The “lemna-gammarid system” as methodological approach for the ecotoxicological evaluation of microplastics on freshwater biota and the assessment of entry points into food chains

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Small plastic debris are a widespread and ubiquitous contaminant of marine and terrestrial ecosystems across the globe because its persistence. Nano and microplastics represent recently emerged issues and a potential threat to the whole biota, because

- they are small enough to be taken up by biota and thus enter the food chain;
- they can sorb pollutants on their surfaces, thus mobilising and releasing them in different environmental matrices.

Even if over last ten years a great increase of researches on these issues has occurred, much remains to be understood concerning the kinetic and the impact of plastic pollutants within the ecosystems. To date, knowledge on the biological impact of small MP is limited and sometimes conflicting, making difficult a risk assessment in water systems.

Monitoring the impacts of plastic litter on biota depends on the availability of indicator species in which to measure the effects of plastic exposure, moreover, a multi-species approach could ensure to assess impact linked to MP different in size and nature, on species with a different trophic role in ecosystems.

The proposed system is aimed at evaluating the effects of microplastics in the freshwater environment using as target species an aquatic plant belonging to the genus *Lemna* and the gammarid *Echinogammarus veneris*. The suitability of using these freshwater organisms for assessing the effects of microplastics on biota has been highlighted (Mateos-Cárdenas et al., 2019). Duckweed (*Lemna* spp.) is a recognized bioindicator of water quality due to its sensitivity to xenobiotic compounds in freshwaters. Gammarid species are increasingly used as model organisms as they are sensitive to environmental stresses and adaptable to laboratory conditions. *E. veneris* is a circum-Mediterranean species, considered sensitive to environmental stress. Due to its ecological traits, it was selected for several ecotoxicological studies.

The methodological approach relies on a two-step assay: first, *Lemna* plants will be exposed to microplastics along a 7-day test (OECD guideline 221, 2002); then, *Lemna* plants will be used as feedstock for the gammarid *Echinogammarus veneris* in a 24h test.

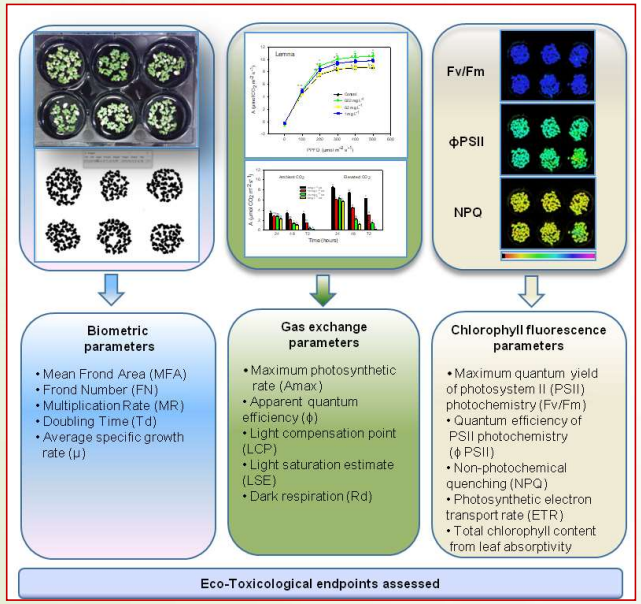
Microplastics exposure of a freshwater primary producer species, *Lemna minor*, and a consumer *Echinogammarus veneris*, a detritivores species having a critical role in debris turnover of all aquatic environments, is expected to provide results useful as a baseline for the understanding of the impacts of microplastics on the lower levels of the freshwater trophic food chain.

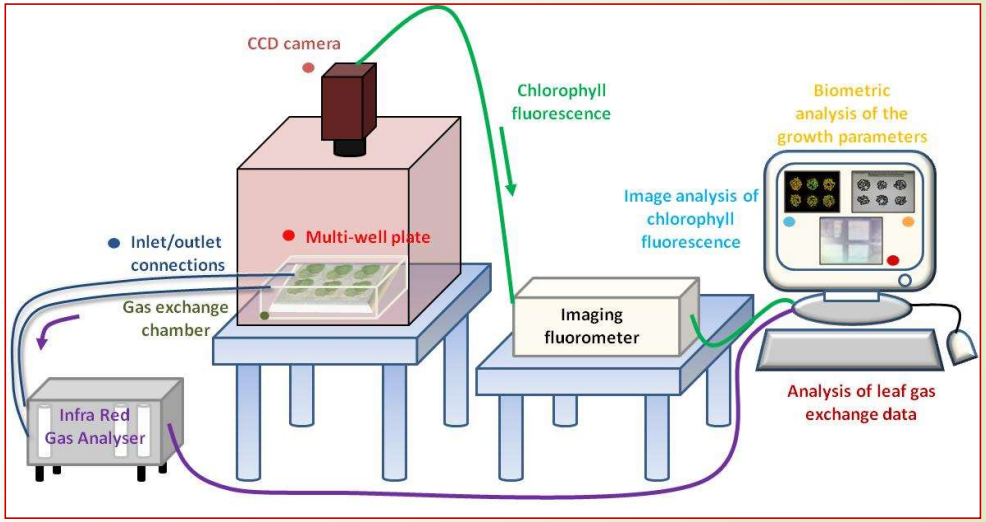
-Mateos-Cárdenas, Alicia, et al. (2019) "Polyethylene microplastics adhere to *Lemna minor* (L.), yet have no effects on plant growth or feeding by *Gammarus duebeni* (Lillj.)." Science of the Total Environment 689 : 413-421.

-OECD (2002) OECD guideline 221. OECD Guidelines for the Testing of Chemicals: Revised Proposal for a New Guideline 221, *Lemna* sp. Growth Inhibition Test. Organization of Economic Cooperation and Development Paris, France

Duckweeds (Lemnaceae) due to their small size (5 mm in length), high multiplication rates, susceptibility to pollutants and importance in the aquatic food web, are used as model aquatic plants in toxicity testing procedures of various inorganic and organic chemicals and their mixtures. Namely, a specific 7-day test for measuring the toxicity caused by a suspected harmful compound in plants has been developed (OECD guideline 221, 2002), targeting biometric endpoints in *Lemna* sp.

At IRET-CNR, an experimental set-up, termed Eco-Tox Photo system Tool (ETPT), was developed to measure in real time and in not destructive way both the plant growth and the physiological state of the photosynthetic apparatus (Pietrini and Zacchini, 2020), providing larger number of indices associated to toxicity endpoints linked to plant biometric, physiology, biochemistry. ETPT allows to detect early toxicity symptoms due to the impairment of the photosynthetic apparatus not visible yet.





-OECD (2002) OECD guideline 221. OECD Guidelines for the Testing of Chemicals: Revised Proposal for a New Guideline 221, *Lemna* sp. Growth Inhibition Test. Organization of Economic Cooperation and Development Paris, France

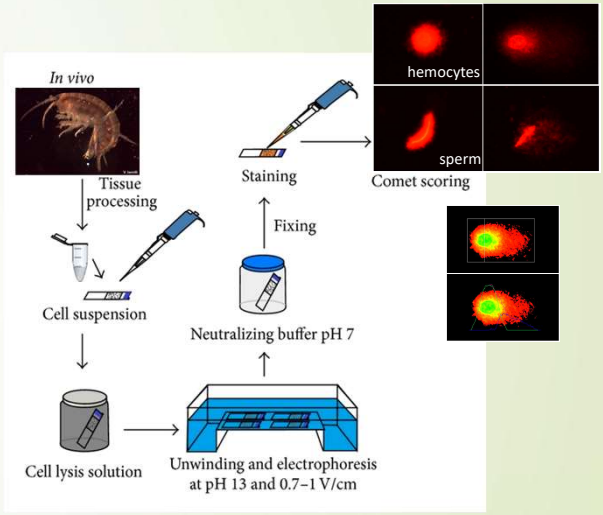
-Pietrini, F., Zacchini, M. (2020). A New Ecotoxicity Assay for Aquatic Plants: Eco-Tox Photosystem Tool (ETPT). Trends in Plant Science. https://doi.org/10.1016/j.tplants.2020.08.012

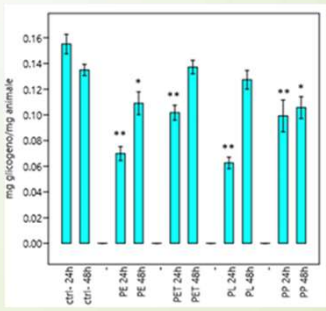
Because ingested MP can trigger molecular, cellular or physiological effects in these species, biomarkers of effect can usefully be applied as early indicators of the body's response to exposure. A biomarkers series consisting of several different endpoints is performed after exposure to investigate the effects of microplastics and related pollutants.

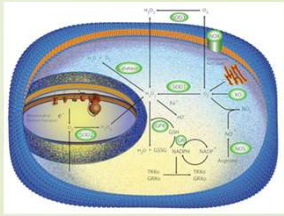
Preliminary tests showed that *E. veneris* can ingest small plastic fragments dispersed in the environment together with organic debris, like other amphipod species (Iannilli *et al*. 2019, 2020)

We examine:

- body's biochemical composition in lipid, glucose, glycogen and proteins to assess the energy reserves of specimens exposed, as a sensitive physiological indicator of stress, because feeding on MP could be related to reduction in organism's energy assimilation and modification of the feeding rate
- reduced glutathione (GSH), the major free thiol in most living cells involved in many biological processes such as detoxification of xenobiotics, it is the key antioxidant in animal tissues, GSH determination is used as an indicator of oxidant stress and injury caused by microplastic exposure.
- DNA damage as single-strand breakage by alkaline comet assay, on somatic and germ cells (hemocytes and spermatozoa), because genotoxicity is one of the most important endpoints in the environmental risk assessment







- Iannilli, V., Pasquali, V., Setini, A., Corami, F., 2019. First evidence of microplastics ingestion in benthic amphipods from Svalbard. Environ. Res. 179, 108811. https://doi.org/10.1016/J.ENVRES.2019.108811
- Iannilli, V., Corami, F., Grasso, P., Lecce, F., Buttinelli, M., Setini, A., 2020. Plastic abundance and seasonal variation on the shorelines of three volcanic lakes in Central Italy: can amphipods help detect contamination? Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-07954-7