Dottorato di Ricerca in Biologia Ambientale ed Evoluzionistica – *Curriculum Scienze Ecologiche*

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Title: *Understanding pathways of microplastics through marine food webs: Developing a system perspective to achieve a more suitable risk assessment and to drive an effective management policy.*

Project Presentation

In the XX century *Homo sapiens* became able of producing a new class of totally synthetic materials, which he called plastics. All plastic materials are organic polymers that share low production costs, malleability and durability. Since 1950s plastic waste accumulates in natural environments and today contaminates almost all places on earth.

Plastic pollution is one of the greatest environmental threats of contemporary times and for the future. The lack of natural analogues makes plastic polymers resistant to biodegradation. Therefore, plastic waste may persist and accumulate for centuries. In the meantime, chemical, physical and biological drivers break plastic litter into particles of ever smaller sizes.

Thompson *et al.*[1] first used the term "microplastic" (MP) to describe plastic fragments less than 5 mm in size. From that moment, many studies began to focus on the presence of MPs in every kind of environmental samples. Since 2014, the number of publications about MP contamination rapidly increased, and the list is daily updated.

Most studies about MP emphasize contamination of the marine environment. However, many of the papers merely describe MP contamination without providing an ecological perspective. Then, the comprehension of the pathways that MPs follow from sources to sinks and the dynamic of their transfer through the food web are important knowledge gaps to fill.

The analysis of MP ingested by living organisms or occurring in their tissues can give interesting information. MPs overlaps the size range of prey of a wide variety of marine animals. Therefore, MP ingestion may happen either accidently, or intentionally (*i.e.*, by confusing plastic particles with natural preys), as well as a result of secondary ingestion (*i.e.*, items already ingested by prey). Extending Thayer's theory[2], MP ingestion maybe not depend only on its detectability, but likely also on the foraging decision-rules of organisms. Furthermore, feeding habits and specific biological traits could influence the retention time of ingested plastics, affecting their recovery during the analyses[3-4].

Both positive and negative selection mechanisms likely determine unknown pathways of MPs through the food web. Some authors speculate on the relationship between MP ingestion and feeding habits of different species[5-6]. Nevertheless, no previous studies combine a detailed diets' analysis with an accurate characterization of the MPs isolated both from the gastrointestinal tract of different species and from sediment and water samples.

The proposed research aims at filling this gap by comparing the diet composition and the diversity of MPs ingested by different fish species considering the background knowledge of water and sediment contamination.

To hit this goal and considering the collaboration of the local fishery[3-4;7-9], the food web architecture covering the bathymetric layer from the continental shelf to the shelf break and the upper slope (50 – 600 m) off Anzio coast (Tyrrhenian Sea, Western Mediterranean) could be modelled to select different target species.

This selection may be based on the foraging arena theory[10], which claims that the availability of resources in an area influences the consumers living there. According to Ricci *et al.*[11], the trophic similarity and the bathymetric distribution of fish species could be considered as the main driving-forces in the definition of functional groups within the marine food webs. Two species from each functional group may be selected for the analyses, in order to subsequently verify the goodness of the aggregation criteria and the ecological reality of the groups.

Sampling activities will be made on board of professional fishing vessels and oceanographic vessels. Fish samples will be collected during single (or few) fishing trips in predetermined areas. Taking advantage that fish are organisms with continuous growth, samples into predetermined species-specific size ranges will be selected, to avoid variability caused by ontogenetic shifts. To deal with seasonal variations of the food web structure, four sampling campaigns will be organized in the four seasons that characterize the latitudes in which the study area is included.

General information on the biological and ecological traits of the selected species will be acquired from the scientific literature. Thereafter, specific information on their diet composition within the study area will be achieved by integrating complementary methods for diet analysis, namely the visual identification from stomach contents, DNA metabarcoding and Stable Isotope Analysis.

Afterwards, the amount, characteristics and composition of MP ingested by the different fish species will be analyzed considering differences between and within guilds and functional groups. Unbalances in the distribution of MPs between stomach and intestinal contents will be used to gather information on the relative retention time of MPs in each species[3]. Furthermore, MPs isolated from the gastrointestinal tract of fish will also be compared to MPs extracted from sediment and water samples collected in the same area during the same period.

MPs will be characterized considering their color, size, shape, and chemical composition. MPs are almost always classified according to shape categories and size classes, but the use of categorical variables may limit the understanding of selection mechanisms occurring in MP ingestion events. Since classification means loss of information, new tools will be used to translate categorical data into quantitative variables. Pictures of MPs will be collected using a camera-equipped dissecting microscope. Then, size and shape of MPs will be characterized using two different approaches for image processing, namely the shapeR package[12] for R≥3.0.2[13] and ImageJ≥1.52v (https://imagej.nih.gov/ij/). The automate use of ImageJ with Macros and Batch Processing will be employed to smooth the pictures and get shape descriptors (*e.g.*, aspect ratio, compactness, solidity, and convexity). The shapeR package will be used to get surface area measurements of each microplastic and 64 Walvelet coefficients computed on 10 Wavelet levels using the Daubechies least-asymmetric Wavelet. The polymeric characterization of MPs will be made using micro-ftir spectroscopy.

The proposed methodological framework introduces several innovations in the study of MPs. Most of previous studies provide a one-time description of MP contamination[14], while the main goal of this project is to investigate its functioning. The outcomes of this project will help to clarify some mechanisms that determine the fate of MPs within marine ecosystems, partially filling the knowledge gaps and proposing a new ecological perspective in this research field.

A full comprehension of mechanisms and patterns that define the fate of MPs will be useful to support the global action plans to mitigate the impact of plastic pollution, such as the Global Partnership on Marine Litter (GPML, https://www.gpmarinelitter.org/), the Honolulu strategy and the G7 countries agenda. Even within the UN sustainable development agenda, 4 of 17 Sustainable Development Goals (SDGs) pose targets concerning reduction and mitigation of plastic pollution of marine environments by 2030.

Furthermore, an integrative approach is crucial to plan a monitoring program. Several legal and policy frameworks address the “marine litter” topic, both globally and at European and Mediterranean levels. The 2008/56/EC Marine Strategy Framework Directive (MSFD), with the new Commission Decision 2017/848/EU defines the criteria D10C3 “The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned”, with the aim of achieving the Good Environmental Status. The seabird *Fulmarus glacialis* (Linnaeus, 1761) in the Northern European seas[15] and the loggerhead sea turtle *Caretta caretta* (Linnaeus, 1758) in the Mediterranean basin[16] are the target species for monitoring macro-litter (> 5 mm) ingestion. In contrast, a micro-litter monitoring strategy is not yet defined, though fish species are regarded as good candidates for assessing this kind of impact.
This project would also be useful to realize a marine micro-litter monitoring system with an ecosystem-based approach for the European and the Mediterranean countries. Although the ecological and biological criteria for the selection of sentinel species are fixed (*e.g.* background knowledge concerning biology, ecology, habitat and trophic information, feeding behavior, spatial distribution, commercial importance, conservation status and documented ingestion of marine litter)[17], there are not many species covering all the MSFD and RSC areas. Therefore, this study could suggest that guilds and functional groups may be the subject of weighted assessments.

Developing a system perspective is crucial to achieve a more suitable risk assessment and to drive an effective management policy. This challenge requires a multidisciplinary approach, as well as the contribution of a holistic vision of the set of environmental problems posed by human activities, such as climate change, habitat and biodiversity loss, interaction of different pollutants and their cumulative impacts. In this view, the proposed research may clear the way for finding links between microplastics, environmental contamination, pollution, trophic interactions, biodiversity conservation and ecosystems management.

Main References

1. Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russel, A.E. (2004). Lost at sea: Where is all the plastic? *Science*, 304(5672), 838.
2. Thayer AH, 1896. The law which underlies protective coloration. *Auk*, 13, 124-129. https://doi.org/10.2307/4068693
3. Valente, T., Sbrana, A., Scacco, U., Jacomini, C., Bianchi, J., Palazzo, L., de Lucia, G.A., Silvestri, C., Matiddi, M. (2019). Exploring microplastic ingestion by three deep-water elasmobranch species: A case study from the Tyrrhenian Sea. *Environ Pollut*, 253, 342-350. https://doi.org/10.1016/j.envpol.2019.07.001
4. Valente, T., Scacco, U., Matiddi, M. (2020). Macro-litter ingestion in deep-water habitats: Is an underestimation occurring? *Environ Res*, 186, 109556. https://doi.org/10.1016/j.envres.2020.109556
5. Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M. (2017). Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Sci Total Environ*, 586, 430-437. https://doi.org/10.1016/j.scitotenv.2017.01.175
6. Lopes, C., Raimundo, J., Caetano, M., Garrido, S. (2020). Microplastic ingestion and diet composition of planktivorous fish. *Limnol Oceanogr-Letters*, 5(1), 103-112. https://doi.org/10.1002/lol2.10144
7. Bianchi, J., Valente, T., Scacco, U., Cimmaruta, R., Sbrana, A., Silvestri, C., Matiddi, M. (2020). Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. *Mar Pollut Bull*, 154, 111050. https://doi.org/10.1016/j.marpolbul.2020.111050
8. Sbrana, A., Valente, T., Scacco, U., Bianchi, J., Silvestri, C., Palazzo, L., de Lucia, G.A., Valerani, C., Ardizzone, G., Matiddi, M. (2020). Spatial variability and influence of biological parameters on microplastic ingestion by *Boops boops* (L.) along the Italian coasts (Western Mediterranean Sea). *Environ Pollut*, 263, 114429. https://doi.org/10.1016/j.envpol.2020.114429
9. Tsangaris, C., Digka, N., Valente, T., Aguilar, A., Borrell, A., de Lucia, G.A., Gambaiani, D:, Garcia-Garin, O., Kaberi, H., Martin, J., Maurino, E., Miaud, C., Palazzo, L., Pérez del Olmo, A., Raga, J.A., Sbrana, A., Silvestri, C., Skylaki, E., Vighi, M., Wongdontree, P., Matiddi, M. (2020). Using *Boops boops* (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. *Mar Pollut Bull*, 158, 111397. https://doi.org/10.1016/j.marpolbul.2020.111397
10. Ahrens, R.N.M., Walter, C.J., Christensen, V. (2012). Foraging arena theory. *Fish Fish*, 13, 41-59. https://doi.org/10.1111/j.1467-2979.2011.00432.x
11. Ricci, P., Libralato, S., Capezzuto, F., D'Onghia, G., Maiorano, P., Sion, L., Tursi, A., Solidoro, C., Carlucci, R. (2019). Ecosystem functioning of two marine food webs in the North-Western Ionian Sea (Central Mediterranean Sea). *Ecol Evol*, 9, 10198-10212. https://doi.org/10.1002/ece3.5527
12. Libungan LA, Pálsson S (2015). shapeR: Collection and Analysis of Otolith Shape Data. R package version 0.1-5.
13. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
14. Borja, A., Elliott, M. (2019). So when will have enough papers on microplastics and ocean litter? *Mar Pollut Bull*, 146, 312-316. https://doi.org/10.1016/j.marpolbul.2019.05.069
15. van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M. (2011). Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. *Environ Pollut*, 159(10), 2609-2615. https://doi.org/10.1016/j.envpol.2011.06.008
16. Matiddi, M., Hochsheid, S., Camedda, A., Baini, M., Cocumelli, C., Serena, F., Tomassetti, P., Travaglini, A., Marra, S., Campani, T., Scholl, F., Mancusi, C., Amato, E., Briguglio, P., Maffucci, F., Fossi, M. C., Bentivegna, F., de Lucia, G. A. (2017). Loggerhead sea turtles (Caretta caretta): A target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environ Pollut, 230, 199-209. https://doi.org/10.1016/j.envpol.2017.06.054
17. Fossi, M. C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M. (2018). Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ Pollut, 237, 1023-1040. https://doi.org/10.1016/j.envpol.2017.11.019