PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

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

Name of PhD student: Michela Panni

Title of PhD research:
Presence, behaviour and risk assessment of pharmaceutical products in aquatic ecosystems

Name of PhD supervisor: Prof. Stefania Gorbi

Research lab name: Ecotoxicology and Environmental Chemistry

Cycle:
XXXVII

PhD Curriculum:
Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
Actea Mobile Laboratory
Advanced Instrumentation lab
Aquarium

ABSTRACT:
In recent years, interest on the potential consequences of Active Pharmaceutical Ingredients (APIs) in aquatic ecosystem is increased, due to their ubiquitous presence in water systems and their potential adverse long-term effects on non-target species. Since the information on occurrence, bioaccumulation and biological consequences in coastal areas are still limited, the overall aim of my PhD study is to clarify the ecotoxicological impact of APIs, integrating field studies with laboratory experiments on single APIs and mixtures in different model species. Results on field investigation will provide the first survey on spatial and temporal distribution on APIs occurrence in seawater, sediments and wild organisms in the Adriatic Sea. Outcomes on *in vivo* tests on *M. galloprovincialis* highlighted interactive mechanisms between tested drugs and the ability of single compounds to modulate various metabolic pathways as the oxidative and the immune systems. Results on *in vitro* and *ex vivo* tests will help to define the molecular target of different therapeutic classes. Overall results will be finally integrated through a Weight of Evidence model, to develop the first pharmaceuticals environmental risk assessment procedure in marine ecosystems.

Part 1. Scientific case of the PhD Research

- BACKGROUND
Active Pharmaceutical Ingredients (APIs) are well documented contaminants of emerging concern due to their ubiquitous presence in aquatic ecosystems. Compounds belonging to different therapeutic classes, as Non-

Steroidal Anti- Inflammatory drugs (NSAIDs), lipid-lowering agents, cardiovascular and psychiatric drugs, were detected in various environmental matrices, including sea water, sediments and biota at concentrations ranging from ng to µg per L or Kg (Almeida et al., 2021, Madikizela et al., 2020; Mezzelani and Regoli, 2022). Although the evidence of APIs uptake in microalgae, mollusks, crustaceans and fishes (Swiacka et al., 2019, Mezzelani et al., 2020) their capability to interfere with physiological and biochemical processes detailed information on distribution along European coastal areas and long-term adverse to marine species is still limited (Trombini et al., 2019, Cravo et al., 2022). Furthermore, wild organisms are continuously exposed to a complex mixture of pollutants, including pharmaceuticals, which, based on their specific chemical-physical proprieties and modes of action (MOAs), can act on the same metabolic targets causing interactions which lead to antagonistic, additive or synergistic effects (Mezzelani and Regoli, 2022).
All these aspects have been so far overshadowed and limited investigated.
For this reason, the aim of my PhD study is to clarify the ecotoxicological impact of APIs, both characterizing their occurrence in seawater, sediments and uptake in wild species and investigating under laboratory conditions the onset of adverse effects of both single molecules and mixtures, toward a final development of an environmental risk assessment procedure.

- SCIENTIFIC AIMS

The overall aim of my PhD project is to study the ecotoxicological impact of APIs in marine ecosystems: (a) investigating the occurrence, spatial and temporal distribution of pharmaceuticals in seawater, sediments and marine wild species along the Adriatic Sea; (b) evaluating the bioaccumulation and the onset of sublethal adverse effects of both single molecules and mixtures, by using different approaches under laboratory conditions, including *in vivo*, *ex vivo* and *in vitro* tests, on a wide array of model species belonging to different trophic levels; (c) to finally develop the first comprehensive environmental risk assessment procedure for pharmaceuticals in marine ecosystems.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Field investigation: pharmaceuticals along the Adriatic Coasts

Objective: the aim of the field investigation is to study the APIs occurrence along the Adriatic Sea, characterizing temporal and spatial variation in water column, sediment and wild marine species.
Methods: three areas characterized by different environmental and anthropogenic pressure were selected in the North, Central and South-Adriatic Sea, in which were collected water samples with pump by filling-up two bottles (500mL) in each sampling site; sediments with the Van Veen grab (500g); and species of commercial interest belonging to different trophic levels (*Merluccius merluccius*, *Mullus barbatus*, *Solea solea*, *Squilla mantis, Mytilus galloprovincalis*) by local fisherman. Water and sediments were stored at 4°C and -20°C; while liver and muscle tissues (n= 20) from fishes, and the entire soft tissues (n=20) from invertebrates were stored at -20°C. All samples will be processed for the extraction, detection and quantification of APIs through liquid chromatography-mass spectrometry (LC/MS) techniques.
Expected/Obtained Results: the adaption and development of new analytical methods through liquid chromatography-mass spectrometry (LC/MS) techniques allowing the simultaneously extraction, detection and quantification of various APIs from both abiotic and biotic matrices, are still ongoing. Results will provide the first investigation on spatial and temporal distribution on pharmaceuticals occurrence in seawater, sediments and selected species in the Adriatic Sea.

WP 2. Laboratory studies: bioaccumulation and sublethal effects of pharmaceuticals in marine species

Objective: the overall aim is to investigate short and long-term adverse effects caused by single pharmaceuticals and APIs mixtures in various aquatic species, by using different approaches under laboratory conditions, including *in vivo*, *ex vivo* and *in vitro* tests.

***In vivo* tests: mussel experimental plans.** *Ecotoxicological potential of single pharmaceuticals and their mixture in model organism M. galloprovincialis.*

Methods: Four different experimental plans were set up exposing the marine mussel *M. galloprovincialis* to environmentally realistic concentrations (ranging from 0.1 - 2.5 μg/L) of APIs belonging to the main therapeutic classes detected in the marine environment. Pharmaceuticals were tested alone and in mixtures. The main aims of these studies were to investigate (1) the interaction among different therapeutic classes of APIs, (2) influence of single APIs on mussels' lipid metabolism, and (3) the toxicity of APIs mixtures in relation to climate change scenario (marine heatwaves, ocean acidification) in order to provide novel insights on the mechanisms that can modulate bioaccumulation, excretion and onset of sublethal effects in *M. galloprovincialis*. A multidisciplinary approach was applied integrating drugs bioaccumulation with RNA-sequencing of transcriptomic responses and a wide panel of biochemical, cellular and histological markers, including immunological responses (lysosomal membrane stability, phagocytosis rate and granulocytes/hyalinocytes ratio), genotoxic damage (loss of DNA integrity and micronuclei frequency), neurotoxic effects (acetylcholinesterase activity), peroxisomal proliferation (Acyl-CoA oxidase activity), single antioxidant enzymes (catalase, glutathione S-transferases, Se-dependent glutathione peroxidases, the sum of Se-dependent and Se-independent glutathione peroxidases and glutathione reductase), total glutathione, the total oxyradical scavenging capacity toward peroxyl (ROO•) and hydroxyl (HO•) radicals, accumulation of malondialdehyde, lipofuscin and neutral lipids (Bocchetti et al., 2008). Overall results will finally be integrated through a quantitative Weight Of Evidence model (WOE, Sediqualsoft), that elaborates specific hazard indices based on the number, magnitude and toxicological relevance of the observed responses (Regoli et al., 2019).

Expected/Obtained Results:

Experiment 1. *Mussels' 30-day exposure to the NSAIDs ibuprofen (1μg/L), the antidepressant paroxetine (1μg/L) and their mixture followed by 14 days in pharmaceuticals-free seawater.*

Results of chemical analysis highlighted a different bioaccumulation pattern in single compared to mixture-exposed organisms. Co-exposure lowered IBU accumulation while did not influence PAR levels, suggesting a possible competing mechanism between the two molecules for the same cellular transporters with PAR having a higher affinity for them compared to IBU. Although clear interactive effects were not measured, most of the biological pathways in organisms exposed to the mixture showed a pattern of variation similar to organisms treated with Par alone, corroborating the results from bioaccumulation. In general, obtained outcomes highlighted a modulation of the immune system, lipid and oxidative metabolisms, such effects were maintained even after the depuration phase highlighted a long-lasting effect of tested pharmaceuticals, despite the tissue concentrations of both compounds were <LOD. Overall results elaborated with the WOE approach revealed a "MODERATE" risk for all treatments during the exposure phase, confirming the lack of clear synergisms between tested compounds, and a "SLIGHT" risk after the depuration phase highlighting the mussels' ability to recover from the harmful effects caused by tested pharmaceuticals in a relatively short time (Fig.1).

Experiment 2. *Mussels' 30-day exposure to the antidepressant venlafaxine (1μg/L), the lipid regulating agent gemfibrozil (1μg/L), the antihypertensive ramipril (1μg/L), the antidiabetic metformin (1μg/L) and their mixture followed by 14 days in pharmaceuticals-free seawater.*

The overall results on biological parameters highlighted the immune and the antioxidant systems as the main pathways modulated by all tested pharmaceuticals, with evidence of interactive effects for the mixture. Outcomes of the immune system showed the lysosomal membrane stability and the phagocytosis capacity of hemocytes as the most sensitive endpoints for the tested drugs, indicating the lipid regulating agent gemfibrozil and the antihypertensive ramipril as the most effective among the investigated pharmaceuticals. Such effects were measured both after the exposure and depuration phases, indicating a long-term effect on the immune system modulation by tested drugs. Results on antioxidant system highlighted an induction of glutathione-S-

transferase (GST) and a consequent decrease in level of total glutathione (GSH) for all treatments, suggesting phase II conjugation reactions as one of the key processes for tested pharmaceuticals metabolism in *M. galloprovincialis*. These results will be integrated with the ongoing analyses on bioaccumulation.

Experiment 3. *Mussels' 14-day exposure to the lipid regulating agent gemfibrozil (2.5µg/L), the antidiabetic metformin (2.5µg/L) and the antidiabetic rosiglitazone (2.5µg/L).*

Analysis on bioaccumulation and biological responses are still ongoing. Overall results will allow to clarify the interactions between tested pharmaceuticals and mussels' lipid metabolism.

Experiment 4. *Mussels' 42-day exposure to clean or pharmaceuticals contaminated artificial seawater either at constant seasonal environmental temperature and/or pH or under marine heatwave and/or reduced-pH scenario, according to the seven following treatments: CTRL, control condition at seasonal climatological sea surface temperature and pH (TCSST = 18 °C; pH = 8.10/pCO_2= ~400 µatm); MIX, pharmaceuticals mixtures exposure at TCSST = 18 °C and pH = 8.10/pCO_2= ~400 µatm; MHW, marine heatwave scenario (two consecutive heatwaves, peaking T=23°C, duration = 10 days); reduced pH/hypercapnia, ACD (pH= 7.6, pCO_2=1700µatm); MHW + MIX, pharmaceuticals mixture exposure under marine heatwave scenario; ACD + MIX, pharmaceuticals mixture exposure under reduced-pH condition; MHW+ ASD, marine heatwave and reduced-pH scenario; MHW+ACD+MIX, pharmaceuticals mixture exposure under marine heatwave and reduced-pH scenario.*

APIs mixture was composed of environmentally realistic concentrations of the main pharmaceuticals detected in marine environment: carbamazepine (1.5µg/L), ibuprofen (1.5µg/L), metformin (1.5µg/L), venlafaxine (0.3 µg/L), ramipril (0.1 µg/L) and gemfibrozil (0.4µg/L).

Analysis on bioaccumulation and biological responses are still ongoing. Obtained results will provide novel information about the effects of climate change on pharmaceutical mixtures toxicity in non-target marine organisms.

A

TREATMENT	LOE 3 BIOAVAILABILITY	LOE 4 BIOMARKER	WOE INTEGRATION	LEVEL OF RISK
IBU	MAJOR HQ: 7.96	MODERATE HQ: 212.61	MODERATE	
PAR	MAJOR HQ: 12.82	MODERATE HQ: 163.68	MODERATE	
IBU + PAR	MAJOR HQ: 7.3	MODERATE HQ: 176.92	MODERATE	

B

TREATMENT	LOE 3 BIOAVAILABILITY	LOE 4 BIOMARKER	WOE INTEGRATION	LEVEL OF RISK
IBU	ABSENT HQ: 0	MODERATE HQ: 203.21	SLIGHT	
PAR	ABSENT HQ: 0	MODERATE HQ: 184.62	SLIGHT	
IBU + PAR	ABSENT HQ: 0	MODERATE HQ: 192.31	SLIGHT	

Figure 1. Experiment 1: weighted elaboration of whole dataset for each treatment at (A) exposure phase (day 30) and (B) depuration phase (day 44).

***In vivo* tests: ecotoxicology bioassays.** *Application of a battery of bioassays within the assessment procedure for the ecotoxicological impact of environmental pharmaceuticals.*

Methods: a panel of 15 pharmaceuticals belonging to different therapeutic classes (Non-steroidal Antinflammatory drugs, psychiatric drugs, cardiovascular drugs and lipid lowering agents) were tested at two different environmentally realistic concentrations (1µg/L and 10 µg/L) through a battery of bioassays which includes the algal growth inhibition of *Phaeodactylum tricornutum*, the embryotoxicity assay with *Paracentrotus lividus* and *Crassostea gigas* and the bioluminescence test with *Aliivibrio fischeri*. For some of these molecules their metabolites have also been tested. Pharmaceuticals were also tested as mixtures, choosing six different combinations based on: (a) MOA of single molecule, (b) combinations of pharmaceuticals

typically measured in the environment and (c) mixtures used in human therapy. Obtained results were elaborated through the quantitative Weight of Evidence (WOE) model, which assign to each bioassay specific thresholds and weights based on the measured endpoint and sensitivity of the tested species, to finally provide a cumulative level of hazard of the entire bioassays battery.
Obtained Results: results highlighted different sensitivity of tested species toward selected pharmaceuticals and mixtures, with major effects on *P. tricornutum* and *P. lividus*. The overall elaboration with the WOE model revealed a higher hazard level for gemfibrozil (lipid lowering agent), paroxetine, carbamazepine and its metabolite (psychiatric drugs), compared to all the other tested APIs. Furthermore, higher level of hazard was measured for mixtures compared to single compounds. Quite interesting, in mixtures higher toxicity was not related to the exposure dose or to the number of combined drugs, revealing the need of future investigations to address mechanisms of interaction among these molecules.

***In vitro* tests: fish cell lines.** *Toxicity of single pharmaceuticals and mixtures in PLHC-1 and RT-gillW1 cell lines*

Methods: The experimental plan included the exposure to single APIs and mixtures at two environmental realistic concentrations (1μg/L and 10 μg/L) in two fish cell lines (PLHC-1 liver cells, derived from Topminnow *Poeciliopsis lucida*, and RT-gillW1 gill cells, derived from the Rainbow trout *Oncorhynchus mykiss)* to investigate the cell viability, the Reactive Oxygen Species (ROS) production and the mitochondrial membrane stability. Only for PLHC-1 was investigated also the capability of cells migration and proliferation with the wound healing test. Tested molecules and mixtures were the same used for the ecotoxicological bioassays, with the addition of the antidiabetic metformin and the same mixture of the second mussels' *in vivo* experiment (composed by venlafaxine, gemfibrozil, ramipril and metformin).
Obtained Results: Obtained results highlighted major effects for all measured endpoints in PLHC-1 liver cells compared to Rt-gillW1 gill cells, suggesting this tissue as the most sensitive to tested drugs. In general, the mixtures showed lower effects compared to the single molecules, with a significant variation only for the higher exposure dose. Among all tested therapeutic classes, Non-Steroidal Anti-Inflammatory drugs appear to be the least effective compared to the others. Outcomes revealed paroxetine, naproxen and atenolol as the most effective molecules for psychiatric drugs, NSAIDs and cardiovascular drugs respectively.

***Ex vivo* tests: Precision cut tissues slices (PCTS).** *Toxicity of single pharmaceuticals and mixtures PCTS of mussels' digestive glands.*

Methods: A panel of pharmaceuticals belonging to different therapeutic classes and their mixtures were tested in PCTS of mussels' digestive glands. Standardized method (Giuliani et al., 2019) involved the excised of mussels' digestive glands, the inclusion of the tissue in agarose (2.5%) and the cut in slices of 300μm thickness slices using a motorized vibrating blade vibratome. This experimental plan was aimed to study the mechanisms of action of single drugs and the possible mechanisms of interaction between APIs mixtures, investigating the transcriptome profile and the modulation of the activity of main enzymes involved in oxidative and lipid metabolisms.
Expected Results: Analysis of this experimental plan are still ongoing and will allow to provide novel insight on the ecological consequences of single APIs and mixtures for marine ecosystems.

WP 3. Outreach activities

In these two years of PhD, I participated to various dissemination events: SHARPER night (the European night of researchers, September 2021, 2022 and 2023), SostenibilMente (event on sustainability organized by Ancona Municipality, November 2021), Sealogy (The European Blue Economy Show, November 2021) and laboratories for high schools. During these events, I had the opportunity to talk about aims and activities of my PhD project to citizen, students, public and private sector experts also through the preparation of poster,

banner and brochures. My PhD activities are within a European project called PHARMASEA, founded by AquaticPollutants ERA-NET. For this reason, I had the opportunity to participate in monthly and annual meetings with the project partners which aimed to discuss about the activities carried out by the various research groups.

- REFERENCES

Almeida, A., Soares, A.M.V.M., Esteves, V.I., Freitas, R., 2021. **Occurrence of the antiepileptic carbamazepine in water and bivalves from marine environments: a review.** Environ. Toxicol. Pharmacol. 86. https://doi.org/10.1016/j. etap.2021.103661.

Bocchetti R., Lamberti C.V., Pisanelli B., Razzetti E.M., Maggi C., Catalano B., Sesta G., Martuccio G., Gabellini M., Regoli F., 2008. **Seasonal variations of exposure biomarkers, oxidative stress responses and cell damage in the clams,** *Tapes philippinarum***, and mussels,** *Mytilus galloprovincialis***, from Adriatic Sea**. Mar. Environ. Res., 66 (1), pp. 24-26, 10.1016/j.marenvres.2008.02.013

Cravo A., Silva S., Rodrigues J., Cardoso V.V., Benoliel M.J., Correia C., Coelho M.R., Rosa M.J., Almeida C.M.M., 2022. **Understanding the bioaccumulation of pharmaceutical active compounds by clams Ruditapes decussatus exposed to a UWWTP discharge**. Environ. Res., 208, Article 112632, 10.1016/j.envres.2021.112632

Giuliani, M. E., Sparaventi, E., Lanzoni, I., Pittura, L., Regoli, F., Gorbi, S., 2019. **Precision-Cut Tissue Slices (PCTS) from the digestive gland of the Mediterranean mussel *Mytilus galloprovincialis*: An *ex vivo* approach for molecular and cellular responses in marine invertebrates**. Toxicology in Vitro, 61, 104603. https://doi.org/10.1016/j.tiv.2019.104603

Madikizela, L.M., Ncube, S., Tutu, H., Richards, H., Newman, B., Ndungu, K., Chimuka, L., 2020. **Pharmaceuticals and their metabolites in the marine environment: sources, analytical methods and occurrence.** Trends Environ. Anal. Chem. 28. https://doi.org/10.1016/ j.teac.2020.e00104.

Mezzelani M., Fattorini D., Gorbi S., Nigro M., Regoli F., 2020. **Human pharmaceuticals in marine mussels: evidence of sneaky hazard along Italian coasts**. Mar. Environ. Res., 162, Article 105137, 10.1016/j.marenvres.2020.105137

Mezzelani M, Regoli F. 2022. **The biological effects of pharmaceuticals in the marine environment.** Annual Review of Marine Science 14:105–128. https://doi.org/10.1146/annurev-marine-040821-075606.

Regoli, F., d'Errico, G., Nardi, A., Mezzelani, M., Fattorini, D., Benedetti, M., Di Carlo, M., Pellegrini, D., Gorbi, S., 2019. **Application of a weight of evidence approach for monitor- ing complex environmental scenarios: the case-study of offshore platforms.** Front. Mar. Sci. 6. https://doi.org/10.3389/fmars.2019.00377.

Swiacka, K., Maculewicz, J., Smolarz, K., Szaniawska, A., Caban, M., 2019. Mytilidae as model organisms in the marine ecotoxicology of pharmaceuticals - a review. Environ. Pollut. 254, 113082. https://doi.org/10.1016/j.envpol.2019.113082.

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, pp. 146-156

Part 2. PhD student information on the overall year activity

List of attended courses/seminars/schools

1. "The resolution revolution in Cryo-electron-microscopy, in Structural Biology and in Life Sciences", Martino Bolognesi, Department of Biosciences, University of Milan, 7 June 2022.

2. "Current threats to research ethics and how to cope with them", Marco Seeber, Department of Political Science and Management, University of Agder, Norway, 9 June 2022.
3. “Cambiamenti climatici, comunità e Sindaci resilienti: contributi e riflessioni con il mondo universitario”, Università Politecnica Marche, Facoltà Di Ingegneria, 12 October 2022.
4. “Ecotoxicology under global changes: impacts in marine wildlife”, Rosa Freitas, Departamento de Biologia, Universidade de Aveiro. 27 April 2023.
5. 2nd Workshop of SETAC Italian Language Branch. Consiglio Nazionale delle Ricerche, Roma 11 October 2023.

List of periods spent abroad

1. Exchanging period (17th March- 23rd June 2023) at the University of Stavanger (UiS), Norway.
Activities: *In vitro* test in fish cell lines (PLHC-1 liver cells; RTgill-W1 gill cells): exposure to single pharmaceuticals and mixtures to evaluate cell viability, ROS production and mitochondrial membrane stability.

List of conferences/workshops attended and of contributions eventually presented

1. **M. Panni**, M. Mezzelani, G. d’Errico, M. Benedetti, S. Gorbi, F. Regoli (2023). Application of a battery of bioassays within the assessment procedure for the ecotoxicological impact of environmental pharmaceuticals. SETAC Europe, Dublin, 30 April - 4 May 2023.
2. M. Mezzelani, **M. Panni**, G. d’Errico, M. Benedetti, S. Gorbi, F. Regoli (2023). Molecular, biochemical and cellular effects of single and combined pharmaceuticals in *Mytilus galloprovincialis*. SETAC Europe, Dublin, 30 April - 4 May 2023.
3. M. del Mar Garcìa Pimentel, J.A. Campillo, J. Valdés, J.M. Castano-Ortiz, S. Rodriguez-Mozaz, M. Mezzelani, S. Gorbi, **M. Panni**, F. Regoli, V.M.Leòn (2023). Biological effects of simultaneous and separated exposure of mussels to citalopram/bezafibrate and polyethylene microplastics in seawater. SETAC Europe, Dublin, 30 April - 4 May 2023.
4. M.del Mar Garciìa Pimentel, J.A. Campillo, V.M. Leòn, J. Valdés, J.M. Castano-Ortiz, S. Rodriguez-Mozaz, M. Mezzelani, **M. Panni**, S. Gorbi, F. Regoli (2023). Biological responses in digestive gland of mussels (*Mytilus galloprovincialis*) exposed to citalopram/bezafibrate and polyethylene microplastics in seawater. SETAC Europe, Dublin, 30 April - 4 May 2023.
5. Regoli, T. Braunbeck, D. M. Pampanin, S. M. Lorenzo, V. M. León, M. Mezzelani, S. Gorbi, **M. Panni** et al. (2023). Presence, Behavior and Risk Assessment of Pharmaceuticals in European Marine Ecosystem. SETAC Europe, Dublin, 30 April - 4 May 2023.
6. **M. Panni**, M. Mezzelani, G. d’Errico, M. Benedetti, S. Gorbi, F. Regoli (2022). Application of a battery of bioassays within the assessment procedure for the ecotoxicological impact of environmental pharmaceuticals. SETAC Italia, Siena, 15 September 2022.
7. M. Mezzelani, L. Peruzza, I. Bernardini, F. Buttari, S. Gorbi, G. d’Errico, **M. Panni**, D. Fattorini, M. Milan, F. Regoli (2022). Interactive effects of pharmaceuticals’ mixtures in *Mytilus galloprovincialis.* Symposium on Pollutant Responses In Marine Organisms (PRIMO21), Gothenburg, Sweden, 22-25 May 2022.

Part 3. PhD student information on publications

List of publications on international journals

1. **M. Panni**, M. Mezzelani, G. d’Errico, S. Gorbi, F. Regoli. Evidence on modulation of *M. galloprovincialis* metabolic pathways by environmental pharmaceuticals: first insights on ibuprofen, paroxetine and their mixture.
In preparation for Environmental Research

2. **M. Panni**, M. Mezzelani, G. d'Errico, S. Gorbi, F. Regoli, D.M. Pampanin. Toxicity assessment of environmental pharmaceuticals and their mixture using a cell-based bioassays from fish.
   In preparation for Environmental Toxicology and Pharmacology
3. M.E. Giuliani, M. Mezzelani, **M. Panni**, S. Gorbi. Evidence of similar hypolipidemic function of Fenofibrate and the natural bioactive compound Caulerpin in Precision Cut-Tissue Slices (PCTS) of *Mytilus galloprovincialis*.
   In preparation for the Special issue in Marine drugs (MDPI)
4. **M. Panni**, M. Mezzelani, G. d'Errico, S. Gorbi, F. Regoli. Application of a battery of bioassays within the assessment procedure for the ecotoxicological impact of environmental pharmaceuticals.
   In preparation for Environmental Research

Ancona, 13 ottobre 2023

Student signature

Supervisor signature