Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Presence, behavior and risk assessment of pharmaceutical products in aquatic ecosystems

PhD student: Michela Panni

DiSVA, Laboratorio di Ecotossicologia e Chimica Ambientale

Tutor: Prof. Stefania Gorbi

INTRODUCTION

Active Pharmaceutical Ingredients (APIs) are considered contaminants of emerging concern. The massive use in human and veterinary medicine and a limited removal by wastewater treatment plants (WWTPs) [1] are the causes of their simultaneous occurrence in seawater, sediment and aquatic biota [2]. Designed to be effective at very low concentrations, APIs can interfere with biochemical and physiological processes in non-target species [3] with virtually unknown long - term effects. The investigation on their occurrence, bioaccumulation and adverse effects in coastal ecosystems are still limited and represent a research priority to characterize the risk for aquatic species.

AIMS

The overall aim of this PhD study is to clarify the ecotoxicological impact of APIs: (i) characterizing their occurrence in wild species, sediments and seawater from the Adriatic Sea and (ii) investigating the onset of adverse effects under laboratory conditions, toward a final (iii) development of a pharmaceutical environmental risk assessment procedure.

FIELD STUDIES: PHARMACEUTICALS ALONG THE ADRIATIC COSAT

METHODS: SAMPLING AREA

High anthropic impact site
Porto Garibaldi

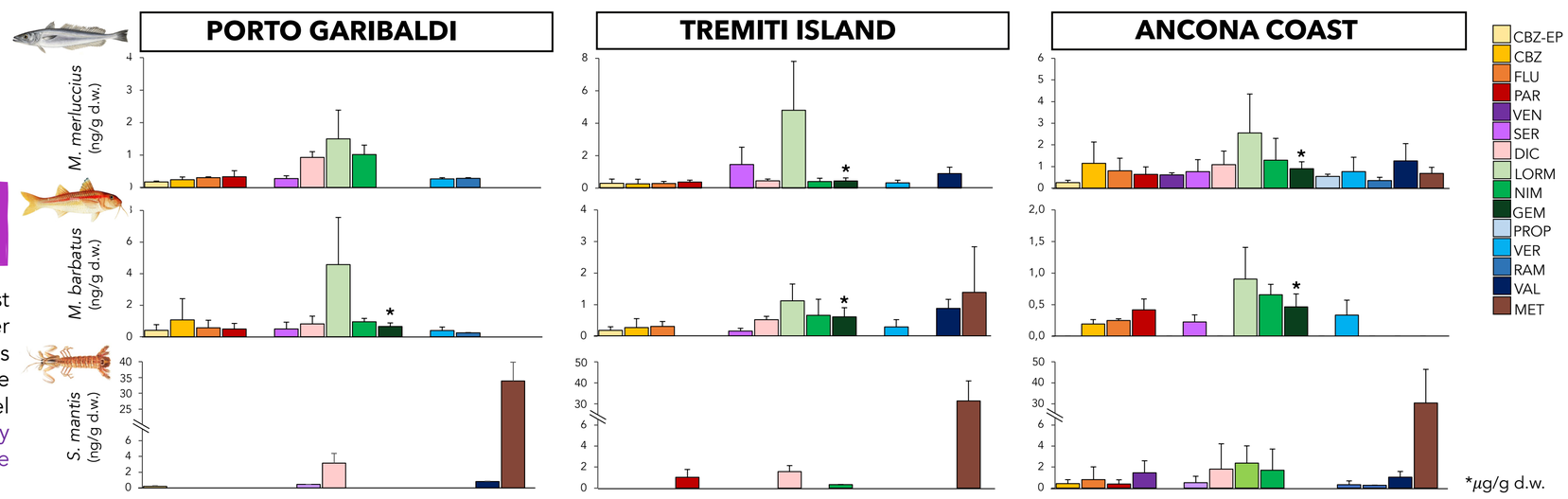
Ancona coast
WWTPs area and Civitanova Marche

Reference site
Tremiti Island

SAMPLING PERIODS: WINTER AND SUMMER

Sediments and seawater were sampled, while species of commercial interest belonging to different trophic levels were obtained from local fishermen. Liver and muscle tissues were dissected from the benthopelagic and benthic fishes including *Merluccius merluccius*, *Mullus barbatus* and *Solea solea*, while the entire soft tissues were collected from crustaceans *Squilla mantis* and mussel *Mytilus gallorpovincialis*. All samples were analyzed. In the present poster only results on levels of APIs measured in marine biota sampled in winter 2022 will be shown.

RESULTS: BIOACCUMULATION IN MARINE BIOTA - WINTER 2022



LABORATORY INVESTIGATION: BIOACCUMULATION AND SUBLETHAL EFFECTS IN NON-TARGET SPECIES

METHODS: EXPERIMENTAL APPROACHES

In vivo experiment: mussels exposure and ecotoxicological bioassays

MUSSEL EXPOSURES

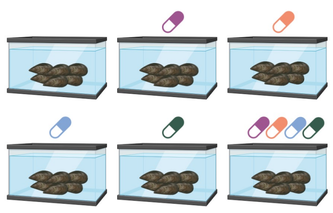
Four different experimental plans were set up:

1) Ibuprofen, paroxetine and mixture
2) Venlafaxine, gemfibrozil, ramipril, metformin and mixture
(1 µg/L, 30-days of exposure + 14-days depuration)

3) Metformin, rosiglitazone and gemfibrozil
(2.5 µg/L, 14-days of exposure)

4) APIs mixture under marine heatwave and/or reduced-pH scenario
(42-days of exposure)

APIs mixture: Carbamazepine (1.5µg/L), ibuprofen (1.5µg/L), metformin (1.5µg/L), venlafaxine (0.3µg/L), ramipril (0.1µg/L), gemfibrozil (0.4µg/L).



BIOCHEMICAL AND CELLULAR RESPONSES

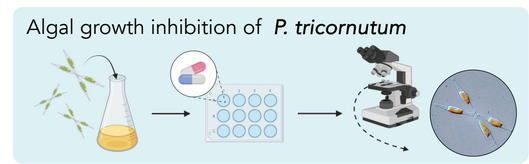
DRUGS BIOACCUMULATION

Results elaboration on mussel exposures is still ongoing.

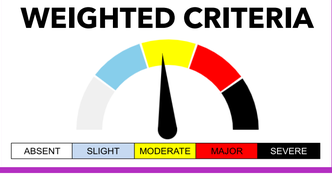
ECOTOXICOLOGICAL BIOASSAYS

The effects of a panel of 15 APIs belonging to different therapeutic classes (psychiatric drugs, Non-Steroidal Anti-Inflammatory drugs, cardiovascular drugs, lipid lowering agents and antidiabetics) and their mixtures were investigated through a battery of ecotoxicological bioassays. Pharmaceuticals were dosed at two different environmentally realistic concentrations (1µg/L and 10 µg/L).

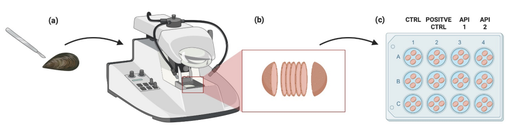
Obtained results on the ecotoxicological bioassays were shown in previous posters.



ELABORATION WITH WEIGHTED CRITERIA



Ex vivo experiment: Precision cut tissue slices (PCTS) of mussels digestive glands

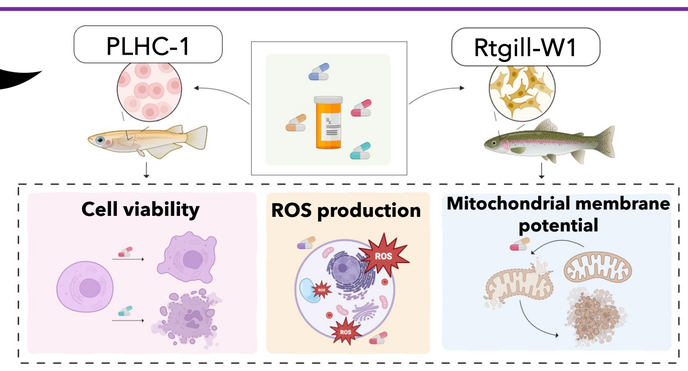


This approach includes (a) the excision of the digestive glands, (b) its inclusion in agarose and the cut of slices of 300µm thickness using a motorized vibrating blade vibratome. The PCTS produced (c) were exposed to different concentrations of APIs to investigate their mechanisms of action.

Analysis for these experimental plans are still ongoing.

In vitro experiment: fish cell lines

The mechanisms of action and toxicity of the same APIs and mixtures (1-10µg/L) tested through the ecotoxicological bioassays were investigated in fish hepatic **PLHC-1** and gill **RTgill-W1** cell lines. Cell viability, ROS production and mitochondrial membrane potential were assessed to evaluate respectively the cytotoxicity, the capability to induce oxidative stress and cell apoptosis of tested conditions.



RESULTS AND DISCUSSIONS: PLHC-1 CELL LINE

Obtained results on **PLHC-1 fish cell line** revealed antidepressants as the most reactive therapeutic class, and paroxetine, naproxen and atenolol as the most toxic molecules. In mixtures the higher toxicity was not related to the number of the combined drugs, but rather to their interactions, with evidence of antagonistic mechanisms between the class of NSAIDs and antidepressants. Elaboration of the overall biological effects with weighted criteria provided quantitative hazard indices which summarize the toxicity of several pharmaceuticals and their mixtures, allowing an easier comparison between different molecules and exposure conditions. More marked effects were measured in the branchial cell line (RTgill-W1) than in the liver one (PLHC-1) for most treatments (data not showed), suggesting the importance of an integrated approach to a more comprensive environmental risk assessments of these pollutants in aquatic ecosystems.

SINGLE PHARMACEUTICALS

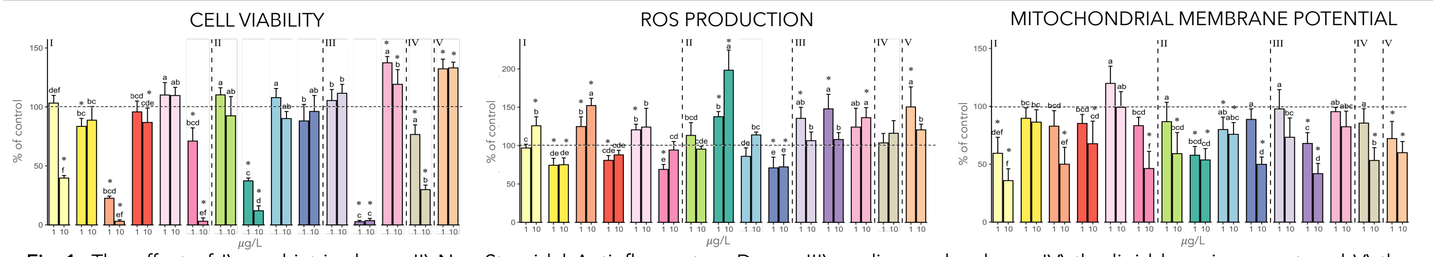


Fig 1. The effect of I) psychiatric drugs, II) Non-Steroidal Antinflammatory Drugs, III) cardiovascular drugs, IV) the lipid lowering agent and V) the antidiabetic on cell viability, ROS production and mitochondrial membrane potential in fish liver cell culture PLHC-1.

PHARMACEUTICALS MIXTURES

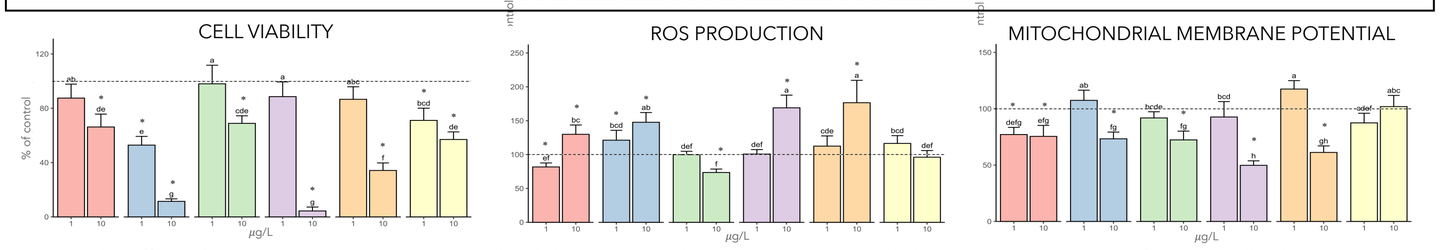


Fig 2. The effect of pharmaceutical mixtures on cell viability, ROS production and mitochondrial membrane potential in fish liver cell culture PLHC-1.

A)

Treatment	Class of hazard (1µg/L)	Class of hazard (10µg/L)
Psychiatric drugs		
VEN	ABSENT	MAJOR
O-DES	ABSENT	ABSENT
PAR	MAJOR	SEVERE
FLU	ABSENT	ABSENT
CBZ	ABSENT	ABSENT
CBZ EP	MODERATE	MAJOR
NSAIDs		
IBU	ABSENT	ABSENT
NAP	MAJOR	SEVERE
DIC	ABSENT	ABSENT
NIM	ABSENT	SLIGHT
Cardiovascular drugs		
VAL	ABSENT	ABSENT
ATE	MAJOR	MAJOR
RAM	SLIGHT	SLIGHT
Lipid lowering agents		
GEM	ABSENT	MAJOR
Antidiabetic		
MET	MODERATE	MODERATE

B)

Treatment	Class of hazard (1µg/L)	Class of hazard (10µg/L)
MIX A	ABSENT	MODERATE
MIX B	MODERATE	MAJOR
MIX C	ABSENT	MODERATE
MIX D	ABSENT	SEVERE
MIX E	ABSENT	MAJOR
MIX F	ABSENT	ABSENT

Tab 1. Weighted elaboration of results on cell viability, ROS production and mitochondrial membrane electrochemical potential damage in in PLHC-1 exposed to A) single pharmaceuticals and B) pharmaceutical mixtures.

MIX A: IBU + PAR;
MIX B: CBZ + VAL;
MIX C: IBU + PAR + CBZ + VAL;
MIX D: VEN + RAM + GEM + MET;
MIX E: VEN + NAP;
MIX F: VAL + ATE + GEM + RAM.

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