UNIVERSITÀ POLITECNICA DELLE MARCHE

Dipartimento di Scienze della Vita e dell'Ambiente DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Cattaneo Nico
Title of PhD research: Microplastics in aquaculture: accumulation and physiological effects on experimental models and species of commercial interest

Name of PhD supervisor: Prof. Olivotto Ike
Research lab name: Reproductive and Developmental Biology Lab, DiSVA

Cycle:
[ ] XXXVI
[X] XXXVII

PhD Curriculum:
[X] Marine biology and ecology
[ ] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[X] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):
The presence of microplastics (size<5mm; MPs) has been detected in oceans constituting a source of pollution for aquatic organisms and they have been found in wild marine animals and in sea and land-farmed aquatic species. Furthermore, it has been shown that the MPs concentration in fishmeal is higher than that found in the raw materials from which it is derived, because of the processing and packaging procedures that use plastic. MPs contamination can have negative effects on both wild and farming fish during different life-cycle stage. To better understand the negative effects of MPs on fish and their possible translocation among organs, this study proposes a first part on model species zebrafish (*Danio rerio*) fed on experimental diets formulated with two different MPs polymers (sizes: 1-5µm and 40-47µm) implemented in fish feed at 0.05g/kg and 0.5g/kg. These first results will be used to perform a study on a fish species of commercial interest, the sea bass (*Dicentrarchus labrax*).

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- BACKGROUND

The presence of microplastics (MPs; size<5mm) has been detected in several different environments (Dris et al., 2018; Gasperi et al., 2018; Strungaru et al., 2019; Wang et al., 2019), marine ecosystem included (Sundt et al., 2014; Tanhua et al., 2020), making them a worldwide threat. The presence of MPs has been detected in marine animals from the lowest trophic level to the top of the food chain, including fish (Ding et al., 2019; Lusher et al., 2013; Thiele et al., 2021). The presence of MPs was also detected in sea-farmed aquatic species that showed similar rates of MPs accumulation to those evidenced by wild specimens (Chen et al., 2021; Lusher et al., 2017). This is due to the environmental pollution but also to the fish feed contamination, in which MPs has been widely found (Castelvetro et al., 2021). In addition, fish feed represents the main source of pollution also in the land-based aquaculture farms (Wang et al., 2022). In fact, MPs have been found in the principal ingredients used for aquafeed formulation, including both marine (like fish meal) and plant-derived ones.

Furthermore, it has been shown that the concentration of MPs in the ingredients is higher than in the raw materials since the processing procedures and packaging (PE is one of the most widely used materials to produce storage bags for fishmeal) can significantly contribute to the contamination process (Gündoğdu et al., 2021).

In this context, MPs contamination can have negative effects on farmed fish during the different life-cycle stages (Guzzetti et al., 2018). Many of these effects have been found primarily in the larval stages during which fish can mistake zooplankton for MPs resulting in gastrointestinal tract obstruction, a reduction in predatory activity caused by an apparent feeling of satiety, reduced growth and swimming capacity, and induction of inflammatory responses in the intestinal tract and other tissues because of translocation processes (Campos et al., 2021; Q. Wang et al., 2022). In addition, further adverse effects due to both physical and chemical mechanisms of action of MPs included in the diet, such as induction of oxidative stress and pro-inflammatory response in target organs such as the liver, intestine and encephalon, and alterations in social and feeding behaviour, have also been recently found in juvenile specimens of commercially interesting species such as gilthead seabream (*Sparus aurata*) (Capó et al., 2021; Rios-fuster et al., 2021; Solomando et al., 2020). Finally, a recent study conducted on juvenile European seabass (*Dicentrarchus labrax*) demonstrated the translocation of the dietary MPs (with a size range of 1-5 µm) into the fillet, highlighting attention to potential effects on human health (Zeytin et al., 2020).

To assess the effects of MPs on growth and welfare of teleosts, Zebrafish (*Danio rerio*) can represent an excellent experimental model being a fish widely used in fish nutrition and toxicology studies, with a relative short life cycle that allows to easily study its different phases under controlled conditions (Ribas & Piferrer, 2013). Additionally, zebrafish has been widely used to study the effects of MPs exposure due to the transparency of embryos and larvae, features that represent a significant advantage for studying the tissue localization of MPs (Trevisan et al., 2019). Zebrafish has been extensively used to study the effects of exposure to MPs from both the environment and diet of different polymers, highlighting a possible accumulation in target organs such as the gastrointestinal tract with the appearance of inflammatory processes and consequent alterations in the gene expression of markers involved in the immune response, oxidative stress, metabolic and microbiome alterations (Bhagat et al., 2020; Zhang et al., 2022). However, only a few studies have been conducted on MPs effects comparing different phases of zebrafish life-cycle (Tarasco et al., 2022) and to the best of our knowledge none was made using different MPs sizes in the same experiment.

- SCIENTIFIC AIMS

In the present study, diets including microplastics of different sizes and at different concentrations were prepared. The aim was to test the effects of such diets through a multidisciplinary approach: (i) on the larval stage, which is a critical phase of the life cycle; (ii) on the juvenile stage to determine the effects of long-term exposure; (iii) make a comparison of the physiological responses of fish to MPs between the two developmental stages.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1.

Objective

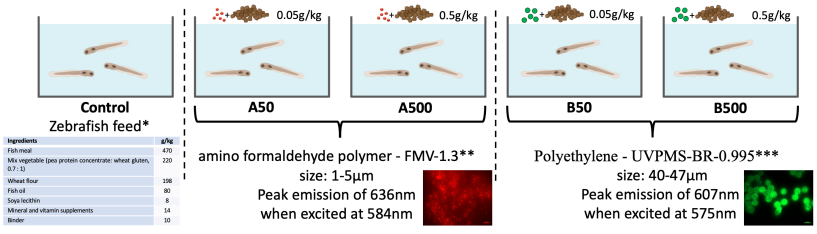
The objective is to assess the effects of a feed-induced MPs contamination during zebrafish larval development, giving particular emphasis to growth and welfare, by monitoring the gut health status and the stress and immune response, and to the MPs fate to other target organs in relation to their size.

Methods

A control diet containing fish meal as major protein source was prepared according to a commercially available standard diet for zebrafish (Zebrafeed, Sparos ltd, Portugal). Four experimental diets were prepared by adding to the basal diets two different sized fluorescent MPs (Polymer A:amino formaldehyde polymer, 1-5 µm; Polymer B: Polyethylene, 40-47 µm), both from Cospherics (Santa Barbara, USA) at two different concentrations (0.05 g/kg and 0.5 g/kg). Zebrafish were fed the experimental diets (daily dose corresponding to the 3% of the body weight) from 5 to 21 days post fertilization for larvae, until 2 months post fertilization for juveniles, and six months post fertilization for adults.

Larval stage: At the end of the trial, survival rate and specific growth rate were measured. To detect the presence of the fluorescent MPs ingested by the fish and their possible translocation from the gastrointestinal tract to other organs, the whole specimens were analysed through Nikon A1R confocal microscope. For the quantification of the MPs accumulated by fish, individuals were chemically digested, the product of digestion was filtered. Filters were then analysed through fluorescent microscopy to detect and count the number of MPs per individual. Histological analyses were performed to assess a possible damage to intestine absorptive epithelium and liver induced by the MPs while molecular analyses were performed to analyse the relative expression of genes involved in immune response (*il1b*, *tnfa*, and *il10*), appetite regulation (*npy* and *cb1*) and oxidative stress response (*SOD* and *CAT*).

Experimental design:



Obtained Results-larval stage

Both types of MPs were detected in fish feed. Zebrafish larvae were able to ingest both MPs polymers. Growth and survival rate were not affected by the two different polymers. As regards the liver, no MPs were detected inside this organ, consequently no alterations in hepatic parenchyma were found, as well as no differences in oxidative stress.

In the intestinal tract, the small polymer A did not induce inflammation, but it was absorbed by the intestinal villi in a dose-dependent manner. As regards the bigger polymer B no inflammation was detected; however, differently from polymer A it was not absorbed at the villi level and simply transited inside the intestinal

lumen. , As a consequence of this transit in the fish fed on diet B a mucosal folds height reduction and an increase in goblet cells abundance was observed.

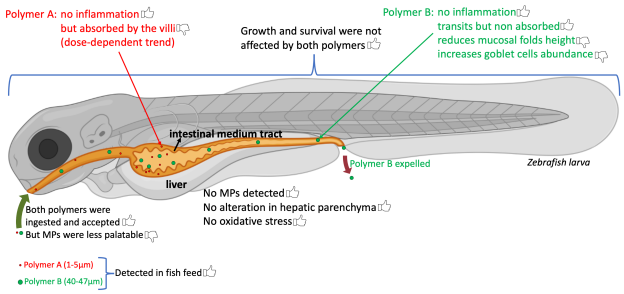


Figure 1. Summary of the results.

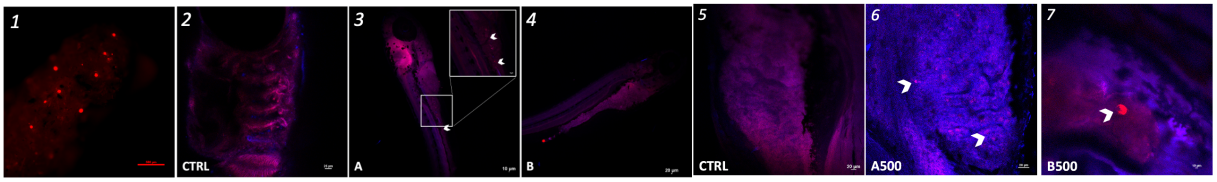


Figure 2. Identification of fluorescent MPs internalised in the fish feed and ingested by larvae. *1)* MPs (bright red) detected in diet B (MPs size 40-47µm) through fluorescent Zeiss microscope; *2)* Control group larva; *3)* Group fed on diet A (MPs size 1-5µm); *4)* Group fed on diet B (MPs size 40-47µm); *5)* Intestine of a larvae of control group; *6)* Intestine of group fed with the MPs A size 1-5 µm at 0.5g/kg; *7)* Intestine of group fed with the MPs B size 40-47µm at 0.5g/kg. The MPs are represented by the bright red beads (arrow-heads). The MPs are represented by the bright red beads (arrow-heads). Confocal microscope: Nikon A1R.

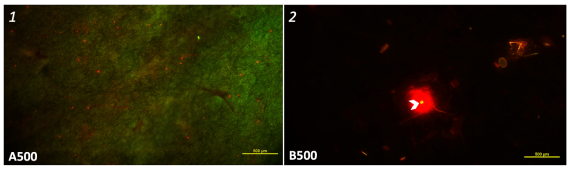


Figure 3: MPs quantification. Quantification of MPs done on a fiberglass filter (pore size 0.7µm) after KOH 10% mediated digestion of the whole specimen through Confocal microscope (Nikon A1R). *1)* quantification of MPs presence of the group fed on diet A (MPs size 1-5µm, red dots) at 0.05 g/kg; *2)* quantification of MPs presence of the group fed on diet B (MPs size 40-47µm, white arrow-head) at 0.05 g/kg.

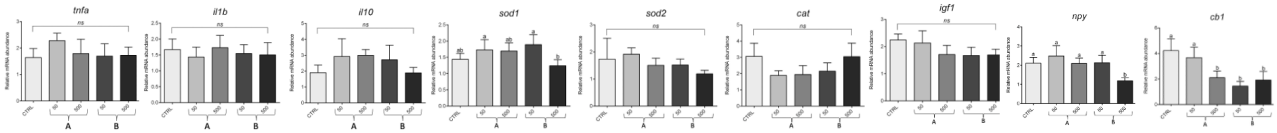


Figure 3: qPCR analyses. *tnfa*: tumor necrosis factor-alpha; *il1b*: interleukin 1 beta; *il10*: interleukin 10; *sod1*: Superoxide Dismutase 1; *sod2*: Superoxide Dismutase 2; *cat*: Catalase; *igf1*: Insulin-like growth factor 1; *npy*: Neuropeptide Y; *cb1*: Cannabinoid receptor type 1.

WP 2

Objective

The objective is to assess the effects of a feed-induced MPs contamination during zebrafish juveniles and adult phases, giving particular emphasis to growth and welfare, by monitoring the gut health status and the stress and immune response, and to the MPs fate to other target organs in relation to their size.

Methods

Same methods used for WP1.

Expected Results

Expected results comparable with WP1.

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Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Design of Research (European Projects) – Prof. Paone Nicola

2. Technology Transfer and Innovation – Prof. Iacobucci Donato

3. Lyfe Cycle Assessment (LCA) – PhD Amato Alessia

4. "The resolution revolution in Cryo-electron-microscopy, in Structural Biology and in Life Sciences", Martino Bolognesi, Department of Biosciences, University of Milan - 7.06.22: 11h00, Aula Azzurra

5. Final examination (PhD students cycle XXXIV) - 8.06.22: 10h00, room s2 (BEM)

6. Inshore: second conference Project PSR (InShore) - 23.06.22: 10h-12h00, Aula Magna Agraria

7. Seminario: Circular economy

8. Seminario: Donne nella pesca

List of periods spent abroad

List of conferences/workshops attended and of contributions eventually presented

1. Aquaculture Europe 2022 (EAS – European Aquaculture Society) – 27-30.09.22, Rimini (Italy). Oral presentation: Cattaneo, N., Zarantoniello, M., Conti, F., Randazzo, B., Frontini, A., Cardinaletti, G., Gioacchini, G., Olivotto, I. "The fate of dietary microplastics: a multidisciplinary laboratory approach to evaluate localization and physiological responses of zebrafish (*Danio rerio*) larvae"

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

In preparation

List of publications on conference proceedings

List of other publications (books, book chapters, patents)

[14.10.22]

Student signature

Supervisor signature

Prof. Ike Olivotto