The EU Interreg Project "GEREMIA" on waste management for the improvement of port waters: results on monitoring the health status of fish as bioindicator

A. Reboa[1,*], G. Besio[2], L. Cutroneo[1], I. Geneselli[1], S. Gorbi[3], A. Nardi[3], M.E. Piccione[4], F. Regoli[3], M. Capello[1]

[1] DISTAV, University of Genoa, Genoa, I-16132, Italy
[2] DICCA, University of Genoa, Genoa, I-16145, Italy
[3] DISVA, Polytechnic University of Marche, Ancona, I-60131, Italy
4 ISPRA, Rome, I-00144 Italy

Keywords: monitoring, port, marine waters, fish
* Presenting author email: anna.reboa@edu.unige.it

Marine waters pollution became a trend topic for environmental research in the last decade, due to the role of our seas as collectors and deposit of several contaminants. The inputs of marine pollution are mostly land related, but also come from navigation issues, such as oil spills, ballast water disposal, use of anti-fouling products, vessel scrapping, air-pollution, and accidental loss of solid waste (Homsombat *et al.*, 2013). Ports represent a peculiar environment where it is necessary to monitor and manage marine water health, because they are strictly connected with cities, and lot of commercial and industrial activities take place within their basins.

The Project "GEREMIA" (http://interreg-maritime.eu/web/geremia), part of the European Interreg Italy-France Maritime 2014-2020 Programme, focused on the development of strategies and tools for improving the waste management and the quality of port waters. The project involved Italian and French partners such as DISTAV University of Genoa (IT), University of Toulon (FR), Institute for Coastal Marine Environment – CNR (IT), Italian Institute for Environmental Protection and Research – ISPRA (IT), Port System Authority of the Eastern Ligurian Sea (IT), Ecological Services of the Port of Genoa (IT). The objectives were the following: the development of a Decision Support System for port operators dealing with environmental emergencies; the creation of a new integrated index for assessing the quality of port waters; the use of floating devices as myco-remediation system. Several monitoring actions were carried out during the Project on different abiotic and biotic matrices, and fish from Mugilidae family were chosen as bioindicator for assessing the health of port waters, due to their ecological role, wide diffusion, feeding habits and commercial importance. (Whitfield *et al.*, 2012)

The Port of Genoa (IT) was selected as the study site for the fish biomonitoring campaigns, since it is the one of the main Mediterranean ports for commercial traffic, and several industrial and touristic activities are present. In addition, the city directly faces the port waters, there are lots of waste waters discharging points and two river mouths insist on the area. The health status of fish collected in the Port of Genoa was compared with the results of fish sampled at the natural fishpond of S'Ena Arrubia (Sardinia, IT), which was chosen as an area less affected by human activities.

Two sampling campaigns took place both in the Port of Genoa and at the fishpond of S'Ena Arrubia, in May/June 2019 and in July 2020. The same number of samples were caught at the two sites within the same annual campaign, that is 20 during the 2019 campaign and 18 during the 2020 campaign. Specimens were all from the Mugilidae family, and the following species: *Liza aurata*, *Liza ramada* and *Mugil cephalus*. Fish were captured using a purse seine with the help of a professional fisherman, and kept alive until sacrifice by cervical dislocation, as suggested by European Guidelines (Directive 2010/63/EU). Different organs were excised and stored as requested by the following analysis. The histopathological investigation was carried out on 4 µm thick samples of liver and gills, fixed in Bouin solution just after excision, then embedded in paraffin, cut with microtome, and stained with haematoxylin and eosin. Histological alterations were investigated under optical microscope and quantified by their reversibility and extent within the section; a final score was assigned to each sample, and the higher the score, the worse the health status of the organ. Liver, gills, and muscle samples were stored at -20°C, and then investigated by chemical analysis on their content of the following metals: aluminium (Al), antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu), zinc (Zn). Finally, small liver samples were immediately frozen by liquid nitrogen, and then stored at -80°C for molecular analysis about ethoxyresorufin-O-deethylase (EROD) activity and biliary metabolites of three polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP), naphthalene and pyrene. EROD is a complex of enzymes which role is the detoxification of metabolism products; if the activity of these enzymes is higher than basic level, it means that the organism has been exposed to a stressor. EROD activity was indirectly measured by a spectrofluorometer, as nanomoles of resorufin produced in one minute per mg of enzyme in the sample.

Different histological alterations were investigated in liver and gills samples. Some alterations were more related to a possible inflammatory state of the tissue, such as blood vessels congestion, hemorrhages, aneurysms, and presence of granulocytes, while others were typical of an altered status of the specific tissue. Gills distinctive

alterations were epithelial lifting, hyperplasia/hypertrophy, shortening of secondary lamellae, and fusion/adhesion of secondary lamellae. On the other side, some specific alterations were analyzed on liver samples, such as steatosis, melanomacrophage centers, hydropic change, hyalinization, pyknotic nuclei, loss of the typical hepatic cords structure, and hepatic stroma degeneration. Finally, the presence of necrotic portion of tissue were considered both for liver and gills. Quantification of the presence of different alterations resulted in a numerical score, that represented the health status index of the organ. Both in 2019 and in 2020, fish from the Port of Genoa revealed a higher index than the ones from S'Ena Arrubia, either in gills or liver tissue. These results highlighted a worse health status in organisms from the more impacted area than in the natural one, but only few samples presented very high values of the index.

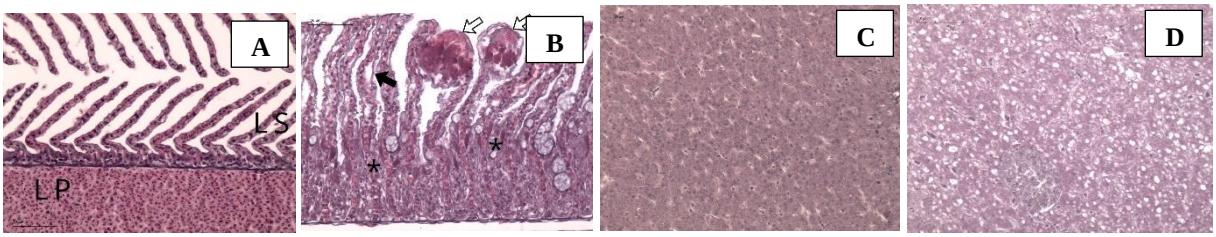

Fig.1: examples of results from histopathological analysis. A) gills of fish from S'Ena Arrubia with non-altered primary (LP) and secondary (LS) lamellae; B) gills of fish from the Port of Genoa, presenting alteration as aneurysms (blank arrow), epithelial lifting (black arrow) and hyperplasia (asterisk); C) liver of fish from S'Ena Arrubia with normal structure; D) liver of fish from the Port of Genoa showing an altered structure with severe presence of steatosis.

In a similar way, results from the EROD analysis showed significantly higher values of the catalytic activity in liver samples from the Port of Genoa compared to the ones from S'Ena Arrubia, in both campaigns. These results were partially in accordance with the analysis of the biliary metabolites, which were higher in samples from the Port of Genoa than in S'Ena Arrubia, except for a slightly higher level of BaP in S'Ena Arrubia samples in the second campaign.

On the other hand, the chemical analysis on metals content in tissue did not show the same strong contrast between sites. A particular attention was paid to the concentration of Pb, Cd and Hg, since the exposure to these metals is stated as potentially dangerous for the organisms (Yilmaz *et al*., 2018). The most relevant difference between sites was presented by the concentration of Pb, which was higher in fish form the port than from the fishpond. It is also interesting to notice that Pb was the only analysed metal that showed the highest concentrations in gills, while it is usually liver the organ that mostly accumulates metals.

In conclusion, the results obtained from this study confirm, also in our case, that biomonitoring on fish is an interesting and useful tool to define the health status of an environment affected by anthropic activities, and these data can be exploited to improve the management of quality of marine waters in specific areas, such as ports.

Acknowledgements: This research was funded by the Interreg Italy-France Maritime 2014-2020 Project "GEREMIA - Gestione dei reflui per il miglioramento delle acque portuali" (CUP D41I18000600005).

References

European Commission, 2010. Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. Off J Eur Union 276, 33-79.

Yilmaz, A.B., Yanar, A., Alkan, E.N., 2018. Review of heavy metal accumulation in aquatic environment of northern east Mediterranean Sea part II: some non-essential metals. Pollution 4, 143-181. https://doi.org/10.22059/poll.2017.236121.287

Homsombat, W., Yip, T.L., Yang, H., Fu, X., 2013. Regional cooperation and management of port pollution. Marit. Policy Manag. 40, 451-466. http://dx.doi.org/10.1080/03088839.2013.797118

Whitfield, A. K., Panfili, J., Durand, J.D., 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. Rev Fish Biol Fisheries 22, 641–681. https://doi.org/10.1007/s11160-012-9263-9