



The Use of Mussels in Environmental Toxicology

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Editorial

Volume 5 Issue 1

Received Date: January 28, 2022

Published Date: February 07, 2022

DOI: [10.23880/izab-16000351](https://doi.org/10.23880/izab-16000351)

Editorial

The continuous global increasing in terms of industrialization and urbanization has produced a severe impact in various environments and related biota. Hence, the anthropogenic activities are to blame for the release of high pollution levels into various environmental compartments (soil, air, and water). Particularly among the aquatic environments, the marine coastal areas, like the harbours, suffer the harmful effects of this continuous discharge of toxic compounds. In these enclosed areas, the constant pollutant emission leads to a reduction of water quality, with low level of oxygen and biodiversity [1]. Therefore the measurement of the various pollutants effects and the development of related remediation strategies for these impacted sites become fundamental tasks for the international environmental risk management [2]. In this scenario, the purpose of a multidisciplinary scientific field, like the environmental toxicology, is to assess the effect of potential harmful agents (biological, chemical, physical), often revealed in the various environmental media (soil, air, water) on living organisms [3].

In several ecotoxicological studies, different species were employed to investigate the impact of various contaminants detected in marine environment. The list of employed species comprise fishes [4-6], invertebrates like echinoderms [7,8] and polychaetes [9], as well as huge cetaceans like whales [10]. All these research emphasized the tendency of several pollutants to cause significant alteration in the various biological processes, weakening the health and survival rate of the biota.

Among the organisms already mentioned, the mussels (Mollusca, Bivalvia) represent surely ones of the most widely applied species in the environmental toxicology [2,11], since they exhibit most of the main features of bioindicators,

e.g. global spread, good knowledge of their biology, easy to collect and high sensitivity to stress condition [11-13]. Mussels, which include both freshwater and saltwater species, are small invertebrates with two shells called valves and a basic and well-known anatomy [14]. Due their filter-feeding habits, they can easily interact with every kind of chemical compound dissolved in the water, even in low concentrations. As confirmed by different authors [15-17] the bioaccumulation of harmful compound in the various tissues is common in these species. Moreover, they are sessile organism with a wide diffusion throughout the different geographies, and as a result, they may be employed on a worldwide scale to assess the environmental situation of specific points in areas affected by anthropogenic pollution [13]. For all these reasons, they are widely used in biomonitoring programs, guaranteeing the increase of knowledge regarding the biological alterations induced by the harmful stressors, detectable in the polluted areas [18-21]. Furthermore, because of their small size and low biological complexity, they may be easily housed for laboratory studies. The use of acclimated mussels in laboratory tests allows to gather more information about specific stressful conditions, both biotic and abiotic. In these experimental conditions, it was possible to estimate the impact of different abiotic stress, such as chemical compounds or particles [16,22-24], changes in the level of oxygen, temperature and salinity [25-29] but also biotic stress, like the exposure to bacteria [30-32]. The easiness with which they may be maintained under controlled condition, like tanks or aquaria, allows their use for more particular and elaborate experiments. Hence several authors [33-36] used the mussels to assess the efficacy of recovery strategies against petrochemical pollutant by mesocosm scale-up experiments. In general, a mesocosm is an experimental setup that may integrate the complexity of an environmental experiment with the

controllability given by a laboratory setting [37]. The biological responses in mussels, elicited by the experimental conditions reproduced in the mesocosms, allow to provide useful information for optimizing recovery methods against polluted areas. The use of mussels under the aforementioned conditions demonstrates their applicability in the different environmental research fields.

The variety of pollutants in the natural environment is able to elicit a wide range of reactions in the species. Therefore it is important to assess this plethora of biological responses (biomarker) to better elucidate the real impact on the various biological processes [38].

In all the several experimental conditions adopted, it was demonstrated how the different stress conditions may cause disparate types of alterations in the mussels, which can be detected using a multi-biomarker approach. As just observed in various research [6,9,39], the ability of pollutants to provoke several morphological alterations in the aquatic organisms is a very common effect as much as the tissue morphology assay became a standard endpoint in the environmental toxicology, widely applied also on mussels [1,20]. In these species, exposure to various kinds of contaminants is able to induce significant impairment in different important tissues, like gills [1,20,23,40], digestive gland [19,36,41-44] or gonad [24,45-56]. The tissue injury in mussels are revealable by histological [20,30] and ultrastructure [36,42] analysis.

In several cases, histological impairments have often associated with functional alterations. The gills, for example, perform a wide range of activities (gas exchange, osmoregulation, uptake of nutrients), which are regulated by a neurotransmission system (serotonergic and cholinergic) [18,17]. In the cholinergic system, the acetylcholinesterase (AChE) is a key enzyme [48] in the neuronal signalling. Its activity, measurable by spectrophotometric analysis, can be influenced by various classes of pollutant, as observed in several aquatic species [5,9,49] and in different species of bivalves [20,22,50-53]. The AChE assay proved to be a useful endpoint for evaluating the neurotoxicity of several environmental contaminants. In regards to the gonads, the tissue impairments can be induced by some classes of pollutants, such as the endocrine disrupting compounds (EDCs). The EDCs can affect the reproductive activity at several levels, such as gonad maturation and gamete development [54]. In regards to this final point, mussel gonads, like those of other aquatic animals [55,56], may be used to assess the impact of the environmental challenges on reproduction [45,57].

The stress conditions can harm biota reproductive activities by affecting also the embryonic development. Hence

compared to adults, aquatic species in their early life stages are more sensitive to stressors [58], resulting in a decrease of the biota survival rate [39]. Although in embryotoxicity tests fishes, in particular zebrafish (*Danio rerio*), are the wide used species [59-61], the use of mussels [62-66] provides a viable option in this specific field of the environmental toxicology.

It is well known how exposure to environmental challenges can provoke the rise of reactive oxygen species (ROS) and oxidative stress in the aquatic organisms [67,68]. Therefore the assay of the antioxidant activity for mitigating this stress condition has become an extremely common endpoint in the ecotoxicology [9,69-71]. Also, in mussels changes in the antioxidant enzymes activities (catalase, glutathione peroxidase, superoxide dismutase and glutathione *S*-transferase) can be revealed by using spectrophotometric techniques. The use of these approach permit to enlarge the knowledge regarding the pro-oxidant effect of several contaminants [22,72-75] in an aquatic organism model like the mussel.

Other common targets for the stressors in biological systems can be the gene and protein expression [76]. The use of PCR technique and western blotting permit to estimate the gene transcription and protein synthesis in different aquatic species, exposed to chemical compounds [7,39,77-79]. Also the various biological pathways in mussels, exposed to various types of biotic and abiotic stress [20,25,26,30,35,57,80,81] showed a good susceptibility in terms of gene and protein expression.

As aforementioned, the responses to external factors can be extremely complex, involving multiple metabolic pathways [38]. The use of “-omics” methodologies such as proteomics, transcriptomics, and metabolomics facilitated the development of the “systems biology,” which can investigate numerous biological processes as interconnected and interactive systems [82,83]. In particular, metabolomics, based on the study of endogenous, low molecular weight metabolites (<1000 Da), is one of the pivotal technique for the application of the “systems biology” in the environmental toxicology [84]. The use of protonic nuclear magnetic resonance spectroscopy (¹H-NMR) permit to successfully investigate the alteration of metabolomic profiles in several aquatic organisms exposed to different environmental challenges [6,85-88]. In mussels, NMR-based metabolomics proven to be a very sensitive tool analysing the baseline levels of metabolites in various tissues, both qualitatively and quantitatively [85]. In ecotoxicology, ¹H-NMR has undoubtedly contributed to further elucidating the impacts of pollutants on the metabolic profile recorded on these bivalves used in various experimental plans. Hence, metabolomics was successfully employed on mussels in biomonitoring programmes [1,18,89], under controlled

laboratory condition during exposure to various stress condition [16,54,90,91] and in mesocosm experiment [35,36,92].

In light of the wide range of experimental approaches and methods of investigation used on this species, mussels have proven to be excellent organisms in the field of environmental toxicology. Thus, it is reasonable to expect in the future their further employment in this area of research in order to continue contributing significantly to the understanding of the environmental impact of various anthropogenic stressors in aquatic invertebrates.

References

- Cappello T, Mauceri A, Corsaro C, Maisano M, Parrino V, et al. (2013) Impact of environmental pollution on caged mussels *Mytilus galloprovincialis* using NMR-based metabolomics. *Mar Pollut Bull* 77: 132-139.
- Fasulo S, Guerriero G, Cappello S, Colasanti M, Schettino T, et al. (2015) The "SYSTEMS BIOLOGY" in the study of xenobiotic effects on marine organisms for evaluation of the environmental health status: biotechnological applications for potential recovery strategies. *Rev Environ Sci Bio Technology* 14: 339-345.
- Agathokleous E, Calabrese EJ (2020) Environmental toxicology and ecotoxicology: How clean is clean? Rethinking dose-response analysis. *Sci Total Environ* 746: 138769.
- Cappello T, Giannetto A, Parrino V, De Marco G, Mauceri A, et al. (2018) Food safety using NMR-based metabolomics: Assessment of the Atlantic bluefin tuna, *Thunnus thynnus*, from the Mediterranean Sea. *Food Chem Toxicol* 115.
- Parrino V, De Marco G, Minutoli R, Lo Paro G, Giannetto A, et al. (2021) Effects of pesticides on *Chelon labrosus* (Risso, 1827) evaluated by enzymatic activities along the north eastern Sicilian coastlines (Italy). *Eur Zool J* 88: 540-548.
- Vignet C, Cappello T, Fu Q, Lajoie K, De Marco G, et al. (2019) Imidacloprid induces adverse effects on fish early life stages that are more severe in Japanese medaka (*Oryzias latipes*) than in zebrafish (*Danio rerio*). *Chemosphere* pp: 225.
- Giannetto A, Cappello T, Oliva S, Parrino V, De Marco G, et al. (2018) Copper oxide nanoparticles induce the transcriptional modulation of oxidative stress-related genes in *Arbacia lixula* embryos. *Aquat Toxicol* pp: 201: 187-197.
- Maisano M, Cappello T, Catanese E, Vitale V, Natalotto A, et al. (2015) Developmental abnormalities and neurotoxicological effects of CuO NPs on the black sea urchin *Arbacia lixula* by embryotoxicity assay. *Mar Environ Res* 111: 121-127.
- Missawi O, Bousserhine N, Zitouni N, Maisano M, Boughattas I, et al. (2021) Uptake, accumulation and associated cellular alterations of environmental samples of microplastics in the seaworm *Hediste diversicolor*. *J Hazard Mater* 406: 124287.
- Baini M, Panti C, Fossi MC, Tepsich P, Jiménez B, et al. (2020) First assessment of POPs and cytochrome P450 expression in Cuvier's beaked whales (*Ziphius cavirostris*) skin biopsies from the Mediterranean Sea *Sci Rep* 10: 21891.
- Li J, Lusher AL, Rotchell JM, Deudero S, Turra A, et al. (2019) Using mussel as a global bioindicator of coastal microplastic pollution. *Environ Pollut* 244: 522-533.
- Schöne BR, Krause RA (2016) Retrospective environmental biomonitoring—Mussel Watch expanded. *Glob. Planet. Change* 144: 228-251.
- Yancheva VS, Stoyanova SG, Georgieva ES, Velcheva IG (2018) Mussels in Ecotoxicological Studies—Are They Better Indicators for Water Pollution Than Fish?. *Ecol Balk* 10.
- Lutz RA (2004) Bivalve Molluscs: Biology, Ecology and Culture. By Elizabeth Gosling. *Q Rev Biol* 79: 317.
- Álvarez Ruiz R, Picó Y, Campo J (2021) Bioaccumulation of emerging contaminants in mussel (*Mytilus galloprovincialis*): Influence of microplastics. *Sci Total Environ* 796: 149006.
- Cappello T, De Marco G, Oliveri Conti G, Giannetto A, Ferrante M, et al. (2021) Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. *Ecotoxicol Environ Saf* 209: 111780.
- Mata MC, Castro V, Quintana JB, Rodil R, Beiras R, Vidal-Liñán L (2022) Bioaccumulation of organophosphorus flame retardants in the marine mussel *Mytilus galloprovincialis*. *Sci Total Environ* 805: 150384.
- Cappello T, Maisano M, Giannetto A, Parrino V, Mauceri A, Fasulo S (2015) Neurotoxicological effects on marine mussel *Mytilus galloprovincialis* caged at petrochemical contaminated areas (eastern Sicily, Italy): 1H NMR and immunohistochemical assays. *Comp. Biochem. Physiol C Toxicol Pharmacol* 169: 7-15.

19. Cappello T, Maisano M, D'Agata A, Natalotto A, Mauceri A, et al. (2013) Effects of environmental pollution in caged mussels (*Mytilus galloprovincialis*). *Mar Environ Res* 91: 52-60.
20. Maisano M, Cappello T, Natalotto A, Vitale V, Parrino V, et al. (2017) Effects of petrochemical contamination on caged marine mussels using a multi-biomarker approach: Histological changes, neurotoxicity and hypoxic stress. *Mar Environ Res* 128: 114-123.
21. Parrino V, Costa G, Giannetto A, De Marco G, Cammilleri G, et al. (2021) Trace elements (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in *Mytilus galloprovincialis* and *Tapes decussatus* from Faro and Ganzirri Lakes (Sicily, Italy): Flow cytometry applied for hemocytes analysis. *J Trace Elem Med Biol* 68: 126870.
22. Capolupo M, Valbonesi P, Kiwan A, Buratti S, Franzellitti S, et al. (2016) Use of an integrated biomarker-based strategy to evaluate physiological stress responses induced by environmental concentrations of caffeine in the Mediterranean mussel *Mytilus galloprovincialis*. *Sci Total Environ* 563-564 (1): 538-548.
23. D Agata A, Fasulo S, Dallas LJ, Fisher AS, Maisano M, et al. (2014) Enhanced toxicity of "bulk" titanium dioxide compared to "fresh" and "aged" nano-TiO₂ in marine mussels (*Mytilus galloprovincialis*). *Nanotoxicology* 8: 549-558.
24. Pinto J, Costa M, Leite C, Borges C, Coppola F, et al. (2019) Ecotoxicological effects of lanthanum in *Mytilus galloprovincialis*: Biochemical and histopathological impacts. *Aquat Toxicol* 211: 181-192.
25. Giannetto A, Maisano M, Cappello T, Oliva S, Parrino V, et al. (2015) Hypoxia-Inducible Factor α and Hif-prolyl Hydroxylase Characterization and Gene Expression in Short-Time Air-Exposed *Mytilus galloprovincialis*. *Mar Biotechnol* 17: 768-781.
26. Giannetto A, Maisano M, Cappello T, Oliva S, Parrino V, et al. (2017) Effects of Oxygen Availability on Oxidative Stress Biomarkers in the Mediterranean Mussel *Mytilus galloprovincialis*. *Mar Biotechnol* 19.
27. Freitas R, Silvestro S, Coppola F, Meucci V, Battaglia F, et al. (2020) Combined effects of salinity changes and salicylic acid exposure in *Mytilus galloprovincialis*. *Sci Total Environ* 715: 136804.
28. Freitas R, Silvestro S, Pagano M, Coppola F, Meucci V, et al. (2020) Impacts of salicylic acid in *Mytilus galloprovincialis* exposed to warming conditions. *Environ. Toxicol Pharmacol* 80: 103448.
29. Feidantsis K, Georgoulis I, Giantsis IA, Michaelidis B (2021) Treatment with ascorbic acid normalizes the aerobic capacity, antioxidant defence, and cell death pathways in thermally stressed *Mytilus galloprovincialis*. *Comp Biochem Physiol Part B Biochem Mol Biol* 255: 110611.
30. Parisi MG, Maisano M, Cappello T, Oliva S, Mauceri A, et al. (2019) Responses of marine mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae) after infection with the pathogen *Vibrio splendidus*. *Comp Biochem Physiol Part C Toxicol Pharmacol* 221: 1-9.
31. Nguyen TV, Alfaro AC, Young T, Ravi S, Merien F (2018) Metabolomics Study of Immune Responses of New Zealand Greenshell™ Mussels (*Perna canaliculus*) Infected with Pathogenic *Vibrio* sp. *Mar Biotechnol* 20: 396-409.
32. Yang Q, Guo K, Zhou X, Tang X, Yu X, et al. (2021) Histopathology, antioxidant responses, transcriptome and gene expression analysis in triangle sail mussel *Hyriopsis cumingii* after bacterial infection. *Dev Comp Immunol* 124: 104175.
33. Pirrone C, Rossi F, Cappello S, Borgese M, Mancini G, et al. (2018) Evaluation of biomarkers in *Mytilus galloprovincialis* as an integrated measure of biofilm-membrane bioreactor (BF-MBR) system efficiency in mitigating the impact of oily wastewater discharge to marine environment: a microcosm approach. *Aquat Toxicol* 198: 49-62.
34. Ancora S, Rossi F, Borgese M, Pirrone C, Caliani I, Cappello S, et al. (2020) Assessing the Effect of Contaminated and Restored Marine Sediments in Different Experimental Mesocosms Using an Integrated Approach and *Mytilus galloprovincialis* as a Model *Mar Biotechnol* 22(3): 411-422.
35. Caliani I, De Marco G, Cappello T, Giannetto A, Mancini G, et al. (2022) Assessment of the effectiveness of a novel BioFilm-Membrane BioReactor oil-polluted wastewater treatment technology by applying biomarkers in the mussel *Mytilus galloprovincialis*. *Aquat Toxicol* 243: 106059.
36. Gornati R, Maisano M, Pirrone C, Cappello T, Rossi F, et al. (2019) Mesocosm System to Evaluate BF-MBR Efficacy in Mitigating Oily Wastewater Discharges: an Integrated Study on *Mytilus galloprovincialis*. *Mar Biotechnol* 21: 773-790.
37. Sagarin RD, Adams J, Blanchette CA, Brusca RC, Chorover J, et al. (2016) Between control and complexity: opportunities and challenges for marine mesocosms.

- Front Ecol Environ 14: 389-396.
38. Brooks S, Lyons B, Goodsir F, Bignell J, Thain J (2009) Biomarker Responses in Mussels, an Integrated Approach to Biological Effects Measurements. *J Toxicol Environ Heal Part A* 72: 196-208.
 39. De Marco G, Conti GO, Giannetto A, Cappello T, Galati M, et al. (2022) Embryotoxicity of polystyrene microplastics in zebrafish *Danio Rerio* *Environ Res* 208: 112552.
 40. Ciacci C, Barmo C, Gallo G, Maisano M, Cappello T, et al. (2012) Effects of sublethal, environmentally relevant concentrations of hexavalent chromium in the gills of *Mytilus galloprovincialis*. *Aquat Toxicol* 120-121: 109-118.
 41. Banni M, Sforzini S, Balbi T, Corsi I, Viarengo A, et al. (2016) Combined effects of n-TiO₂ and 2,3,7,8-TCDD in *Mytilus galloprovincialis* digestive gland: A transcriptomic and immunohistochemical study. *Environ Res* 145: 135-144.
 42. Gornati R, Longo A, Rossi F, Maisano M, Sabatino G, et al. (2016) Effects of titanium dioxide nanoparticle exposure in *Mytilus galloprovincialis* gills and digestive gland. *Nanotoxicology* 10: 807-817.
 43. Von Moos N, Burkhardt Holm P, Kohler A (2012) Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. *Environ Sci Technol* 46: 11327-11335.
 44. Stara A, Pagano M, Capillo G, Fabrello J, Sandova M, et al. (2020) Acute effects of neonicotinoid insecticides on *Mytilus galloprovincialis*: A case study with the active compound thiacloprid and the commercial formulation calypso 480 SC. *Ecotoxicol Environ Saf* 203: 110980.
 45. Koagouw W, Stewart NA, Ciocan C (2021) Long-term exposure of marine mussels to paracetamol: is time a healer or a killer?. *Environ Sci Pollut Res* 28: 48823-48836.
 46. Arrighetti F, Landro SM, Lambre ME, Penchaszadeh PE, Teso V (2019) Multiple-biomarker approach in the assessment of the health status of a novel sentinel mussel *Brachidontes rodriguezii* in a harbor area. *Mar Pollut Bull* 140: 451-461.
 47. Catapane E, Aiello E, Stefano GB (1974) Ganglionic mediation mechanism of lateral cilia in *Mytilus edulis* gill. *Physiologist* 17: 372.
 48. Matozzo V, Tomei A, Marin MG (2005) Acetylcholinesterase as a biomarker of exposure to neurotoxic compounds in the clam *Tapes philippinarum* from the Lagoon of Venice. *Mar Pollut Bull* 50: 1686-1693.
 49. Cappello T, Vitale V, Oliva S, Villari V, Mauceri A, Fasulo S, Maisano M (2017) Alteration of neurotransmission and skeletogenesis in sea urchin *Arbacia lixula* embryos exposed to copper oxide nanoparticles. *Comb Biochem Physiol Part C Toxicol Pharmacol* 199: 20-27.
 50. Aguirre Martínez GV, André C, Gagné F, Martín Díaz LM (2018) The effects of human drugs in *Corbicula fluminea*. Assessment of neurotoxicity, inflammation, gametogenic activity, and energy status. *Ecotoxicol Environ Saf* 148: 652-663.
 51. Trombini C, Hampel M, Blasco J (2019) Assessing the effect of human pharmaceuticals (carbamazepine, diclofenac and ibuprofen) on the marine clam *Ruditapes philippinarum*: An integrative and multibiomarker approach. *Aquat Toxicol* 208: 146-156.
 52. Choi JS, Kim K, Hong SH, Park KI, Park JW (2021) Impact of polyethylene terephthalate microfiber length on cellular responses in the Mediterranean mussel *Mytilus galloprovincialis*. *Mar Environ Res* 168: 105320.
 53. Dellali M, Hedfi A, Ali M, Ben Noureldeen A, Darwish H, et al. (2021) Multi-biomarker approach in *Mytilus galloprovincialis* and *Ruditapes decussatus* as a predictor of pelago-benthic responses after exposure to Benzo[a]Pyrene. *Comp Biochem Physiol Part C Toxicol Pharmacol* 249: 109141.
 54. Tolussi CE, Gomes ADO, Kumar A, Ribeiro CS, Lo Nostro FL, et al. (2018) Environmental pollution affects molecular and biochemical responses during gonadal maturation of *Astyanax fasciatus* (Teleostei: Characiformes: Characidae). *Ecotoxicol Environ Saf* 147: 926-934.
 55. Song W, Xie C, Hao S (2021). Developmental and Histopathological Changes in the Gonads of Female Japanese Medaka Following Exposure to Water from the Yellow River, China. *Bull Environ Contam Toxicol* 106: 765-772.
 56. Han Y, Shi W, Tang Y, Zhou W, Sun H, et al. (2022) Microplastics and bisphenol A hamper gonadal development of whiteleg shrimp (*Litopenaeus vannamei*) by interfering with metabolism and disrupting hormone regulation. *Sci Total Environ* 810: 152354.
 57. Fernández González LE, Sánchez Marín P, Gestal C, Beiras R, Diz AP (2021) Vitellogenin gene expression in marine mussels exposed to ethinylestradiol: No induction at the transcriptional level. *Mar Environ Res* 168: 105315.
 58. Mohammed A (2013) Why are Early Life Stages of Aquatic Organisms more Sensitive to Toxicants than

- Adults?. In: *New Insights into Toxicity and Drug Testing*. Intech Open.
59. Orozco Hernández JM, Gómez Oliván LM, Elizalde Velázquez GA, Heredia García G, Cardoso Vera JD, et al. (2022) Effects of oxidative stress induced by environmental relevant concentrations of fluoxetine on the embryonic development on *Danio rerio*. *Sci Total Environ* 807(3): 151048.
 60. Wang WQ, Chen HH, Zhao WJ, Fang KM, Sun HJ, et al. (2022) Ecotoxicological assessment of spent battery extract using zebrafish embryotoxicity test: A multi-biomarker approach. *Chemosphere* 287: 132120.
 61. González González ED, Gómez Oliván LM, Islas Flores H, Galar Martínez M (2021) Developmental Effects of Amoxicillin at Environmentally Relevant Concentration Using Zebrafish Embryotoxicity Test (ZET). *Water Air Soil Pollut* 232: 196.
 62. Capolupo M, Franzellitti S, Valbonesi P, Lanzas CS, Fabbri E (2018) Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. *Environ. Pollut* 241: 1038-1047.
 63. Tato T, Salgueiro González N, León VM, González S, Beiras R (2018) Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (*Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Acartia clausi*). *Environ Pollut* 232: 173-182.
 64. Markich SJ (2021) Comparative embryo/larval sensitivity of Australian marine bivalves to ten metals: A disjunct between physiology and phylogeny. *Sci Total Environ* 789: 147988.
 65. Fabbri R, Montagna M, Balbi T, Raffo E, Palumbo F, et al. (2014) Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in *Mytilus galloprovincialis*. *Mar Environ Res* 99: 1-8.
 66. Passarelli MC, Riba I, Cesar A, DelValls TA (2018) What is the best endpoint for assessing environmental risk associated with acidification caused by CO₂ enrichment using mussels?. *Mar Pollut Bull* 128: 379-389.
 67. Benedetti M, Giuliani ME, Mezzelani M, Nardi A, Pittura L, et al. (2022) Emerging environmental stressors and oxidative pathways in marine organisms: Current knowledge on regulation mechanisms and functional effects. *BIOCELL*.
 68. Regoli F, Giuliani ME (2014) Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Mar Environ Res* 93: 106-117.
 69. Muñoz Peñuela M, Lo Nostro FL, Dal Olivo Gomes A, Tolussi CE, Branco GS, et al. (2021) Diclofenac and caffeine inhibit hepatic antioxidant enzymes in the freshwater fish *Astyanax altiparanae* (Teleostei: Characiformes). *Comp Biochem Physiol Part C Toxicol Pharmacol* 240: 108910.
 70. Aguirre Martínez GV, Del Valls TA, Martín Díaz ML (2013) Identification of biomarkers responsive to chronic exposure to pharmaceuticals in target tissues of *Carcinus maenas*. *Mar Environ Res* 87-88: 1-11.
 71. Natalotto A, Sureda A, Maisano M, Spanò N, Mauceri A, et al. (2015) Biomarkers of environmental stress in gills of *Pinna nobilis* (Linnaeus 1758) from Balearic Island. *Ecotoxicol Environ Saf* 122: 9-16.
 72. Freitas R, Silvestro S, Coppola F, Meucci V, Battaglia F, et al. (2019) Biochemical and physiological responses induced in *Mytilus galloprovincialis* after a chronic exposure to salicylic acid. *Aquat Toxicol* 214: 105258.
 73. Gonçalves JM, Sousa VS, Teixeira MR, Bebianno MJ (2022) Chronic toxicity of polystyrene nanoparticles in the marine mussel *Mytilus galloprovincialis*. *Chemosphere* 287: 132356.
 74. Magara G, Khan FR, Pinti M, Syberg K, Inzirillo A, et al. (2019) Effects of combined exposures of fluoranthene and polyethylene or polyhydroxybutyrate microplastics on oxidative stress biomarkers in the blue mussel (*Mytilus edulis*). *J Toxicol Environ Heal Part A* 82: 616-625.
 75. Coppola F, Bessa A, Henriques B, Russo T, Soares AMVM, et al. (2020) Oxidative stress, metabolic and histopathological alterations in mussels exposed to remediated seawater by GO-PEI after contamination with mercury. *Comp. Biochem. Physiol. Part A Mol. Integr Physiol* 243: 110674.
 76. Milan M, Pauletto M, Patarnello T, Bargelloni L, Marin MG, et al. (2013) Gene transcription and biomarker responses in the clam *Ruditapes philippinarum* after exposure to ibuprofen. *Aquat Toxicol* 126: 17-29.
 77. De Domenico E, Mauceri A, Giordano D, Maisano M, Giannetto A, et al. (2013) Biological responses of juvenile European sea bass (*Dicentrarchus labrax*) exposed to contaminated sediments. *Ecotoxicol Environ Saf* 97: 114-123.

78. Costa C, Semedo M, Machado SP, Cunha V, Ferreira M, et al. (2021) Transcriptional analyses reveal different mechanism of toxicity for a chronic exposure to fluoxetine and venlafaxine on the brain of the marine fish *Dicentrarchus labrax*. *Comp Biochem Physiol Part C Toxicol Pharmacol* 250: 109170.
79. Casini S, Caliani I, Giannetti M, Marsili L, Maltese S, et al. (2018) First ecotoxicological assessment of *Caretta caretta* (Linnaeus, 1758) in the Mediterranean Sea using an integrated nondestructive protocol. *Sci Total Environ* 631-632: 1221-1233.
80. Barranger A, Rance GA, Aminot Y, Dallas LJ, Sforzini S, et al. (2019) An integrated approach to determine interactive genotoxic and global gene expression effects of multiwalled carbon nanotubes (MWCNTs) and benzo[a]pyrene (BaP) on marine mussels: evidence of reverse 'Trojan Horse' effects. *Nanotoxicology* 13: 1324-1343.
81. Kournoutou GG, Giannopoulou PC, Sazakli E, Leotsinidis M, Kalpaxis DL, et al. (2020) Oxidative Damage of Mussels Living in Seawater Enriched with Trace Metals, from the Viewpoint of Proteins Expression and Modification. *Toxics*.
82. Zhang X, Xia P, Wang P, Yang J, Baird DJ (2018) Omics Advances in Ecotoxicology. *Environ Sci Technol* 52: 3842-3851.
83. Garcia Reyero N, Perkins EJ (2011) Systems biology: Leading the revolution in ecotoxicology. *Environ Toxicol Chem* 30: 265-273.
84. Cappello T (2020) NMR-Based Metabolomics of Aquatic Organisms. eMagRes, Major Reference Works.
85. Cappello T, Giannetto A, Parrino V, Maisano M, Oliva S, et al. (2018) Baseline levels of metabolites in different tissues of mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). *Comp Biochem Physiol Part D Genomics Proteomics* 26.
86. De Marco G, Brandão F, Pereira P, Pacheco M, Cappello T (2022) Organ-Specific Metabolome Deciphering Cell Pathways to Cope with Mercury in Wild Fish (Golden Grey Mullet *Chelon auratus*). *Anim*.
87. Nagato EG, D'eon JC, Lankadurai BP, Poirier DG, Reiner EJ, et al. (2013) ¹H NMR-based metabolomics investigation of *Daphnia magna* responses to sub-lethal exposure to arsenic, copper and lithium. *Chemosphere* 93: 331-337.
88. Liu F, Lu Z, Wu H, Ji C (2019) Dose-dependent effects induced by cadmium in polychaete *Perinereis aibuhitensis*. *Ecotoxicol Environ Saf* 169: 714-721.
89. Fasulo S, Iacono F, Cappello T, Corsaro C, Maisano M, et al. (2012) Metabolomic investigation of *Mytilus galloprovincialis* (Lamarck 1819) caged in aquatic environments. *Ecotoxicol Environ Saf* 84: 139-146.
90. Ellis RP, Spicer JI, Byrne JJ, Sommer U, Viant MR, et al. (2014) ¹H NMR Metabolomics Reveals Contrasting Response by Male and Female Mussels Exposed to Reduced Seawater pH, Increased Temperature, and a Pathogen. *Environ Sci Technol* 48: 7044-7052.
91. May MA, Bishop KD, Rawson PD (2017) NMR Profiling of Metabolites in Larval and Juvenile Blue Mussels (*Mytilus edulis*) under Ambient and Low Salinity Conditions. *Metab*.
92. Sanchís J, Llorca M, Olmos M, Schirinzi GF, Bosch Orea C, et al. (2018) Metabolic Responses of *Mytilus galloprovincialis* to Fullerenes in Mesocosm Exposure Experiments. *Environ. Sci Technol* 52: 1002-1013.

