead in the gut lumen of zebrafish larva fed B500 diet; (**c**) whole larva fed A50 diet; (**d**,**e**) focus on intestine and liver from larvae fed A50 diet; (**f**,**g**) z-stack images of intestine from zebrafish larvae fed A50 and B50 diets, respectively. Asterisks indicate polymer A microbeads; arrowheads indicate polymer B microbeads; arrow indicate the direction of the gut tract, from cranial to caudal region. Abbreviations: M, muscle tissue; G, gut tract.



Representative images of (**a**,**d**) **intestine**, (**b**,**e**) **liver**, and (**c**,**f**) **muscle** samples of zebrafish juveniles fed Control (**a-c**) and A500 (**d-f**) diets. Asterisks indicate polymer A microbeads.

Histopathological analyses



Representative histological images (**a-e**) larvae and (**f-g**) juveniles, and (**k-o**) example of Ab+ goblet cells in intestine villus. (**a**,**f**,**k**) Control; (**b**,**g**,**l**) A50; (**c**,**h**,**m**) A500; (**d**,**i**,**n**) B50; (**e**,**j**,**o**) B500. Scale bars: (**a-e**) 200 µm; (**f-g**) 100 µm; (**k-o**) 50 µm.

		Control	A50	A500	B50	B500
Larvae	Mucosal fold height	102.9 ± 15.0 ^{a}	86.7 ± 8.4 ^{ab}	88.0 ± 5.8 ^{ab}	73.2 ± 4.6 ^{bc}	65.7 ± 6.0 ^{c}
	Ab+ goblet cells relative abundance	+	+	+	++	++
Juveniles	Mucosal fold height	94.9 ± 5.7 ^{a}	96.4 ± 8.8 ^{a}	88.2 ± 9.4 ^{a}	69.7 ± 7.9 ^{b}	70.1 ± 5.4 ^{b}
	Ab+ goblet cells relative abundance	++	++	++	+++	+++
Adults	Mucosal fold height	123.8 ± 9.0 ^{a}	125.2 ± 11.2 ^{a}	123.0 ± 26.8 ^{a}	91.2 ± 8.7 ^{b}	90.9 ± 14.3 ^{b}
	Ab+ goblet cells relative abundance	+	++	++	+++	+++

Histopathological index. Data are reported as mean ± standard deviation (n = 15). ^{a,b,c} Different letters denote statistically significant differences among the experimental groups ($p < 0.05$).

Quantification

		Control	A50	A500	B50	B500
Larvae	whole specimen	0	0.5 ± 0.2 ^{a}	3.5 ± 0.8 ^{b}	0	0
Juveniles	intestine	0	1.15 ± 0.45 ^{a}	61.93 ± 14.30 ^{b}	0.14 ± 0.01 ^{a}	0.64 ± 0.15 ^{a}
	liver	0	5.4 ± 1.6 ^{a}	231.1 ± 47.1 ^{b}	0	0
	muscle	0	0.3 ± 0.1 ^{a}	4.7 ± 1.2 ^{b}	0	0
Adults	intestine	0	2.8 ± 0.3 ^{a}	170.9 ± 20.6 ^{b}	0.6 ± 0.3 ^{a}	0.8 ± 0.2 ^{a}
	liver	0	6.6 ± 1.7 ^{a}	821.1 ± 95.5 ^{b}	0	0
	muscle	0	2.0 ± 0.2 ^{a}	8.0 ± 2.4 ^{b}	0	0

MPs quantification after 10% KOH mediated digestion (number of microbeads/mg of tissue). Data are reported as mean ± standard deviation (n = 9). ^{a,b} Different letters denote statistically significant differences among the experimental groups ($p < 0.05$).

Molecular analyses

zebrafish larvae

zebrafish juveniles

zebrafish adults

Relative mRNA abundance of genes involved in immune response (*il1b*, *il10*, and *litaf*) and oxidative stress (*sod1*, *sod2*, and *cat*) analysed in zebrafish larvae, juveniles and adults. ^{a,b} Different letters denote statistically significant differences among the experimental groups ($p < 0.05$); *ns*, no significant differences.

Discussion

- Both type of polymers are ingested by all zebrafish life cycle stages.
- **No significant differences in survival and specific growth rates** were detected among the experimental groups for all life cycle stages of zebrafish.
- **Polymer A: was detected in zebrafish larvae, juveniles and adults organs in a dose-dependent trend. Polymer A was absorbed at intestinal level in all the life cycle stages, but reached liver and muscle only in zebrafish juveniles and adults**. The MPs were not trapped by the intestine (no alteration in the expression of genes involved in immune response was detected) but tended to accumulate in liver causing oxidative stress. The MPs retention by the liver resulted in in a reduced amount of polymer A in the muscles.
- **Polymer B simply transited in the intestine** causing a reduction in mucosal fold height in all zebrafish life cycle stages. The fish reacted increasing the goblet cells number in order to secrete more mucus to facilitate the transition of polymer B microbeads. However, polymer B caused an upregulation of the gene *sod1* in both groups B50 and B500.

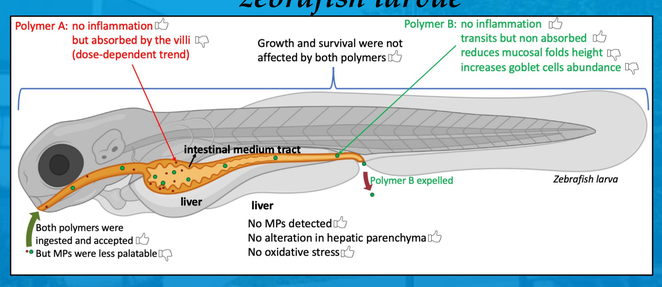
Conclusions

Zebrafish **have biological barriers against dietary MPs** acting in relation to size, concentration, and exposure time, leading to different scenarios during the different fish life cycle stages. **MPs microbeads of 40-47 µm in size were not absorbed** at the intestinal level and they simply transited through the gut lumen, progressively causing a shortening of mucosal folds and an increase in mucous cells, in all life cycle stages. Differently, **MPs microbeads of 1-5 µm in size were able to pass the intestinal barrier** and, only in juveniles and adults, to translocate from the gut to other target organs and tissues like the liver and the muscle, in a dose-dependent way. However, the reduced amount of polymer A microbeads detected in the juveniles' muscle samples indicated that the **liver is a key organ in retaining these MPs**.

Future perspectives: These results are important for the aquaculture sector and underline the need of further research to promote animal welfare by mitigating MPs negative side effects in fish, as well as the necessity of further studies on other finfish species of commercial interest.

Summary

zebrafish larvae



zebrafish juvenile / adults