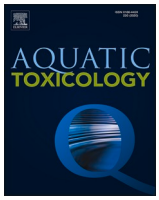
Aquatic Toxicology 279 (2025) 107255

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox



Transcriptional modulation in Mediterranean Mussel *Mytilus galloprovincialis* following exposure to four pharmaceuticals widely distributed in coastal areas

Ilaria Bernardini [a,c,†], Marica Mezzelani [b,†], Michela Panni [b], Giulia Dalla Rovere [a], Alessandro Nardi [b,c], Ouafa El Idrissi [d], Luca Peruzza [a], Stefania Gorbi [b,c], Serena Ferraresso [a], Luca Bargelloni [a,c], Tomaso Patarnello [a,c], Francesco Regoli [b,c,*], Massimo Milan [a,c,*]

[a] Dipartimento di Biomedicina Comparata e Alimentazione, Università di Padova, Viale dell'Università, 16, 35020 Legnaro (PD), Polo di Agripolis, Italy
[b] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche 60131 Ancona, Italy
[c] NBFC, National Biodiversity Future Center, Palermo, Italy
[d] Université de Corse Pasquale Paoli, UMR CNRS 6134 Sciences pour l'Environnement, 20250 Corte, France

ARTICLE INFO

Keywords:
Pharmaceuticals
Bivalves
Mechanisms of action
Transcriptomics
Microbiota
Recovery

ABSTRACT

Ecotoxicological risk and the mode of action of human drugs on non-target marine animals remain unclear, keeping a gap of knowledge on risks related to ecosystem disruption and chemical contamination of food chains.

Understanding these impacts is critical to developing proper waste management practices and regulatory frameworks to prevent long-term environmental and human health problems. This study investigates the impacts of Gemfibrozil, Metformin, Ramipril, and Venlafaxine, individually and combined on *Mytilus galloprovincialis* over 30 days and assesses persistent effects post-recovery using RNA-seq and 16S rRNA microbiota profiling. All pharmaceuticals caused few changes in the microbiota while gene expression analyses highlighted drug-specific alterations. Gemfibrozil exposure led to alterations in lipid and fatty acid metabolism, suggesting a similar mode of action to that observed in target species. Metformin significantly impacted the mussels' energy metabolism, with disruptions in specific genes and pathways potentially related to glucose uptake and insulin signaling. Metformin was also the treatment leading to the most significant changes in predicted functional profiles of the microbiota, suggesting that it may influence the microbiota's potential to interact with host glucose metabolism. Ramipril exposure resulted in the up-regulation of stress response and cell cycle regulation pathways and Venlafaxine induced changes in serotonin and synapse pathways, indicating potential similarities in mechanisms of action with target species. Mixture of the four pharmaceuticals severely impacted mussel physiology, including impairment of oxidative phosphorylation and compensatory activation of several pathways involved in energy metabolism. Despite recovery after depuration, changes in stress and energy related metabolism pathways suggests potential persistent effects from combined pharmaceutical exposure. Notably, the up-regulation of mTOR1 signaling in all treatments after 30 days underscores its key role in coordinating bivalve stress responses. The Transcriptomic Hazard Index (THI) calculated for each treatment indicates major/severe hazards after exposure that decreased to slight/moderate hazards after depuration.

1. Background

The widespread use of human and veterinary pharmaceuticals and their subsequent release into environmental compartments represent a growing environmental problem worldwide. Due to the limited efficiency of sewage treatment processes, pharmaceuticals are systematically introduced into soils, water bodies, groundwater and coastal areas, affecting ecosystems and poses possible threats for aquatic species (Heberer, 2002; Desbiolles et al., 2018; Ojemaye and Petrik, 2019). For these reasons, pharmaceuticals are considered a class of Contaminants of Emerging Concern (CECs), recently included in the EU Watch list (EPRS, 2023) including antibiotics, antidepressants,

* Corresponding authors.
E-mail addresses: f.regoli@staff.univpm.it (F. Regoli), massimo.milan@unipd.it (M. Milan).
† These authors contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.aquatox.2025.107255
Received 19 November 2024; Received in revised form 5 January 2025; Accepted 19 January 2025
Available online 23 January 2025

antidiabetics, anti-inflammatories, analgesics, blood-pressure medications, lipid regulators, hormonal compounds, among others. Each class has unique chemical and physical properties that determine its environmental fate. Although pharmaceuticals are specifically developed and produced for humans, animals or crops, once in the environment, they can also accumulate in non-target marine animals, with unpredictable effects on their health (e.g., Ojemaye and Petrik, 2019; Mezzelani et al., 2020; Madikizela and Ncube, 2022; Mezzelani and Regoli, 2022). Despite the considerable number of studies that have confirmed the presence of pharmaceuticals in the tissues of non-target aquatic species, the ecotoxicological effects and modes of action on non-target animals are still poorly investigated.

This study aims to point out the potential the Mode of Action (MoA) and effects of four common human pharmaceuticals, belonging to the main classes of assumed drugs and known to be present in marine ecosystem in recent years, in the Mediterranean mussel (*Mytilus galloprovincialis*), a species inhabiting coastal areas and covering important ecological and economic roles. To achieve this goal, we combined gene expression profiling (RNA-sequencing) and microbiota characterization (16S rRNA amplicon sequencing) in mussels exposed for 30 days to Gemfibrozil (GEM; used for treating high cholesterol and triglycerides levels treatment), Metformin (MET; used for treating type 2 diabetes), Ramipril (RAM; used for treating high blood pressure), Venlafaxine (VEN; anti-depressant) and their combination. These compounds are representative of the pharmaceuticals most frequently and simultaneously detected in coastal waters (Fernandes et al., 2021; Ślósarczyk et al., 2021; García-Blasco et al., 2024; Castaño-Trias et al., 2023; Castaño-Ortiz et al., 2023a). Their concentrations typically range from ng/L to mg/L worldwide (Mezzelani and Regoli, 2022), mainly due to their high stability and persistence. In this context, GEM's measured degradation/photochemical half-life ranges from 100 to over 200 days under sunlight (Araujo et al., 2011; Fabbri et al., 2017), while MET cannot be completely removed by WWTPs due to its physicochemical properties such as high solubility, low octanol-water partition coefficient, and low biodegradation rate (Mahmoud et al., 2020), also showing a 3-day stability in water (Prajaputra and Isnaini, 2023). RAM persistence in solution was assessed under laboratory conditions, highlighting the lack of degradation for up to 25 days (De Diego et al., 2010). VEN is not efficiently removed in WWTPs even with advanced technologies such as membrane bioreactors. In surface water, VEN half-life ranges between 50 and 62 days, considering all possible mechanisms of degradation such as direct/indirect photodegradation and biotic degradation (Rúa-Gómez and Püttmann, 2013; Souza et al., 2022). In terms of biological targets, GEM interacts with the peroxisome proliferator-activated receptor-alpha (PPAR-alpha) promoting fatty acid oxidation and reducing triglycerides, cholesterol, and low-density lipoproteins levels in humans (Saku et al 1985; Valentovic, 2007). Few studies investigated the effects on non-target species, revealing changes in endocrine and lipid metabolism, reproduction, immune and oxidative stress in fishes and *Mytilus* spp. (Mimeault et al., 2005; Prindiville et al., 2011; Skolness et al., 2012; Oliveira et al., 2018; Canesi et al., 2007; Quinn et al., 2011; Schmidt et al., 2011). MET is a biguanide antihyperglycemic agent whose mechanism of action involves multiple pathways, such as the suppression of gluconeogenesis (Foretz et al. 2023). Following ingestion, MET is not metabolized and is excreted unchanged in the urine (Rena et al., 2017). Effects on freshwater and marine animals have already been reported in literature. Among them, impacts on the endocrine, neuronal, immune systems and cell cycle regulation (Niemuth et al., 2015; Niemuth and Klaper, 2015; MacLaren et al., 2018; Lee et al., 2019; Ambrosio-Albuquerque et al., 2021; Koagouw and Ciocan, 2018; Koagouw et al. 2021). RAM belongs to the angiotensin-converting enzyme (ACE) inhibitor class acting by blocking the conversion of angiotensin I to angiotensin II, involved in blood-pressure modulation within the renin-angiotensin-aldosterone system (RAAS; Frampton and Peters, 1995). Lastly, VEN is an antidepressant that inhibits the reuptake of serotonin and norepinephrine at the presynaptic terminal of nervous system (Holliday and Benfield, 1995). Recent studies indicated possible effects on behavior, development, morphology and reproduction in aquatic non-target species (Bisesi et al., 2014; Salahinejad et al., 2022; Sehonova et al., 2018). Moreover, DNA damage and consequences on reproduction, lysosomal stability, and locomotion have been also observed in invertebrates (Lacaze et al., 2015; Rafiq et al., 2023). In light of the pervasive presence of pharmaceuticals in coastal waters and their documented but poorly understood effects on non-target species (Mezzelani and Regoli, 2022), this study employed gene expression analyses to fill critical gaps in our understanding of the mechanisms of action of these pharmaceuticals in the non-target marine species *M. galloprovincialis* and to elucidate possible interaction between them.

An additional, not negligible point concerns the potential consequences of PPCPs on the gut microbiota of non-target organisms. The gut microbiota plays a pivotal role in regulating the homeostasis of various host physiological functions (Van de Water et al., 2018; Paillard et al., 2022; Ma et al., 2023; Balasubramanian et al., 2024). However, this symbiotic relationship can be disrupted by external stressors, including environmental contaminants and pharmaceuticals (e.g., Bernardini et al., 2023; Bernardini et al., 2022; Li et al., 2024; Kong et al., 2023; Claus et al., 2016; Evariste et al., 2019; Peruzza et al., 2023; Liu et al., 2023; Milan et al. 2018; Iori et al. 2020), leading to alterations in microbial composition that may have lasting impacts on the host's fitness (Evariste et al., 2019). Other studies have demonstrated that impaired host health status following chemical exposure reduces the animal's ability to regulate its microbiota, ultimately leading to imbalances in microbial communities (Gaulke et al., 2016; Jin et al., 2017; Bernardini et al., 2023; Milan et al., 2018). Accordingly, understanding how pharmaceutical exposure influences host-microbiota dynamics is essential for gaining a broader and more detailed understanding of the potential effects of PPCPs.

To conclude, we also assessed the persistent effects of the investigated pharmaceuticals by applying transcriptional analyses and microbiota characterization after 14 days of recovery from pharmaceutical exposure.

2. Material and methods

2.1. Animal collection and experimental plan

Mussels, *Mytilus galloprovincialis* (5.5 ± 1.00 cm shell length), were collected from a from a shellfish farm in Senigallia (Ancona, Adriatic Sea), and were acclimatized for 7 days with aerated artificial seawater (ASW; Istant Ocean), at local seasonal environmental conditions of temperature (18 ± 1 °C), salinity (33 practical salinity units) and pH (8.20). Collection and experimental use of mussels is not subjected to ethical review permissions according to both European and Italian normative (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, 2010; Italian Legislative Decree n. 26, 2014), while monitoring guidelines recommend this species as appropriate bioindicator organism for assessing bioavailability of ecotoxicological effects of environmental pollutants in marine environments (Mezzelani et al., 2023). Organisms were randomly distributed into 6 glass-tanks (n = 60 per tank) in a volume of 17 L of ASW, and exposed for 30 days to the six following treatments: CTL, control condition (0.0029% of dimethyl sulfoxide, DMSO); GEM, Gemfibrozil exposure (1 μg/L); MET, Metformin exposure (1 μg/L); RAM, Ramipril exposure (1 μg/L); VEN, Venlafaxine exposure (1 μg/L); MIXT, mixture exposure of GEM, MET, RAM and VEN (1 μg/L for each drug). After the exposure phase, mussels were maintained for additional 14 days in pharmaceuticals-free artificial seawater intended as a depuration phase. The selected active principles are representative of the most frequently and simultaneously detected pharmaceuticals in marine ecosystems (Mezzelani et al., 2020; Fernandes et al., 2021; Ślósarczyk et al., 2021; García-Blasco et al.,

2024; Castaño-Trias et al., 2023; Castaño-Ortiz et al., 2023). However, their interactive effects have not yet been investigated in marine species. Exposure doses of each pharmaceutical were selected within environmentally realistic concentrations possibly occurring in coastal areas (Gaw et al., 2014; Birch et al., 2015; Ghoshdastidar et al., 2015; Freitas et al., 2016; Tao et al., 2018; Castillo-Zacarías et al., 2020; Mezzelani and Regoli, 2022). Exposure doses of each pharmaceutical were selected within environmentally realistic concentrations for coastal areas (Birch et al., 2015; Freitas et al., 2016; Mezzelani and Regoli, 2022). Stock solutions of GEM (Sigma Aldrich, CAS 25,812–30–0), MET (Sigma Aldrich, CAS 1115–70–4), RAM (Sigma Aldrich, CAS 87,333–19–5) and VEN (Sigma Aldrich, CAS 99,300–78–4) were obtained by dissolving drug powder in DMSO and stored at + 4 °C, while working solutions were prepared daily by diluting the stock solution in ASW. Water was changed every two days and, during the exposure phase, pharmaceuticals were re-dosed. All tested pharmaceuticals are considered stable pollutants in surface waters as supported by the available Literature (Araujo et al.,2011; De Diego et al., 2010; Fabbri et al.,2017, Prajaputra and Isnaini, 2023; Rúa-Gómez and Püttmann, 2013; Souza et al., 2022) and the 48h elapsed between each redosing guaranteed the stability and bioavailability of all tested pharmaceuticals. The choice of 30 days exposure was considered appropriate to highlight the onset of biological alterations as also demonstrated by previous studies (Mezzelani et al., 2018; Nardi et al., 2022), thus providing insights on possible chronic effects similar to those occurring in field conditions, with an increased prognostic and diagnostic value on the impact of these ubiquitous molecules in marine biota.

Mussels were fed with 500 μL of a commercial mixture of marine plankton (EasySPS EVO, size range 0.2- 400 μm) before the water change. At the end of exposure and depuration phases (days 30 and 44, respectively), 15 digestive glands were collected from 15 individuals of each treatment, pooled in 5 separate samples, each constituted by tissues of 3 individuals, rapidly frozen in liquid nitrogen and maintained at −80 °C for the transcriptional and microbiota analyses.

2.2. RNA extraction

Total RNA of digestive gland was extracted from samples collected at 30 and 44 days (5 individuals/experimental group, total 60 samples) by using RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA quantification and quality check were performed through Agilent 2100 Expert system and Qubit. Extracted RNA from each sample was used both for gene expression and microbiota characterization analyses.

2.3. Gene expression analysis

Extracted RNA was sent to a sequencing platform (CRIBI, University of Padova) for library preparation using QuantSeq 3′ mRNA-Seq Library Prep Kit FWD and sequencing by Illumina Novaseq 6000 (single-end 75 bp setup (sequences available in NCBI SRA; BioProject PRJNA1018482). Raw reads were processed for quality check through FastQC/v0.11.9 and low-quality reads and residual adaptors were removed with the program BBDuk of the suite BBTools (program specific options indicated in the Lexogen's website). After checking the average length and standard deviation of samples' sequences, mapping was carried out using the reference transcriptome by Salmon v1.10.0 (Patro et al., 2017). Reference transcriptome was obtained from Iso-Seq long RNA sequencing by pooling two groups of digestive glands and one of gills of mussel individuals (each composed of 4 animals) obtaining high-quality full--length mRNA sequences (335,861 total). CD-HIT est (clustering threshold > 90%) was used to obtain the final transcriptome (98,359 sequences) used as reference. Finally, "abundance estimates to matrix.pl" script from Trinity suite (Haas et al., 2013) was employed to generate the count table. Following, the count table was imported on R/v3.6.0 (R Development Core Team, 2014) and filtered: contigs with the minimum counts in a sample <5 reads, in a total minimum count < minimum count*(number of samples/2) in at least the 70% libraries. Filtered counts were then normalized by RUVs function from the RUVSeq/v1.18 library (Gerstner et al., 2016; Pradhan et al., 2020) and considered for the further Principal Component Analysis and pairwise comparison. These analyses were performed using both whole samples together (number of total samples=60, RUVs' k= 1) and each sampling time separately (number of total samples=30, RUVs' k= 5 for samples of the 30th day of treatment and RUVs' k= 6 for samples of the 44th day of treatment). Pairwise comparison (set parameters: p. adjusted≤0.05, Fold Change≥2) was performed by comparing each group to the control within each sampling time using the Likelihood approach. Moreover, an "A posteriori filter" was adopted in order to exclude genes whose expression level was not deemed biologically meaningful because they had a CPM value ≤ 0, in more than 70% of the biological replicates of all treatments considered in the pairwise comparison. For a deeper restrictiveness, we considered DE genes that FDR < 0.05.

Data obtained were employed for the functional analysis studied through the Over-Representation Analysis (ORA; at biological process and KEGG pathway levels) where up- and down-regulated pathways were deemed significant with FDR < 0.1 and the number of genes count > 4. Furthermore, we performed the GSEA applied to Biological Process, KEGG pathways and HALLMARK databases was also performed (p-adjusted < 0.05). In addition, according to Cecchetto et al. (2023), GSEA was applied to a reduced Hallmark gene sets (p-adjusted < 0.2) (Supplementary File 3) to calculate the transcriptomic hazard index (THI). The THI quantitatively assesses the transcriptomic response of organisms by integrating changes occurring in Hallmark gene sets through Gene Set Enrichment Analysis (GSEA). The results are then classified into five hazard classes, ranging from "absent" to "severe", providing a comprehensive evaluation of the whole-transcriptome effects of chemical exposure.

2.4. Microbiota characterization

Extracted RNA (1000 ng) was reverse transcribed to cDNA using the Superscript IV Kit (Invitrogen, Life Technologies, Monza, Italy). cDNA was shipped to the BMR Genomics (Padova, Italy) for library construction, using specific reverse and forward primers (10 μM) for the V3-V4 gene region of bacterial 16S rRNA, as described by Milan et al. (2018) and the following sequencing with Illumina MiSeq (2 × 300bp). Microbiome sequencing generated 4158,844 million reads, approximately 74,265 reads on average per sample (sequences available in the NCBI Sequence Read Archive: BioProject PRJNA1018482). Raw sequences obtained were imported on QIIME 2 (Bolyen et al., 2019) for barcode removing in all sequences using cutadapt tool (Martin, 2011). To optimize merging of the forward and reverse reads, low-quality portions of the reads were removed using DADA2 (Katoh and Standley, 2013). High-quality representative sequences were merged and chimeric fragments were eliminated, obtaining a total of 2020,436 reads, yielding 2107 features. Alignment of remaining representative sequences was performed using MAFFT software (Katoh and Standley, 2013) and classified using the Python library Scikit-Learn. Taxonomy classification was assigned using the trained SILVA-138 database for V3-V4 region. Finally, all samples were rarefied to 19,756 reads in order to normalize the analysis. The following statistical analyses were performed using Microbiome Analyst 2.0 (Lu et al., 2023). Microbiota characterization was performed through Principal coordinate analysis (PCoA). Richness's, Simpson's and Shannon's diversity indices and pairwise comparison were also performed to compare each treatment with control group to define differently represented species/genera (FDR<0.05). Pairwise comparisons were also performed within the same treatment to compare microbiota community after 30 days and after depuration period. After the normality and homoscedasticity of data observation, significant differences in calculated indices were detected by Wilcoxon-Mann-Whitney and Tuckey Tests basing on data distribution characteristics. For these analyses, not rarefied and not

transformed data were filtered basing on the low count (minimum reads count=4, % prevalence in sample=10), the low variance (% to remove= 10%, based on inter-quantile range) and operating the Total Sum Scaling (TSS).

Tax4fun tool of Microbiome analyst software was then used to predict functional capabilities of microbial community by comparing each treatment against control group within the same sampling time (Aßhauer et al., 2015). Differentially expressed pathways (p-value Bonferroni correction < 0.05) were graphically represented using STAMP (Parks et al., 2014).

3. Results

3.1. Gene expression analyses

From the RNA-sequencing, we obtained a total of 369,226,471 reads with an average of 6153,775 raw reads across all samples. Principal Component Analysis (PCA) was performed separately at day 30 and day 44. At the end of the 30-day exposure, CTL mussels were separated from all treatments along both the X-axis (6.58%) and Y-axis (5.56%), with a weak separation among treatments also observed along the X-axis (Fig. 1A). After depuration on day 44 (Fig. 1B), three main clusters were observed: i) CTL, GEM, RAM, MIX; ii) VEN; and iii) MET.

Pairwise comparisons at both sampling times revealed the highest number of differentially expressed genes (DEGs) in MET-exposed mussels (393 DEGs after 30 days and 325 DEGs after 44 days; Fig. 2 and Supplementary File 1). Notably, for all treatments, the majority of DEGs were up-regulated after exposure (Day 30) (86.9%), while an opposite trend was observed after depuration, with the majority of DEGs being down-regulated (77.7%). VEN was the only treatment showing a higher number of DEGs after depuration than after 30 days of exposure. A total of 9 transcripts were commonly found differentially regulated in all treatments (VEN, GEM, MET and RAM) after 30 days, while no common DEGs were found after depuration. The full lists of DEGs (including genes commonly found differentially expressed) are provided in Supplementary File 1.

Functional analyses, including ORA and GSEA, were performed. The full lists of significant pathways and biological processes are reported in Supplementary File 2 (KEGG pathways and GO terms obtained with ORA and GSEA) and Supplementary File 3 (Hallmark terms obtained with GSEA), while a summary of the most important significant GO, KEGG, and Hallmark terms for each treatment is schematically shown in Fig. 3.

Among the most important findings, GEM led to the up-regulation of "peroxisome" and pathways and genes involved in lipid and fatty acid metabolism (e.g., "fatty acid metabolism"; "lipid metabolism"; "adipogenesis"; "fatty acid catabolic processes"; *Estrogen related receptor gamma, ESRRG; hydroxysteroid 17-beta dehydrogenase 7, HIS17B7*), energy metabolism (e.g. "AMPK signaling pathways"; "Notch signaling pathway"; *Succinate dehydrogenase complex flavoprotein subunit A*, SDHA), cell cycle regulation (e.g., "cell cycle phase transition"), detoxification ("response to xenobiotic stimulus"), repair of cellular components and maintenance of homeostasis (e.g., "regulation of DNA repair"; "protein repair"; "response to unfolded protein"; "tissue homeostasis"; *three prime repair exonuclease 2, TREX2; OXA1L mitochondrial inner membrane protein, OXAIL; peptidylprolyl isomerase H,PPIH; heat shock protein 90 beta family member 1, HSP90B1*). Up-regulation of several pathways involved in immune response, inflammation, apoptosis (including *caspase 9; CASP9*), neurotransmission, and synapse was also observed. After depuration, in addition to the up-regulation of "peroxisome", "fatty acid metabolic process", and "response to xenobiotic stimulus", we observed the down-regulation of several terms involved in cell cycle regulation and immune response, most of them showing opposite trends compared to Day 30. Among them, "positive regulation of cell cycle", "G2M_checkpoint", "E2F target", "defense response to virus", "defense response to symbiont" and "interferon response" supported by several DEGs as *Cyclin dependent kinase 2 associated protein 1* (*CDK2AP1*), *Signal transducer and activator of transcription 5B* (*STAT5B), Septin 14* (*SEPT14), Septin 2* (*SEPT2)* and *RAS-like proto-oncogene A* (*RALA)*.

MET exposure led to the upregulation of several pathways involved in energy metabolism (e.g., "ATP metabolic process", "glycolysis", "pyruvate metabolism", "acetyl-CoA biosynthetic process from pyruvate"). Increased energy metabolism is also suggested by the upregulation of *Isocitrate dehydrogenase (NADP+) 2* (*IDH2*), *sirtuin 6* (*SIRT6*), and *amylase alpha 2B* (*AMY2B*). MET exposure also led, after 30 days, to increased oxidative stress response, as indicated by the upregulation of "oxidative stress response", "reactive oxygen species pathway", and *glutathione peroxidase 1* (*GPX1*). Pairwise comparisons and functional analyses also revealed the upregulation of several pathways involved in cell cycle regulation (e.g., "MYC targets", "cell cycle phase transition"), xenobiotic metabolism (e.g. "response to toxic substances", "xenobiotic metabolism"; *cytochrome P450 family 3 subfamily A member 4, CYP3A4; carboxylesterase 2, CES2*), cell signaling (e.g., "MAPK signaling", "NF-kB signaling pathways"), "DNA repair", "response to unfolded protein" and several serine/threonine kinases, such as *Serine/threonine-protein kinase*

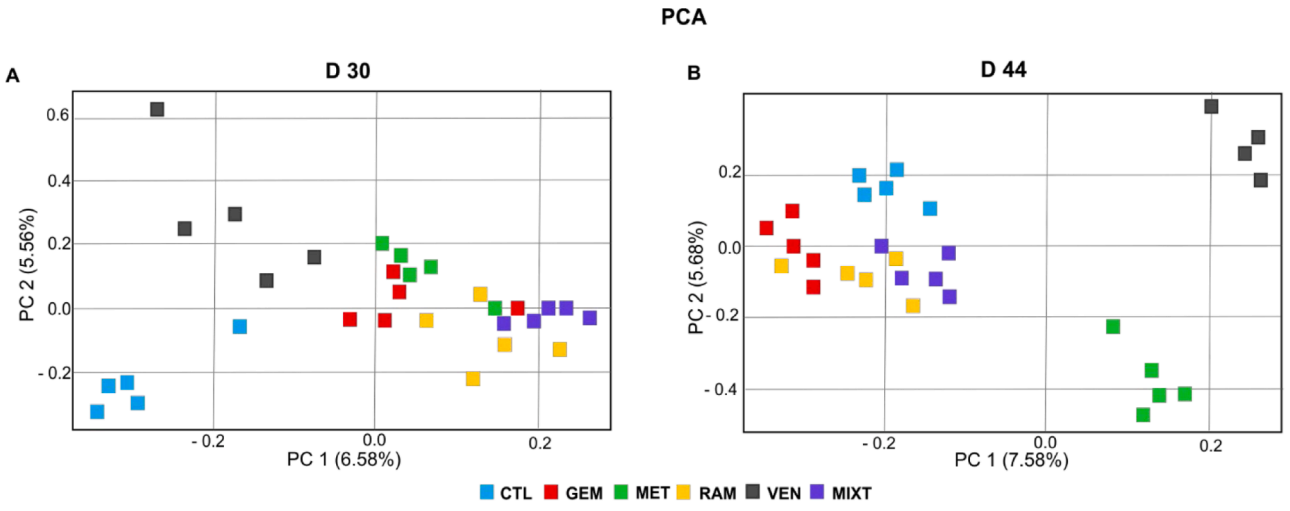


Fig. 1. Principal Component Analyses (PCA) charts showing spatial distribution of samples collected after 30 days of exposure (Fig. 1A, D 30) and 14 days of depuration (Fig. 1B, D 44). Squares represent samples of different treatments: CTL (Control group), GEM (Gemfibrozil), MET (Metformin), RAM (Ramipril), VEN (Venlafaxine), MIXT (combined exposure of GEM, MET, RAM, VEN).

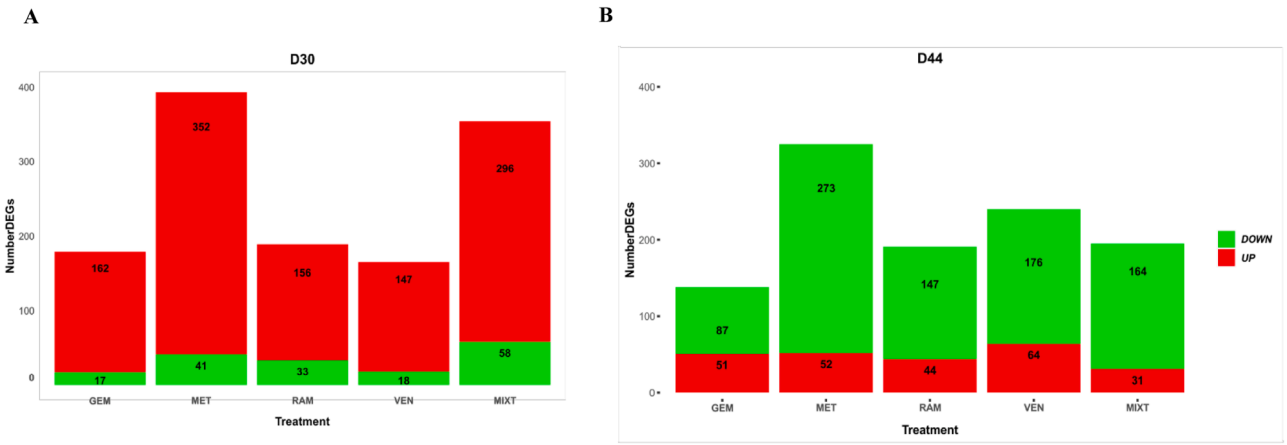


Fig. 2. Barplots showing the number of Differently Expressed Genes (DEGs) in samples of the different treatments GEM (Gemfibrozil), MET (Metformin), RAM (Ramipril), VEN (Venlafaxine) and MIXT (combined exposure of GEM, MET, RAM and VEN) collected after 30 days of exposure (Fig. 2A, D30) and 14 days of depuration (Fig. 2B, D44). DEGs resulted from the Pairwise Comparison Analysis by contrasting each treatment to the control group at the same sampling time (Day 30 and Day 44).

(*AKT2*), *NIMA-related kinase 2* (*NEK2*), and *Serine/threonine-protein kinase RIO3* (*RIOK3*). After depuration, all significant terms were down-regulated. Most of them were involved in immune response and inflammation, as "defense response to other organisms", "toll-like receptor signaling pathway", "NOD-like receptor signaling pathway", supported by several DEGs (e.g. *interferon induced protein 44 like, IFI44L; immunity-related GTPase, IRGC; toll-like receptor 6, TLR6; toll-like receptor 2, TLR2*).

RAM showed the lowest number of significant biological processes and molecular pathways. The most important transcriptional changes were related to cell cycle regulation. In addition to the up-regulation of several pathways (e.g., "positive regulation of cell cycle", "MYC targets V1", "G2M checkpoint", among others indicated in Fig. 3), differentially expressed genes involved in this process were also identified. Among them, *E2F transcription factor 7 (E2F7), chromatin licensing and DNA replication factor 1 (CDT1), cell division cycle 45 (CDC45), cell division cycle 7 (CDC7)* and *Centriolin (CNTRL)*. RAM led also to the up-regulation of several pathways involved in the stress response, such as "apoptosis", "unfolded protein response", "DNA repair", "reactive oxygen species pathways", "DNA damage response" and "DNA repair." After depuration, a few significant down-regulated pathways were identified, mostly involved in immune response and inflammation (e.g., "defense response to organism", "complement", "interferon response", and "NOD-like receptor signaling pathway").

Mussels exposed to VEN showed the upregulation of "serotonin metabolic process" and "synapse organization", as well as the up-regulation of *Sirtuin 2* (*SIRT2)* and *ATPase H+ transporting V1 subunit F* (*ATP6V1F*). Up-regulation of several pathways involved in xenobiotic metabolism after 30 days was also observed (e.g., "Drug metabolism - cytochrome P450", "Metabolism of xenobiotics by cytochrome P450", "Drug metabolism - other enzymes", "response to xenobiotic stimulus"). After depuration, the most significant transcriptional changes were related to the downregulation of processes involved in immune response and inflammation (e.g. "cytokine production", "interferon response", "innate immune response", *toll-like receptor 6, TLR6; hematopoietic prostaglandin D synthase, HPGDS*), as well as "xenobiotic metabolism" and "wound healing". VEN was the only treatment that led to the upregulation of terms and genes involved in cell cycle regulation on day 44, including "E2F targets", "G2M checkpoint", "mitotic spindle", *cyclin B2* (*CCNB2*), *structural maintenance of chromosomes* (SMC5 and SMC6) and *XPC complex subunit* (*DNA damage recognition and repair factor*), among others.

Enrichment analyses (ORA) applied to mussels exposed to the combination of MET, GEM, VEN, and RAM (MIXT) indicated the down-regulation of "oxidative phosphorylation", while GSEA indicated the up-regulation of several molecular pathways and genes related to energy metabolism (e.g., "TCA cycle," "glucose import," "glycolysis," "fatty acid metabolism," *phosphoenolpyruvate carboxykinase 1; PCK1; ADP-ribosylserine hydrolase*, ADPRS; *ATPase H+ transporting V1 subunit H*, ATP6V1H). The activation of several terms involved in cell cycle regulation, "DNA repair", "unfolded protein response", "response to oxidative stress", "apoptosis", "response to toxic substances", "tissue homeostasis", "inflammatory response" and "xenobiotic metabolism" was also observed. These findings were supported by the up-regulation of genes involved in apoptosis (e.g. *caspase 9*, CAS9; *baculoviral IAP repeat containing 7, BIRC7; death associated protein, DAP*), drug metabolism (*carboxylesterase 2*;CES2), cell cycle regulation and stress response (e.g. *cell division cycle 37*, CDC37; *MAPK regulated corepressor interacting protein 2, MCRIP2; NFKB inhibitor alpha, NFKBIA; heat shock protein family A member 5, HSPA5; sirtuin 6, SIRT6*). After depuration, as observed in single exposures, most significant pathways were down-regulated, playing roles in immune response, energy metabolism, and signaling (see Fig. 3).

Noteworthy, the mTOR1 signaling pathway was up-regulated in all treatments after 30 days, as were several processes involved in cell cycle regulation (e.g. "mitotic spindle", "MYC targets 1″), "apoptosis", "endocytosis", "cellular response to toxic substances", "protein secretion", "drug metabolism – other enzymes", protein turnover and transcription (e.g., "spliceosome", "ubiquitin-mediated proteolysis", "protein processing in the endoplasmic reticulum").

3.2. Microbiota characterization

PCoA showed a separation along the y-axis between the two sampling times, while no separations between treatments were detected at either time point (Fig. 5 and Supplementary File 4). A significant decrease in Shannon's Index was identified in MIXT-exposed mussels on Day 30, while GEM and MET exhibited lower diversity than the control group after the recovery period (Simpson's and Shannon's Index; Supplementary File 4). The hepatopancreas microbial composition indicated that the *Vibrio* genus was the most represented in all treatments except in GEM-exposed mussels (Supplementary File 4). Pairwise comparisons between CTL and different treatments were also performed at both sampling times. All treatments showed a few significantly over-represented and underrepresented taxa, suggesting that all treatments led to minor changes in digestive gland microbiota composition (Table 1). Full lists of significant taxa detected at the species and genus levels are provided in Supplementary File 4. Among all treatments, MET

Fig. 3. Representation of selected enriched KEGG, GO, Hallmark (HM) pathways (p-adj<0.05) in samples of the treatments with GEM (Gemfibrozil), MET (Metformin), RAM (Ramipril), VEN (Venlafaxine) and MIXT (combined exposure of GEM, MET, RAM, VEN) collected after 30 days of exposure (D30) and 14 days of depuration (D44). Results were obtained by comparing each treatment with the control group at the same sampling time, separately at Day 30 and Day 44. Full lists of significant pathways are reported in Supplementary File 2. Red and green cells indicate significant up- and down- regulation of enriched pathways; the different color intensity reflects the Normalized Enrichment Scores (NES) value obtained for the corresponding terms.

exposure yielded the most substantial alterations in predicted metabolic pathways after depuration (Supplementary file 4), with notable changes in carbohydrate metabolism ("Fructose and mannose metabolism", "Starch and sucrose metabolism", “carbohydrate digestion and absorption”, “insulin resistance”, “insulin signaling pathway”), fatty acid metabolism (e.g. “fatty acid metabolism”, “fatty acid biosynthesis”), bacterial metabolic processes (e.g. “carbon fixation process”, “propanoate metabolism”, “butanoate metabolism”, “carbon metabolism”), xenobiotic degradation (atrazine, toluate and benzoate degradation) suggesting potential effects on mussel physiology. The exposure to GEM for 30 days revealed significant alterations in few microbial metabolic pathways, as “fluorobenzoate degradation”, significantly increased also in RAM-exposed mussels compared to control. Not significant terms were highlighted for other treatments at both sampling time.

4. Discussion

The present study highlighted the strong modulation of specific drug-related pathways in treatments exposed to single pharmaceuticals, with evidence of rather general and non-specific activation of biological responses in the mixture treatment. In the following sections treatment-specific modulated pathways are singularly discussed in order to disentangle complexity and to specifically explore all the relevant aspects highlighted by obtained results.

4.1. Gemfibrozil

Gemfibrozil (GEM) is a fibrate amphipathic carboxylic acid molecule used to regulate lipid levels by reducing triglyceride concentrations in humans (Saku et al 1985; Valentovic, 2007). The mechanism of action of GEM in mammals is mainly based on the activation of the PPARα receptor, which leads to the transcription of genes involved in lipid metabolism, increased fatty acid oxidation, and enhanced lipoprotein lipase activity, thereby lowering serum triglycerides (Cancio et al., 1999; Valentovic, 2007). Our findings indicate that exposure of mussels to GEM led to significant alterations in lipid and fatty acid metabolism,

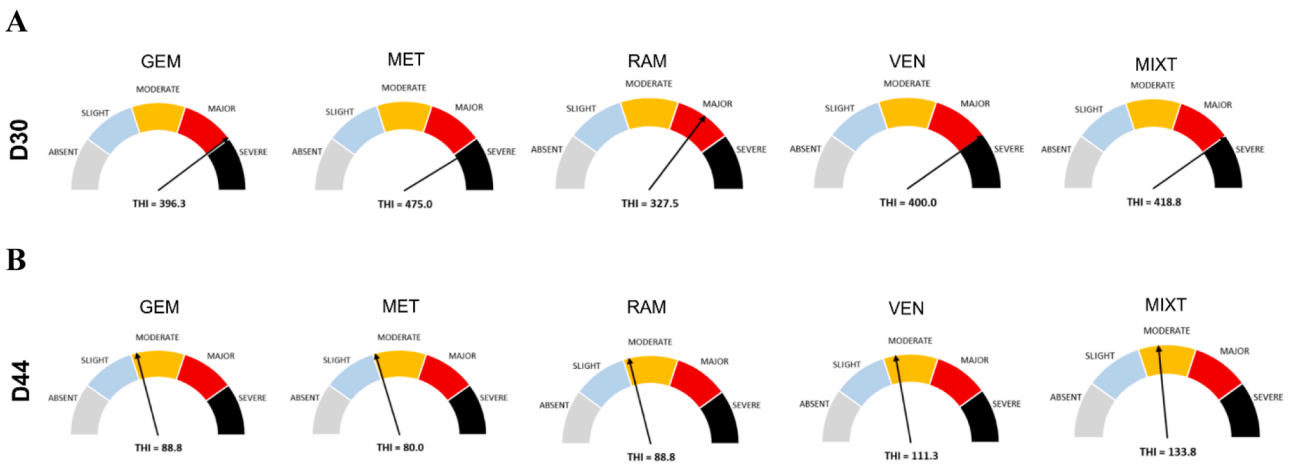


Fig. 4. Transcriptional Hazard Index (THI) for samples of different treatments GEM (Gemfibrozil) MET (Metformin), RAM (Ramipril) VEN (Venlafaxine) and MIXT (combined exposure of GEM, MET, RAM and VEN) collected after 30 days of exposure (Fig. 4A, D30) and after 14 days of depuration (Fig. 4B, D44). Colors represent the different hazard classes: Absent, Slight, Moderate, Major, Severe.

suggesting mechanisms of action similar to those in humans. In detail, we observed the dysregulation of "fatty acid metabolism", "adipogenesis", "insulin signalling", "lipid metabolism", "lipid and atherosclerosis" and "adipogenesis", among others. While most of these transcriptional changes were also observed in response to other treatments, significant up-regulation of "fatty acid catabolic processes", "peroxisome" and other pathways related to peroxisome regulation was uniquely found in GEM-exposed mussels. Notably, the effects of GEM exposure persisted even after depuration. At Day 44, "fatty acid metabolic process" and "peroxisome" were among the few terms that remained up-regulated, indicating a lasting impact on lipid metabolism. This persistent effect is further evidenced by the up-regulation of *Sterol O-acyltransferase 1* (*SOAT1*), a gene crucial in maintaining cellular cholesterol homeostasis (Chang et al., 2009). The impact on lipid metabolism at day 30, was also suggested by the up-regulation of *HSD17B7*, involved in cholesterol biosynthesis (Ohnesorg et al., 2006), and *ESRRG*, crucial in controlling lipid-mediated metabolic processes and playing a major role in glucose metabolism and energy homeostasis (Sadasivam et al., 2024).

These findings align with the observed activation of energy metabolism after 30 days of GEM exposure, characterized by the up-regulation of pathways and DEGs involved in maintaining energetic homeostasis. This metabolic shift likely reflects a compensatory response to ensure adequate ATP and other resources necessary for the activation of key cellular processes. Indeed, we observed the triggering of cell proliferation, signaling, and repair of cellular components at 30 days, suggesting a coordinated adaptation to the metabolic challenges posed by GEM exposure. Importantly, the up-regulation of "AMPK signaling pathways", observed exclusively in GEM-exposed mussels, provides further support for this interpretation. AMPK, a key energy sensor and regulator of cellular homeostasis, influences glucose uptake, fatty acid oxidation, and mitochondrial biogenesis (Mihaylova et al., 2011). Its activation suggests that cells were detecting and responding to a low energy status. This is further corroborated by the specific up-regulation of "glucose import" in GEM-exposed mussels, underscoring the organism's efforts to maintain energy balance.

Transcriptional changes of several pathways involved in immune response, inflammation and apoptosis were also observed, in particular after 30 days. Immune and inflammation modulatory effects of GEM have already been widely described in mammals (Jana et al. 2007; Roy and Pahan, 2009; Cámara-Lemarroy et al., 2015), as well as in mussels (Canesi et al., 2007). Concerning microbiota characterization, GEM led to the highest number of significant taxa after 30 days. However, there were few changes in predicted metabolic pathways. Moreover, potential pathogens such as *Vibrio* and *Pseudomonas* were found to be under-represented. These changes on microbial communities could potentially be attributed to a significant upregulation of the immune response observed at 30 days.

Among the four investigated treatments, GEM led also to the most important transcriptional changes in neurotransmission and synapse pathways and was the only treatment leading to the up-regulation of the "Notch signaling pathway". While the potential effects of GEM on neurotransmission have been recently proposed by Ivraghi et al. (2024), to our knowledge changes in the Notch signaling pathway have never been described in the literature.

After depuration, the number of DEGs decreased, with most of them being down-regulated in GEM-exposed mussels. In addition to the few pathways related to peroxisome, energy, and xenobiotic metabolism (maintained up-regulated after depuration), a few pathways and genes involved in cell cycle regulation and the immune system were down-regulated, showing opposite trends compared to 30 days. Noteworthy, among down-regulated genes showing higher fold changes at Day 44, a couple of genes coding for *SEPT14* and *SEPT2*, putatively involved in cell cycle progression (Fung et al., 2014). *SEPT2* is essential for cytokinesis, stabilizing the mitotic spindle, and organizing the actin cytoskeleton (Kremer et al., 2007), while *SEPT14* is involved in gametogenesis and may support asymmetric cell division and cytoskeletal dynamics, particularly in reproductive tissues (Vahabi Barzi et al., 2020). However, their specific roles in bivalves remain unclear and require further investigation. Also *STAT5B*, promoting G1 to S phase transition (Liang et al., 2009), was found down-regulated. Deceleration of the cell cycle after depuration was also suggested by the up-regulation of *CDK2AP1* (up-regulated also following MET and RAM exposure), playing a role in both cell-cycle and epigenetic regulation (Wong et al., 2012). Overall, these findings suggest that recovery after the cessation of GEM exposure was significant but not complete, with the THI indicating a reduction in hazard from "major/severe" to "moderate".

4.2. *Metformin*

Metformin (MET) is a biguanide molecule representing the first-choice drug for treatment of type 2 diabetes. MET influences a wide spectrum of biological processes like the glucose and fatty-acid metabolism, immune system and antioxidant system (Abdulla Mahmood,

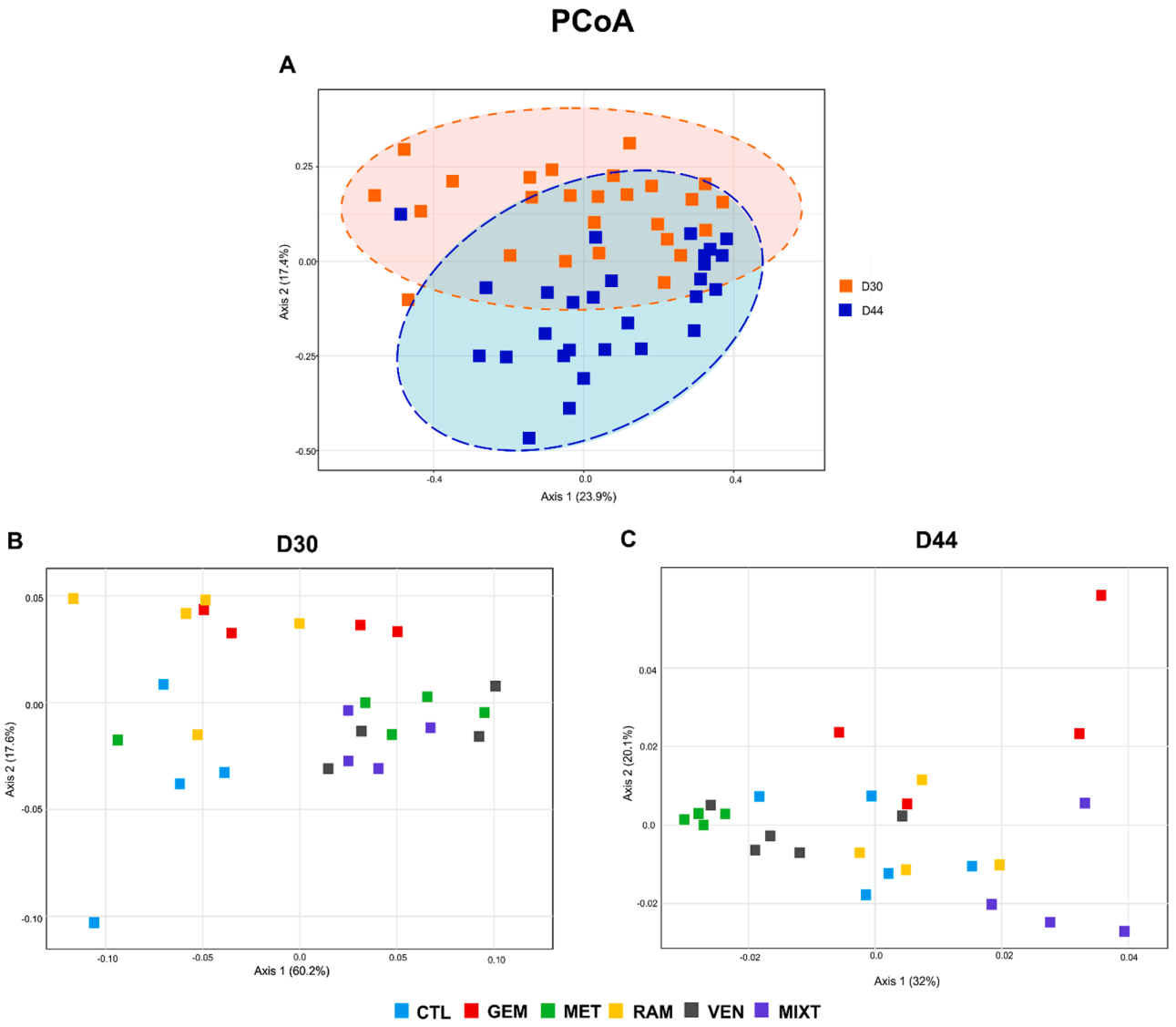


Fig. 5. Principal Coordinate Analyses (PCoA) A) showing spatial distribution of samples collected after ■ 30 days of exposure (D30) and ■ 14 days of depuration (D44); including B) samples collected after 30 days of exposure (D30); C) samples collected after 14 days of depuration (D44). Squares represent samples of different treatments: ■ CTL (Control group), ■ GEM (Gemfibrozil), ■ MET (Metformin), ■ RAM (Ramipril), ■ VEN (Venlafaxine), ■ MIXT (combined exposure of GEM, MET, RAM, VEN).

Table 1
Number of the over- (↑) and down- (↓) represented taxa (genus and species level) after 30 days of exposure (D30) and after the 14 days of depuration (D44) in treatments GEM (Gemfibrozil), MET (Metformin), Ramipril (RAM), VEN (Venlafaxine) and MIXT (combined exposure of GEM, MET, RAM and VEN) compared to the control group of the same sampling time.

	GENUS						SPECIES					
	D 30			D44			D 30			D44		
	↑	↓	TOT	↑	↓	TOT	↑	↓	TOT	↑	↓	TOT
GEM	10	6	**16**	0	6	**6**	3	4	**7**	0	1	**1**
MET	8	5	**13**	2	3	**5**	0	3	**3**	0	1	**1**
RAM	3	5	**8**	5	3	**8**	0	3	**3**	2	2	**4**
VEN	1	4	**5**	2	3	**5**	1	4	**5**	0	1	**1**
MIXT	3	7	**10**	0	6	**6**	1	3	**4**	0	1	**1**

2021; Du et al., 2022). In detail, MET has pleiotropic effects in humans and a complex mechanism of action mainly represented by *i)* the inhibition of Complex I in the respiratory chain leading to decreased ATP production and an increased AMP ratio, which activates AMP-activated protein kinase (AMPK) (Owen and Nemeroff, 1998); *ii)* the stimulation of AMPK, leading to repression of anabolic processes (e.g., gluconeogenesis; Hasanvand, 2022) and activation of catabolic processes, which increases fatty acid oxidation and glucose uptake to restore cellular energy homeostasis (Abdulla Mahmood, 2021); *iii)* the suppression of enzymes involved in gluconeogenesis, lowering blood glucose levels (Rena et al. 2017). A significant area of recent scientific investigation is also related to the potential impact of metformin (MET)

on the gut microbiome (Ma et al. 2018; Mueller et al. 2021; Petakh et al. 2023a; Petakh et al., 2023b). Although the effects on gut microbiota diversity have not been consistent, necessitating further research to understand their clinical significance (Petakh et al. 2023a), several studies have highlighted that metformin can alter gut microbiota composition and function, suggesting possible beneficial changes for metabolic and immune health (Pollak et al. 2017).

Mussels exposed to MET showed up-regulation of several pathways involved in energy metabolism at Day 30, related to glycolysis, fatty acid metabolism, and respiratory chain. As suggested for GEM-exposed mussels, the up-regulation of these pathways may function to support oxidative stress response, repair, and homeostasis maintenance (Goodchild et al., 2015). However, the up-regulation of "acetyl-CoA biosynthetic process from pyruvate" and "pyruvate metabolism", uniquely observed in MET-exposed mussels, as well as the over-expression of *IDH2* (a key enzyme in the Krebs cycle) and *SIRT6* (a stress-responsive protein influencing energy production and metabolic stress resistance; Klein and Denu (2020), suggest possible adaptive responses to metabolic changes caused by MET. Notably, *AMY2B*, involved in the digestion of dietary starch and glycogen (Li et al., 2020), was also found up-regulated at Day 30, while an opposite trend was observed after depuration at Day 44. This indicates possible metabolic adaptation following direct or indirect effects of MET on mussels' carbohydrate metabolism. In detail, following the inhibition of mitochondrial Complex I, with consequent reduced ATP production and altered metabolic states, cells may up-regulate pathways related to acetyl-CoA production and genes involved in carbohydrate metabolism. This could ensure a steady supply of key metabolites required for energy production and biosynthesis. Notably, also *AKT2*, a key kinase in the insulin signaling pathway responsible for regulating glucose uptake (Cho et al. 2001; Garofalo et al. 2003; Tassi et al. 2008), was found to be differentially expressed only in MET exposed mussels at Day 30. Its up-regulation could indicate a cellular adaptation aimed at increasing glucose uptake and utilization. Although MET and *AKT2* operate through different pathways and mechanisms, both contribute to increased glucose uptake. Specifically, *AKT2* is essential in the insulin signaling pathway for insulin-stimulated GLUT4 translocation. On the other hand, MET enhances glucose uptake through AMPK activation, which subsequently increases GLUT4 translocation to the cell membrane and improves insulin sensitivity (Garabadu and Krishnamurthy, 2017; Herman et al., 2024). Consequently, we can speculate that AMPK activation mediated by MET might indirectly affect *AKT2* by improving insulin sensitivity and influencing insulin signaling.

MET was the treatment leading to most important changes on microbiota predicted functional profiles. These changes, observed after depuration, were related to carbon metabolism, fatty acid metabolism and carbohydrate processing. In particular, changes in fructose, mannose, and starch metabolism suggest possible shifts in the community's potential for carbohydrate utilization. Microbiota functional analysis also revealed alterations in pathways associated with insulin signaling and type II diabetes mellitus. These changes, including modifications in the insulin resistance and signaling pathways, suggest that MET exposure may influence the microbiota's potential to interact with host glucose metabolism, indicating that the microbial community might play a role in mediating some of MET's effects on glucose homeostasis, even after depuration. Overall, while our findings should be considered preliminary evidence and further studies are required to deepen these aspects, they confirm, along with the significant decrease in microbial diversity detected at day 44, that MET alters the microbiota of the mussels' digestive glands, consistent with previous observations in model species (Pollak et al. 2017).

In addition to *AKT2*, activation of others serine/threonine kinases has been also observed (i.e. *NEK2, RIOK3*). *NEK2* is mainly involved in cell cycle regulation, while *RIOK3* plays a role in both cell cycle regulation and the modulation of type I interferon (IFN)-dependent immune responses (Fry et al. 2012; Shen et al. 2021; Fang and Zhang, 2016). Disruptions in several pathways involved in cell cycle regulation (e.g., "MYC target", "cell cycle phase transition") and cell signaling (e.g., "MAPK signaling", "NF-kB signaling pathways") were also highlighted by functional analyses. While possible links between MET exposure and modulation of most of these key pathways/biological processes need further investigation, possible effects on immune system and inflammation were already reported in freshwater fishes (*Labeo rohita* and *Danio rerio* (Demarco et al., 2023) and mussels (Koagouw and Ciocan, 2018).

Overall, MET was the investigated treatment leading to the highest number of differentially expressed genes (DEGs) at both sampling time. However, after depuration, only a few significant pathways and biological processes resulted from both GSEA and enrichment analysis (ORA). Most of these significant terms were involved in immune response and inflammation. Notably, all significant terms identified after depuration were down-regulated, showing opposite trends compared to Day 30.

In conclusion, our findings reveal that MET exposure induces a complex array of molecular and metabolic adaptations, potentially affecting energy metabolism, glucose homeostasis, cell cycle regulation, and immune responses. These changes are accompanied by significant alterations in the microbiota functions, suggesting a multifaceted impact of MET on both host physiology and associated microbial communities.

The significant transcriptional shifts from end-exposure to depuration were confirmed by the THI, which showed the highest and lowest values compared to other treatments at 30 and 44 days, respectively. Specifically, a "severe" hazard was observed after 30 days (THI = 475), while after depuration the THI dropped to "slight/moderate" (THI = 80). This suggests that MET exposure led to transient changes in the regulation of molecular pathways.

4.3. Ramipril

Ramipril (RAM), a member of the angiotensin-converting enzyme (ACE) inhibitors class, is among the most widely used antihypertensive drugs, commonly prescribed for the management of hypertension (high blood pressure), heart failure, and for reducing the occurrence of cardiovascular events in patients at high risk (Frampton and Peters, 1995; Wu et al. 2021). Despite the urgent need to obtain insights on the effects of pharmaceuticals in non-target aquatic organisms, this is, to our knowledge, the first study investigating the potential consequences of RAM exposure on marine species.

RAM blocks the ACE enzyme responsible for converting angiotensin I into angiotensin II, a peptide that causes blood vessel constriction. As a result, lower levels of angiotensin II lead to blood vessel dilation which reduces blood pressure, as well to a decreased aldosterone secretion, resulting in less sodium and water retention by the kidney (Salzet et al., 2001; Herman et al., 2024).

Homologues of angiotensin peptides, enzymes, and receptors have been documented in invertebrate species (Laurent et al. 1997; Salzet et al., 2001; Filice et al., 2024), confirming the evolutionary importance of the Renin-Angiotensin System (RAS). The presence of an ACE-like enzyme has been described in *Mytilus edulis* by Laurent et al. (1997) who suggested that this enzyme has properties potentially similar to human ACE, with the ability to convert angiotensin I to angiotensin II and a kinetic profile close to that observed in mammals. Overall, while the presence of RAS components in invertebrates has been demonstrated, the system has likely adapted for various functions beyond its classic role in vertebrates, which are still far from being fully understood.

Here, GSEA suggested fewer changes in signaling pathways, immune response, and inflammation compared to other treatments at Day 30. Conversely, several pathways involved in cell cycle regulation (e.g., "MYC targets V1", "MYC targets V2", "E2F targets", "G2M checkpoint") and stress response (e.g., "apoptosis", "unfolded protein response", "DNA repair", "reactive oxygen species pathways") were found to be up-

regulated, similar to other treatments. Disruption of cell cycle regulation was also suggested by several genes specifically found up-regulated in RAM exposed mussels at Day 30. Among them, *CNTRL* which plays a key role in cytokinesis (Salas et al., 2023); *E2F7*, a transcription factor that regulates cell cycle progression and the G1/S phase transition (de Bruin et al., 2003); *CDC45* and *CDC7* which are involved in the initiation of DNA replication and are essential for S phase progression (Petojevic et al., 2015); *CDT1* which regulates the initiation of DNA replication during the S phase and is putatively involved in the interlink between cell cycle regulation and DNA damage response pathways (Kanellou et al., 2020). Notably, enrichment analyses highlighted the significant up-regulation of "DNA damage response" (16 DEGs) and "DNA repair" (13 DEGs), suggesting possible cell damage. Following depuration (Day 44), GSEA indicated a disruption of the immune response, with all significant terms involved in this function ("NOD-like receptor signaling pathway", "defense response to other organism", "defense response to virus", "defense response to symbiont", "autophagy" and "innate immune response"). The potential impairment of the immune response following RAM depuration may have favored the relative over-representation of *Vibrio fortis* in RAM samples compared to the control, as well as the increase of *Tenacibaculum aiptasiae* in mussels sampled at Day 44 compared to Day 30 (Wang et al., 2008). While *Vibrio fortis* has already been associated with disease in many aquatic organisms (Romalde et al., 2014; Austin et al., 2005; Ding et al., 2014; Dai et al., 2023), to our knowledge, the potential pathogenicity of these taxa has never been described in mussels.

Calculation of THI indicates the lower value among treatments after exposure (THI=327.5; major), while a "moderate" hazard was calculated after depuration, mainly due to the down-regulation of pathways involved in immune response.

4.4. Venlafaxine

VEN is an antidepressant that acts as serotonin-norepinephrine reuptake inhibitor (SNRI) in the presynaptic terminal. In detail, VEN binds to the serotonin transporter (SERT) and norepinephrine transporter (NET), inhibiting the reuptake of serotonin and norepinephrine back into the presynaptic neuron. VEN is widely used for the treatment of moderate-to-severe depression and is also prescribed for patients with treatment-refractory depression (Holliday and Benfield, 1995; Andrews et al. 1996; Harvey et al. 2000).

Serotonin in bivalve species is involved in multiple physiological roles, including modulation of cardiovascular activity (Paciotti et al. 1985), gametogenesis and spawning, oocyte maturation and sperm motility (Alavi et al. 2017; Díaz Enrich et al., 2002; Deguchi and Osanai, 1995), feeding behavior and gill ciliary beating (Carroll and Catapane, 2007; Gainey, 2007), contraction and relaxation of the mantle and other muscles (Fabbri and Capuzzo, 2010; Dyachuk et al. 2012), as well as immune responses (Cloëz-Tayarani and Changeux, 2007; Jia et al. 2018). Accordingly, serotonin shows pleiotropic functions in bivalves and is considered a main actor in the neuroendocrine-immune regulation, with a key role in efficient responses to environmental stressors, including temperature changes, acidification and pollution (Alavi et al. 2017; Canesi et al. 2022). Norepinephrine is less studied in bivalve species than serotonin. However, possible involvement in heart rate regulation, as well as in stress responses and behavior have been proposed (Stefano and Catapane, 1980; Beiras et al. 1995; Malham et al. 2002).

Previous studies have highlighted the impact of various emerging contaminants on serotonergic components in bivalves (e.g. Ciacci et al., 2011). Of particular concern is the environmental exposure to antidepressants (e.g., fluoxetine, venlafaxine and duloxetine), which are among the most commonly detected human pharmaceuticals in aquatic environments. These antidepressants target neurotransmitters that are conserved in invertebrate groups (Lazzara et al., 2012; Fong and Ford, 2014; Franzellitti et al., 2014).

After 30 days of VEN exposure, we observed an upregulation of "serotonin metabolic process" and "synapse organization", suggesting a similarity in VEN's mechanism of action with the target species. Additionally, upregulation of *SIRT2* and *ATP6V1F* was noted. *SIRT2* can modulate depression-like behaviors. Recent studies have described SIRT2 upregulation in depressed patients and down-regulation following monoaminergic antidepressant administration, highlighting its potential as a target for therapeutic intervention (Liu et al., 2015; Wang et al., 2019; Erburu et al., 2015; Zhang et al., 2021). Polymorphisms in the *SIRT2* gene have also been associated with depression in Alzheimer's disease patients (Porcelli et al., 2013). Furthermore, Erburu and colleagues (2015) have described modulation of *SIRT2* expression following treatment with the selective noradrenaline reuptake inhibitor reboxetine and the selective serotonin reuptake inhibitor fluoxetine, suggesting that this could be a shared mechanism of action among monoaminergic antidepressants. *ATP6V1F* encodes a component of vacuolar ATPase (V-ATPase), which is involved in the storage of various neurotransmitters (Morel et al. 2023). A recent study suggested that V-ATPase are potentially impacted by Venlafaxine Souza (da Silva et al., 2023). Overall, these findings suggest that the MoA of VEN in mussels is similar to that observed in the target species.

Gomez and colleagues (2021) demonstrated the rapid decrease of VEN bioaccumulation in Mediterranean mussel after 7 days of depuration, while metabolites as N-desmethylvenlafaxine which contributes to the therapeutic effects of VEN in humans, persist in mussel tissues (Gomez et al., 2021). Here, sustained transcriptional activation of several pathways involved in xenobiotic metabolism (see Fig. 3) confirm the mussels' ability to metabolize VEN (Ariza-Castro et al., 2021). However, the higher number of differentially expressed genes (DEGs) detected after depuration compared to 30 days (240 and 165 DEGs, respectively) and the highest THI among treatments detected after 44 days (THI = 125; "moderate" hazard), suggest that VEN and/or its metabolites led to persistent changes at transcriptional level. Similar to MET and RAM treatments, major changes detected after depuration were related to the down-regulation of processes/pathways involved in immune response and inflammation as "cytokine production", "interferon response", "innate immune response" and "response to bacterium" among others. After depuration, we observed distinctive transcriptional changes. These included the down-regulation of pathways related to "xenobiotic metabolism", "wound healing" and "response to wounding". Concurrently, we noted an up-regulation of pathways involved in cell cycle regulation. While the down-regulation of xenobiotic metabolism and wound healing indicate that mussels were recovering from the exposure and no longer need to actively metabolize the drug or repair tissue damage, up-regulation of pathways and genes involved in cell cycle regulation and DNA repair was also observed (e.g. *structural maintenance of chromosomes, SMC5* and *SMC6; XPC; CCNB2; Protein Phosphatase 4 Regulatory Subunit 1, PPP4R1)*. Among them, *XPC*, contribute to the removal of oxidative DNA damage and prevent cell cycle arrest caused by genetic damage (Melis et al., 2011), while *SMCs* are key organizers of chromosome architecture and are essential for genome integrity (Laflamme et al. 2014). Overall, these findings indicate lasting effects at the molecular level and possible shift toward cellular proliferation, that could be related to an attempt to substitute cells damaged or lost during VEN exposure.

To conclude, while recent studies indicate possible modulation of mice gut microbiota following VEN administration (Du et al., 2024), we didn't observe significant changes in microbial diversity, composition and functions in the mussel digestive gland.

4.5. Combined exposure to Venlafaxine, Metformin, Ramipril and Gemfibrozil

The marine environment ultimately becomes a repository for all human-made products, including a diverse range of pharmaceuticals that often accumulate in aquatic ecosystems. However, the potential

additive or synergistic effects of these pharmaceutical mixtures remain insufficiently explored (Vasquez et al., 2014; Mezzelani et al. 2023). In this context, the MIXT treatment provides valuable insights into the potential effects of combined exposure to four different pharmaceuticals frequently detected in coastal waters.

MIXT and MET exposures were the only treatments showing "severe" hazard after 30 days. In addition, MIXT showed the highest THI after depuration, indicating a "moderate" hazard. Notably, ORA revealed the significant down-regulation of "oxidative phosphorylation", the crucial mitochondrial pathway for ATP production. In contrast, GSEA indicated the up-regulation of several key molecular pathways and biological processes involved in energy metabolism, including the "TCA cycle", "carbohydrate catabolic process", "glucose import", "glycolysis" and "fatty acid metabolism". These findings suggest that the cell may up-regulate these pathways to increase the availability of intermediates and cofactors, thereby partially compensating for reduced ATP production. This adaptation likely supports detoxification and stress responses, even when oxidative phosphorylation is less efficient. While the activation of molecular mechanisms maintaining energetic homeostasis suggests a potential synergistic effect of the investigated drugs, based on results obtained from single-drug exposures, a significant contribution from MET and GEM can be hypothesized. Noteworthy, MIXT treatment was unique in showing down-regulation of numerous energy metabolism pathways after depuration, a pattern not observed with individual drug exposures. This indicates that the combination of VEN, MET, RAM, and GEM might have induced more severe cellular stress, necessitating a longer recovery period. We speculate that the down-regulation of energy metabolism after depuration likely represents an adaptive response to conserve energy following multi-drug exposure stress.

GSEA revealed at Day 30 the up-regulation of nearly all terms related to cell cycle regulation included in this functional analysis. The up-regulation of processes involved in cell cycle regulation, along with several pathways and DEGs involved in damage repair (e.g., "DNA repair", "unfolded protein response", "regulation of DNA repair") and stress response (e.g., "response to oxidative stress", "apoptosis", "response to toxic substances", "tissue homeostasis", "inflammatory response", "xenobiotic metabolism", *CES2, CAS9, BIRC7* among others), indicates that the mussels experienced stress and damage from chemical exposure. The increased regulation of cell cycle processes, often observed in bivalve species following exposure to chemical stress, may reflect an attempt to maintain homeostasis, promote damage repair, and manage altered metabolic demands (Iannello et al., 2021; Mezzelani et al., 2023; Bernardini et al., 2023; Iori et al., 2020). Overall, after 30 days of exposure, the MIXT treatment led to transcriptional modifications also observed in one or more single-drug exposures. The only exception was "Toll-like receptor signaling pathway", which was found to be up-regulated exclusively following the combined exposure to the investigated pharmaceuticals.

After the depuration, all significant pathways identified were down-regulated as already described for energy metabolism. Among them, several pathways involved in immune response, signaling and xenobiotic metabolism were described in at least one single treatment. Conversely, "apoptosis" (both KEGG and Hallmark) were observed only in mussels exposed to MIXT, suggesting that mussels are still experiencing some degree of stress, albeit reduced compared to during exposure. Concerning digestive gland microbiota characterization, MIXT exposure led to similar changes to those detected in exposure to single pharmaceuticals, while no significant shifts were observed in microbiota functional profiles. In detail, at the end of exposure, 3 out of 4 significant taxa (species level) were commonly under-represented in VEN exposed mussels, while after depuration we found a unique significant microbial species common to VEN, MET and RAM. Overall, these findings confirm that all investigated pharmaceuticals led to limited changes in digestive gland microbiota, also following combined exposure.

4.6. Common responses among treatments

Molecular pathways commonly found to be differentially regulated across treatments can provide valuable insights into the key mechanisms that coordinate stress responses and help identify molecular markers of chemical stress. Among the signaling pathways consistently found to be differentially expressed, particular attention should be given to the upregulation of the "mTOR1 signaling pathway" observed across all treatments after 30 days. Indeed, mTORC1 signaling is a central regulator in eukaryotic cells, integrating various environmental signals including nutrient availability, energy status, and diverse stressors such as metabolic, genotoxic, and oxidative stress (Su and Dai, 2017). In detail, mTORC1 plays a crucial role in maintaining cellular homeostasis by modulating a wide array of fundamental cellular processes in response to environmental stressors (Saxton and Sabatini, 2017). These processes include protein synthesis and folding, proteome homeostasis, autophagy, vesicle and protein trafficking, glycolysis, endosome and lysosome distribution, apoptosis, and cell cycle regulation (Zhu et al., 2022; Su and Dai, 2017; Kaeser-Pebernard et al., 2022; Liu et al., 2017; Pourdehnad et al., 2013; Bae et al., 2022). The differential regulation of many of these processes observed across all single treatments and following MIXT exposure (e.g. "spliceosome", "protein export", "ubiquitin mediated proteolysis", "protein processing in ER", "endocytosis", "lipid transport", "carbohydrate catabolic process", "glucose catabolic process", "apoptosis", "cellular response to toxic substance", "protein secretion", "unfolded protein response", "tissue homeostasis"), supports the pivotal role of mTORC1 signaling in orchestrating mussels' stress response. This aligns with recent findings in other bivalve species exposed to chemical stress, such as the Manila clam *Ruditapes philippinarum* and the Pacific oyster *Magallana gigas* (Bernardini et al., 2023; Bernardini et al., 2024), confirming that mTORC1 signaling may be a conserved mechanism for coordinating cellular responses to environmental stressors also in bivalve species. In detail, the upregulation of mTORC1 signaling, along with pathways involved in drug metabolism, likely represents the mussels' attempt to maintain homeostasis under pharmaceutical exposure by monitoring cellular energy status and activating appropriate adaptive responses. This also makes mTORC1 signaling a potential key molecular marker for assessing chemical stress in bivalves and possibly other aquatic organisms.

A total of 9 DEGs were found commonly differentially regulated after single exposure to GEM, MET, RAM and VEN (see Supplementary File 1). Among them, *cystathionine beta-synthase* (*CBS*) is a key enzyme in the transsulfuration pathway, which converts homocysteine to cystathionine. This pathway is crucial for maintaining proper levels of homocysteine and for producing cysteine, a precursor to glutathione, one of the body's most important antioxidants (Zuhra et al., 2020). However, to our knowledge, *CBS* function in bivalves has never been characterized. Others commonly differentially regulated genes were represented by the up-regulated *GLI Pathogenesis Related 2* (GLIPR2), an evolutionarily conserved Golgi-associated protein recently proposed to negatively regulate autophagy (Eberle et al., 2002; Shoji-Kawata et al., 2013; Zhao et al., 2021), *NA Binding Motif Protein 25* (*RBM25*) and *Serine and Arginine Rich Splicing Factor 4* (*SRSF4*) involved in RNA processing and splicing (Carlson et al., 2017; Tan et al., 2018), and *SEC63 Homolog, Protein Translocation Regulator* (*SEC63*) that being part of the SEC complex is essential for proper folding and assembly of newly synthesized proteins within the ER (Jung and Kim, 2021).

5. Conclusion

Aquatic organisms are continually exposed to various classes of pharmaceuticals and personal care products (PPCPs) in their environment. Studying the effects and mechanisms of action of these pharmaceuticals on non-target species, particularly those detected in marine ecosystems, is crucial for developing effective monitoring plans to protect these ecosystems and also human health. In this study, by

combining gene expression analyses with microbiota characterization, we assessed the effects of four pharmaceuticals with different therapeutic properties on the Mediterranean mussel *M. galloprovincialis*. Exposure to the investigated pharmaceuticals led to significant alterations in mussel physiology, particularly in energy pathways, stress responses, signaling, cell cycle regulation, and immune response, with gemfibrozil, metformin, and venlafaxine showing potential similarities with the modes of action described in target species. Gene expression analyses confirmed the potential role of mTOR1 signaling as a key coordinator in the bivalve stress response. While host-microbiota characterization suggested few changes after exposure, occurring mainly following MET exposure, the Transcriptomic Hazard Index (THI) indicated a "severe" hazard for metformin and combined exposure after 30 days. THI decreased for all treatments by day 44, indicating "slight/moderate" hazards after a relatively short recovery period. Such partial recovery after a depuration period, with persistent impacts on stress-related and energy metabolism pathways, was particularly evident following combined exposure, further highlighting the urgent need to characterize the long-term effects of such mixtures for proper risk assessment of environmental pharmaceuticals.

CRediT authorship contribution statement

Ilaria Bernardini: Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marica Mezzelani:** Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Michela Panni:** Methodology, Investigation. **Giulia Dalla Rovere:** Investigation. **Alessandro Nardi:** Visualization, Data curation. **Ouafa El Idrissi:** Investigation. **Luca Peruzza:** Software. **Stefania Gorbi:** Visualization, Methodology. **Serena Ferraresso:** Investigation, Data curation. **Luca Bargelloni:** Supervision. **Tomaso Patarnello:** Supervision. **Francesco Regoli:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Massimo Milan:** Writing – original draft, Visualization, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors would like to thank the European Commission and the Ministry of University and Research (MUR, Italy), the Federal Ministry of Education and Research (BMBF, Germany), the Research Council of Norway (RCN, Norway) and State Research Agency (AEI, Spain) for funding in the frame of the collaborative international consortium PHARMASEA financed under the 2020 AquaticPollutants Joint call of the AquaticPollutants ERA-NET Cofund (GA N° 869178). This ERA-NET is an integral part of the activities developed by the Water, Oceans and AMR JPIs.

In addition, this work has been financially supported by the and under the framework of the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of MUR by the European Union – NextGenerationEU (Award number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the MUR and Research, CUP I33C22001300007, Project title "National Biodiversity Future Center—NBFC"). Marica Mezzelani benefited from a Research position co-funded by "Programma Operativo Nazionale (PON) Ricerca e Innovazione 2014–2020″ (MUR, Italy), by European Union REACT-EU and PHARMASEA.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2025.107255.

Data availability

We wrote in Data Availability section the link and the accession number of our data reference

References

Abdulla Mahmood, S., 2021. Mechanisms of Action of Metformin. In: Akhtar, J., Ahmad, U., Badruddeen, Irfan Khan, M. (Eds.), Metformin - Pharmacology and Drug Interactions. IntechOpen. https://doi.org/10.5772/intechopen.99189.

Alavi, S.M.H., Nagasawa, K., Takahashi, K.G., Osada, M., 2017. Pharmacology and Molecular Identity of Serotonin Receptor in Bivalve Mollusks. In: Shad, K.F. (Ed.), Serotonin - A Chemical Messenger Between All Types of Living Cells. InTech. https://doi.org/10.5772/intechopen.69680.

Ambrosio-Albuquerque, E.P., Cusioli, L.F., Bergamasco, R., Sinópolis Gigliolli, A.A., Lupepsa, L., Paupitz, B.R., Barbieri, P.A., Borin-Carvalho, L.A., De Brito Portela-Castro, A.L., 2021. Metformin environmental exposure: A systematic review. Environ. Toxicol. Pharmacol. 83, 103588. https://doi.org/10.1016/j.etap.2021.103588.

Andrews, J.M., Ninan, P.T., Nemeroff, C.B., 1996. Venlafaxine: A novel antidepressant that has a dual mechanism of action 4, 48–56.

Araujo, L., Villa, N., Camargo, N., Bustos, M., Garcia, T., Prieto, A, J., 2011. Persistence of gemfibrozil, naproxen and mefenamic acid in natural waters. Environ. Chem. Lett. 9, 13–18. https://doi.org/10.1007/s10311-009-0239-5.

Ariza-Castro, N., Courant, F., Dumas, T., Marion, B., Fenet, H., Gomez, E., 2021. Elucidating venlafaxine metabolism in the Mediterranean mussel (Mytilus galloprovincialis) through combined targeted and non-targeted approaches. Sci. Total Environ. 779, 146387. https://doi.org/10.1016/j.scitotenv.2021.146387.

Aßhauer, K.P., Wemheuer, B., Daniel, R., Meinicke, P., 2015. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics. 31, 2882–2884. https://doi.org/10.1093/bioinformatics/btv287.

Bae, S., Oh, B., Tsai, J., Park, P.S.U., Greenblatt, M.B., Giannopoulou, E.G., Park-Min, K. H., 2022. The crosstalk between MYC and mTORC1 during osteoclastogenesis. Front. Cell Dev. Biol. 10, 920683. https://doi.org/10.3389/fcell.2022.920683.

Balasubramanian, B., Liu, W.C., 2024. Editorial: Gut microbiota: allied with livestock nutrition, health, and welfare. Front. Vet. Sci. 11, 1413671. https://doi.org/10.3389/fvets.2024.1413671.

Beiras, R., Widdows, J., 1995. Effect of the neurotransmitters dopamine, serotonin and norepinephrine on the ciliary activity of mussel (Mytilus edulis) larvae. Mar. Biol. 122, 597–603. https://doi.org/10.1007/BF00350681.

Bernardini, I., Fabrello, J., Vecchiato, M., Ferraresso, S., Babbucci, M., Peruzza, L., Rovere, G.D., Masiero, L., Marin, M.G., Bargelloni, L., Gambaro, A., Patarnello, T., Matozzo, V., Milan, M., 2022. Effects of environmental concentrations of the fragrance amyl salicylate on the mediterranean mussel Mytilus galloprovincialis. Environ. Pollut. 307, 119502. https://doi.org/10.1016/j.envpol.2022.119502.

Bernardini, I., Quagliariello, A., Peruzza, L., Martino, M.E., Dalla Rovere, G., Iori, S., Asnicar, D., Ciscato, M., Fabrello, J., Corami, F., Cecchetto, M., Giubilato, E., Carrer, C., Bettiol, C., Semenzin, E., Marcomini, A., Matozzo, V., Bargelloni, L., Milan, M., Patarnello, T., 2023. Contaminants from dredged sediments alter the transcriptome of Manila clam and induce shifts in microbiota composition. BMC. Biol. 21, 234. https://doi.org/10.1186/s12915-023-01741-9.

Bernardini, I, Tallec, K, Paul-Pont, I, Peruzza, L, Dalla Rovere, G, Huber, M, Di Poi, C, Koechlin, H, Quéré, C, Quillien, V, Le Grand, J, Le Goïc, N, Lambert, C, Lagarde, F, Détrée, C, Trevisan, R, Corporeau, C, Patarnello, T, Milan, M, Huvet, A, 2024. Effects of tire particles and associated-chemicals on the Pacific oyster (Magallana gigas) physiology, reproduction and next-generation. J. Hazard. Mater. 480, 135742. https://doi.org/10.1016/j.jhazmat.2024.135742. Dec 5Epub 2024 Sep 10. PMID: 39276742.

Bisesi, J.H., Bridges, W., Klaine, S.J., 2014. Reprint of: Effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. Aquat. Toxicol. 151, 88–96. https://doi.org/10.1016/j.aquatox.2014.02.015.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y.,

Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Birch, G.F., Drage, D.S., Thompson, K., Eaglesham, G., Mueller, J.F., 2015. Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary. Australia. Mar. Pollut. Bull. 97, 56–66. https://doi.org/10.1016/j.marpolbul.2015.06.038.

Cámara-Lemarroy, C.R., Guzman-De La Garza, F.J., Cordero-Perez, P., Ibarra-Hernandez, J.M., Muñoz-Espinosa, L.E., Fernandez-Garza, N.E., 2015. Gemfibrozil attenuates the inflammatory response and protects rats from abdominal sepsis. Exp. Ther. Med. 9, 1018–1022. https://doi.org/10.3892/etm.2015.2190.

Cancio, I., Ibabe, A., P. Cajaraville, M., 1999. Seasonal variation of peroxisomal enzyme activities and peroxisomal structure in mussels Mytilus galloprovincialis and its relationship with the lipid content. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 123, 135–144. https://doi.org/10.1016/S0742-8413(99)00019-5.

Canesi, L., Lorusso, L.C., Ciacci, C., Betti, M., Regoli, F., Poiana, G., Gallo, G., Marcomini, A., 2007. Effects of blood lipid lowering pharmaceuticals (bezafibrate and gemfibrozil) on immune and digestive gland functions of the bivalve mollusc, Mytilus galloprovincialis. Chemosphere 69, 994–1002. https://doi.org/10.1016/j.chemosphere.2007.04.085.

Canesi, L., Miglioli, A., Balbi, T., Fabbri, E., 2022. Physiological Roles of Serotonin in Bivalves: Possible Interference by Environmental Chemicals Resulting in Neuroendocrine Disruption. Front. Endocrinol. 13, 792589. https://doi.org/10.3389/fendo.2022.792589.

Carlson, S.M., Soulette, C.M., Yang, Z., Elias, J.E., Brooks, A.N., Gozani, O., 2017. RBM25 is a global splicing factor promoting inclusion of alternatively spliced exons and is itself regulated by lysine mono-methylation. J. Biol. Chem. 292, 13381–13390. https://doi.org/10.1074/jbc.M117.784371.

Carroll, M.A., Catapane, E.J., 2007. The nervous system control of lateral ciliary activity of the gill of the bivalve mollusc, Crassostrea virginica. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 148, 445–450. https://doi.org/10.1016/j.cbpa.2007.06.003.

Castaño-Ortiz, J.M., Gil-Solsona, R., Ospina-Alvarez, N., García-Pimentel, M.M., León, V. M., Santos, L.H.M.L.M., Barceló, D., Rodríguez-Mozaz, S., 2023a. Bioaccumulation and fate of pharmaceuticals in a Mediterranean coastal lagoon: Temporal variation and impact of a flash flood event. Environ. Res. 228, 115887. https://doi.org/10.1016/j.envres.2023.115887.

Castaño-Trias, M., Rodríguez-Mozaz, S., Buttiglieri, G., 2023. A decade of water monitoring in a Mediterranean region: pharmaceutical prioritisation for an upgraded analytical methodology. Environ. Nanotechnol. Monit. Manag. 20. https://doi.org/10.1016/j.enmm.2023.100850.

Castillo-Zacarías, C., Barocio, M.E., Hidalgo-Vázquez, E., Sosa-Hernández, J.E., Parra-Arroyo, L., López-Pacheco, I.Y., et al., 2020. Antidepressant drugs as emerging contaminants: occurrence in urban and non-urban waters and analytical methods for their detection. Sci. Total Environ. 757. https://doi.org/10.1016/j.scitotenv.2020.143722. Article 143722.

Cecchetto, M., Peruzza, L., Giubilato, E., Bernardini, I., Rovere, G.D., Marcomini, A., Regoli, F., Bargelloni, L., Patarnello, T., Semenzin, E., Milan, M., 2023. An innovative index to incorporate transcriptomic data into weight of evidence approaches for environmental risk assessment. Environ. Res. 227, 115745. https://doi.org/10.1016/j.envres.2023.115745.

Chang, TY, Li, BL, Chang, CC, Urano, Y., 2009. Acyl-coenzyme A:cholesterol acyltransferases. Am. J. Physiol. Endocrinol. Metab. 297 (1), E1–E9. https://doi.org/10.1152/ajpendo.90926.2008. JulEpub 2009 Jan 13. PMID: 19141679; PMCID: PMC2711667.

Cho, H., Mu, J., Kim, J.K., Thorvaldsen, J.L., Chu, Q., Crenshaw, E.B., Kaestner, K.H., Bartolomei, M.S., Shulman, G.I., Birnbaum, M.J., 2001. Insulin Resistance and a Diabetes Mellitus-Like Syndrome in Mice Lacking the Protein Kinase Akt2 (PKBβ). Science (1979) 292, 1728–1731. https://doi.org/10.1126/science.292.5522.1728.

Ciacci, C., Barmo, C., Fabbri, R., Canonico, B., Gallo, G., Canesi, L., 2011. Immunomodulation in Mytilus galloprovincialis by non-toxic doses of hexavalent Chromium. Fish. Shellfish. Immunol. 31, 1026–1033. https://doi.org/10.1016/j.fsi.2011.09.002.

Claus, S.P., Guillou, H., Ellero-Simatos, S., 2016. The gut microbiota: a major player in the toxicity of environmental pollutants? NPJ. Biofilms. Microbiomes. 2. https://doi.org/10.1038/npjbiofilms.2016.3.

Cloëz-Tayarani, I., Changeux, J.P., 2007. Nicotine and serotonin in immune regulation and inflammatory processes: a perspective. J. Leukoc. Biol. 81, 599–606. https://doi.org/10.1189/jlb.0906544.

Da Silva, A.A.S., De Santi, F., Hinton, B.T., Cerri, P.S., Sasso-Cerri, E., 2023. Venlafaxine increases aromatization, reduces apical V-ATPase in clear cells and induces increased number of mast cells and smooth muscle cells death in rat cauda epididymis. Life Sci. 315, 121329. https://doi.org/10.1016/j.lfs.2022.121329.

de Bruin, A, Maiti, B, Jakoi, L, Timmers, C, Buerki, R, Leone, G., 2003. Identification and characterization of E2F7, a novel mammalian E2F family member capable of blocking cellular proliferation. J. Biol. Chem. 278 (43), 42041–42049. https://doi.org/10.1074/jbc.M308105200. Oct 24Epub 2003 Jul 31. PMID: 12893818.

De Diego, M., Godoy, G., Mennickent, S., Olivares, M., Godoy, R., 2010. Stress degradation studies of ramipril by a validated stability-indicating liquid chromatographic method. J. Chil. Chem. Soc. 55, 450–453. https://doi.org/10.4067/S0717-97072010000400008.

Deguchi, R., Osanai, K., 1995. Serotonin-Induced Meiosis Reinitiation from the First Prophase and from the First Metaphase in Oocytes of the Marine Bivalve Hiatella flaccida: Respective Changes in Intracellular Ca2+ and pH. Dev. Biol. 171, 483–496. https://doi.org/10.1006/dbio.1995.1298.

Demarco, N., Andrade, B., Zazula, M., Sobjak, T., D'Arce, L., Guimarães, A., 2023. Tissue Damage, Mutagenic Effect, and Alteration in Antioxidant Defense in Danio rerio (Cypriniformes: Cyprinidae) after Chronic Exposure to Metformin Hydrochloride. Ecotoxicol. Environ. Contam. 17, 115–125. https://doi.org/10.5132/eec.2022.02.12.

Desbiolles, F., Malleret, L., Tiliacos, C., Wong-Wah-Chung, P., Laffont-Schwob, I., 2018. Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment? Sci. Total Environ. 639, 1334–1348. https://doi.org/10.1016/j.scitotenv.2018.04.351.

Díaz Enrich, M.J., Ramos Martínez, J.I., Ibarguren Arizeta, I., 2002. Implication of Guanosine 3',5'-Cyclic Monophosphate, Adenosine 3',5'-Cyclic Monophosphate, Adenosine 5'-Mono-, Di- and Triphosphate and Fructose-2,6-Bisphosphate in the Regulation of the Glycolytic Pathway in Hypoxic/Anoxic Mussel. Mytilus Galloprovincialis. Mol. Cell. Biochem. 240, 111–118. https://doi.org/10.1023/A:1020666623094.

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, 2010. TextWith EEA Relevance.

Du, Y., Zhu, Y.J., Zhou, Y.X., Ding, J., Liu, J.Y., 2022. Metformin in therapeutic applications in human diseases: its mechanism of action and clinical study. Mol. Biomed. 3, 41. https://doi.org/10.1186/s43556-022-00108-w.

Du, C., Zhang, T., Feng, C., Sun, Q., Chen, Z., Shen, X., Liu, Y., Dai, G., Zhang, X., Tang, N., 2024. The effects of venlafaxine on depressive-like behaviors and gut microbiome in cuprizone-treated mice. Front. Psychiatry 15, 1347867. https://doi.org/10.3389/fpsyt.2024.1347867.

Dyachuk, V., Wanninger, A., Voronezhskaya, E., 2012. Innervation of bivalve larval catch muscles by serotonergic and FMRF AMIDERGIC neurons. Acta Biol. Hung. 63, 221–229. https://doi.org/10.1556/ABiol.63.2012.Suppl.2.30.

Eberle, H.B., Serrano, R.L., Füllekrug, J., Schlosser, A., Lehmann, W.D., Lottspeich, F., Kaloyanova, D., Wieland, F.T., Helms, J.B., 2002. Identification and characterization of a novel human plant pathogenesis-related protein that localizes to lipid-enriched microdomains in the Golgi complex. J. Cell Sci. 115, 827–838. https://doi.org/10.1242/jcs.115.4.827.

EPRS, 2023. Pollutants in EU waters. Update of chemicalsubstances listed for control.

Erburu, M., Muñoz-Cobo, I., Domínguez-Andrés, J., Beltran, E., Suzuki, T., Mai, A., Valente, S., Puerta, E., Tordera, R.M., 2015. Chronic stress and antidepressant induced changes in Hdac5 and Sirt2 affect synaptic plasticity. Eur. Neuropsychopharmacol. 25, 2036–2048. https://doi.org/10.1016/j.euroneuro.2015.08.016.

Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., Pinelli, E., 2019. Gut microbiota of aquatic organisms: A key endpoint for ecotoxicological studies. Environ. Pollut. 248, 989–999. https://doi.org/10.1016/j.envpol.2019.02.101.

Fabbri, E., Capuzzo, A., 2010. Cyclic AMP signaling in bivalve molluscs: an overview. J. Exp. Zool. Part Ecol. Genet. Physiol. 313A, 179–200. https://doi.org/10.1002/jez.592.

Fabbri, D, Maurino, V, Minella, M, Minero, C, Vione, D., 2017. Modelling the photochemical attenuation pathways of the fibrate drug gemfibrozil in surface waters. Chemosphere. Mar 170, 124–133. https://doi.org/10.1016/j.chemosphere.2016.11.135.

Fang, Y., Zhang, X., 2016. Targeting NEK2 as a promising therapeutic approach for cancer treatment. Cell Cycle 15, 895–907. https://doi.org/10.1080/15384101.2016.1152430.

Filice, M., Caferro, A., Amelio, D., Impellitteri, F., Iovine, M.A., Porretti, M., Faggio, C., Gattuso, A., Cerra, M.C., Imbrogno, S., 2024. The effects of ACE inhibitor Enalapril on Mytilus galloprovincialis: Insights into morphological and functional responses. Aquat. Toxicol. 273, 107014. https://doi.org/10.1016/j.aquatox.2024.107014.

Fernandes, JP, Almeida, CMR, Salgado, MA, Carvalho, MF, Mucha, AP., 2021. Pharmaceutical Compounds in Aquatic Environments-Occurrence, Fate and Bioremediation Prospective. Toxics. 9 (10), 257. https://doi.org/10.3390/toxics9100257. Oct 9PMID: 34678953; PMCID: PMC8537644.

Fong, P.P., Ford, A.T., 2014. The biological effects of antidepressants on the molluscs and crustaceans: A review. Aquat. Toxicol. 151, 4–13. https://doi.org/10.1016/j.aquatox.2013.12.003.

Foretz, M., Guigas, B., Viollet, B., 2023. Metformin: update on mechanisms of action and repurposing potential. Nat. Rev. Endocrinol. 19, 460–476. https://doi.org/10.1038/s41574-023-00833-4.

Fung, KY, Dai, L, Trimble, WS., 2014. Cell and molecular biology of septins. Int. Rev. Cell Mol. Biol. 310, 289–339. https://doi.org/10.1016/B978-0-12-800180-6.00007-4. PMID: 24725429.

Frampton, J.E., Peters, D.H., 1995. Ramipril: An Updated Review of its Therapeutic Use in Essential Hypertension and Heart Failure. Drugs 49, 440–466. https://doi.org/10.2165/00003495-199549030-00008.

Franzellitti, S., Buratti, S., Capolupo, M., Du, B., Haddad, S.P., Chambliss, C.K., Brooks, B. W., Fabbri, E., 2014. An exploratory investigation of various modes of action and potential adverse outcomes of fluoxetine in marine mussels. Aquat. Toxicol. 151, 14–26. https://doi.org/10.1016/j.aquatox.2013.11.016.

Freitas, R., Almeida, A., Calisto, V., Velez, C., Moreira, A., Schneider, R.J., Esteves, V.I., Wrona, F.J., Figueira, E., Soares, A.M.V.M., 2016. The impacts of pharmaceutical drugs under ocean acidification: new data on single and combined long-term effects of carbamazepine on Scrobicularia plana. Sci. Total Environ. 541, 977–985. https://doi.org/10.1016/j.scitotenv.2015.09.138.

Fry, A.M., O'Regan, L., Sabir, S.R., Bayliss, R., 2012. Cell cycle regulation by the NEK family of protein kinases. J. Cell Sci. jcs. 111195. https://doi.org/10.1242/jcs.111195.

Gainey, L.F., 2007. Seasonal control of particle clearance by isolated gills from the clam Mercenaria mercenaria. J. Exp. Biol. 210, 2518–2525. https://doi.org/10.1242/jeb.005637.

Garabadu, D., Krishnamurthy, S., 2017. Metformin attenuates hepatic insulin resistance in type-2 diabetic rats through PI3K/Akt/GLUT-4 signalling independent to bicuculline-sensitive GABA A receptor stimulation. Pharm. Biol. 55, 722–728. https://doi.org/10.1080/13880209.2016.1268635.

García-Blasco, C, Domingo-Echaburu, S, Orive, G, Lertxundi, U., 2024. Considering environmental impact of medicines in healthcare. Sci. Total. Environ. 908, 168526. https://doi.org/10.1016/j.scitotenv.2023.168526. Jan 15Epub 2023 Nov 13. PMID: 37967630.

Garofalo, R.S., Orena, S.J., Rafidi, K., Torchia, A.J., Stock, J.L., Hildebrandt, A.L., Coskran, T., Black, S.C., Brees, D.J., Wicks, J.R., McNeish, J.D., Coleman, K.G., 2003. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKBβ. J. Clin. Invest. 112, 197–208. https://doi.org/10.1172/JCI16885.

Gaulke, C.A., Barton, C.L., Proffitt, S., Tanguay, R.L., Sharpton, T.J., 2016. Triclosan Exposure Is Associated with Rapid Restructuring of the Microbiome in Adult Zebrafish. PLoS. One 11, e0154632. https://doi.org/10.1371/journal.pone.0154632.

Gaw, S., Thomas, K., Hutchinson, T., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. T. R. Soc. B. 369 (1656), 20130572. https://doi.org/10.1098/rstb.2013.0572.

Gerstner, J.R., Koberstein, J.N., Watson, A.J., Zapero, N., Risso, D., Speed, T.P., Frank, M. G., Peixoto, L., 2016. Removal of unwanted variation reveals novel patterns of gene expression linked to sleep homeostasis in murine cortex. BMC. Genomics. 17, 727. https://doi.org/10.1186/s12864-016-3065-8.

Ghoshdastidar, A.J., Fox, S., Tong, A.Z., 2015. The presence of the top prescribed pharmaceuticals in treated sewage effluents and receiving waters in Southwest Nova Scotia. Canada. Environ Sci Pollut Res 22, 689–700. https://doi.org/10.1007/s11356-014-3400-z.

Gomez, E., Boillot, C., Martinez Bueno, M.J., Munaron, D., Mathieu, O., Courant, F., Fenet, H., 2021. In vivo exposure of marine mussels to venlafaxine: bioconcentration and metabolization. Environ. Sci. Pollut. Res. 28, 68862–68870. https://doi.org/10.1007/s11356-021-14893-4.

Goodchild, C.G., Frederich, M., Zeeman, S.I., 2015. AMP-activated protein kinase is a biomarker of energetic status in freshwater mussels exposed to municipal effluents. Sci. Total Environ. 512–513, 201–209. https://doi.org/10.1016/j.scitotenv.2015.01.065.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8, 1494–1512. https://doi.org/10.1038/nprot.2013.084.

Harvey, A.T., Rudolph, R.L., Preskorn, S.H., 2000. Evidence of the Dual Mechanisms of Action of Venlafaxine. Arch. Gen. Psychiatry 57, 503. https://doi.org/10.1001/archpsyc.57.5.503.

Hasanvand, A., 2022. The role of AMPK-dependent pathways in cellular and molecular mechanisms of metformin: a new perspective for treatment and prevention of diseases. Inflammopharmacology. 30, 775–788. https://doi.org/10.1007/s10787-022-00980-6.

Herman, L.L., Padala, S.A., Ahmed, I., Bashir, K., 2024. Angiotensin-Converting Enzyme Inhibitors (ACEI). StatPearls. StatPearls Publishing, Treasure Island (FL).

Holliday, S.M., Benfield, P., 1995. Venlafaxine: A Review of its Pharmacology and Therapeutic Potential in Depression. Drugs 49, 280–294. https://doi.org/10.2165/00003495-199549020-00010.

Iannello, M., Mezzelani, M., Dalla Rovere, G., Smits, M., Patarnello, T., Ciofi, C., Carraro, L., Boffo, L., Ferraresso, S., Babbucci, M., Mazzariol, S., Centelleghe, C., Cardazzo, B., Carrer, C., Varagnolo, M., Nardi, A., Pittura, L., Benedetti, M., Fattorini, D., Regoli, F., Ghiselli, F., Gorbi, S., Bargelloni, L., Milan, M., 2021. Long-lasting effects of chronic exposure to chemical pollution on the hologenome of the Manila clam. Evol. Appl. 14, 2864–2880. https://doi.org/10.1111/eva.13319.

Iori, S., Rovere, G.D., Ezzat, L., Smits, M., Ferraresso, S.S., Babbucci, M., Marin, M.G., Masiero, L., Fabrello, J., Garro, E., Carraro, L., Cardazzo, B., Patarnello, T., Matozzo, V., Bargelloni, L., Milan, M., 2020. The effects of glyphosate and AMPA on the mediterranean mussel Mytilus galloprovincialis and its microbiota. Environ. Res. 182, 108984. https://doi.org/10.1016/j.envres.2019.108984.

Italian Legislative Decree n. 26, 2014. Implementation of Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, 2014.

Ivraghi, M.S., Zamanian, M.Y., Gupta, R., Achmad, H., Alsaab, H.O., Hjazi, A., Romero-Parra, R.M., Alwaily, E.R., Hussien, B.M., Hakimizadeh, E., 2024. Neuroprotective effects of gemfibrozil in neurological disorders: Focus on inflammation and molecular mechanisms. CNS Neurosci. Ther. 30, e14473. https://doi.org/10.1111/cns.14473.

Jana, M., Jana, A., Liu, X., Ghosh, S., Pahan, K., 2007. Involvement of Phosphatidylinositol 3-Kinase-Mediated Up-Regulation of IκBα in Anti-Inflammatory Effect of Gemfibrozil in Microglia. J. Immunol. 179, 4142–4152. https://doi.org/10.4049/jimmunol.179.6.4142.

Jia, Y., Yang, B., Dong, W., Liu, Z., Lv, Z., Jia, Z., Qiu, L., Wang, L., Song, L., 2018. A serotonin receptor (Cg5-HTR-1) mediating immune response in oyster Crassostrea gigas. Dev. Comp. Immunol. 82, 83–93. https://doi.org/10.1016/j.dci.2017.12.029.

Jin, Y., Wu, S., Zeng, Z., Fu, Z., 2017. Effects of environmental pollutants on gut microbiota. Environ. Pollut. 222, 1–9. https://doi.org/10.1016/j.envpol.2016.11.045.

Jung, S., Kim, H., 2021. Emerging View on the Molecular Functions of Sec62 and Sec63 in Protein Translocation. Int. J. Mol. Sci. 22, 12757. https://doi.org/10.3390/ijms222312757.

Kaeser-Pebernard, S., Vionnet, C., Mari, M., Sankar, D.S., Hu, Z., Roubaty, C., Martínez-Martínez, E., Zhao, H., Spuch-Calvar, M., Petri-Fink, A., Rainer, G., Steinberg, F., Reggiori, F., Dengjel, J., 2022. mTORC1 controls Golgi architecture and vesicle secretion by phosphorylation of SCYL1. Nat. Commun. 13, 4685. https://doi.org/10.1038/s41467-022-32487-7.

Kanellou, A, Giakoumakis, NN, Panagopoulos, A, Tsaniras, SC, Lygerou, Z., 2020. The Licensing Factor Cdt1 Links Cell Cycle Progression to the DNA Damage Response. Anticancer Res. 40 (5), 2449–2456. https://doi.org/10.21873/anticanres.14214. MayPMID: 32366388.

Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Klein, MA, Denu, JM., 2020. Biological and catalytic functions of sirtuin 6 as targets for small-molecule modulators. J. Biol. Chem. 295 (32), 11021–11041. https://doi.org/10.1074/jbc.REV120.011438. Aug 7Epub 2020 Jun 9. PMID: 32518153; PMCID: PMC7415977.

Koagouw, W., Ciocan, C., 2018. Impact of Metformin and Increased Temperature on Blue Mussels Mytilus edulis - Evidence for Synergism. J. Shellfish Res. 37, 467–474. https://doi.org/10.2983/035.037.0301.

Koagouw, W., Hazell, R.J., Ciocan, C., 2021. Induction of apoptosis in the gonads of Mytilus edulis by metformin and increased temperature, via regulation of HSP70, CASP8, BCL2 and FAS. Mar. Pollut. Bull. 173, 113011. https://doi.org/10.1016/j.marpolbul.2021.113011.

Kong, C., Pan, T., Chen, X., Junaid, M., Liao, H., Gao, D., Wang, Q., Liu, W., Wang, X., Wang, J., 2023. Exposure to polystyrene nanoplastics and PCB77 induced oxidative stress, histopathological damage and intestinal microbiota disruption in white hard clam Meretrix lyrata. Sci. Total Environ. 905, 167125. https://doi.org/10.1016/j.scitotenv.2023.167125.

Kremer, BE, Adang, LA, Macara, IG., 2007. Septins regulate actin organization and cell-cycle arrest through nuclear accumulation of NCK mediated by SOCS7. Cell 130 (5), 837–850. https://doi.org/10.1016/j.cell.2007.06.053. Sep 7PMID: 17803907; PMCID: PMC2085444.

Lacaze, E., Pédelucq, J., Fortier, M., Brousseau, P., Auffret, M., Budzinski, H., Fournier, M., 2015. Genotoxic and immunotoxic potential effects of selected psychotropic drugs and antibiotics on blue mussel (Mytilus edulis) hemocytes. Environ. Pollut. 202, 177–186. https://doi.org/10.1016/j.envpol.2015.03.025.

Laflamme, G, Tremblay-Boudreault, T, Roy, MA, Andersen, P, É, Bonneil, Atchia, K, Thibault, P, D'Amours, D, Kwok, BH, 2014. Structural maintenance of chromosome (SMC) proteins link microtubule stability to genome integrity. J. Biol. Chem. 289 (40), 27418–27431. https://doi.org/10.1074/jbc.M114.569608. Oct 3Epub 2014 Aug 18. PMID: 25135640; PMCID: PMC4183782.

Laurent, V., Stefano, G., Salzet, M., 1997. Presence and biochemical properties of a molluscan invertebrate angiotensin-converting enzyme. Regul. Pept. 69, 53–61. https://doi.org/10.1016/S0167-0115(96)02121-0.

Lazzara, R., Blázquez, M., Porte, C., Barata, C., 2012. Low environmental levels of fluoxetine induce spawning and changes in endogenous estradiol levels in the zebra mussel Dreissena polymorpha. Aquat. Toxicol. 106–107, 123–130. https://doi.org/10.1016/j.aquatox.2011.11.003.

Lee, J.W., Shin, Y.J., Kim, Hokyun, Kim, Heejung, Kim, J., Min, S.A., Kim, P., Yu, S.D., Park, K., 2019. Metformin-induced endocrine disruption and oxidative stress of Oryzias latipes on two-generational condition. J. Hazard. Mater. 367, 171–181. https://doi.org/10.1016/j.jhazmat.2018.12.084.

Li, F., Gong, X., Zhou, Y., Geng, Q., Jiang, Y., Yao, L., Qu, M., Tan, Z., 2024. Integrated evidence of transcriptional, metabolic, and intestinal microbiota changes in Ruditapes philippinarum due to perfluorooctanoic acid-induced immunotoxicity. Sci. Total Environ. 916, 170341. https://doi.org/10.1016/j.scitotenv.2024.170341.

Li, X, Yang, J, Zhu, S, Li, Y, Chen, W, Hu, Z., 2020. Insight into the combinatorial transcriptional regulation on α-amylase gene in animal groups with different dietary nutrient content. Genomics 112 (1), 520–527. https://doi.org/10.1016/j.ygeno.2019.04.004. JanEpub 2019 Apr 6. PMID: 30965097.

Liang, QC, Xiong, H, Zhao, ZW, Jia, D, Li, WX, Qin, HZ, Deng, JP, Gao, L, Zhang, H, Gao, GD., 2009. Inhibition of transcription factor STAT5b suppresses proliferation, induces G1 cell cycle arrest and reduces tumor cell invasion in human glioblastoma multiforme cells. Cancer Lett. 273 (1), 164–171. https://doi.org/10.1016/j.canlet.2008.08.011. Jan 8Epub 2008 Sep 14. PMID: 18793823.

Liu, P., Ge, M., Hu, J., Li, X., Che, L., Sun, K., Cheng, L., Huang, Y., Pilo, M.G., Cigliano, A., Pes, G.M., Pascale, R.M., Brozzetti, S., Vidili, G., Porcu, A., Cossu, A., Palmieri, G., Sini, M.C., Ribback, S., Dombrowski, F., Tao, J., Calvisi, D.F., Chen, L., Chen, X., 2017. A functional mammalian target of rapamycin complex 1 signaling is indispensable for c-Myc-driven hepatocarcinogenesis. Hepatology 66, 167–181. https://doi.org/10.1002/hep.29183.

Liu, R., Dang, W., Du, Y., Zhou, Q., Jiao, K., Liu, Z., 2015. SIRT2 is involved in the modulation of depressive behaviors. Sci. Rep. 5, 8415. https://doi.org/10.1038/srep08415.

Liu, S., Wen, D., Feng, C., Yu, C., Gu, Z., Wang, L., Zhang, Z., Li, W., Wu, S., Liu, Y., Duan, C., Zhuang, R., Xue, L., 2023. Alteration of gut microbiota after heat acclimation may reduce organ damage by regulating immune factors during heat stress. Front. Microbiol. 14, 1114233. https://doi.org/10.3389/fmicb.2023.1114233.

Lu, Y., Zhou, G., Ewald, J., Pang, Z., Shiri, T., Xia, J., 2023. MicrobiomeAnalyst 2.0: comprehensive statistical, functional and integrative analysis of microbiome data. Nucleic. Acids. Res. 51, W310–W318. https://doi.org/10.1093/nar/gkad407.

Ma, L., Zhao, H., Wu, L.B., Cheng, Z., Liu, C., 2023. Impact of the microbiome on human, animal, and environmental health from a One Health perspective. Sci. One Health 2, 100037. https://doi.org/10.1016/j.soh.2023.100037.
Ma, W., Chen, J., Meng, Y., Yang, J., Cui, Q., Zhou, Y., 2018. Metformin alters gut microbiota of healthy mice: implication for its potential role in gut microbiota homeostasis. Front. Microbiol. 9, 1336. https://doi.org/10.3389/fmicb.2018.01336.
MacLaren, R.D., Wisniewski, K., MacLaren, C., 2018. Environmental concentrations of metformin exposure affect aggressive behavior in the Siamese fighting fish, Betta splendens. PLoS. One 13, e0197259. https://doi.org/10.1371/journal.pone.0197259.
Madikizela, L.M., Ncube, S., 2022. Health effects and risks associated with the occurrence of pharmaceuticals and their metabolites in marine organisms and seafood. Sci. Total Environ. 837, 155780. https://doi.org/10.1016/j.scitotenv.2022.155780.
Mahmoud, M.E., El-Ghanam, A.M., Saad, S.R., Mohamed, R.H.A., 2020. Promoted removal of metformin hydrochloride anti-diabetic drug from water by fabricated and modified nanobiochar from artichoke leaves. Sustain. Chem. Pharm. 18, 100336. https://doi.org/10.1016/j.scp.2020.100336.
Malham, S., 2002. A first insight into stress-induced neuroendocrine and immune changes in the octopus Eledone cirrhosa. Aquat. Living Resour. 15, 187–192. https://doi.org/10.1016/S0990-7440(02)01173-7.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17, 10. https://doi.org/10.14806/ej.17.1.200.
Melis, JP, Luijten, M, Mullenders, LH, van Steeg, H., 2011. The role of XPC: implications in cancer and oxidative DNA damage. Mutat. Res. 728 (3), 107–117. https://doi.org/10.1016/j.mrrev.2011.07.001. Nov-DecEpub 2011 Jul 7. PMID: 21763452; PMCID: PMC3203325.
Mezzelani, M., Fattorini, D., Gorbi, S., Nigro, M., Regoli, F., 2020. Human pharmaceuticals in marine mussels: evidence of sneaky environmental hazard along Italian coasts. Mar. Environ. Res 162. https://doi.org/10.1016/j.marenvres.2020.105137.
Mezzelani, M., Regoli, F., 2022. The biological effects of pharmaceuticals in the marine environment. Annu. Rev. Mar. Sci. 14. https://doi.org/10.1146/annurev-marine-040821-075606.
Mezzelani, M., Peruzza, L., d'Errico, G., Milan, M., Gorbi, S., Regoli, F., 2023. Mixtures of environmental pharmaceuticals in marine organisms: Mechanistic evidence of carbamazepine and valsartan effects on Mytilus galloprovincialis. Sci. Total Environ. 860, 160465. https://doi.org/10.1016/j.scitotenv.2022.160465.
Mezzelani, M., Gorbi, S., Fattorini, D., d'Errico, G., Consolandi, G., Milan, M., Bargelloni, L., Regoli, F., 2018a. Long-term exposure of Mytilus galloprovincialis to Diclofenac, Ibuprofen and Ketoprofen: insights into bioavailability, biomarkers and transcriptomic changes. Chemosphere 198, 238–248. https://doi.org/10.1016/j.chemosphere.2018.01.148.
Mihaylova, M.M., Shaw, R.J., 2011. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat. Cell Biol. 13, 1016–1023. https://doi.org/10.1038/ncb2329.
Milan, M., Carraro, L., Fariselli, P., Martino, M.E., Cavalieri, D., Vitali, F., Boffo, L., Patarnello, T., Bargelloni, L., Cardazzo, B., 2018. Microbiota and environmental stress: how pollution affects microbial communities in Manila clams. Aquat. Toxicol. 194, 195–207. https://doi.org/10.1016/j.aquatox.2017.11.019.
Mimeault, C., Woodhouse, A.J., Miao, X.S., Metcalfe, C.D., Moon, T.W., Trudeau, V.L., 2005. The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, Carassius auratus. Aquat. Toxicol. 73, 44–54. https://doi.org/10.1016/j.aquatox.2005.01.009.
Morel, N., 2003. Neurotransmitter release: the dark side of the vacuolar-H + ATPase. Biol. Cell 95, 453–457. https://doi.org/10.1016/S0248-4900(03)00075-3.
Mueller, NT, Differding, MK, Zhang, M, Maruthur, NM, Juraschek, SP, Miller 3rd, ER, Appel, LJ, Yeh, HC., 2021. Metformin Affects Gut Microbiome Composition and Function and Circulating Short-Chain Fatty Acids: A Randomized Trial. Diabetes Care 44 (7), 1462–1471. https://doi.org/10.2337/dc20-2257. JulEpub 2021 May 18. PMID: 34006565; PMCID: PMC8323185.
Nardi, A., Mezzelani, M., Costa, S., d'Errico, G., Benedetti, M., Gorbi, S., Freitas, R., Regoli, F., 2022. Marine heatwaves hamper neuro-immune and oxidative tolerance toward carbamazepine in Mytilus galloprovincialis. Environ. Pollut. 300, 118970. https://doi.org/10.1016/j.envpol.2022.118970.
Niemuth, N.J., Jordan, R., Crago, J., Blanksma, C., Johnson, R., Klaper, R.D., 2015. Metformin exposure at environmentally relevant concentrations causes potential endocrine disruption in adult male fish. Environ. Toxicol. Chem. 34, 291–296. https://doi.org/10.1002/etc.2793.
Niemuth, N.J., Klaper, R.D., 2015. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere 135, 38–45. https://doi.org/10.1016/j.chemosphere.2015.03.060.
Ojemaye, C.Y., Petrik, L., 2019. Pharmaceuticals in the marine environment: a review. Environ. Rev. 27, 151–165. https://doi.org/10.1139/er-2018-0054.
Oliveira, M., Franco, L., Balasch, J.C., Fierro-Castro, C., Tvarijonaviciute, A., Soares, A. M.V.M., Tort, L., Teles, M., 2018. Tools to assess effects of human pharmaceuticals in fish: A case study with gemfibrozil. Ecol. Indic. 95, 1100–1107. https://doi.org/10.1016/j.ecolind.2017.12.051.
Ohnesorg, T, Keller, B, Hrabé de Angelis, M, Adamski, J, 2006. Transcriptional regulation of human and murine 17beta-hydroxysteroid dehydrogenase type-7 confers its participation in cholesterol biosynthesis. J. Mol. Endocrinol. 37 (1), 185–197. https://doi.org/10.1677/jme.1.02043. Aug.
Owen, J.R., Nemeroff, C.B., 1998. New antidepressants and the cytochrome P450 system: focus on venlafaxine, nefazodone, and mirtazapine. Depress. Anxiety 7 (Suppl 1), 24–32. https://doi.org/10.1002/(SICI)1520-6394(1998)7:1+%3C24::AID-DA7%3E3.0.CO;2-F.
Paciotti, G.F., Higgins, W.J., 1985. Potentiation of the 5-hydroxytryptamine-induced increases in myocardial contractility in Mercenaria mercenaria ventricle by forskolin. Comp. Biochem. Physiol. Part C Comp. Pharmacol. 80, 325–329. https://doi.org/10.1016/0742-8413(85)90064-7.
Paillard, C., Gueguen, Y., Wegner, K.M., Bass, D., Pallavicini, A., Vezzulli, L., Arzul, I., 2022. Recent advances in bivalve-microbiota interactions for disease prevention in aquaculture. Curr. Opin. Biotechnol. 73, 225–232. https://doi.org/10.1016/j.copbio.2021.07.026.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 30, 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.
Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419. https://doi.org/10.1038/nmeth.4197.
Petakh, P, Kamyshna, I., Kamyshnyi, A., 2023a. Effects of metformin on the gut microbiota: A systematic review. Mol. Metab. 77, 101805. https://doi.org/10.1016/j.molmet.2023.101805.
Pradhan, P.K., Verma, D.K., Peruzza, L., Gupta, S., Haq, S.A., Shubin, S.V., Morgan, K.L., Trusch, F., Mohindra, V., Hauton, C., van West, P., Sood, N., 2020. Molecular insights into the mechanisms of susceptibility of Labeo rohita against oomycete Aphanomyces invadans. Sci. Rep. 10, 19531. https://doi.org/10.1038/s41598-020-76278-w.
Prajaputra, V., Isnaini, N., 2023. Assessment of Metformin Stability and Its Removal from Water by Pumice-Based Zeolite. Jurnal Penelitian Pendidikan IPA 9 (7), 4909–4916. https://doi.org/10.29303/jppipa.v9i7.3965.
Porcelli, S., Salfi, R., Politis, A., Atti, A.R., Albani, D., Chierchia, A., Polito, L., Zisaki, A., Piperi, C., Liappas, I., Alberti, S., Balestri, M., Marsano, A., Stamouli, E., Mailis, A., Biella, G., Forloni, G., Bernabei, V., Ferrari, B., Lia, L., Papadimitriou, G.N., De Ronchi, D., Serretti, A., 2013. Association between Sirtuin 2 gene rs10410544 polymorphism and depression in Alzheimer's disease in two independent European samples. J. Neural Transm. 120, 1709–1715. https://doi.org/10.1007/s00702-013-1045-6.
Peruzza, L., Tucci, C.F., Frizzo, R., Riello, T., Quagliariello, A., Martino, M.E., Manuzzi, A., Dalla Rovere, G., Bonsembiante, F., Gelain, M.E., Smits, M., Borgheresi, O., Camerani, F., Panin, M., Venier, P., Mammi, S., Hauton, C., Patarnello, T., Milan, M., Bargelloni, L., 2023. Impaired reproduction, energy reserves and dysbiosis: The overlooked consequences of heatwaves in a bivalve mollusc. Mar. Pollut. Bull. 193, 115192. https://doi.org/10.1016/j.marpolbul.2023.115192.
Petakh, P., Oksenych, V., Kamyshnyi, A., 2023. The F/B ratio as a biomarker for inflammation in COVID-19 and T2D: impact of metformin. Biomed. PharmacOther 163. https://doi.org/10.1016/j.biopha.2023.114892.
Petojevic, T, Pesavento, JJ, Costa, A, Liang, J, Wang, Z, Berger, JM, Botchan, MR., 2015. Cdc45 (cell division cycle protein 45) guards the gate of the Eukaryote Replisome helicase stabilizing leading strand engagement. Proc Natl Acad Sci U S A. 112 (3), E249–E258. https://doi.org/10.1073/pnas.1422003112. Jan 20Epub 2015 Jan 5. PMID: 25561522; PMCID: PMC4311868.
Pollak, M., 2017. The effects of metformin on gut microbiota and the immune system as research frontiers. Diabetologia 60 (9), 1662–1667. https://doi.org/10.1007/s00125-017-4352-x.
Pourdehnad, M., Truitt, M.L., Siddiqi, I.N., Ducker, G.S., Shokat, K.M., Ruggero, D., 2013. Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. Proc. Natl. Acad. Sci. 110, 11988–11993. https://doi.org/10.1073/pnas.1310230110.
Prindiville, J.S., Mennigen, J.A., Zamora, J.M., Moon, T.W., Weber, J.M., 2011. The fibrate drug gemfibrozil disrupts lipoprotein metabolism in rainbow trout. Toxicol. Appl. Pharmacol. 251, 201–208. https://doi.org/10.1016/j.taap.2010.12.013.
Quinn, B., Schmidt, W., O'Rourke, K., Hernan, R., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on biomarker expression in the zebra mussel (Dreissena polymorpha) and their comparison with standardised toxicity tests. Chemosphere 84, 657–663. https://doi.org/10.1016/j.chemosphere.2011.03.033.
Rafiq, A., Capolupo, M., Addesse, G., Valbonesi, P., Fabbri, E., 2023. Antidepressants and their metabolites primarily affect lysosomal functions in the marine mussel. Mytilus galloprovincialis. Sci. Total Environ. 903, 166078. https://doi.org/10.1016/j.scitotenv.2023.166078.
Rena, G., Hardie, D.G., Pearson, E.R., 2017. The mechanisms of action of metformin. Diabetologia 60, 1577–1585. https://doi.org/10.1007/s00125-017-4342-z.
Roy, A., Pahan, K., 2009. Gemfibrozil, stretching arms beyond lipid lowering. Immunopharmacol. Immunotoxicol. 31, 339–351. https://doi.org/10.1080/08923970902785253.
Rúa-Gómez, P.C., Püttmann, W., 2013. Degradation of lidocaine, tramadol, venlafaxine and the metabolites O-desmethyltramadol and O-desmethylvenlafaxine in surface waters. Chemosphere 90 (6), 1952–1959. https://doi.org/10.1016/j.chemosphere.2012.10.039, 2013 Feb.
Sadasivam, N, Park, WR, Choi, B, Seok Jung, Y, Choi, HS, Kim, DK, 2024. Exploring the impact of estrogen-related receptor gamma on metabolism and disease. Steroids. 211, 109500. https://doi.org/10.1016/j.steroids.2024.109500. NovEpub 2024 Aug 17. PMID: 39159854.
Saku, K., Gartside, P.S., Hynd, B.A., Kashyap, M.L., 1985. Mechanism of action of gemfibrozil on lipoprotein metabolism. J. Clin. Invest. 75, 1702–1712. https://doi.org/10.1172/JCI111879.
Salahinejad, A., Attaran, A., Meuthen, D., Chivers, D.P., Niyogi, S., 2022. Proximate causes and ultimate effects of common antidepressants, fluoxetine and venlafaxine, on fish behavior. Sci. Total Environ. 807, 150846. https://doi.org/10.1016/j.scitotenv.2021.150846.

Salas, J, Garcia, A, Zora, V, Dornbush, S, Mousa-Ibrahim, F, Fogg, H, Gromley, Z, Gromley, A., 2023. Centriolin interacts with HectD1 in a cell cycle dependent manner. BMC. Res. Notes. 16 (1), 375. https://doi.org/10.1186/s13104-023-06670-y. Dec 19PMID: 38115153; PMCID: PMC10731774.

Salzet, M., Deloffre, L., Breton, C., Vieau, D., Schoofs, L., 2001. The angiotensin system elements in invertebrates. Brain Res. Rev. 36, 35–45. https://doi.org/10.1016/S0165-0173(01)00063-7.

Saxton, R.A., Sabatini, D.M., 2017. mTOR Signaling in Growth, Metabolism, and Disease. Cell 168, 960–976. https://doi.org/10.1016/j.cell.2017.02.004.

Schmidt, W., O'Rourke, K., Hernan, R., Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar. Pollut. Bull. 62, 1389–1395. https://doi.org/10.1016/j.marpolbul.2011.04.043.

Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., Faggio, C., 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: A review. Sci. Total Environ. 631–632, 789–794. https://doi.org/10.1016/j.scitotenv.2018.03.076.

Shen, Y., Tang, K., Chen, D., Hong, M., Sun, F., Wang, S., Ke, Y., Wu, T., Sun, R., Qian, J., Du, Y., 2021. Riok3 inhibits the antiviral immune response by facilitating TRIM40-mediated RIG-I and MDA5 degradation. Cell Rep. 35, 109272. https://doi.org/10.1016/j.celrep.2021.109272.

Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G.R., Zou, Z., Kinch, L., Wilkins, A. D., Sun, Q., Pallauf, K., MacDuff, D., Huerta, C., Virgin, H.W., Helms, J.B., Eerland, R., Tooze, S.A., Xavier, R., Lenschow, D.J., Yamamoto, A., King, D., Lichtarge, O., Grishin, N.V., Spector, S.A., Kaloyanova, D.V., Levine, B., 2013. Identification of a candidate therapeutic autophagy-inducing peptide. Nature 494, 201–206. https://doi.org/10.1038/nature11866.

Skolness, S.Y., Durhan, E.J., Jensen, K.M., Kahl, M.D., Makynen, E.A., Villeneuve, D.L., Ankley, G.T., 2012. Effects of gemfibrozil on lipid metabolism, steroidogenesis, and reproduction in the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 31, 2615–2624. https://doi.org/10.1002/etc.1989.

Ślósarczyk, K., Jakóbczyk-Karpierz, S., Różkowski, J., Witkowski, A.J., 2021. Occurrence of Pharmaceuticals and Personal Care Products in the Water Environment of Poland: A Review. Water. (Basel) 13, 2283. https://doi.org/10.3390/w13162283.

Souza, L.P.de, Sanches-Neto, F.O., Junior, G.M.Y., Ramos, B., Lastre-Acosta, A.M., Carvalho-Silva, V.H., Teixeira, A.C.S.C., 2022. Photochemical environmental persistence of venlafaxine in an urban water reservoir: a combined experimental and computational investigation. Process Saf. Environ. Protect. 166, 478–490. https://doi.org/10.1016/j.psep.2022.08.049.

Stefano, G.B., Catapane, E.J., 1980. Norepinephrine: Its presence in the central nervous system of the bivalve mollusc, Mytilus edulis. J. Exp. Zool. 214, 209–213. https://doi.org/10.1002/jez.1402140213.

Su, K., Dai, C., 2017. mTORC1 senses stresses: Coupling stress to proteostasis. Bioessays 39, 1600268. https://doi.org/10.1002/bies.201600268.

Tan, W., Wang, W., Ma, Q., 2018. Physiological and Pathological Function of Serine/Arginine-Rich Splicing Factor 4 and Related Diseases. BioMed Res. Int. 2018, 3819719. https://doi.org/10.1155/2018/3819719.

Tao, Y., Chen, B., Zhang, B.H., Zhu, Z.J., Cai, Q., 2018. Occurrence, impact, analysis and treatment of metformin and guanylurea in coastal aquatic environments of Canada, USA and Europe. Adv. Mar. Biol. 81, 23–58. https://doi.org/10.1016/bs.amb.2018.09.005.

Tassi, V., Coco, A., Padovano, L., De Bonis, C., De Cosmo, S., Trischitta, V., 2008. Akt2 Gene common allelic variants in insulin resistance and the metabolic syndrome. Nutr. Metab. Cardiovasc. Dis. 18, 263–270. https://doi.org/10.1016/j.numecd.2007.01.005.

Vahabi Barzi, N, Kakavand, K, Sodeifi, N, Ghezelayagh, Z, Sabbaghian, M., 2020. Expression and localization of Septin 14 gene and protein in infertile men testis. Reprod. Biol. 20 (2), 164–168. https://doi.org/10.1016/j.repbio.2020.03.007. JunEpub 2020 Apr 3. PMID: 32249155.

Valentovic, M., 2007. Gemfibrozil, in: xPharm: The Comprehensive Pharmacology Reference. Elsevier 1–5. https://doi.org/10.1016/B978-008055232-3.61817-3.

Van De Water, J.A.J.M., Allemand, D., Ferrier-Pagès, C., 2018. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. Microbiome 6, 64. https://doi.org/10.1186/s40168-018-0431-6.

Vasquez, M.I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D., 2014. Environmental side effects of pharmaceutical cocktails: What we know and what we should know. J. Hazard. Mater. 279, 169–189. https://doi.org/10.1016/j.jhazmat.2014.06.069.

Wang, J.T., Chou, Y.J., Chou, J.H., Chen, C.A., Chen, W.M., 2008. Tenacibaculum aiptasiae sp. nov., isolated from a sea anemone Aiptasia pulchella. Int. J. Syst. Evol. Microbiol. 58, 761–766. https://doi.org/10.1099/ijs.0.65437-0.

Wang, S.E., Ko, S.Y., Jo, S., Jo, H.R., Han, J., Kim, Y.S., Son, H., 2019. Downregulation of SIRT2 by Chronic Stress Reduces Expression of Synaptic Plasticity-related Genes through the Upregulation of Ehmt2. Exp. Neurobiol. 28, 537–546. https://doi.org/10.5607/en.2019.28.4.537.

Wong, DT, Kim, JJ, Khalid, O, Sun, HH, Kim, Y., 2012. Double edge: CDK2AP1 in cell-cycle regulation and epigenetic regulation. J. Dent. Res. 91 (3), 235–241. https://doi.org/10.1177/0022034511420723. MarEpub 2011 Aug 24. PMID: 21865592; PMCID: PMC3275332.

Wu, J., Hall, A.S., Gale, C.P., 2021. Long-term survival benefit of ramipril in patients with acute myocardial infarction complicated by heart failure. Heart. 107, 389–395. https://doi.org/10.1136/heartjnl-2020-316823.

Zhang, Y., Anoopkumar-Dukie, S., Davey, A.K., 2021. SIRT1 and SIRT2 Modulators: Potential Anti-Inflammatory Treatment for Depression? Biomolecules. 11, 353. https://doi.org/10.3390/biom11030353.

Zhao, Y., Zou, Z., Sun, D., Li, Y., Sinha, S.C., Yu, L., Bennett, L., Levine, B., 2021. GLIPR2 is a negative regulator of autophagy and the BECN1-ATG14-containing phosphatidylinositol 3-kinase complex. Autophagy. 17, 2891–2904. https://doi.org/10.1080/15548627.2020.1847798.

Zhu, J., Wang, H., Jiang, X., 2022. mTORC1 beyond anabolic metabolism: Regulation of cell death. J. Cell Biol. 221, e202208103. https://doi.org/10.1083/jcb.202208103.

Zuhra, K., Augsburger, F., Majtan, T., Szabo, C., 2020. Cystathionine-β-Synthase: Molecular Regulation and Pharmacological Inhibition. Biomolecules. 10, 697. https://doi.org/10.3390/biom10050697.

Further reading

Li, W., Chen, X., Li, M., Cai, Z., Gong, H., Yan, M., 2022. Microplastics as an aquatic pollutant affect gut microbiota within aquatic animals. J. Hazard. Mater. 423, 127094. https://doi.org/10.1016/j.jhazmat.2021.127094.

Li, D., Miao, J., Pan, L., Zhou, Y., Gao, Z., Yang, Y., Xu, R., Zhang, X., 2021. Impacts of benzo(a)pyrene exposure on scallop (Chlamys farreri) gut health and gut microbiota composition. Sci. Total Environ. 799, 149471. https://doi.org/10.1016/j.scitotenv.2021.149471.

Petersen, J.M., Osvatic, J., 2018. Microbiomes In Natura : Importance of Invertebrates in Understanding the Natural Variety of Animal-Microbe Interactions. mSystems 3, e00179–17. https://doi.org/10.1128/mSystems.00179-17.

Shen, M., Song, B., Zeng, G., Zhang, Y., Huang, W., Wen, X., Tang, W., 2020. Are biodegradable plastics a promising solution to solve the global plastic pollution? Environ. Pollut. 263, 114469. https://doi.org/10.1016/j.envpol.2020.114469.