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An alien metabolite vs. a synthetic chemical hazard: An ecotoxicological comparison in the Mediterranean blue mussel

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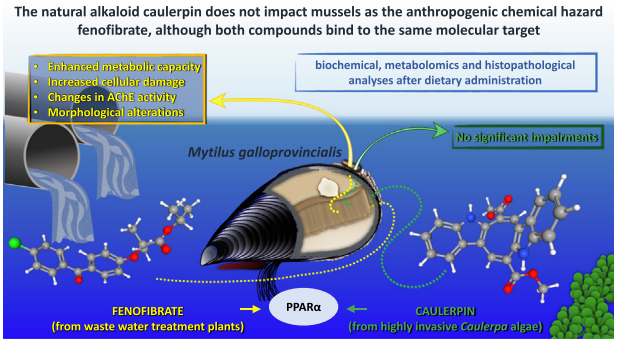
HIGHLIGHTS

- Metabolism and energy reserves increased in mussels fed with fenofibrate.
- Mussels fed with caulerpin or fenofibrate increased catalase activity.
- Fenofibrate induced cellular damage and loss of redox homeostasis in mussels.
- Levels of malonate, homarine and amino acids were higher in mussels fenofibrate-fed.
- Higher histopathological alterations were observed in fenofibrate-treated mussels.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioactive natural products from marine invasive species may dramatically impact native communities, while many synthetic pharmaceutical drugs are released into the marine environment and have long-lasting harmful effects on aquatic life. Sometimes, metabolites from alien species and synthetic compounds share similar mechanisms of action, suggesting comparable ecotoxicological impacts. This applies to the alkaloid caulerpin (CAU) from the green algae *Caulerpa cylindracea,* highly invasive in the Mediterranean Sea, and to the synthetic lipid-lowering drug fenofibrate (FFB), both acting as agonists of peroxisome proliferator-activated receptors (PPARs). Analogies with FFB, which is widely considered hazardous to the aquatic environment, have led to concerns about the ecotoxicological potential of CAU. The problem has implications for public health as CAU is well known to enter the food web accumulating in fish of commercial importance. Here, we compared the effects of FFB and CAU through biochemical and histopathological analysis on a relevant bioindicator molluscan species, the mussel *Mytilus galloprovincialis*. Under laboratory conditions, mussels were fed with food enriched with CAU or FFB. After treatment, biochemical markers were analyzed revealing metabolic capacity impairments, cellular damage, and changes in acetylcholinesterase activity in mussels fed with FFB-enriched food. NMR-based metabolomic studies also showed significant alterations in the metabolic profiles of FFB-treated mussels. In addition, dietary administration of FFB produced morphological alterations in the mussels' gills and digestive tubules. Obtained results confirm that FFB is harmful to aquatic life and that its release into the environment should be avoided. Conversely, dietary treatment with CAU did not produce any significant alterations in the mussels. Overall, our results pave the way for the possible valorization of the huge biomass from one of the world's worst invasive species to obtain CAU, a natural product of interest in drug discovery.

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1. Introduction

Biological invasions represent a major driver of ecosystem and biodiversity changes, along with habitat loss, climate change, pollution, and natural resource overexploitation (Caro et al., 2022; Nelson et al., 2006). In particular, the Mediterranean Sea is one of the marine regions most impacted by invasive species, especially due to the opening and ongoing expansion of the Suez Canal (Galil et al., 2018; Katsanevakis et al., 2013). Beyond the several impacts that invasive species can have on native communities (Shea and Chesson, 2002; Simberloff et al., 2013), growing attention in the marine literature is currently also directed to the so-called "alien metabolites": the bioactive molecules that marine invasive species carry in the new environment with potential dramatic ecological effects (Defranoux and Mollo, 2020; Mollo et al., 2015; Mollo et al., 2008). A special focus has been placed on the bisindolic red pigment caulerpin (CAU) isolated from the highly invasive green algae *Caulerpa cylindracea*, which accumulates in the tissues of native fish feeding on the exotic algae, thus entering the food chain (Felline et al., 2012, 2014, 2017; Gorbi et al., 2014; Magliozzi et al., 2017, 2019; Raniello et al., 2007; Terlizzi et al., 2011). Evidence has been recently provided for the direct binding and the activation by CAU of the peroxisome proliferator-activated receptors (PPARs) α and γ, which are nuclear transcription factors modulating the expression of genes involved in the regulation of metabolism, behavior, reproduction, cellular differentiation, embryonic development, inflammation, and tumorigenesis (Vázquez-Carrera and Wahli, 2022; Vitale et al., 2018). This seems consistent with a direct involvement of CAU in metabolic and behavioral alterations observed in fish-eating *C. cylindracea* (Del Coco et al., 2018; Gorbi et al., 2014; Magliozzi et al., 2017; Terlizzi et al., 2011). PPAR activation, in fact, has also been associated with reproductive toxicity and endocrine disruptor activity (Nepelska et al., 2017). In the frame of a still open question, however, the harmfulness of CAU has recently been questioned, since it increases fish voracity and reproductive performance when administered via food to *Danio rerio* (zebrafish). The discovery of these properties has led proposing CAU as a possible ingredient to add to aquaculture feed (Schiano et al., 2022). This controversial issue gives urgency to proceed to an effective comparison of any health impairments induced by CAU in aquatic animal models with those produced by a standard compound of ecotoxicological interest. Moving in this direction, here we evaluated the effects of CAU on the filter-feeder mussel *Mytilus galloprovincialis*, one of the most relevant bioindicator species in the coastal area (Coppola et al., 2020a; Kanduč et al., 2011; Pinto et al., 2019), in comparison with fenofibrate (FFB), a synthetic drug used in the treatment of hypertriglyceridemia, mixed dyslipidemia, hypercholesterolemia, type 2 diabetes and metabolic syndromes (Rosenson, 2008). There are essentially three reasons behind this decision:

- CAU and FFB share the same molecular target (PPARα) having agonist properties, while PPAR homologs were identified in a marine bivalve mollusk (Ran et al., 2021);
- FFB can be effectively chosen for comparative evaluations in ecotoxicology studies since it is released into the coastal waters from wastewater treatment plants (WWTPs) and it is widely considered harmful to aquatic life posing a major threat to aquatic ecosystems (Andreozzi et al., 2003; Du et al., 2004, 2008; Hering et al., 2021; Ido et al., 2017; Isidori et al., 2007; Jung et al., 2021; Rosal et al., 2010). The concentrations of FFB detected in the effluents from WWTPs range from 0.08 ng/L to 160 ng/L, reaching 70.3 ng/L in coastal areas (Afsa et al., 2020; Andreozzi et al., 2003; Ido et al., 2017; Solé and Sanchez-Hernandez, 2018; Tete et al., 2020). Moreover, FFB as well as its active form, the fenofibric acid, have been found in groundwater, surface water and drinking water in concentrations up to 1 ng/L (Ido et al., 2017; Jung et al., 2021);
- both CAU and FFB have the potential for bioaccumulation in marine organisms and persistence in the environment. Indeed, FFB has been isolated and quantified in mussels and oysters at concentrations of 0.01 and 0.03 ng/g, respectively (Maskrey et al., 2021), while CAU was found in the tissues of fish, including edible species (Felline et al., 2017; Schiano et al., 2022; Vitale et al., 2018).

Overall, the present study aims at unambiguously clarifying whether CAU can be safely used as a fish feed supplement, opening new and interesting perspectives for the exploitation of the invasive algae *C. cylindracea* in aquaculture, or whether it should be considered a risk to aquatic life, as has been widely established for FFB. For this purpose, CAU and FFB have been administered to mussels together with suspended particulate. The effects of the compound on *M. galloprovincialis* have been then compared by means of biochemical markers, metabolomics and histopathological analyses, to provide new insights into two of the major sources of biodiversity disturbance in aquatic systems: biological invasions and chemical pollution.

2. Materials and methods

2.1. Sampling of mussels and breeding conditions

Mussels *(Mytilus galloprovincialis)* with a mean length of 5.6 ± 0.3 cm and a mean width of 3.4 ± 0.2 cm, were collected in October 2021 in the Ria de Aveiro lagoon, Portugal. After sampling, the bivalves were transported to the laboratory and subjected to a two-weeks period of depuration/acclimation with artificial seawater (salinity 30 ‰, 17 ± 1 °C, pH 8.0 ± 0.1) prepared with reverse osmosis water and artificial salt (Tropic Marin® SEA SALT from Tropic Marine Center), and constant aeration. Seawater was changed each 2–3 days, and mussels were fed with Algamac protein plus (150.000 cells/animal/day) starting from three days after their arrival to the laboratory.

2.2. Artificial food preparation

Control food was prepared by soaking a combination of microalgae and probiotics (RotiBomb dry food, Algova) in acetone and then evaporating the organic solvent under reduced pressure, while treated food was made in the same manner but after dissolving CAU (1 mg/g dry food) or FFB (1 mg/g dry food) in an equal volume of acetone. Previous studies carried out on fish models (*Diplodus sargus, Danio rerio*) have shown significant changes in behavioral, metabolic, and molecular responses when CAU was administered at a concentration of 1 mg/g dry food (Del Coco et al., 2018; Magliozzi et al., 2019; Schiano et al., 2022; Vitale et al., 2018). Therefore, the same dose of CAU has been employed in the present study to facilitate comparisons between the effects in vertebrate and invertebrate models. The use of acetone during the food preparation procedure ensured a homogeneous distribution of CAU and FFB (two compounds almost insoluble in water) within the food. In parallel, to guarantee that the preliminary treatment with acetone did not affect the organoleptic properties of the food, plain dry food was also separately administered to mussels.

2.3. Feeding treatments

A total of 72 mussels were devoted to 4 different feeding treatments, including plain food, control food (CTL), food added with CAU, food added with FFB. For each treatment, three aquaria with 6 mussels each were used for a 28-days chronic dietary treatment. Mussels were fed three times per week. During the whole experiment, temperature, salinity and mortality were monitored daily, and seawater was changed each week.

2.4. Biochemical markers

After treatment, 3 mussels from each aquarium (9 per treatment) were immediately frozen in liquid nitrogen. Then, the soft tissues were homogenized, and aliquots of 0.5 g fresh weight (FW) were used to perform biochemical analysis. The sample extraction from each aliquot of homogenized tissue was performed by using specific buffers in a proportion of 1:2

(*w/v*, tissue/buffer) (Coppola et al., 2018, 2020a). Samples were sonicated, using TissueLyser II (Qiagen) for 90 s and centrifuged for 20 or 10 min at 10,000*g* or 3,000*g* depending on the biomarker (Coppola et al., 2020a), at 4 °C. After the samples' centrifugation, about 1 mL of supernatants were collected and stored at −80 °C or immediately used.

The electron transport system (ETS) was selected to assess the metabolic capacity, following the De Coen and Janssen (1997) method. Absorbance was measured during 10 min at 490 nm with intervals of 25 s and the extinction coefficient (Ɛ) of 15,900 $(mol/L)^{-1}$ cm^{-1} was used to calculate the amount of formazan formed and results were expressed in nmol per min per g FW.

To assess energy reserves, glycogen (GLY) and total protein (PROT) contents were measured. For GLY quantification the sulfuric acid method was applied as described by DuBois et al. (1956). Glucose standards at a concentration between 0 and 5 mg/mL were used to obtain a calibration curve. Absorbance was measured at 492 nm after incubation during 30 min at room temperature. The PROT content was measured following the Biuret method (Robinson and Hogden, 1940). The calibration curve was obtained by using bovine serum albumin (BSA) as standards from 0 to 40 mg/mL and the absorbance was read at 540 nm. Both results were expressed in mg per g FW.

Mechanisms of antioxidant defenses were assessed determining the activity of the superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) enzymes. The activity SOD was analyzed following Beauchamp and Fridovich (1971) method with adaptations accomplished by Carregosa et al. (2014). The standard curve was obtained with SOD standards between 0 and 60 U/mL. The absorbance was read at 560 nm after 20 min of incubation at room temperature. The results were expressed in U (one unit: quantity of the enzyme that catalyzes the conversion of 1 μmol of substrate per min) per g FW. The activity of CAT was quantified according to the Johansson and Borg (1988) method and adaptations accomplished by Carregosa et al. (2014). The standard curve was determined using formaldehyde standards between 0 and 150 μmol/L and the absorbance was read at 540 nm. The results were expressed in U per g FW (one unit: the quantity of enzyme that generates the formation of 1.0 nmol formaldehyde per min). The activity of GR was determined according to Carlberg and Mannervik (1985). Absorbance was measured at 340 nm, during 5 min in intervals of 15 s, using the extinction coefficient (Ɛ) 6,220 $(mol/L)^{-1}$ cm^{-1} and the activity was expressed in U (oxidation of 1.0 μmol of NADPH per min) per g FW.

Mussels' detoxification capacity was evaluated by measuring glutathione S-transferases (GSTs) and carboxylesterases (CbEs) activities. The activity of GSTs was determined based on Habig et al. (1974) method by reading the absorbance at 340 nm during 5 min in intervals of 15 s, with an extinction coefficient Ɛ = 9,600 $(mol/L)^{-1}$ cm^{-1}. The activity was expressed in U per g FW, where U represents the amount of enzyme necessary to catalyze the formation of 1 μmol of dinitrophenyl thioether per min. The activities of CbEs were determined following Hosokawa and Satoh (2001) and using the colorimetric substrates *p*-nitrophenyl acetate (pNPA) and *p*-nitrophenyl butyrate (pNPB). Absorbance was measured at 405 nm for 5 min in intervals of 15 s and the extinction coefficient (Ɛ) 18,000 $(mol/L)^{-1}$ cm^{-1} was used to determine the activity. The hydrolysis rate of pNPA and pNPB were expressed in nmol per min per g FW.

Redox balance was assessed by calculating the oxidized glutathione (GSSG) content, while cellular damage was investigated through lipid peroxidation levels (LPO) determination. The content of GSSG was determined as described in Rahman (2007) using GSSG as standard at a concentration from 0 to 90 μmol/L. Absorbance was read at 412 nm for 2 min in intervals of 30 s and the GSSG content was expressed in μmol per g FW. LPO levels were measured through the quantification of malondialdehyde (MDA) as reported in Ohkawa et al. (1979). The amount of MDA formed was quantified at an absorbance of 535 nm using the extinction coefficient Ɛ = 156,000 $(mol/L)^{-1}$ cm^{-1}. Results were expressed in nmol of malondialdehyde formed per g of FW.

The acetylcholinesterase (AChE) activity was evaluated to assess neurotoxicity following Ellman et al. (1961). The activity was measured using acetylthiocholine iodide (ATChI 5 mmol/L) substrates and reading the absorbance for 5 min with 25 s intervals at 412 nm, using the extinction coefficient Ɛ = 13,600 $(mol/L)^{-1}$ cm^{-1}. The activity was expressed in nmol per min per g FW.

All biochemical parameters were run in duplicate and analyzed with the use of a microplate reader (Biotek).

2.5. NMR sample preparation and spectra acquisition

At the end of the exposure assay, three mussels per treatment (one per aquarium) were homogenized in liquid nitrogen and stored at −80 °C and used for NMR analyses. Then, tissues were lyophilized and processed to extract metabolites of interest (e.g., lipids, amino acids, carbohydrates and other small metabolites; see supplementary materials Table 1 s). Combined extraction of polar and lipophilic metabolites was carried out by using methanol/water/chloroform as suggested by Beckonert et al. (2007). Polar and nonpolar fractions were transferred into different glass vials and the solvents were removed by using a rotary vacuum evaporator at room temperature. For NMR analysis, polar fractions were resuspended in 630 μL of phosphate buffer saline (PBS, pH 7.4), adding 70 μL of $^{2}H_2O$ solution [containing 1 mM sodium 3-trimethylsylyl [2,2,3,3-2H4] propionate (TSP) as a chemical shift reference for ^{1}H spectra] to provide a field frequency lock, reaching 700 μL of total volume. Samples were loaded into the autosampler and NMR spectra were acquired on a Bruker Avance III–600 MHz spectrometer (BrukerBioSpin GmbH, Rheinstetten, Germany), equipped with a TCI CryoProbe fitted with a gradient along the *Z*-axis, at a probe temperature of 27 °C. In particular, standard 1D proton spectra and 2D experiments (clean total-correlation spectroscopy TOCSY and heteronuclear single quantum coherence HSQC) were acquired providing monodimensional metabolic profiles and homonuclear and heteronuclear spectra for metabolites identification. Metabolites assignments were achieved by comparing signal chemical shifts with literature and online databases. All acquired spectra were automatically reduced down to 500 integral segments of 0.02 ppm each between the 0.50–10.50 ppm spectral region, excluding the water resonance (4.50–5.15 ppm) using the AMIX 3.9.15 software package (Bruker Biospin GmbH, Rheinstetten, Germany). After reducing NMR data, bins were normalized to the total spectrum area. The obtained data format, expressed by a matrix (X matrix), was then imported into SIMCA-P + 14 package (Umetrics, Umeå, Sweden) where multivariate statistical analysis was performed.

2.6. Histopathological analysis

One mussel from each aquarium (three per treatment) was used for histological analysis. Mussels were fixed in Davidson's solution and gills and digestive tubules were dehydrated in ascendant ethanol, clarified in methyl benzoate and included in paraffin (Coppola et al., 2022). Sections of 5 μm were obtained with the microtome (Leica Biosystems) and stained with hematoxylin to observe the presence of morphological alterations. Histopathological indices were calculated using the following formula:

$$I_h = \frac{\Sigma_1^j w_j a_{jh}}{\Sigma_1^j M_{j.}}$$

where I_h is the histopathological index for the individual h; w_j the weight of the j_{th} histopathological alteration; a_{jh} is the score attributed to the h_{th} individual for the j_{th} alteration and M_j is the maximum attributable value for the j_{th} alteration (in the case in which all the alterations are present at the maximum diffusion). The I_h was determined following the concepts of the differential biological significance of each analyzed alteration (weight) and its diffusion (score). The weights range from 1 (minimum severity) to 3 (maximum severity) while the score varies from 0 (not present) to 6 (diffuse) (Costa et al., 2013). Six pictures from each tissue and sample were randomly taken with a camera (Canon, PowerShot s 50) connected to an optical microscope (Leica, DM RB) through the acquisition tool RemoteCapture, and observed to determine the diffusion score of each analyzed alteration.

2.7. Statistical analyses

Biomarkers and histopathological analysis, obtained for each treatment, were submitted to a non-parametric permutational analysis of variance (PERMANOVA + add-on in PRIMER v6) (Anderson et al., 2008). Values lower than 0.05 ($p < 0.05$) were considered significantly different. The null hypothesis tested was, for biomarkers and histopathological indices analysis, no significant differences were observed among treatments (plain food, CTL, CAU, FFB). Because no significant differences were observed between plain food and CTL treatments (see supplementary materials Table 2 s), regardless of the biological response, this treatment was not represented in the graphs.

Multivariate statistical analysis was performed for metabolomic data as first approach, the unsupervised principal component analysis (PCA) was applied to assess class homogeneity, uncover data trends and detect outliers (data not shown). Then, Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to visualize class separation, clusters and the spectral variables influencing sample distribution according to the alteration of the metabolic profiles. Data visualization was achieved through scores and loadings plots, which also highlighted specific compounds as putative markers useful for classification. OPLS-DA models were validated by internal iterative cross-validation with 7 rounds of permutation test response (800 repeats), and CV-ANOVA (ANOVA testing of Cross-Validated predictive residuals). Selected isolated signals and bins with $|pcorr| \geq 0.7$ were considered for univariate statistical analysis elaborated with the OriginPro 9.1 software package (OriginLab Corporation, Northampton, USA). Statistical significance for selected metabolites was determined by parametric (ANOVA with Bonferroni correction) or non-parametric (Mann-Whitney U) tests according to the results of the normality test performed on data to evaluate each distribution (Shapiro-Wilk, Kolgomorov-Smirnov test). p values <0.05 were considered as statistically significant.

3. Results

3.1. Biochemical markers

3.1.1. Metabolic capacity and energy reserves

The ETS activity showed significantly higher levels in FFB mussels compared to CTL and CAU treatments (Fig. 1A). Regarding GLY content, significantly higher levels were found in FFB organisms compared to the CTL (Fig. 1B). Similarly, significantly higher levels of PROT were found in FFB organisms compared to CTL and CAU treatments (Fig. 1C).

3.1.2. Antioxidant defenses and biotransformation isoenzymes

No significant differences were found among treatments in terms of SOD and GR activities (Figs. 2A and B). Regarding CAT, significantly higher activity was observed in CAU and FFB mussels compared to CTL ones (Fig. 2C).

Regarding GSTs activity, CAU mussels showed significantly higher GSTs activities compared to the CTL ones (Fig. 2D). The activity of CbEs – pNPA and CbEs – pNPB enzymes showed no differences among treatments (Figs. 2E and F).

3.1.3. Redox balance and cellular damage

FFB induced a significant increase in GSSG content compared to CTL and CAU treatments (Fig. 3A). Similarly, LPO levels showed a significant increment in FFB organisms compared to the CTL mussels (Fig. 3B).

3.1.4. Neurotoxicity

The activity of AChE was significantly higher in FFB mussels compared to CTL and CAU ones (Fig. 4).

3.2. NMR-based metabolomics

OPLS-DA performed on NMR spectra resulted in one predictive and one orthogonal component with parameters $R^2 = 0.44$ and $Q^2 = 0.001$. The scores plot in Fig. 5A shows sample projection onto the principal components. The first component t[1] accounts for the main differences between FFB mussels group at t[1] negative coordinates, and the CTL class, placed at positive t[1], while the CAU category appeared in the middle. The orthogonal component to[1] expresses the intraclass inhomogeneity, mainly due to betaine variation (3.93, 3.29 ppm). The related loadings plot in Fig. 5B shows the NMR variables responsible for sample projection and clustering in the model. Assigning metabolites to the variables expressed in the associated loadings plot in Fig. 5B, FFB group resulted in significantly higher levels of malate, asparagine, histidine, tryptophan compared to CTL as well as in significantly higher levels of homarine compared to CTL and CAU mussels (Table 1). A considerably higher content of inosine

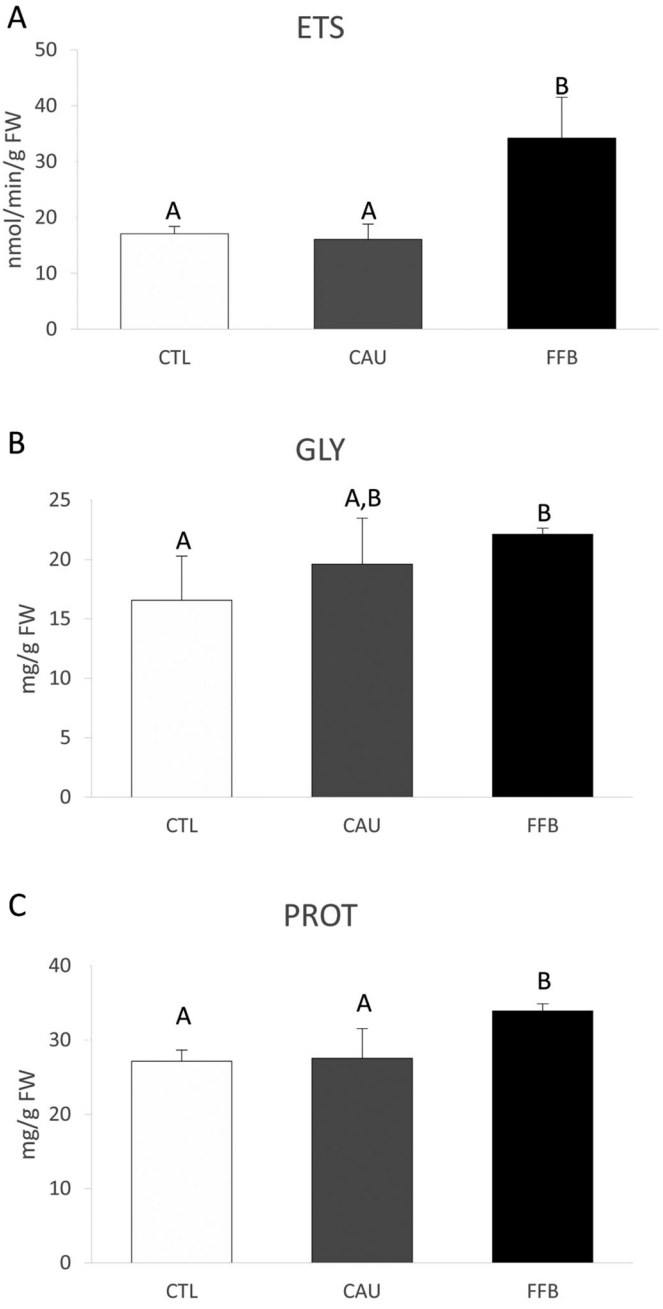

Fig. 1. Metabolic capacity and energy reserve biomarkers in mussels treated with caulerpin (CAU) and fenofibrate (FFB) compared to control mussels (CTL). A: Electron transport system activity (ETS), B: Glycogen content (GLY) and C: total protein content (PROT). Results are mean + standard deviation. Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. $n = 9$.

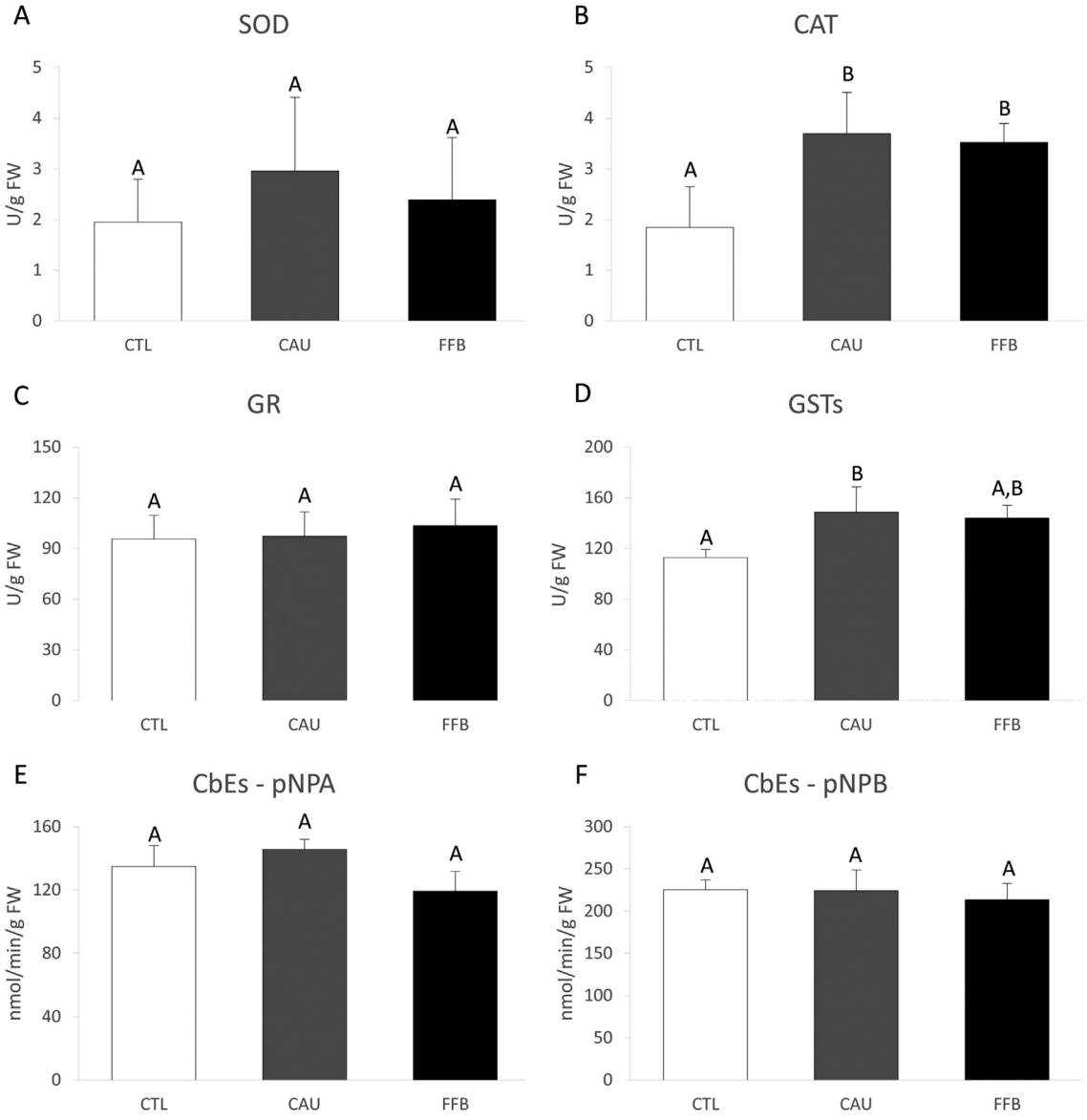

Fig. 2. Antioxidant and biotransformation enzyme activities in mussels treated with caulerpin (CAU) and fenofibrate (FFB) compared to control mussels (CTL). A: Superoxide dismutase activity (SOD), B: Catalase activity (CAT), C: Glutathione reductase activity (GR), D: Glutathione S-transferases activity (GSTs), E: Carboxylesterases pNPA activity (cBES-pNPA), F: Carboxylesterases pNPB activity (cBES-pNPB). Results are mean + standard deviation. Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. n = 9.

monophosphate was found in CTL compared to CAU and FFB mussels (Table 1). Total fumarate, malonate, choline and glutathione, resulted higher but not significant in FFB mussels (Table 1).

3.3. Histopathological indices

Histopathological analysis showed a significantly higher histopathological index (I_h) in the gills of FFB mussels compared to CTL (Fig. 6A), especially in terms of accumulation of lipofuscin and infiltration of hemocytes (Fig. 7). In digestive tubules FFB induced significantly higher histological alterations compared to CTL and CAU treatments (Fig. 6B), in particular, more lipofuscin aggregates and atrophy were found (Fig. 7).

4. Discussion

The present study aimed to compare the effects of the natural alkaloid caulerpin (CAU) from the invasive green algae *Caulerpa cylindracea* with those of the synthetic drug fenofibrate (FFB) which is well-known for its ecotoxic potential in the aquatic environment. The two compounds share similar mechanisms of action, both acting as agonists of peroxisome proliferator-activated receptors (PPARs) (Vitale et al., 2018), suggesting comparable toxicological effects. The toxicological evaluation was carried out on the Mediterranean mussel *Mytilus galloprovincialis*, to which the compounds were administered together with food at the concentration of 1 mg/g dry food.

Obtained results showed an FFB-mediated increment in metabolic capacity, measured with high ETS activity. This finding is consistent with the high levels of malate, an intermediate of the KREBS cycle whose activity provides electrons to the ETS (Yi et al., 2015), which were revealed by the NMR-based metabolomic profile of the FFB-treated group. Accordingly, malate was found to accumulate in *M. californianus* under hypoxic stress (Bayne et al., 1976; Connor and Gracey, 2012). Instead, the treatment with CAU did not induce any significant alterations in both ETS activity and malate levels, indicating that CAU does not impact mussels' metabolic capacity. Furthermore, both FFB and CAU-treated mussels showed low levels of inosine monophosphate (IMP), a precursor of adenosine 5′-

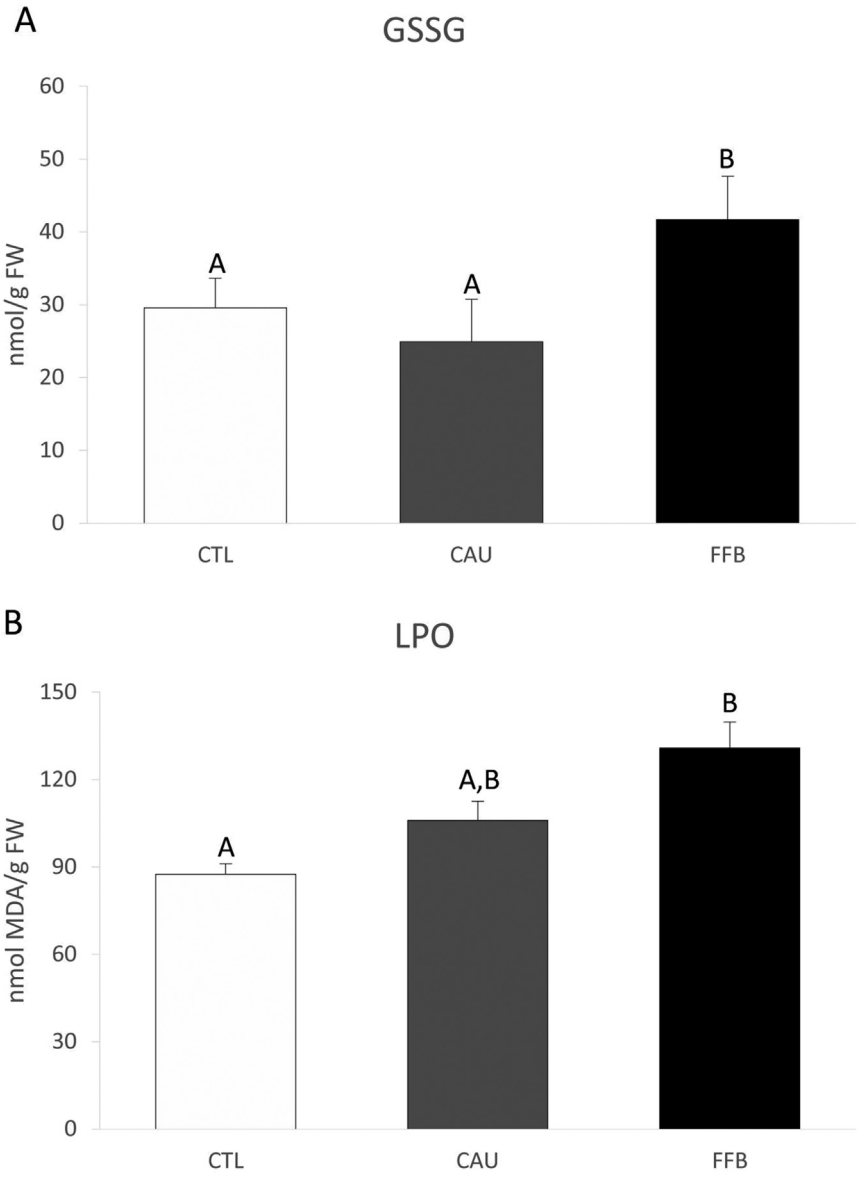

Fig. 3. Redox balance and cellular damage biomarkers in mussels treated with caulerpin (CAU) and fenofibrate (FFB) compared to control mussels (CTL). A: Oxidized glutathione levels (GSSG), B: Lipid peroxidation levels (LPO). Results are mean + standard deviation. Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. n = 9.

monophosphate (AMP) and guanosine 5′-monophosphate (GMP) (Lovászi et al., 2021) suggesting an increased purine expenditure during treatments with CAU and FFB. Increased metabolic capacity in mussels treated with FFB was not accompanied by a higher expenditure of GLY and PROT which, in turn, increased content under this treatment. In parallel, the high levels of free amino acids detected in FFB-treated mussels through NMR-analysis are consistent with mussels' effort to produce defensive enzymes under treatment with the drug. Accordingly, Teixeira et al. (2017) proposed that increased protein content in mussels exposed to the antihistamine cetirizine was possibly associated with the induction of defensive mechanisms. Furthermore, increased GLY content was observed in FFB-treated mussels, suggesting that GLY was not the preferential energy reserve used to fuel up the defense mechanisms of mussels that probably stored energy to fight the stressors (Cunha et al., 2022). In addition, NMR metabolic profiles revealed high levels of homarine, a crucial osmolyte in marine bivalves (Jones et al., 2008), in FFB-treated mussels, as also occurred in *M. galloprovincialis* exposed to cadmium (Wu et al., 2017). This could be related to the fact that osmolytes, among other functions, stabilize proteins (Yancey and Siebenaller, 2015). Overall, the above findings support that bivalves use first lipids to meet their energy requirements when under stress, preserving PROT and GLY levels (Andrade et al., 2018; Velez et al., 2016). This hypothesis meets the literature, where the hypolipidemic activity of FFB was also observed in aquatic organisms (Du et al., 2004, 2008). Among fibrates, the PPAR agonist clofibrate is also known to decrease triglyceride levels in the bivalve *Dreissena polymorpha* (Lazzara et al., 2012). Instead, the lack of alteration in GLY and PROT contents in mussels treated with CAU indicates that the algal alkaloid does not affect mussels' energy reserves.

Since reactive oxygen species (ROS) are commonly associated with oxidative stress and pathologies caused by the oxidation of lipids, proteins, and DNA (Schieber and Chandel, 2014), further comparisons between CAU and FFB included the study of the antioxidant defenses. ROS generation was reported to be induced by FFB in immature rainbow trout hepatocyte cultures (Laville et al., 2004). In normal conditions, ROS levels are balanced by antioxidant defenses, including the enzymes SOD, CAT and GR (Schieber and Chandel, 2014). In the present study, although the administration of food enriched with FFB increased mussel's metabolic capacity, SOD and GR were unaltered, while CAT activity was enhanced in

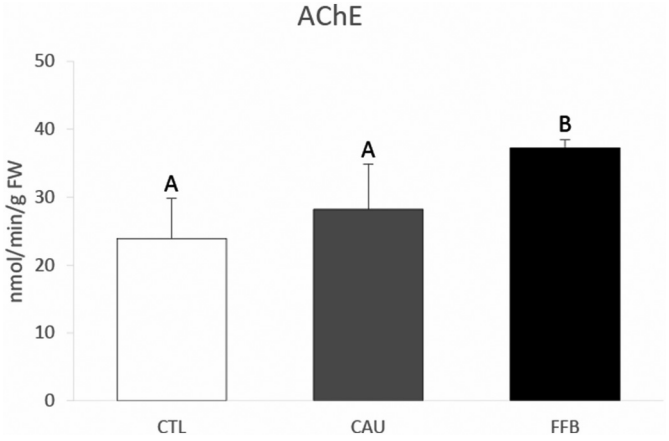

Fig. 4. Acetylcholinesterase activity (AChE, neurotoxicity biomarker) in mussels treated with caulerpin (CAU) and fenofibrate (FFB) compared to control mussels (CTL). Results are mean + standard deviation. Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. n = 9.

Table 1
Normalized bin mean ± standard deviation of metabolites found in *Mytilus galloprovincialis* after different feeding treatments: control (CTL), caulerpin (CAU), fenofibrate (FFB). Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. n = 3.

Metabolites	Normalized bin mean ± standard deviation		
	CTL	CAU	FFB
Malate	$4.25E^{-4} \pm 6.98E^{-5}$ A	$6.80E^{-4} \pm 1.31E^{-4}$ A,B	$7.44E^{-4} \pm 9.87E^{-5}$ B ↑
Asparagine	$4.32E^{-4} \pm 1.69E^{-4}$ A	$8.55E^{-4} \pm 2.78E^{-4}$ A,B	$10.4E^{-4} \pm 2.87E^{-4}$ B ↑
Histidine	$4.39E^{-5} \pm 3.24E^{-5}$ A	$1.29E^{-4} \pm 6.26E^{-5}$ A,B	$1.59E^{-4} \pm 4.14E^{-5}$ B ↑
Tryptophan	$9.91E^{-5} \pm 3.13E^{-5}$ A	$1.99E^{-4} \pm 2.85E^{-5}$ A,B	$1.93E^{-4} \pm 5.53E^{-5}$ B ↑
IMP	$8.87E^{-7} \pm 5.40E^{-7}$ A ↑	$1.47E^{-7} \pm 2.37E^{-7}$ B	$4.33E^{-8} \pm 3.78E^{-8}$ B
Homarine	$1.48E^{-4} \pm 3.94E^{-5}$ A	$2.05E^{-4} \pm 2.95E^{-5}$ A	$3.70E^{-4} \pm 1.13E^{-5}$ B ↑
Fumarate	$3.02E^{-6} \pm 2.96E^{-6}$ A	$1.13E^{-5} \pm 6.13E^{-6}$ A	$1.24E^{-5} \pm 5.87E^{-6}$ A
Choline	$22.6E^{-4} \pm 1.91E^{-4}$ A	$29.5E^{-4} \pm 8.19E^{-4}$ A	$31.8E^{-4} \pm 7.40E^{-4}$ A
Malonate	$12.50E^{-4} \pm 1.94E^{-4}$ A	$17.80E^{-4} \pm 2.20E^{-4}$ A	$17.70E^{-4} \pm 4.63E^{-4}$ A
GSH	$3.85E^{-4} \pm 1.26E^{-4}$ A	$5.32E^{-4} \pm 1.76E^{-4}$ A	$6.52E^{-4} \pm 9.77E^{-5}$ A

mussels treated with CAU or FFB. This is in line with previous findings showing that PPARα activation increases catalase expression (Shin et al., 2016). A similar result was observed in mice fed with a 0.1 % FFB diet with an increment in CAT activity (Harano et al., 2006). Conversely, Terlizzi et al. (2011) found a negative correlation between CAT and CAU as well as Gorbi et al. (2014) did not find changes in CAT activity in the Mediterranean white sea bream *Diplodus sargus* feeding on *C. cylindracea*. These contradictions can be explained by the fact that above studies were conducted on fish that had consumed *C. cylindracea*, an algae containing various bioactive secondary metabolites beyond CAU. Similarly, regarding detoxification capacity, CAU treatment enhanced the activity of GSTs, most probably due to its known antioxidant proprieties (De Souza et al., 2009). An enhanced GSTs activity was observed in *D. sargus* fish consuming *C. cylindracea* (Felline et al., 2012), while a study on the same species showed that the consumption of the algae did not affect GSTs activity (Gorbi et al., 2014). Nevertheless, several contradictory responses of GSTs have been observed in organisms depending on the treatment time and concentration (Almeida et al., 2014; Carregosa et al., 2014; Felline et al., 2012).

Scavengers like reduced glutathione (GSH) behave as antioxidants when ROS levels rise in the cells, directly reducing reactive species and being converted to GSSG (Regoli and Giuliani, 2014). In fact, the considerable rise in GSSG levels in FFB-treated organisms suggests that a rise in ROS levels and in glutathione peroxidase activity led to the oxidation of GSH into GSSG. The scarce activation of the antioxidant system in FFB-treated mussels also induced cellular damage, highlighted by an increment in LPO. Similarly, an increase of LPO was observed both in the grass carp *Ctenopharyngodon idella* treated with FFB-enriched food (Du et al., 2008), and in zebrafish exposed to clofibric acid (Rebelo et al., 2020) demonstrating the lipids-oxidation capacity of fibrates. In the present study, treatment with CAU did not affect mussels' redox status and did not produce cellular damage. The low capacity of CAU to induce oxidative stress in mussels is consistent with previous studies showing that CAU does not stimulate ROS release in normal cells, although it was able to induce a significant increase in ROS levels in ovarian cancer cells (Ferramosca et al., 2016).

Despite the fact that AChE activity usually decreases in the presence of a neurotoxic compound (Coppola et al., 2020a; Pinto et al., 2019), FFB induced an increment in the activity of this enzyme as a possible inflammatory response, since AChE increases in inflamed tissues or cells (Rodrigues et al., 2022). Similar results were observed in mussels contaminated with Pb and the increment in AChE activity was interpreted as an attempt to hydrolyze accumulated neurotransmitters in synaptic clefts (Freitas et al., 2019). Conversely, the treatment with CAU did not produce neurotoxic effects in mussels, supporting its safety in bivalve species.

Finally, the application of classical histology techniques evidenced histopathological alterations at the level of the gills and digestive tubules of mussels treated with FFB. The increase in LPO recorded in FFB mussels was confirmed by the histological observations, with an accumulation of lipofuscin in tissues which is associated with the lipidic peroxidation process (Viarengo et al., 1990). Moreover, alterations commonly related to inflammation processes, such as the abundance of hemocytes in gills and the presence of atrophied digestive tubules (Cuevas et al., 2015), were detected in mussels treated with FFB, further confirming the harmfulness of this drug for aquatic species. Although this is the first study assessing histopathological alterations induced in bivalves by FFB and CAU, alterations similar to those induced by FFB were, however, found in bivalves (*M. galloprovincialis, Ruditapes philippinarum* and *R. decussatus*) exposed to

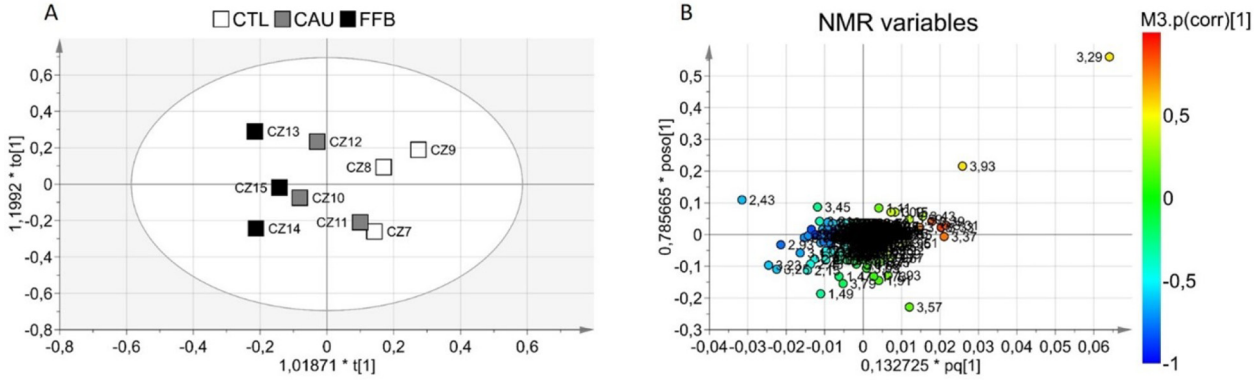

Fig. 5. Metabolomics analysis of mussels treated with caulerpin (CAU, grey squares) and fenofibrate (FFB, black squares) compared to control mussels (CTL, white squares). A: Scores plot showing sample projection onto principal components, B: Loadings plot reporting the NMR variables (chemical shift) responsible for clustering in the model.

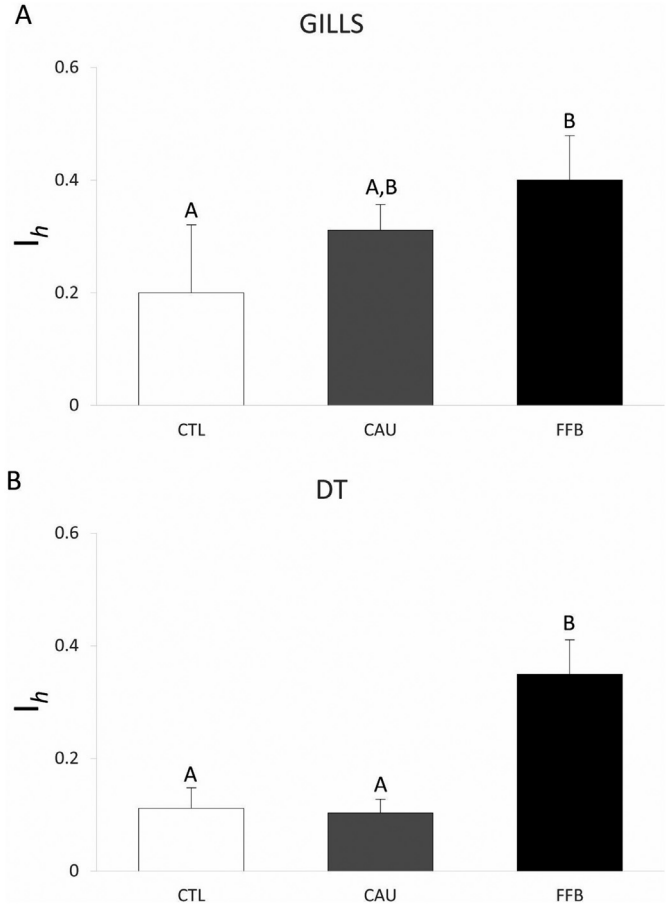

Fig. 6. Histopathological indices in mussels treated with caulerpin (CAU) and fenofibrate (FFB) compared to control mussels (CTL). A: gills, B: digestive tubules (DT). Results are mean + standard deviation. Significant differences ($p < 0.05$) among treatments are presented with different uppercase letters. $n = 3$.

other types of contaminants (Hg, sodium lauryl sulfate, lanthanum, caffeine) (Coppola et al., 2020a, 2020b; Pinto et al., 2019; Piscopo et al., 2021a, 2021b). Conversely, CAU did not significantly impact mussels' gills and digestive tubules morphology, compared to untreated mussels. This finding, along with previous studies showing that CAU has beneficial effects on the whole reproductive process in the zebrafish model (Schiano et al., 2022), strongly supports the harmlessness of CAU when administered via food at a concentration of 1 mg/g to aquatic animals.

5. Conclusions

Our results revealed enhanced metabolic capacity, increased cellular damage, and changes in AChE activity, as well as morphological alterations in gills and digestive tubules after dietary administration of FFB to *M. galloprovincialis*, while no significant impairments were found in CAU-treated mussels. On the one hand, this study confirms that FFB poses serious risks to aquatic organisms. Furthermore, it supports the possible valorization and exploitation of the biomass produced by the green algae *C. cylindracea*, one of the most invasive species along the Mediterranean coasts, to obtain CAU, a non-toxic compound of interest for possible pharmaceutical and nutraceutical applications. Accordingly, CAU has already demonstrated antitumoral and anti-inflammatory properties (Cuomo et al., 2021; De Souza et al., 2009; Yu et al., 2017). However, future challenging studies are needed to elucidate the details of the molecular pathways involved in the effects of CAU in the chosen molluscan model compared to those observed in vertebrates, in which PPAR agonists, such as FFB, regulate different crucial biological processes, including inflammation and tumorigenesis (Augimeri et al., 2020; Jin et al., 2023; Lian et al., 2018; Murphy and Holder, 2000; Vázquez-Carrera and Wahli, 2022).

CRediT authorship contribution statement

Tania Russo: Investigation, Formal analysis, Writing – original draft. **Francesca Coppola:** Investigation. **Carla Leite:** Investigation. **Marianna**

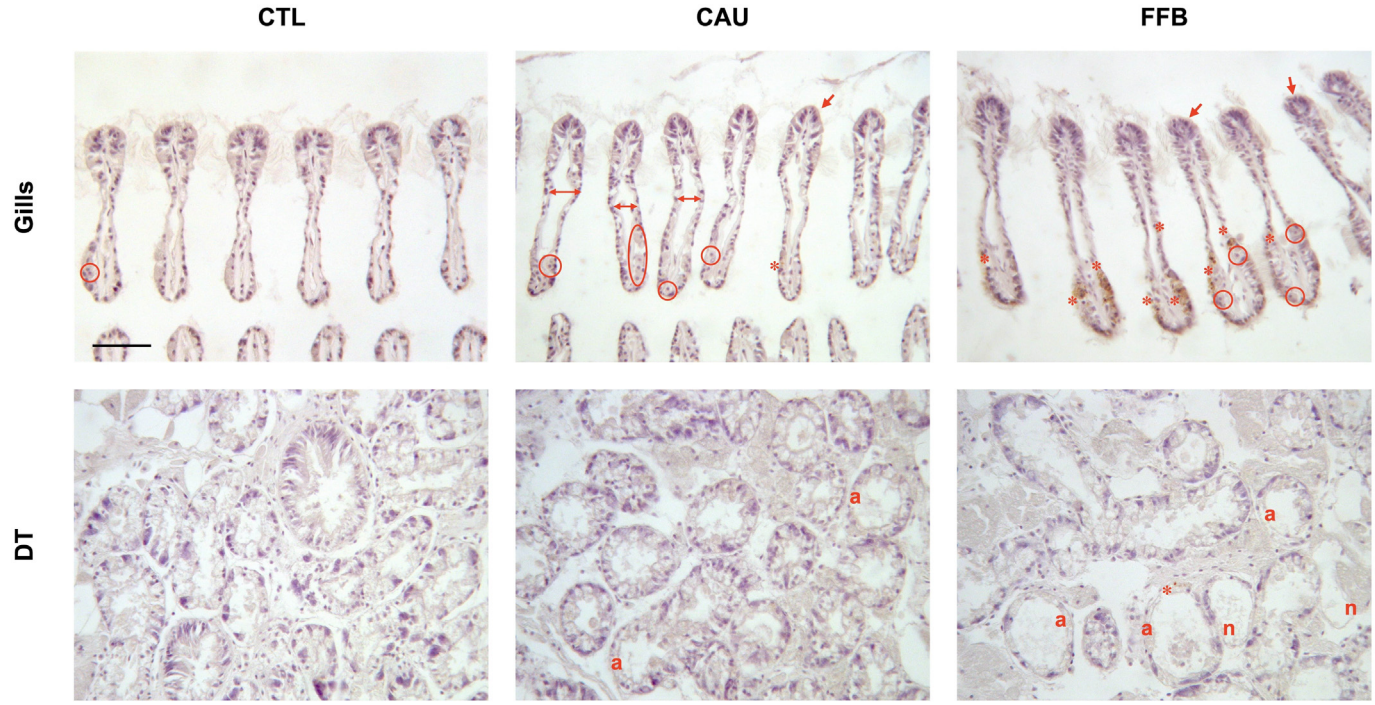

Fig. 7. Micrographs of gills and digestive tubules (DT) sections of mussels after different feeding treatments: control (CTL), caulerpin (CAU), fenofibrate (FFB) stained with hematoxylin. * (lipofuscin aggregates), arrows (cilia lost), double-headed arrows (enlargement of the central vessel), circles (hemocytes infiltration), a (atrophy), n (necrosis). Scale bar = 50 μm. n = 3.

Carbone: Resources, Supervision. **Debora Paris:** Investigation, Formal analysis. **Andrea Motta:** Resources, Supervision. **Anna Di Cosmo:** Resources. **Amadeu M.V.M. Soares:** Funding acquisition. **Ernesto Mollo:** Conceptualization, Supervision, Writing – review & editing. **Rosa Freitas:** Conceptualization, Resources, Supervision, Writing – review & editing. **Gianluca Polese:** Conceptualization, Resources, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164476.

References

Afsa, S., Hamden, K., Lara Martin, P.A., Mansour, H. Ben, 2020. Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ. Sci. Pollut. Res. 27, 1941–1955. https://doi.org/10.1007/s11356-019-06866-5.

Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Presence of the pharmaceutical drug carbamazepine in coastal systems: effects on bivalves. Aquat. Toxicol. 156, 74–87. https://doi.org/10.1016/j.aquatox.2014.08.002.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + Primer V7: User Manual. Primer-E Ltd., Plymouth UK 93. http://updates.primer-e.com/primer7/manuals/PERMANOVA+_manual.pdf.

Andrade, M., Soares, A., Figueira, E., Freitas, R., 2018. Biochemical changes in mussels submitted to different time periods of air exposure. Environ. Sci. Pollut. Res. 25, 8903–8913. https://doi.org/10.1007/s11356-017-1123-7.

Andreozzi, R., Marotta, R., Paxéus, N., 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere 50, 1319–1330. https://doi.org/10.1016/S0045-6535(02)00769-5.

Augimeri, G., Giordano, C., Gelsomino, L., Plastina, P., Barone, I., Catalano, S., Andò, S., Bonofiglio, D., 2020. The role of ppary ligands in breast cancer: from basic research to clinical studies. Cancers (Basel) 12, 1–28. https://doi.org/10.3390/cancers12092623.

Bayne, B.L., Bayne, C.J., Carefoot, T.C., Thompson, R.J., 1976. The physiological ecology of Mytilus californianus Conrad - 2. Adaptations to low oxygen tension and air exposure. Oecologia 22, 229–250. https://doi.org/10.1007/BF00344794.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8.

Beckonert, O., Keun, H.C., Ebbels, T.M.D., Bundy, J., Holmes, E., Lindon, J.C., Nicholson, J.K., 2007. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat. Protoc. 2, 2692–2703. https://doi.org/10.1038/nprot.2007.376.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods Enzymol., 484–490 https://doi.org/10.1016/S0076-6879(85)13062-4.

Caro, T., Rowe, Z., Berger, J., Wholey, P., Dobson, A., 2022. An inconvenient misconception: climate change is not the principal driver of biodiversity loss. Conserv. Lett. 15. https://doi.org/10.1111/conl.12868.

Carregosa, V., Velez, C., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Physiological and biochemical responses of three Veneridae clams exposed to salinity changes. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 177–178, 1–9. https://doi.org/10.1016/j.cbpb.2014.08.001.

Connor, K.M., Gracey, A.Y., 2012. High-resolution analysis of metabolic cycles in the intertidal mussel Mytilus californianus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 302, R103–R111. https://doi.org/10.1152/ajpregu.00453.2011.

Coppola, F., Almeida, Â., Henriques, B., Soares, A.M.V.M., Figueira, E., Pereira, E., Freitas, R., 2018. Biochemical responses and accumulation patterns of Mytilus galloprovincialis exposed to thermal stress and Arsenic contamination. Ecotoxicol. Environ. Saf. 147, 954–962. https://doi.org/10.1016/j.ecoenv.2017.09.051.

Coppola, F., Bessa, A., Henriques, B., Russo, T., Soares, A.M.V.M., Figueira, E., Pereira, E., Marques, P., Polese, G., Freitas, R., 2020b. The role of temperature on the impact of remediated water towards marine organisms. Water (Switzerland) 12. https://doi.org/10.3390/W12082148.

Coppola, F., Russo, T., Soares, A.M.V.M., Marques, P.A.A.P., Polese, G., Pereira, E., Freitas, R., 2022. The influence of salinity on the toxicity of remediated seawater. Environ. Sci. Pollut. Res. 29, 32967–32987. https://doi.org/10.1007/s11356-021-17745-3.

Coppola, Francesca, Bessa, A., Henriques, B., Russo, T., Soares, A.M.V.M., Figueira, E., Marques, P.A.A.P., Polese, G., di Cosmo, A., Pereira, E., Freitas, R., 2020a. Oxidative stress, metabolic and histopathological alterations in mussels exposed to remediated seawater by GO-PEI after contamination with mercury. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 243, 110674. https://doi.org/10.1016/j.cbpa.2020.110674.

Costa, P.M., Carreira, S., Costa, M.H., Caeiro, S., 2013. Development of histopathological indices in a commercial marine bivalve (Ruditapes decussatus) to determine environmental quality. Aquat. Toxicol. 126, 442–454. https://doi.org/10.1016/j.aquatox.2012.08.013.

Cuevas, N., Zorita, I., Costa, P.M., Franco, J., Larreta, J., 2015. Development of histopathological indices in the digestive gland and gonad of mussels: integration with contamination levels and effects of confounding factors. Aquat. Toxicol. 162, 152–164. https://doi.org/10.1016/j.aquatox.2015.03.011.

Cunha, M., Louro, P., Silva, M., Soares, A.M.V.M., Pereira, E., Freitas, R., 2022. Biochemical alterations caused by lanthanum and gadolinium in Mytilus galloprovincialis after exposure and recovery periods. Environ. Pollut. 307. https://doi.org/10.1016/j.envpol.2022.119387.

Cuomo, P., Medaglia, C., Allocca, I., Montone, A.M.I., Guerra, F., Cabaro, S., Mollo, E., Eletto, D., Papaianni, M., Capparelli, R., 2021. Caulerpin mitigates helicobacter pylori-induced inflammation via formyl peptide receptors. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms222313154.

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in Daphnia magna toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. J. Aquat. Ecosyst. Stress. Recover., 43–55 https://doi.org/10.1006/eesa.2000.2009.

De Souza, É.T., Pereira de Lira, D., Cavalcanti de Queiroz, A., Costa da Silva, D.J., Bezerra de Aquino, A., Campessato Mella, E., Prates Lorenzo, V., de Miranda, G.E., de Araújo-Júnior, J.X., de Oliveira Chaves, M.C., Barbosa-Filho, J.M., Filgueiras de Athayde-Filho, P., de Oliveira Santos, B.V., Alexandre-Moreira, M.S., 2009. The antinociceptive and anti-inflammatory activities of caulerpin, a bisindole alkaloid isolated from seaweeds of the genus Caulerpa. Mar. Drugs 7, 689–704. https://doi.org/10.3390/md7040689.

Defranoux, F., Mollo, E., 2020. Molecular interactions as drivers of changes in marine ecosystems. Reference Series in Phytochemistry 121–133. https://doi.org/10.1007/978-3-319-96397-6_64.

Del Coco, L., Felline, S., Girelli, C., Angilè, F., Magliozzi, L., Almada, F., D'Aniello, B., Mollo, E., Terlizzi, A., Fanizzi, F., 2018. 1H NMR spectroscopy and MVA to evaluate the effects of caulerpin-based diet on diplodus sargus lipid profiles. Mar. Drugs 16, 390. https://doi.org/10.3390/md16100390.

Du, Z.Y., Demizieux, L., Degrace, P., Gresti, J., Moindrot, B., Liu, Y.J., Tian, L.X., Cao, J.M., Clouet, P., 2004. Alteration of 20:5n-3 and 22:6n-3 fat contents and liver peroxisomal activities in fenofibrate-treated rainbow trout. Lipids 39, 849–855. https://doi.org/10.1007/s11745-004-1306-3.

Du, Z.Y., Clouet, P., Degrace, P., Zheng, W.H., Frøyland, L., Tian, L.X., Liu, Y.J., 2008. Hypolipidaemic effects of fenofibrate and fasting in the herbivorous grass carp (Ctenopharyngodon idella) fed a high-fat diet. Br. J. Nutr. 100, 1200–1212. https://doi.org/10.1017/S0007114508986840.

DuBois, Michel, Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, Fred, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9.

Felline, S., Caricato, R., Cutignano, A., Gorbi, S., Lionetto, M.G., Mollo, E., Regoli, F., Terlizzi, A., 2012. Subtle effects of biological invasions: cellular and physiological responses of fish eating the exotic pest Caulerpa racemosa. PLoS One 7. https://doi.org/10.1371/journal.pone.0038763.

Felline, S., Mollo, E., Ferramosca, A., Zara, V., Regoli, F., Gorbi, S., Terlizzi, A., 2014. Can a Marine Pest Reduce the Nutritional Value of Mediterranean Fish Flesh? 1275–1283. https://doi.org/10.1007/s00227-014-2417-7.

Felline, S., Mollo, E., Cutignano, A., Grauso, L., Andaloro, F., Castriota, L., Consoli, P., Falautano, M., Sinopoli, M., Terlizzi, A., 2017. Preliminary observations of caulerpin accumulation from the invasive Caulerpa cylindracea in native Mediterranean fish species. Aquat. Biol. 26, 27–31. https://doi.org/10.3354/ab00671.

Ferramosca, A., Conte, A., Guerra, F., Felline, S., Grazia, M., Mollo, E., Zara, V., Terlizzi, A., 2016. Biochemical and biophysical research communications metabolites from invasive pests inhibit mitochondrial complex II: a potential strategy for the treatment of human ovarian carcinoma? Biochem. Biophys. Res. Commun. 473, 1133–1138. https://doi.org/10.1016/j.bbrc.2016.04.028.

Freitas, R., Leite, C., Pinto, J., Costa, M., Monteiro, R., Henriques, B., di Martino, F., Coppola, F., Soares, A.M.V.M., Solé, M., Pereira, E., 2019. The influence of temperature and salinity on the impacts of lead in Mytilus galloprovincialis. Chemosphere 235, 403–412. https://doi.org/10.1016/j.chemosphere.2019.05.221.

Galil, B., Marchini, A., Occhipinti-Ambrogi, A., 2018. East is east and west is west? Management of marine bioinvasions in the Mediterranean Sea. Estuar. Coast. Shelf Sci. 201, 7–16. https://doi.org/10.1016/j.ecss.2015.12.021.

Gorbi, S., Giuliani, M.E., Pittura, L., d'Errico, G., Terlizzi, A., Felline, S., Grauso, L., Mollo, E., Cutignano, A., Regoli, F., 2014. Could molecular effects of Caulerpa racemosa metabolites modulate the impact on fish populations of Diplodus sargus? Mar. Environ. Res. 96, 2–11. https://doi.org/10.1016/j.marenvres.2014.01.010.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

Harano, Y., Yasui, K., Toyama, T., Nakajima, T., Mitsuyoshi, H., Mimani, M., Hirasawa, T., Itoh, Y., Okanoue, T., 2006. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. Liver Int. 26, 613–620. https://doi.org/10.1111/j.1478-3231.2006.01265.x.

Hering, I., Eilebrecht, E., Parnham, M.J., Weiler, M., Günday-Türeli, N., Türeli, A.E., Modh, H., Heng, P.W.S., Böhmer, W., Schäfers, C., Fenske, M., Wacker, M.G., 2021. Microparticle formulations alter the toxicity of fenofibrate to the zebrafish Danio rerio embryo. Aquat. Toxicol. 234. https://doi.org/10.1016/j.aquatox.2021.105798.

Hosokawa, M., Satoh, T., 2001. Measurement of carboxylesterase (CES) activities. Curr. Protoc. Toxicol. 10, 1–14. https://doi.org/10.1002/0471140856.tx0407s10.

Ido, A., Hiromori, Y., Meng, L., Usuda, H., Nagase, H., 2017. Occurrence of fibrates and their metabolites in source and drinking water in Shanghai and Zhejiang. Nat. Publ. Group 1–9. https://doi.org/10.1038/srep45931.

Isidori, M., Nardelli, A., Pascarella, L., Rubino, M., Parrella, A., 2007. Toxic and genotoxic impact of fibrates and their photoproducts on non-target organisms. Environ. Int. 33, 635–641. https://doi.org/10.1016/j.envint.2007.01.006.

Jin, L., Hua, H., Ji, Y., Jia, Z., Peng, M., Huang, S., 2023. Anti-inflammatory role of fenofibrate in treating diseases. Biomol. Biomed. 23, 376–391. https://doi.org/10.17305/bb.2022.8534.

Johansson, L.H., Borg, L.A., 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal. Biochem. 174, 331–336. https://doi.org/10.1016/0003-2697(88)90554-4.

Jones, O.A.H., Dondero, F., Viarengo, A., Griffin, J.L., 2008. Metabolic profiling of Mytilus galloprovincialis and its potential applications for pollution assessment. Mar. Ecol. Prog. Ser. 369, 169–179. https://doi.org/10.3354/meps07654.

Jung, F., Thurn, M., Krollik, K., Gao, G.F., Hering, I., Eilebrecht, E., Emara, Y., Weiler, M., Günday-Türeli, N., Türeli, E., Parnham, M.J., Wacker, M.G., 2021. Predicting the environmental emissions arising from conventional and nanotechnology-related pharmaceutical drug products. Environ. Res. 192, 110219. https://doi.org/10.1016/j.envres.2020.110219.

Kanduč, T., Medaković, D., Hamer, B., 2011. Mytilus galloprovincialis as a bioindicator of environmental conditions: the case of the eastern coast of the Adriatic Sea. Isot. Environ. Health Stud. 47, 42–61. https://doi.org/10.1080/10256016.2011.548866.

Katsanevakis, S., Zenetos, A., Belchior, C., Cardoso, A.C., 2013. Invading European seas: assessing pathways of introduction of marine aliens. Ocean Coast. Manag. 76, 64–74. https://doi.org/10.1016/j.ocecoaman.2013.02.024.

Laville, N., Aït-Ässa, S., Gomez, E., Casellas, C., Porcher, J.M., 2004. Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. Toxicology 196, 41–55. https://doi.org/10.1016/j.tox.2003.11.002.

Lazzara, R., Fernandes, D., Faria, M., López, J.F., Tauler, R., Porte, C., 2012. Changes in lipid content and fatty acid composition along the reproductive cycle of the freshwater mussel Dreissena polymorpha: its modulation by clofibrate exposure. Sci. Total Environ. 432, 195–201. https://doi.org/10.1016/j.scitotenv.2012.05.094.

Lian, X., Wang, G., Zhou, H., Zheng, Z., Fu, Y., Cai, L., 2018. Anticancer properties of fenofibrate: a repurposing use. J. Cancer 9, 1527–1537. https://doi.org/10.7150/jca.24488.

Lovászi, M., Németh, Z.H., Pacher, P., Haskó, G., Gause, W.C., 2021. Inosine Monophosphate and Inosine Differentially Regulate Endotoxemia and Bacterial sepsis 1–16. https://doi.org/10.1096/fj.202100862R.

Magliozzi, L., Almada, F., Robalo, J., Mollo, E., Polese, G., Gonçalves, E.J., Felline, S., Terlizzi, A., D'Aniello, B., 2017. Cryptic effects of biological invasions: reduction of the aggressive behaviour of a native fish under the influence of an "invasive" biomolecule. PLoS One 12, 1–11. https://doi.org/10.1371/journal.pone.0185620.

Magliozzi, L., Maselli, V., Almada, F., di Cosmo, A., Mollo, E., Polese, G., 2019. Effect of the algal alkaloid caulerpin on neuropeptide Y (NPY) expression in the central nervous system (CNS) of Diplodus sargus. J. Comp. Physiol. A 205, 203–210. https://doi.org/10.1007/s00359-019-01322-8.

Maskrey, B.H., Dean, K., Morrell, N., Turner, A.D., 2021. A Simple and Rapid Ultra – High - Performance Liquid Chromatography – Tandem Mass Spectrometry Method for the Quantitation of Pharmaceuticals and Related Compounds in Mussels and Oysters. 40, pp. 3263–3274. https://doi.org/10.1002/etc.5046.

Mollo, E., Gavagnin, M., Carbone, M., Castelluccio, F., Pozone, F., Ghiselin, M.T., Cimino, G., Roussis, V., 2008. Factors Promoting Marine Invasions: A Chemoecological Approach. https://doi.org/10.1073/pnas.0709355105.

Mollo, E., Cimino, G., Ghiselin, M.T., 2015. Alien biomolecules: a new challenge for natural product chemists. Biol. Invasions 17, 941–950. https://doi.org/10.1007/s10530-014-0835-6.

Murphy, G.J., Holder, J.C., 2000. PPAR-γ agonists: therapeutic role in diabetes, inflammation and cancer. Trends Pharmacol. Sci. 21, 469–474. https://doi.org/10.1016/S0165-6147(00)01559-5.

Nelson, G.C., Bennett, E., Berhe, A.A., Cassman, K., DeFries, R., Dietz, T., Dobermann, A., Dobson, A., Janetos, A., Levy, M., Marco, D., Nakicenovic, N., O'Neill, B., Norgaard, R., Petschel-Held, G., Ojima, D., Pingali, P., Watson, R., Zurek, M., 2006. Anthropogenic drivers of ecosystem change: an overview. Ecol. Soc. 11, art29. https://doi.org/10.5751/ES-01826-110229.

Nepelska, M., Odum, J., Munn, S., 2017. Adverse outcome pathway: peroxisome proliferator-activated receptor α activation and reproductive toxicity—development and application in assessment of endocrine disruptors/reproductive toxicants. Appl. In Vitro Toxicol. 3, 234–249. https://doi.org/10.1089/aivt.2017.0004.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., di Cosmo, A., Soares, A.M.V.M., Polese, G., Pereira, E., Freitas, R., 2019. Ecotoxicological effects of lanthanum in Mytilus galloprovincialis: biochemical and histopathological impacts. Aquat. Toxicol. 211. https://doi.org/10.1016/j.aquatox.2019.03.017.

Piscopo, R., Almeida, Â., Coppola, F., de Marchi, L., Esteves, V.I., Soares, A.M.V.M., Pretti, C., Morelli, A., Chiellini, F., Polese, G., Freitas, R., 2021a. How temperature can alter the combined effects of carbon nanotubes and caffeine in the clam Ruditapes decussatus? Environ. Res. 195. https://doi.org/10.1016/j.envres.2021.110755.

Piscopo, R., Coppola, F., Almeida, Â., de Marchi, L., Russo, T., Esteves, V.I., Soares, A.M.V.M., Pretti, C., Chiellini, F., Polese, G., Freitas, R., 2021b. Effects of temperature on caffeine and carbon nanotubes co-exposure in Ruditapes philippinarum. Chemosphere 271. https://doi.org/10.1016/j.chemosphere.2021.129775.

Rahman, K., 2007. Studies on free radicals, antioxidants, and co-factors. Clin. Interv. Aging 2, 219–236.

Ran, Z., Kong, F., Liao, K., Xu, J., Liu, X., Shi, P., Zhang, M., Wu, K., Yan, X., 2021. Identification and expression of PPAR in Sinonovacula constricta and their potential regulatory effects on Δ6 fad transcription. J. Ocean Univ. China 20, 1557–1566. https://doi.org/10.1007/s11802-021-4784-2.

Raniello, R., Mollo, E., Lorenti, M., Gavagnin, M., Buia, M.C., 2007. Phytotoxic activity of caulerpenyne from the Mediterranean invasive variety of Caulerpa racemosa: a potential allelochemical. Biol. Invasions 9, 361–368. https://doi.org/10.1007/s10530-006-9044-2.

Rebelo, D., Correia, A.T., Nunes, B., 2020. Acute and chronic effects of environmental realistic concentrations of clofibric acid in Danio rerio: behaviour, oxidative stress, biotransformation and lipid peroxidation endpoints. Environ. Toxicol. Pharmacol. 80, 103468. https://doi.org/10.1016/j.etap.2020.103468.

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106–117. https://doi.org/10.1016/j.marenvres.2013.07.006.

Robinson, H.W., Hogden, C.G., 1940. The biuret reaction in the determination of serum proteins. J. Biol. Chem. 135, 707–725. https://doi.org/10.1016/S0021-9258(18)73134-7.

Rodrigues, F., Vieira, H., Campos, D., Pires, S., Rodrigues, A., Silva, A., Soares, A., Oliveira, J., Bordalo, M., 2022. Co-exposure with an invasive seaweed exudate increases toxicity of polyamide microplastics in the marine mussel Mytilus galloprovincialis. Toxics 10, 43. https://doi.org/10.3390/toxics10020043.

Rosal, R., Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Gonzalo, S., Petre, A., 2010. Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms. Environ. Sci. Pollut. Res. 17, 135–144. https://doi.org/10.1007/s11356-009-0137-1.

Rosenson, R.S., 2008. Fenofibrate: treatment of hyperlipidemia and beyond. Expert. Rev. Cardiovasc. Ther. 6, 1319–1330. https://doi.org/10.1586/14779072.6.10.1319.

Schiano, V., Cutignano, A., Maiello, D., Carbone, M., Ciavatta, M.L., Polese, G., Fioretto, F., Attanasio, C., Palladino, A., Felline, S., Terlizzi, A., Angelo, L.D., de Girolamo, P., Turano, M., Lucini, C., Mollo, E., 2022. An Alkaloid from a Highly Invasive Seaweed Increases the Voracity and Reproductive Output of a Model Fish Species. https://doi.org/10.3390/md20080513.

Schieber, M., Chandel, N.S., 2014. ROS function in redox signaling. Curr. Biol. 24, 453–462. https://doi.org/10.1016/j.cub.2014.03.034.ROS.

Shea, K., Chesson, P., 2002. Community Ecology Theory as a Framework for Biological Invasions. 17, pp. 170–176. https://doi.org/10.1016/S0169-5347(02)02495-3.

Shin, M.H., Lee, S.R., Kim, M.K., Shin, C.Y., Lee, D.H., Chung, J.H., 2016. Activation of peroxisome proliferator-activated receptor alpha improves aged and UV-irradiated skin by catalase induction. PLoS One 11, 1–15. https://doi.org/10.1371/journal.pone.0162628.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M., 2013. Impacts of biological invasions: what's what and the way forward. Trends Ecol. Evol. 28, 58–66. https://doi.org/10.1016/j.tree.2012.07.013.

Solé, M., Sanchez-Hernandez, J.C., 2018. Elucidating the importance of mussel carboxylesterase activity as exposure biomarker of environmental contaminants of current concern: an in vitro study. Ecol. Indic. 85, 432–439. https://doi.org/10.1016/j.ecolind.2017.10.046.

Teixeira, M., Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Wrona, F.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2017. Toxic effects of the antihistamine cetirizine in mussel Mytilus galloprovincialis. Water Res. 114, 316–326. https://doi.org/10.1016/j.watres.2017.02.032.

Terlizzi, A., Felline, S., Lionetto, M., Caricato, R., Perfetti, V., Cutignano, A., Mollo, E., 2011. Detrimental physiological effects of the invasive alga Caulerpa racemosa on the Mediterranean white seabream Diplodus sargus. Aquat. Biol. 12, 109–117. https://doi.org/10.3354/ab00330.

Tete, V.S., Nyoni, H., Mamba, B.B., Msagati, T.A.M., 2020. Occurrence and spatial distribution of statins, fibrates and their metabolites in aquatic environments. Arab. J. Chem. 13, 4358–4373. https://doi.org/10.1016/j.arabjc.2019.08.003.

Vázquez-Carrera, M., Wahli, W., 2022. PPARs as key mediators in the regulation of metabolism and inflammation. Int. J. Mol. Sci. 23. https://doi.org/10.3390/ijms23095025.

Velez, C., Figueira, E., Soares, A.M.V.M., Freitas, R., 2016. Combined effects of seawater acidification and salinity changes in Ruditapes philippinarum. Aquat. Toxicol. 176, 141–150. https://doi.org/10.1016/J.AQUATOX.2016.04.016.

Viarengo, A., Canesi, L., Pertica, M., Poli, G., Moore, M.N., Orunesu, M., 1990. Heavy metal effects on lipid peroxidation in the tissues of mytilus gallopro vincialis lam. Comp. Biochem. Physiol. Part C Comp. Pharmacol. 97, 37–42. https://doi.org/10.1016/0742-8413(90)90168-9.

Vitale, R., D'Aniello, E., Gorbi, S., Martella, A., Silvestri, C., Giuliani, M., Fellous, T., Gentile, A., Carbone, M., Cutignano, A., Grauso, L., Magliozzi, L., Polese, G., D'Aniello, B., Defranoux, F., Felline, S., Terlizzi, A., Calignano, A., Regoli, F., di Marzo, V., Amodeo, P., Mollo, E., 2018. Fishing for targets of alien metabolites: a novel peroxisome proliferator-activated receptor (PPAR) agonist from a marine pest. Mar. Drugs 16, 431. https://doi.org/10.3390/md16110431.

Wu, H., Xu, L., Yu, D., Ji, C., 2017. Differential metabolic responses in three life stages of mussels Mytilus galloprovincialis exposed to cadmium. Ecotoxicology 74–80. https://doi.org/10.1007/s10646-016-1741-8.

Yancey, P.H., Siebenaller, J.F., 2015. Co-Evolution of Proteins and Solutions: Protein Adaptation Versus Cytoprotective Micromolecules and their Roles in Marine Organisms 1880–1896. https://doi.org/10.1242/jeb.114355.

Yi, G., Grabež, V., Bjelanovic, M., Slinde, E., Olsen, K., Langsrud, O., Phung, V.T., Haug, A., Oostindjer, M., Egelandsdal, B., 2015. Lipid oxidation in minced beef meat with added Krebs cycle substrates to stabilise colour. Food Chem. 187, 563–571. https://doi.org/10.1016/j.foodchem.2015.04.002.

Yu, H., Zhang, H., Dong, M., Wu, Z., Shen, Z., Xie, Y., Kong, Z., Dai, X., Xu, B., 2017. Metabolic reprogramming and AMPKα1 pathway activation by caulerpin in colorectal cancer cells. Int. J. Oncol. 50, 161–172. https://doi.org/10.3892/ijo.2016.3794.