L’attività dell’assegnista è parte del Progetto **PNRR-NBFC-SPOKE 2-ACTIVITY1-ACTION 2 “ZERO POLLUTION” WP3**

**TITOLO:** POLLUTION-MEDIATED LOSS OF BIODIVERSITY IN MEDITERRANEAN AND INTERACTIONS OF EMERGING POLLUTANTS WITH CLIMATE CHANGE.

Tutor: Prof. Elena Fabbri Co-Tutor Prof Enrico Dinelli

The findings on contaminants of emerging concern (CECs) in the marine environment reflect the need for additional research on organisms from different trophic levels and habitats, including the bioaccumulation potential that, due to the lipophilic properties of many pharmaceuticals as well as other CECs, may be increased when pH decreases. We are not aware of any investigation on CECs bioaccumulation or effects in corals naturally living under different acidification conditions. The planned research therefore covers the assessment of environmental concentrations of the below described list of pharmaceuticals and endocrine disrupting chemicals (EDCs) in water and sediments at the selected sites in Panarea, and bioaccumulation in coral tissues. Sediments at the different pH conditions will be also characterized. The Panarea CO2 vent is a natural laboratory for investigating the interaction between emerging pollutants and ocean acidification on marine biodiversity. Contaminants of emerging concern (CECs) are defined as naturally occurring or industrial substances which have recently been discovered or present for a long time but only recently recognized as widely occurring and potentially dangerous (Gunaalan et al. 2020). In general, they are not yet subjected to regulatory criteria or norms for the protection of human health or the environment. CECs include, among others, endocrine disrupting chemicals (EDCs), plastic additives, pharmaceutical and personal care products (PPCPs), flame retardants; some legacy contaminants such as arsenic, lead etc. are also included (Gunaalan et al. 2020). CECs widely occur in the aquatic environment, from surface and marine waters to drinking water. Data on environmental pharmaceuticals clearly indicate their wide occurrence and impact on marine organisms, although they are found at concentrations that were considered below the threshold of toxicity (Fabbri and Franzellitti 2016). Recent studies have also shown interactive effects between seawater acidification and toxicity of pharmaceuticals and personal care products such as triclosan on fish, sea urchins, and bivalves (Freitas et al. 2016; Almeida et al. 2018; Maulvault et al. 2018; Munari et al 2018, 2019; Costa et al. 2020; Mezzelani et al. 2021; Pusceddu et al. 2022). Bisphenol A (BPA) and phthalates, known as potential EDCs (ECHA 2018; UNEP 2017), represent examples of widely investigated plastic additives (Balbi et al. 2016; Canesi and Fabbri 2015). Less studied, though widely used additives include acetophenone, which acts as copolymerization catalyst and is described as a contaminant with potential toxicity to fish and benzothiazoles, used as vulcanizing agent in tire rubber manufacture, also toxic to aquatic species; further organic compounds have been found in leachates from conventional plastics and tire rubber (Capolupo et al. 2020, 2021). To our knowledge, no information is available about the biological impact of these compounds under acidification conditions. Most studies addressed the environmental occurrence or effects of pharmaceuticals and plastic/tire additives as individual compounds, in laboratory studies; field investigations on pharmaceuticals and EDCs in mixtures and acidification conditions are crucial to understand the actual risk for aquatic flora and fauna (Capolupo et al. 2017).

The goal is to investigate the occurrence in water and sediments, and bioaccumulation of emerging contaminants along the natural ocean acidification gradient in a model species, the coral *Balanophyllia europaea*.

**Water and tissues:** chemical analysis (in water and tissues) of a selected battery of emerging contaminants, namely 17-beta-estradiol (E2), estrone (E1), 17-alfa-ethinylestradiol (EE2), 4- nonylphenol (NP), 4-octylphenol (OP), Bisphenol A (BPA), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), diclofenac, ibuprofen, caffeine, carbamazepine and atenolol, (to which we will add venlafaxine and O-desmethylvenlafaxine) will be performed. Here below the analytical methodology description previously reported in details (Valbonesi et al. 2020) with further adaptation to seawater. Seawater sample preparation: 250 mL of sea water is spiked with 50 ng/L of a mixture of labeled internal standards (E2-d2, BPA-d6, 13C4-PFOA, Ibuprofen-d3 and 13C3-Caffeine), filtered with glass microfiber filters (1.60 μm) and then with cellulose acetate filters (0.45 μm). Solid-phase extraction is subsequently performed through Oasis HLB cartridges (6 cm3, 200 mg; Waters S.p.A., Sesto San Giovanni, Milan, Italy). After sample loading, cartridges are washed 4 times with 6 ml of water, then with 5% methanol/water, and finally eluted with 6 mL of methanol. After evaporation under a N2 gentle stream up to a volume of 125 μL, samples are additioned with 125 μL of water (finally 50:50 water/methanol) for the LC-MS-MS analysis, centrifuged (17,000 × g, 5 min), filtered and transferred into glass vials for instrumental analysis. Tissue preparation: Tissues of different organisms will be pooled and homogenized. 1g of homogenate is spiked with 50 µg/L of a mixture of labeled internal standards (E2-d2, BPA-d6, 13C4-PFOA, Ibuprofen-d3 and 13C3- Caffeine), incubated for 10 min, additioned with 25 volumes of extraction buffer (0.01N KOH in methanol), vortexed and shaken (250 rpm, 25 °C, 18h). After centrifugation (15 min 1,500 × g), supernatant was diluted 20× with ultrapure water and loaded on SPE Cartrige. SPE procedure is carried out as previously described for sea water analysis. Chromatographic conditions and mass spectrometry detection. Chemical analyses are carried out with an HPLC system (Agilent 1.200 series, Agilent Technologies Italia S.p.A, Cernusco sul Naviglio, Milan, Italy) coupled with a MS/MS spectrometer, equipped with an electrospray ionization source (Quattro Premier XE Micromass, Waters S.p.A.) operating in multiple reaction monitoring mode. A 20 μL aliquot of sample is injected onto an XBridge C18 3.5μm 2.1 × 150 mm column (Waters S.p.A.) equipped with a precolumn (XBridge BEH C18 VanGuard Cart, 3.9 × 5). For group 1 compounds [17-beta-estradiol (E2), estrone (E1), 17-alfa-ethinylestradiol (EE2), 4-nonylphenol (NP), 4-octylphenol (OP) and Bisphenol A (BPA)] analyses are carried out in negative ion mode using 0.1% ammonium hydroxide in Water (A) and 0.1% ammonium hydroxide in Acetonitrile (B) as mobile phases, with a flow rate of 0.2 mL/min. For group 2 compounds [Perfluorooctane sulfonate (PFOS), Perfluorooctanoate (PFOA), diclofenac and ibuprofen] analyses are conducted in negative ion mode using 10 mM ammonium acetate in Water (A) and Acetonitrile (B) as mobile phases, with a flow rate of 0.2 ml/min. For group 3 compounds (Caffeine, Carbamazepine and Atenolol), analyses are done in positive ion mode using 0.1% formic acid in Water (A) and 0.1% formic acid in Acetonitrile (B) as mobile phases, with a flow rate of 0.3 mL/min. In case of significant occurrence, biological analysis on the effect of the pharmaceutical mixtures will be carried out under controlled conditions at different pH, using mussels (adult and embryo-larval stages) as sentinel organisms according to the methodologies recently reported by the group (Capolupo et al. 2020, 2021).

**Sediments**: CECs may be found in sediments, depending on their chemical features. For a complete evaluation of the environmental status of the site, at the same site of coral collection also bottom sediment could be sampled. A general characterization for grain-size and bulk chemical composition could be performed using XRF. Aqua regia digestion could also be performed for a larger evaluation of the inorganic chemical composition. If an adequate amount of sediment sample is collected then an evaluation of the presence and type of plastic fragments dispersed within the solid matrix. For the separation of plastic material from the sediment, a density separation process could be applied following the method proposed by Thompson et al. (2004) based on the use of a supersaturated NaCl solution (1.202 g cm−3) and collection of the supernatant. After a second filtration, plastic particles could be observed under a microscope, to determine size, shape, color and texture, then eventually be compositionally characterized using Raman spectroscopy. Emerging contaminants can be analysed also in the sediment sample following the method described by Pignotti and Dinelli (2018): concerning sediment samples, 1 g dry weight (dw) of sediment is spiked with 50 μL of a mixture of internal standards (250 μg/L) and left 30 min to reach equilibrium. After that, 10 mL of methanol is added and the CECs are extracted by ultrasonic assisted extraction (UAE) for 1 h. Samples are then centrifuged for 20 min at 4000 rpm at room temperature. The extraction and clean-up procedure is performed by solid phase extraction (SPE) adding 90 mL of ultrapure water to the extracted sample, and following the same procedure described for seawater. Analyses on contaminants of emerging concern (CECs) Sample processing.

REFERENCES

Almeida, Â., R. Freitas, V. Calisto, V.I. Esteves, R.J. Schneider, A.M.V.M. Soares, E. Figueira, B. Campos, and C. Barata. 2018. Effects of carbamazepine and cetirizine under an ocean acidification scenario on the biochemical and transcriptome responses of the clam Ruditapes philippinarum. Environmental Pollution 235:857–868.

Balbi, T., S. Franzellitti, R. Fabbri, M. Montagna, E. Fabbri, L. Canesi. 2016. Impact of bisphenol A (BPA) on early embryo development in the marine mussel Mytilus galloprovincialis: effects on gene transcription. Environmental Pollution 218:996e1004.

Canesi, L., and E. Fabbri. 2015. Environmental effects of BPA: focus on aquatic species. Dose-Response 13:1559325815598304.

Capolupo, M., K. Gunaalan, A. M. Booth, L. Sørensen, P. Valbonesi, and E. Fabbri. 2021. The sub-lethal impact of plastic and tire rubber leachates on the Mediterranean mussel Mytilus galloprovincialis. Environmental Pollution 283:117081.

Capolupo, M., L. Sørensen, K.D.R. Jayasena, A.M. Booth, E. Fabbri. 2020. Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. Water Research 169:115270.

Capolupo, M., S. Franzellitti, A. Kiwan, P. Valbonesi, E. Dinelli, E. Pignotti, M. Birke, E. Fabbri. 2017. A comprehensive evaluation of the environmental quality of a coastal lagoon (Ravenna, Italy): Integrating chemical and physiological analyses in mussels as a biomonitoring strategy. Science of the Total Environment 598:146–159.

Costa, S., F. Coppola, C. Pretti, L. Intorre, V. Meucci, A. M. V. M. Soares, M. Solé, and R. Freitas. 2020.Biochemical and physiological responses of two clam species to Triclosan combined with climate change scenario. Science of the Total Environment 724:138143.

ECHA, 2018. Plastic additives initiative. Available at. <https://echa.europa.eu/plasticadditivesinitiative> Accessed Oct 4th, 2022.

Fabbri, E., and S. Franzellitti. 2016. Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environmental Toxicology and Chemistry 35:799– 812.

Freitas, R., Â. Almeida, V. Calisto, C. Velez, A. Moreira, R.J. Schneider, V.I. Esteves, F.J. Wrona, E. Figueira, and A.M.V.M. Soares .2016. The impacts of pharmaceutical drugs under ocean acidification: New data on single and combined long-term effects of carbamazepine on Scrobicularia plana. Science of the Total Environment 541:977–985.

Gunaalan, K., E. Fabbri, and M. Capolupo. 2020. The hidden threat of plastic leachates: A critical review on their impacts on aquatic organisms. Water Research 184:116170.

Maulvault, A.L., L.H.M.L.M. Santos, C. Camacho, P. Anacleto, V. Barbosa, R. Alves, P. Pousão Ferreira, A. Serra-Compte, D. Barceló, S. Rodriguez-Mozaz, R. Rosa, M. Diniz, and A. Marques .2018. Antidepressants in a changing ocean: Venlafaxine uptake and elimination in juvenile fish (Argyrosomus regius) exposed to warming and acidification conditions. Chemosphere 209:286–297.

Mezzelani, M., A. Nardi, I. Bernardini, M. Milan, L. Peruzza, G. d’Errico, D. Fattorini, S. Gorbi, T. Patarnello, F. Regoli. 2021. Environmental pharmaceuticals and climate change: The case study of carbamazepine in M. galloprovincialis under ocean acidification scenario. Environment International 146:106269.

Munari, M., V. Matozzo, F. Gagné, G. Chemello, V. Riedl, L. Finos, P. Pastore, D. Badocco, M.G. Marin. 2018. Does exposure to reduced pH and diclofenac induce oxidative stress in marine bivalves? A comparative study with the mussel Mytilus galloprovincialis and the clam Ruditapes philippinarum. Environmental Pollution 240:925-937.

Munari, M., V. Matozzo, G. Chemello, V. Riedl, P. Pastore, D. Badocco, M.G. Marin. 2019. Seawater acidification and emerging contaminants: A dangerous marriage for haemocytes of marine bivalves. Environmental Research 175:11–21.

Pignotti, E., and E. Dinelli. 2018. Distribution and partition of endocrine disrupting compounds in water and sediment: Case study of the Romagna area (North Italy). Journal of Geochemical Exploration 195:66- 77.

Pusceddu, F. H., M. M Guimarães, L. O Lopes, L. S. Souza, F. S. Cortez, C. D. S. Pereira, R. B. Choueri, A. Cesar. 2022. Biological effects of the antihypertensive losartan under different ocean acidification scenarios. Environmental Pollution 292:118329.

Thompson, R. C., Y. Olsen, R. P. Mitchell, A. Davis, S. J. Rowland, A. W. G. John, D. McGonigle, A. E. Russell. 2004. Lost at sea: Where is all the plastic? Science 304:838.

UNEP, United Nation Environmental Programme, International Panel on Chemical Pollution (IPCP). 2017. Overview Report II: an Overview of Current Scientific Knowledge on the Life Cycles, Environmental Exposures, and Environmental Effects of Select Endocrine Disrupting Chemicals (EDCs) and Potential EDCs, p. 208.

Valbonesi, P., M. Profita, I. Vasumini, E. Fabbri. 2021. Contaminants of emerging concern in drinking water: Quality assessment by combining chemical and biological analysis. Science of the Total Environment 758:143624.

* **articolazione del progetto e tempi di realizzazione**

In linea con il time course del progetto PNRR, per la parte attribuita all’Università di Bologna PNRR-NBFC-SPOKE 2-ACTIVITY1-ACTION 2 “ZERO POLLUTION” WP3 il lavoro dell’assegnista inizierà nei primi mesi del 2023, appena terminato il Concorso, contribuendo così al raggiungimento delle prime milestones previste. L’assegnista si avvarrà del contributo dell’RTDa BIO07 assegnato al programma per i campionamenti a Panarea. Le attività di studio dei contaminanti emergenti in acqua e tessuti saranno sotto la guida della Prof.ssa Fabbri; le attività di caratterizzazione dei sedimenti e dei contaminanti presenti saranno sotto la guida del Prof. Enrico Dinelli.

Primo anno: sarà condotto lo studio preliminare delle caratteristiche di Panarea, la raccolta campioni di campioni biotici e abiotici nelle aree caratterizzate da differente pH. Seguirà la messa a punto delle condizioni di dosaggio dei CECs all’HPLC-MS/MS e il dosaggio nella matrice acqua. Questo servirà a rendere più mirato il successivo dosaggio nelle matrici tissutali di corallo.

Secondo anno: Sulla base dei dati ottenuti assegnista condurrà anche studi sugli effetti delle miscele di CECs eventualmente riscontrate nelle acque di Panarea, mediante esposizione di embrioni-larve e adulti di organismi sentinella. Avrà inizio la caratterizzazione dei sedimenti, la presenza di inquinanti mediante XRF, e le misure della presenza dei CECs con uso di HPLC-MS/MS. L’attività terminerà con l’analisi statistica dei dati e la pubblicazione del rapporto progettuale, così come di lavori scientifici.

* **PROGRAMMA FORMATIVO (O PIANO DI ATTIVITÀ) DELL’ASSEGNISTA**
* L’assegnista verrà stimolato a progettare, svolgere e riprodurre esperimenti ed analisi in maniera autonoma e ad acquisire esperienza ed indipendenza nella pubblicazione scientifica
* Parteciperà ai Meeting internazionali, corsi e seminari sull’argomento in presenza di un’audience critica
* Contribuirà allo sviluppo di proposte applicative per la valutazione del rischio da contaminanti emergenti in ambiente marino.
* Collaborerà assiduamente con i laboratori coinvolti nel medesimo Progetto PNRR Biodiversità.

POLLUTION-MEDIATED LOSS OF BIODIVERSITY IN MEDITERRANEAN AND INTERACTIONS OF EMERGING POLLUTANTS WITH CLIMATE CHANGE. L’attività è parte del progetto PNRR-NBFC-SPOKE 2-ACTIVITY1-ACTION 2 “ZERO POLLUTION” WP3

La presenza di contaminanti di emergente preoccupazione (CECs) in ambiente marino indica la necessità di ulteriori studi sugli organismi in differenti livelli trofici e habitats, e del potenziale di bioaccumulo conseguente all’elevata natura lipofilica di molti farmaci e di altri CECs. Questo può essere aumentato a fronte di una diminuzione del pH. Panarea è un laboratorio naturale per studiare l’interazione tra i CECs e l’acidificazione, e gli effetti sulla biodiversità marina. L'obiettivo è studiare la presenza nell'acqua e nei sedimenti e il bioaccumulo di contaminanti emergenti lungo il gradiente di acidificazione naturale dell'oceano in una specie modello, il corallo Balanophyllia europaea. Le attività saranno svolte sulla base delle competenze del laboratorio.