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Multi-omic approach to evaluate the response of gilt-head sea bream (*Sparus aurata*) exposed to the UV filter sulisobenzone

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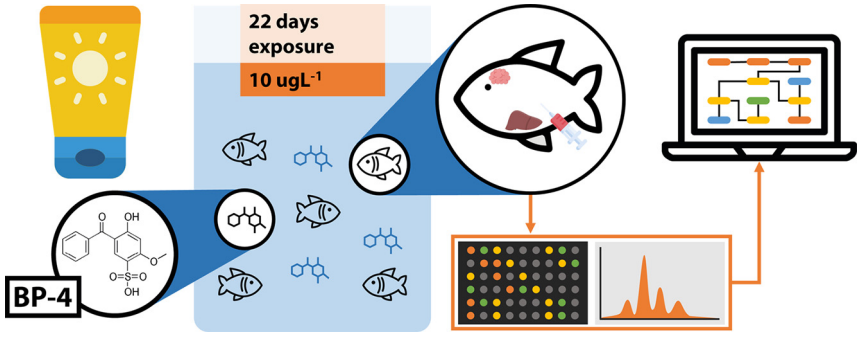
HIGHLIGHTS

- Multiomic approach provides better understanding of BP-4 exposure effects.
- Endocrine disrupting activity of BP-4 demonstrated at realistic concentrations
- Disorders in carbohydrate and amino acid metabolism proved energy disruptio.n
- 15 modulated metabolites and 3 DEGs were found in purine and pyrimidine metabolism.
- Oxidative stress was evidenced by alteration in lipid metabolism.

GRAPHICAL ABSTRACT



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ABSTRACT

Sulisobenzone (BP-4) is one of the benzophenone type UV filters most frequently detected in aquatic ecosystems. As a suspected endocrine disrupting compound, scarce information is available yet about other molecular effects and its mechanism of action. Here, we used an integrated transcriptomic and metabolomic approach to improve the current understanding on the toxicity of BP-4 towards aquatic species. Gilt-head sea bream individuals were exposed at environmentally relevant concentrations (10 μg L^{-1}) for 22 days. Transcriptomic analysis revealed 371 differentially expressed genes in liver while metabolomic analysis identified 123 differentially modulated features in plasma and 118 in liver. Integration of transcriptomic and metabolomic data showed disruption of the energy metabolism (>10 pathways related to the metabolism of amino acids and carbohydrates were impacted) and lipid metabolism (5 glycerophospholipids and the expression of 3 enzymes were affected), suggesting oxidative stress. We also observed, for the first time *in vivo* and at environmental relevant concentrations, the disruption of several enzymes involved in the steroid and thyroid hormones biosynthesis. DNA and RNA synthesis was also impacted by changes in the purine and pyrimidine metabolisms. Overall, the multiomic workflow presented here increases the evidence on suspected effects of BP-4 exposure and identifies additional modes of action of the compounds that could have been overlooked by using single omic approaches.

1. Introduction

Organic ultraviolet (UV) filters are key elements in several personal care products (PCPs) such as sunscreens, soaps, and shampoos (Hauri et al., 2003), as well as in many materials such as adhesives, plastics,

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photographic film, and even food packaging (Latha et al., 2013; Muncke, 2011). Their function is to protect skin from sun damage and to protect products against photo-degradation (Gago-Ferrero et al., 2015; Giokas et al., 2007). Two decades ago, the global production of organic UV filters consisted in approximately 10,000 tons (Danovaro et al., 2008), while nowadays this estimation reaches approximately 27,000 tons (Astel et al., 2020), of which 14,000 tons are calculated to be released into the ocean (Downs et al., 2016). UV filters are continuously released to the environment through a variety of pathways, both directly and indirectly, including wastewater discharges, recreational activities, aquaculture, and shipping (Halpern et al., 2015).

Benzophenone-4 (BP-4) or sulisobenzone (CAS no.: 4065-45-6) is one of the two benzophenone UV filters approved in the European Union for use in cosmetics and PCP formulations (Annex VI, Regulation 1223/2009/EC on Cosmetic Products). In Europe, the maximum amount of BP-4 in commercial products is set to 5%, while in the USA is 10%, according to the U.S. Food and Drug Administration (FDA) (Paredes et al., 2014). Total daily consumption of BP-4 has been estimated to be up to 438 mg/day/1000 people in Spain and 378 mg/day/1000 people in Hong Kong (Tsui et al., 2014), making it one of the UV-filters detected at highest concentrations in wastewater influents and effluents (reaching levels up to 13.3 μg L^{-1} in the UK or 2.1 μg L^{-1} in Spain) and the most frequently detected in surface waters. (Du et al., 2017; Kasprzyk-Hordern et al., 2009; Rodil et al., 2008, 2012; Semones et al., 2017) (Table S1). Additionally, the removal rates of this compound from wastewater in conventional sewage treatment plants (STPs) can be very low (0–20%) (Kasprzyk-Hordern et al., 2009; Rodil et al., 2012), resulting in its ubiquity in many aquatic ecosystems, including coastal and oceanic settings (Lara-Martín et al., 2020; Tsui et al., 2014) and chronic exposure of wildlife organisms. The use of tertiary treatments in STPs such as chlorination (Negreira et al., 2012) would reduce BP-4 loads in wastewater but could also lead to more toxic transformations products via chlorine substitution and oxidation (Xiao et al., 2013).

Previous works that evaluate the toxicological response of BP-4 in aquatic organisms are scarce and have mainly focused on acute toxicity (e.g., $LC50_{48h} = 50$ mg L^{-1} in *Daphnia magna*) (Fent et al., 2010a), morphological endpoints, and hormonal activity (Huang et al., 2018; Li, 2012; Liu et al., 2015; Miller et al., 2001). Benzophenones have been suggested as potential endocrine disruptors in several recent studies (Blüthgen et al., 2012; Fent et al., 2010a, 2010b; Ghazipura et al., 2017; Jeon et al., 2006; Kim et al., 2014). In particular, BP-4 has shown to act as estrogen, antiestrogen, and antiandrogen in *in vitro* assays (Kunz et al., 2006a; Kunz and Fent, 2006), and Lee et al. (2018) found a down regulation of thyroid hormone-regulated genes in rat pituitary cell lines at concentrations between 3.1 mg L^{-1} and 98.7 mg L^{-1}. According to Esperanza et al. (2019), BP-4 exposure at 76 mg L^{-1} caused a decrease in growth rate, cytoplasmic and mitochondrial membrane potentials, metabolic activity, and autofluorescence in the microalga *Chlamydomonas reinhardtii.* These effects can be related to oxidative damage as a response of the formation and release of reactive oxygen species (ROS), which were up to 1.5 times higher in exposed microalgae suggesting that this UV filter has more adverse effects other than only hormonal action (Esperanza et al., 2019; Tsui et al., 2014) mentioned before.

Despite the different toxic effects already reported for BP-4, there is a lack of knowledge regarding its general toxicological mechanism or modes of action in aquatic organisms. Further toxicity assays are thus required at sub-lethal concentration levels for a more precise environmental risk assessment (ERA) of UV filters (Carve et al., 2021; Fent et al., 2010a). Within this context, omics techniques have been proved to be useful tools (Blüthgen et al., 2014; Hampel et al., 2017; Ziarrusta et al., 2019) to globally assess thousands of molecular features, and facilitate a more holistic understanding of the associated adverse outcomes in exposed organisms (Liang et al., 2020; Simmons et al., 2015; Zhang et al., 2018).

Transcriptomic is the most frequently used omic technique for detection and analysis of global gene expression changes (Feswick et al., 2017; Schirmer et al., 2010) and can reveal low concentration effects of contaminants since all responses to toxicants involve disruption of gene expression, both direct (e.g. endocrine disruption) or indirect (e.g. compensatory) (Ankley et al., 2006; Zhu et al., 2020). Metabolomics identifies low-weight molecules involved in all the biological reactions, providing a more integrated assessment of the physiological state of organisms, organs, tissues or even cells (Ankley et al., 2006; Courant et al., 2014).

The present work focuses on the effect of BP-4 towards gilt-head sea bream (*Sparus aurata*), a marine coastal fish with high commercial value and availability widely used as model organism in research (De Magalhães et al., 2020; Raposo De Magalhães et al., 2020). The EU aquaculture industry produces over 200 thousand tons of this species yearly, representing more than 4% of the total production in this sector (Pateiro et al., 2020; Pérez-Sánchez et al., 2019).

To the date, very little is known about the molecular mechanism of UV filters, and other contaminants of emerging concern in exposed gilt-head sea bream. To the best of our knowledge, only four studies have been conducted so far aimed to evaluate the effects of UV filters towards aquatic species, all of them using single omic approaches (Table S2) (Blüthgen et al., 2014; Burkina et al., 2016; Meng et al., 2020; Ziarrusta et al., 2018). As a result, most of the mechanisms of toxicity still remain unclear (Meng et al., 2020). The present work aims to address this issue by combining, for the first time both transcriptomic and metabolomic tools to identify *in vivo* effects at environmentally relevant concentrations (10 μg L^{-1}) of BP-4 in juvenile gilt-head sea bream (*Sparus aurata*).

2. Materials and methods

2.1. Chemicals

Solvents used in sample preparation were pesticide grade (dichloromethane, ethanol, and dimethyl sulfoxide) and LC-MS grade (acetonitrile, methanol, water, and formic acid, purchased either from Carlo Erba (Val de Reuil, France) or from Sigma–Aldrich (Madrid, Spain). Ultrapure high-quality water was produced by a Simplicity® UV Millipore system (Bedford, MA, USA). Individual analytical standards used for determination of BP-4 and metabolite annotation were purchased from different suppliers: LGC Standards, Sigma-Aldrich, Toronto Research Chemicals, and Santa Cruz Biotechnology. Solid phase extraction was performed using Oasis® HLB (200 mg) cartridges from Waters Corporation (Barcelona, Spain).

The RNA extraction kits (RNeasy Mini Kit 740933 and RNase-Free DNase Set 740963) were purchased from Macherey-Nagel (Düren, Germany). Low RNA Input Linear Amplification Kit (5184-3523), Gene expression Hybridization Kit (5188-5242), and Gene Expression Wash Buffers 1 and 2 (5185-5327) were purchase from Agilent Technologies (Cedar Creek, USA).

2.2. Exposure experiments

Juvenile gilt-head sea bream (*Sparus aurata*) specimens were exposed to BP-4 after approval by the Bioethics Committee of the University of Cadiz (UCA, procedure approval 12/11/2018/160). Temperature in the laboratory was kept constant (20 °C) and a 14.5:9.5 h light:dark cycle was used. Gilt-head sea bream individuals were purchased from the fisheries facilities of the UCA in July 2018 and placed in a continuous flow-through seawater system in 75 L tanks at 18.5 ± 0.5 °C, pH ~8.4, salinity 37‰ and dissolved oxygen concentration of 6.6 ± 0.2 mg L^{-1} during a 14-day acclimation period. Fish were fed daily with 0.20 g pellets/fish (D-2 Optibream, 2.5 mm, Skretting, Spain) and the water in the tanks was aerated during the whole experiment using aquarium air

pumps. These physicochemical conditions were maintained during the whole exposure experiment as well.

Once acclimation was over, two tanks containing 11 individuals each were exposed to a nominal concentration of 10 μg L^{-1} of BP-4 using a peristaltic pump. BP-4 stock solutions were prepared in DMSO and working stock solutions prepared and renewed daily in sea water. Two control tanks containing 11 individuals each were exposed to DMSO only. Water was sampled from control and exposure tanks at days 0, 3, 8, and 22 for BP-4 determination (see Text S1 in the Supplementary Material (SM) for more information).

Physicochemical parameters were periodically monitored during the experiment (dissolved oxygen concentration, temperature, and pH). After 22 days of exposure, fish were length measured, weighed, and dissected to collect brain, liver, and plasma. Samples were rapidly submerged into liquid nitrogen and stored at −80 °C for transcriptome and metabolome analyses.

2.3. Transcriptomic analysis

2.3.1. Transcriptome analysis by microarray technology and RT-qPCR validation

Total RNA was extracted and purified from individual brain and liver samples according to the manufacturer's instructions (Macherey-Nagel). Evaluation of RNA quality and quantity was conducted on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and NanoDrop 1000 spectrophotometer (Nanodrop Technologies Inc. Wilmington, DE, USA), respectively.

Gene expression profiling of control and exposure samples was performed using an adaptation of the Agilent-031723 *Sparus aurata* oligo DNA microarray, SAPD_v.3. The array (8 × 15 K format) covers 12,879 annotated transcripts of the gilt-head sea bream. It was developed by Ferraresso et al. (2008) and was manufactured using the SurePrint HD technology. This microarray design was successfully applied to previous transcriptomics analyses (Hampel et al., 2017; Mininni et al., 2014; Vieira et al., 2011). Extra information about the microarray data is accessible with the reference GPL15601at the GEO repository (http://www.ncbi.nlm.nih.gov/geo/). Four biological replicates (arrays) were used for every tissue and treatment. The Agilent One-Color Microarray-Based Gene Expression Analysis protocol was followed for sample preparation, labelling, and hybridization. Microarray slides were scanned with a SureScan Microarray Scanner using the AgilentHD_GX_1color scanner protocol. Raw data were extracted and background was corrected through Agilent Feature Extraction (FE) Software version 12.0.3.1 (Calduch-Giner et al., 2014) to ensure that all entities in each array were measured using the same starting intensity. The same RNA samples used for the microarray experiment underwent quantitative reverse transcription PCR (qPCR) to verify the obtained results from the microarray analysis (Moreira et al., 2014). The sequence design of the primers for the target genes and more detailed qPCR information is presented in Table S3 and Text S2 in the SM.

2.3.2. Microarray data statistical analysis and functional annotation

A workflow diagram is presented in the SM (Fig. S1) for a better understanding of the transcriptomic and metabolomic data analyses performed and the following annotation and interpretation. Extracted transcriptomic data were loaded in GeneSpring GX 14.9.1 software (Agilent Technologies). In order to compare signal intensities between arrays, quantile normalization was performed and baseline transformation was applied to the median value of all samples (Sana et al., 2010). After normalization, quality control (correlation coefficients, plots, metrics, principal component analysis (PCA), Fig. S4) was performed to ensure data quality and comparability. The experiments were grouped non-numerically. A filter was used to keep only the features expressed in at least half of the samples (flagged as detected and set to 50%). In order to obtain the list of differentially expressed genes (DEGs) between the fish in the control and exposure tanks, moderated *t*-test (asymptotic *p*-value) followed by Benjamini–Hochberg false discovery rate (FDR) were applied (Blüthgen et al., 2014; Lunardi et al., 2016). Adjusted p-value (q-value) and fold change were set at 0.05 and 2, respectively. Functional annotation analysis was performed using Blas2Go (Conesa et al., 2005) in all DEGs (up- and down-regulated) as well as in all genes represented on the array. For enrichment analysis, Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp) databases were used to determine the most impacted biological processes (GO-BP), cellular components (GO-CC), molecular functions (GO-MF) and metabolic pathways (Xie et al., 2011).

2.4. Metabolomic analysis

2.4.1. Metabolite extraction and LC-HRMS analysis

The remaining fish tissues after RNA extraction were freeze dried in a Telstar Lyoquest – 55 (Lisbon, Portugal) for 96 h and metabolites were extracted according to Bonnefille et al. (2018). Briefly, 30 mg dry weight (± 0.25 mg) of liver tissue were grounded and later extracted with 75 μL of water and 240 μL of methanol. After homogenization for 60s, a second extraction was performed with 120 μL of water and 240 μL of dichloromethane. Samples were left at 4 °C for 15 min, vortexed and centrifuged at 2000*g* and 4 °C during 15 min to separate the methanol/water and dichloromethane phases. 50 μL of the supernatant containing all hydrophilic metabolites were collected in a glass test tube for further analysis.

Metabolites from plasma samples were extracted from 100 μL aliquots by addition of 400 μL of ice-cold methanol followed by vortexed for 1 min. Samples were then centrifuged at 10000*g* for 15 min at 4 °C to facilitate precipitation of proteins (Bruce et al., 2009; Dunn et al., 2011). 200 μL of supernatant were carefully transferred into vials. Both liver and plasma sample extracts, were evaporated under a gently stream of nitrogen, resuspended in 200 μL water/acetonitrile (95/5; *v*/v), and filtered using 0.22 μm polytetrafluoroethylene (PTFE) filters (Thermo Fischer Scientific). Quality control (QC) samples were prepared by pooling 10 μL of each sample (liver and plasma).

QC, plasma, and liver samples were sorted randomly and analyzed by liquid chromatography – high resolution mass spectrometry (LC-HRMS) in Exactive Orbitrap and Q Exactive Focus Orbitrap instruments (Thermo Fischer Scientific), equipped with an electrospray source (ESI). Injections were performed as described by Dumas et al. (2020a, 2020b). Briefly, ten microliters of sample were injected on a 2.1 × 100 mm × 3 μm particle size, pentafluorophenylpropyl (PFPP) column (Sigma Aldrich, PA, Bellefonte, USA). Chromatographic separation was performed in 31 min at 250 μL/min using water (solvent A) and acetonitrile (solvent B) (both with 0.1% formic acid) and the following gradient: 95% A: 5% B between 0 and 3 min, 60% A: 40% B at 8 min, 50% A: 50% B at 9 min, 30% A: 70% B at 13 min, and 5% A: 95% B from 15 to 18 min, including a re-equilibration time of 10 min.

Samples were simultaneously analyzed in both ionization modes, ESI+ and ESI-, and full range mass spectra were recorded over a mass range of 50 to 1000 *m/z* with a mass resolution of 50,000 FWHM (at m/z 200). For additional confirmation of suspected compounds, samples were reinjected and measured at different collision energies (HCD) for targeted MS/MS fragmentation with a nominal resolving power of 17,500 FWHM.

2.4.2. Processing and statistical analysis of metabolic data

Data processing steps and statistical analyses were performed as described by Dumas et al. (2020b). Briefly, MSConvert command line tool implemented into the ProteoWizard 3.0 open-source software (Chambers et al., 2012) was used to transform raw data files to the mzXML format. Then, XCMS package (Smith et al., 2006) was used in R language to process the previously transformed files using the procedure described by Courant et al. (2009) procedure. To avoid information redundancy, CAMERA Bioconductor package (Kuhl et al., 2012) was also

applied to annotate and remove isotopes and adducts. Additionally, extracted ion chromatograms were visually examined to verify the proper integration of each signal. All features showing relative standard deviation (RSD) > 30% were removed from further analyses.

Welch *t*-test was applied to the data, followed by Benjamini–Hochberg FDR for multiple testing comparisons (*q*-value). Differentially modulated metabolites between liver and plasma samples from control and exposure tanks having a modulation higher than 30% and a statistically significant *q*-value (<0.05) were selected. Multivariate data analysis was performed then to reduce the complexity of the resulting data matrix and to emphasize the similarities and differences between each sample group. In this context PCA was performed using SIMCA 13.0.3 software (Umetrics, Sweden). After unsupervised test, Orthogonal Partial-Least Square Discriminant Analysis (OPLS-DA) was also run in order to evidence the different distribution between groups and to provide better understanding of which variables carried the class separating information (He et al., 2020).

2.4.3. Annotation and identification of metabolites

Human Metabolome Database (http://www.hmdb.ca/) and LipidMaps (https://lipidmaps.org/) were used for the annotation of the LC-HRMS data. The annotation was performed by mass-matching within a 2 mDa window and using different annotation levels according to Sumner et al. (2007). Level 1 annotation corresponded to unambiguous confirmation via measurement of a reference standard showing the same MS spectrum and retention time. Level 2 corresponded to metabolites putatively annotated based on MS and/or MS/MS spectrum matching from two public databases, mzCloud (https://www.mzcloud.org/) and HMDB.

For those metabolites where there were several candidates with very similar (with 2 mDa) exact masses (*m/z*), the next steps were followed to prioritize one option (Gómez-Canela et al., 2017):

a. The minimum mass error value of the measured m/z was chosen to assign a compound to the corresponding metabolite.
b. Protonated $[M+H]^+$ and deprotonated $[M-H]^-$ molecules were prioritized.
c. The candidates must exist in the metabolome database (HMDB) with a biological significance.

KEGG and HMDB were used to understand the metabolic pathways affected by the differentially modulated features. Pathway analysis was carried out by MetaboAnalyst 5.0 (Chong et al., 2019) using the zebrafish database. For highly impacted metabolic pathways, metabolites annotated at level 1 with a modulation amplitude <30% and/or *q*-value <0.1 or analytically less restrictive (RSD QC < 35%) were also considered (e.g. *N*-acetylisoleucine, urea, L-ornithine) in order to obtain a wider view of the observed metabolic alterations involving complex interconnections between metabolites and genes within the metabolic pathways (Bonnefille et al., 2018; Dumas et al., 2020b).

2.5. Biological integration of DEGs and metabolites

DEGs and modified metabolites were used to identify perturbed biological pathways through KEGG (Moriya et al., 2007). Analysis of these pathways is key to understand the mechanisms of metabolic responses in gilt-head sea bream exposed to BP-4. Additionally, we attempted to map and visualize the expression level of genes related to metabolic processes in combination with the metabolomics results (Darzi et al., 2018).

3. Results

No significant changes in the length or weight of fish were observed between control and exposure tanks and there was no mortality during the whole experiment. The mean concentration of BP-4 in water were 8.76 ± 1.89 μg L^{-1} for tank 1 and 9.07 ± 0.44 μg L^{-1} for tank 2 (Fig. S2).

3.1. Transcriptomic analysis

3.1.1. Transcriptomic statistical analysis results

Volcano plots presented in Fig. S4 show the distribution of DEGs after 22 days of exposure of juvenile gilt-head sea bream to a nominal concentration of 10 μg L^{-1} of BP-4, 371 transcripts were differentially expressed (fold-change (FC) > 2, q-value <0.05) in liver (Table S4). No DEGs were observed in brain. From the 371 DEGs in liver, 48 were down-regulated and 323 were up-regulated. The fold change was between 2 (threshold setting) and 9.4 -fold. Among all these differentially expressed transcripts, several of them deserve special attention, because of: a) their known function and involvement in GO terms and metabolic pathways, and b) they have previously been identified as DEGs in transcriptomic experiments on zebrafish after exposure to the UV-filter octocrylene (Blüthgen et al., 2014), suggesting that these transcripts can be involved in the direct modulation of potential biomarkers for UV-filter exposure. These transcripts are: thyroid hormone receptor beta isoform (FC = 2.4), retinol-binding protein 2 (FC = 2.78), cytochrome P450 (FC = 2.2079482 and FC = 2.56), and aryl hydrocarbon receptor nuclear translocator-like protein 1 (FC = 2.4266756) (Table S4). Microarray and qRT-PCR expression data are presented in Fig. S3. The direction of the expression of target genes was always equal in both techniques although the FCs could be slightly different in agreement with other transcriptomics studies (Hampel et al., 2010, 2014; Moreira et al., 2014).

3.1.2. Enrichment analysis of DEGs

After performing enrichment analysis (EA), diverse GO processes, and biological pathways were over-represented. 32 biological processes (GO BP) showed to be enriched, followed by10 cellular components (GO_CC) and 7 molecular functions (GO_MF) (Table S5). Significant GO-BP terms represented by a high number of DEGs in response to BP-4 exposure were related to “complement activation” (6 DEGs), “regulation of small GTPase mediated signal transduction” (6 DEGs), and “cellular response to hormone stimulus” (5DEGs). “Cytoskeleton” (17 DEGs) was the most relevant enriched CC_GO term and “enzyme binding” (11DEGs) and “protein tyrosine kinase activity” (6 DEGs) were the most enriched GO_MF terms (Table 1).

KEGG pathway enrichment analysis (KO) revealed that the majority of the DEGs were involved in pathways related with the following functional categories: (09100) Metabolism, (09130) Environmental Information Processing, (09140) Cellular Processes, and (09150) Organismal Systems (Fig. 1). The most impacted pathways were withing the environmental information category, with up to 6 DEGs involved in Phagosome and Cell adhesion molecules (CAMs). Additionally, focal adhesion, a process involve in cell communication (Hampel et al., 2010), was also a highly impacted pathway, suggesting that certain cellular processes might be affected by BP-4 exposure.

Metabolism (09100) was one of the most impacted functional categories in the liver of gilt-head sea bream exposed to BP-4, suggesting a relationship between DEGs and metabolites. At the transcriptomic level, the most impacted pathways involved in the metabolism category were: (00230) Purine metabolism, (00010) Glycolysis/Gluconeogenesis, (00030) Pentose phosphate pathway, (00140) Steroid hormone biosynthesis, (00830) Retinol metabolism, and (00982) Drug metabolism - cytochrome P450 (Fig. 1). The DEGs associated to these pathways and the encoding enzymes are presented in Table S6.

3.2. Metabolomic analysis

3.2.1. Metabolomic statistical analysis results

The metabolic fingerprint obtained after LC-HRMS analysis of liver and plasma extracts provided 4072 features observed in ESI- and 7098 in ESI+ for plasma, and 3917 features in ESI- and 6880 in ESI+ for liver. The proportions of features with an RSD <30% was 85% in ESI- and 78% in ESI+ for plasma, whereas it was 78% and 77% in ESI- and

Table 1
Most important significantly enriched GO terms in the liver of *Sparus aurata* exposed to BP-4.

GO Category	Term		Count	P-Value
BP	GO:0008360	regulation of cell shape	3	7.42E-04
	GO:0031532	actin cytoskeleton reorganization	3	0.00238772
	GO:0051056	regulation of small GTPase mediated signal transduction	6	0.00286591
	GO:0051091	positive regulation of DNA-binding transcription factor activity	3	0.00321403
	GO:0006956	complement activation	6	0.00385482
	GO:0048741	skeletal muscle fiber development	3	0.00981258
	GO:0045944	positive regulation of transcription by RNA polymerase II	5	0.01060696
	GO:0007160	cell-matrix adhesion	3	0.0267941
	GO:0006338	chromatin remodeling	3	0.02999007
	GO:0071407	cellular response to organic cyclic compound	4	0.03122465
	GO:0071396	cellular response to lipid	4	0.03624138
	GO:0007626	locomotory behavior	3	0.03695526
	GO:0007600	sensory perception	3	0.04467259
	GO:0032870	cellular response to hormone stimulus	5	0.04700084
CC	GO:0005923	bicellular tight junction	4	0.00052198
	GO:0030018	Z disc	4	0.00180515
	GO:0005856	cytoskeleton	17	0.0094493
	GO:0005741	mitochondrial outer membrane	3	0.04072102
MF	GO:0004713	protein tyrosine kinase activity	6	0.00507642
	GO:0019899	enzyme binding	11	0.02511118
	GO:0050661	NADP binding	3	0.03695526

ESI+, respectively, in the case of liver. The PCA score plot (Fig. 2) shows that the QC samples, clustered together, confirming an acceptable analytical repeatability for both plasma and liver samples. PCA score plot of plasma samples (Fig. 2a) (four principal components for a r^2X = 61%) describes a better clustering trend for both control and exposed gilt-head sea bream groups than the PCA score plot of liver samples (Fig. 2b) (four principal components for a r^2X = 59%). A stronger BP-4 exposure signature was then suggested for plasma compared to liver. An OPLS-DA method is additionally presented in SM for a better understanding on the clustering of the plasma dataset (R2X = 0.574, R2Y = 0.995, Q2 = 0.562) and liver (R2X = 0.345, R2Y = 0.919, Q2 = 0.683) (Fig. S5).

3.2.2. Metabolite annotation and modulation

Differentially modulated features were putatively annotated using the online HMDB database. Table 2 shows the metabolites identified at level 1 by confirmation with reference standards in plasma (n = 24) and in liver (n = 30), as well as the putatively annotated features at level 2 belonging to relevant metabolic pathways and confirmed through their MS/MS spectra (Table S7). Additionally, analytical characteristic for the metabolites presented in Table 2 are available in Table S8. All other features of interest not presented in Table 2 are included in the SM (Table S9).

All the metabolites simultaneously detected in plasma and liver (n = 7) showed an opposite trend, being always down-modulated in liver and up-modulated in plasma (Table 2). The significance of the modulation was stronger in liver than in plasma for 6 of these molecules. After annotation of the main modulated metabolites, 36 were involved in 8 amino acid metabolic pathways, 15 in nucleotides metabolic pathways, 3 in fatty acid metabolism and 3 in nicotinate and nicotinamide metabolism (metabolism of cofactors and vitamins).

3.2.3. Pathway analysis of modulated metabolites

Metabolic pathway analysis performed with Metaboanalyst 5.0 software showed that the phenylalanine and tyrosine metabolisms were among the most affected by BP-4 exposure (Figs. S6 and S7). In liver *L*-phenylalanine, L-tyrosine, and 8-methylnonenoate were down modulated, and 3, 4-dihydroxyhydrocinnamic acid was up-modulated. Valine, leucine, and isoleucine metabolisms appear also as impacted pathways in both biological matrices, showing 3 metabolites up-modulated in plasma (L-leucine, N-Acetylisoleucine, and 3-Methyl-2-oxovaleric acid) and 2 down-modulated in liver (L-leucine and L-valine). Some other amino acids were modulated, such as L-arginine, L-serine, L-lysine, L-methionine, and D-glutamine, which are involved in arginine and proline metabolism, glycine, serine and threonine metabolism, lysine metabolism, cysteine and methionine metabolism, and D-glutamine and D-glutamate metabolism, respectively. Although L-tryptophan was very slightly modulated due to BP-4 exposure, 3 other metabolites (acetyl-N-formyl-5-methoxykynurenamine, L-kynurenine, and 5-hydroxyindoleacetaldehyde) were significantly modulated and 2 of them (kynurenic acid, anthranilate) presented modulation with q-value >0.1.

BP-4 exposure also shows effects on nucleotide metabolism. Purine metabolism was mainly impacted by an increase of 2'-deoxyguanosine, inosine, guanosine, and adenine in plasma. These molecules presented the opposite behavior in liver, where additionally, adenosine monophosphate, and inosine-5'-monophosphate were also down-modulated. Concerning the pyrimidine metabolism, the most noticeable effect was observed in the liver, presenting a decrease of uracil and uridine metabolism, and an increase of ureidopropionic acid,

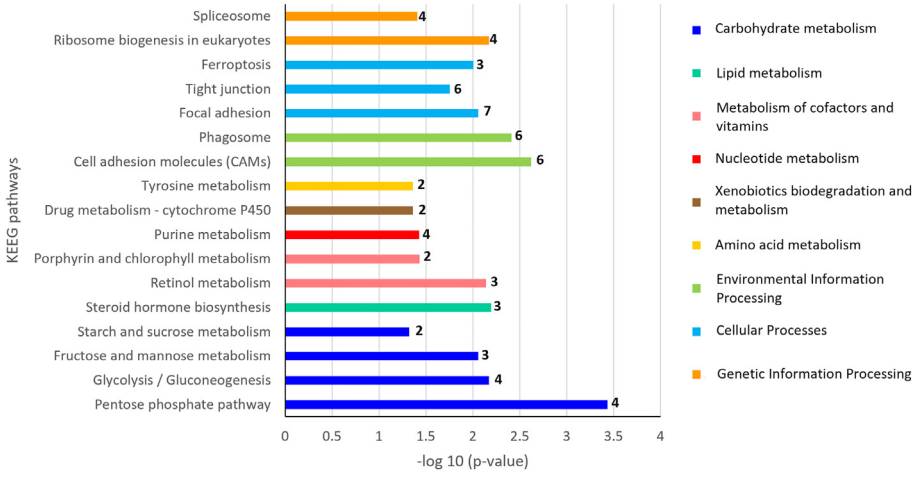

Fig. 1. results of KEGG enrichment analysis of DEGs from gilt head bream liver exposed to BP-4. Numbers next to the bars refer to the number of DEGs involved in each process.

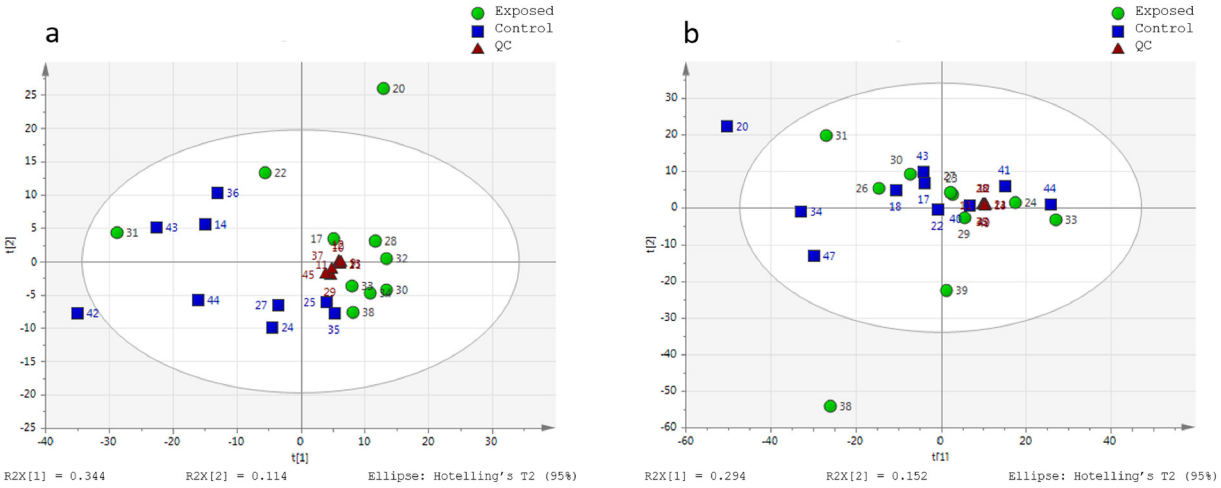

Fig. 2. PCA score plot of metabolic fingerprints from control (blue square) and exposed (green circle) gilt-head bream plasma (a) and liver (b). Red triangles represent QC, that are clustered. The numbers represent the injection order. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dihydrothymine, and L-dihydroorotic acid. The fatty acid metabolism was only disrupted in the liver by the down and up-modulation of L-carnitine and *L*-acetylcarnitine, respectively.

4. Discussion

After integration of both transcriptomic and metabolomic datasets, alterations in the metabolome and transcriptome of gilt-head sea bream were observed following exposure of BP-4 along 22 days. The most relevant results are discussed below.

4.1. Energy disruption

The highest impact at metabolic level was observed in the metabolism of certain amino acids (phenylalanine, tyrosine, and tryptophan metabolism; arginine and proline metabolism; valine, leucine and isoleucine metabolism; alanine, aspartate and glutamate metabolism; and glycine serine and threonine metabolism) in both liver and plasma (Fig. 3). KEGG pathway enrichment analyses, however, revealed that only tyrosine metabolism was significantly disrupted (Fig. 1) at transcriptomic level. Nevertheless, several DEGs encoding enzymes involved in other amino acid pathways were found (Figs. 3 and S8) (e.g., tryptophan metabolism and arginine and proline metabolism).

Among the different effects of the disruption of amino acid metabolism by BP-4, alterations in the energy metabolism are a major one. Lipids and fatty acids in fish constitute, one of the main sources of energy for growth, reproduction, and movement (Tocher, 2003). However, under stressful situations, carbohydrates become essential as quick sources of energy for fish (Rodnick and Planas, 2016). Our results reveal that several glucogenic and ketogenic amino acids (phenylalanine, tyrosine, leucine, valine, methionine, and tryptophan) were down-modulated in liver. Additionally, leucine, tryptophan, arginine, serine, and glutamine were found to be up-modulated in plasma. One of the functions of these amino acids is maintaining the normal nutritional status of the body (Li et al., 2017; Liu et al., 2018; Zhang and Zhao, 2017). A decrease in their levels in liver could be attributed to their consumption due to increase in energy demand. These amino acids are mobilized to other tissues such as brain or gills through the bloodstream where they have been demonstrated to serve as a local energy source during stress conditions (Takei and Hwang, 2016). This mobilization can explain the increase in their levels in plasma.

The hypothesis of energy disruption in fish exposed to BP4, is also supported by the transcriptomics results (Figs. 1 and S8). Several genes encoding enzymes involved in starch/sucrose metabolism, fructose/mannose metabolism, glycolysis/glucogenesis and pentose phosphate pathway were found to be differentially regulated (Fig. 1). The DEGs encoding the enzymes involved in carbohydrate metabolism (fructose-bisphosphate aldolase, alcohol dehydrogenase, phosphoglucomutase, glucose-6-phosphate 1-dehydrogenase, 6-phosphofructo-2-kinase, fructose-2,6-biphosphatase, and glycogen synthase) (Table S6) were all up-regulated. All these enzymes have a function in the glycolysis/gluconeogenesis pathway (Ali et al., 2001; Leskovac et al., 2002; Marsh and Lebherz, 1992; Matsuura et al., 2016; Miclet et al., 2001; Stojkovic et al., 2009; Yalcin et al., 2009), suggesting the constant activation of the energetic metabolism. This leads to the synthesis of glucose in the liver probably from glucogenic amino acids, and its subsequent storage in the form of glycogen (glycogenesis) unless immediately used or transported (Vijayan et al., 2010). Our results also explain the down-modulation of glucogenic amino acids in liver. The disruption of energy metabolism has been previously identified in gilt-head sea bream exposed to other benzophenones (Ziarrusta et al., 2018) and in several other fish exposed to xenobiotics or under stress conditions (Fu et al., 2019; Kokushi et al., 2017; Kovacevic and Simpson, 2020; Wan et al., 2019). Additionally, phosphocreatine was significantly greater in plasma of exposed individuals compared with that of controls. This metabolite plays an essential role in cellular energy metabolism (Chen et al., 2017; Wang et al., 2017), hence its perturbation is consistent with the aforementioned conclusions.

4.2. Oxidative stress

Disruption of energy metabolism and oxidative stress have been previously linked in metabolomic studies (Ling et al., 2014; Yuan et al., 2016). In the present work, oxidative stress was observed mainly by alteration in the metabolism of lipids such as glycerophospholipid and linoleic acid metabolisms.

Glycerophospholipids are key structural components of cell membranes (Ren et al., 2018). In the liver of gilt-head sea bream exposed to BP-4, several enzymes involved in the synthesis and catalysis of these molecules were identified to be encoded by up-regulated genes (Table S6). These results, in combination with the disrupted metabolites putatively identified in lipid metabolism (Table S8), support the theory of oxidative stress through lipid peroxidation, a previously reported adverse effect after xenobiotic exposure (Dumas et al., 2020a; Sotto et al., 2017). Most of the disrupted polar lipids identified are either glycerophospholipids (e.g., lysophosphatidylcholines -LPCs) or unsaturated fatty acids. Several of these lipids have been previously identified by HRMS in gilt-head sea bream exposed to another benzophenone

Table 2
List of the main metabolic pathways modulated in gilt-head bream plasma and liver by exposure to BP-4.

Metabolism pathway	Metabolite	Tissue	RSD QC	Modulation	Difference amplitude %	Q value (FDR)	Annotation level
Phenylalanine and Tyrosine metabolism	*L*-phenylalanine	Plasma	2	↗	21.7	0.058	1
		Liver	5	↘	97.3	0.000	
	L-tyrosine	Liver	19	↘	164.2	0.004	1
	8-methylnonenoate	Liver	26	↘	40.8	0.029	1
	3, 4-dihydroxyhydrocinnamic acid[a]	Liver	3	↗	63.7	0.043	2
Valine, leucine and isoleucine metabolism	L-leucine	Plasma	5	↗	46.8	0.052	1
		Liver	16	↘	64.0	0.008	
	N-acetylisoleucine	Plasma	5	↗	66.4	0.091	1
	L-valine	Liver	15	↘	31.3	0.022	1
	3-methyl-2-oxovaleric acid [a]	Plasma	12	↗	77.5	0.049	2
Arginine and Proline metabolism	Prolylhydroxyproline	Plasma	6	↘	33.3	0.037	1
	L-arginine	Plasma	18	↗	97.1	0.045	1
	Phosphocreatine	Plasma	16	↗	31.9	0.039	1
	Urea	Plasma	8	↗	26.4	0.038	1
	L-ornithine	Liver	12	↘	24.7	0.085	1
	5-aminovaleric acid	Liver	27	↗	35.9	0.043	1
Glycine, serine and threonine metabolism	L-serine	Plasma	12	↗	32.5	0.049	1
	Betaine	Plasma	8	↗	31.5	0.048	1
	Aminoacetone	Plasma	25	↗	73.9	0.036	1
	Glycine	Liver	6	↗	20.0	0.053	1
	Betaine aldehyde [a]	Liver	7	↘	37.8	0.006	2
Lysine metabolism	L-lysine	Plasma	25	↗	73.9	0.056	1
	Aminoadipic acid	Liver	12	↗	47.3	0.014	1
	Diaminopimelic acid	Liver	12	↘	57.0	0.031	1
	N6,N6,N6-trimethyl-L-lysine [a]	Plasma	27	↗	63.8	0.048	2
	Homocitric acid [a]	Liver	17	↗	93.4	0.029	2
Cysteine and methionine metabolism	Adenosine	Plasma	13	↗	283.2	0.014	1
	L-methionine	Liver	3	↘	62.6	0.007	1
	O-acetylserine	Liver	31	↗	93.7	0.030	1
	S-adenosylhomocysteine [a]	Plasma	6	↗	35.5	0.046	2
Alanine, Aspartate and Glutamate metabolism	L-glutamine[b]	Plasma	5	↗	33.1	0.042	1
	N-acetylaspartylglutamic acid [a]	Liver	12	↗	59.3	0.021	2
Tryptophan metabolism	Acetyl-N-formyl-5-methoxykynurenamine	Plasma	11	↗	73.2	0.032	1
	Kynurenic acid	Plasma	3	↘	63.8	0.076	1
	L-kynurenine	Liver	8	↘	249.6	0.015	1
	Anthranilate	Liver	29	↘	35.3	0.078	1
	L-tryptophan	Plasma	1	↗	21.8	0.041	1
		Liver	6	↘	17.8	0.023	
	5-hydroxyindoleacetaldehyde [a]	Liver	12	↗	43.6	0.016	2
Purine metabolism	2'-deoxyguanosine	Plasma	7	↗	50.0	0.037	1
		Liver	33	↘	258.5	0.014	
	Inosine	Plasma	4	↗	49.1	0.039	1
		Liver	11	↘	56.5	0.039	
	Guanosine	Plasma	7	↗	47.3	0.028	1
		Liver	11	↘	128.4	0.003	
	Deoxyinosine	Plasma	14	↗	44.7	0.037	1
	Adenine	Plasma	13	↗	50.9	0.076	1
		Liver	4	↘	36.8	0.008	
	Adenosine monophosphate	Liver	5	↘	48.0	0.000	1
	dGDP or ADP	Liver	4	↗	73.4	0.000	1
	Inosine-5'-monophosphate	Liver	5	↘	202.9	0.000	1
	Cyclic GMP	Liver	6	↗	22.8	0.078	1
Pyrimidine metabolism	Cytidine	Plasma	12	↗	27.6	0.070	1
	Deoxycytidine	Plasma	11	↗	71.6	0.079	1
	Uracil	Liver	28	↘	35.8	0.036	1
	Uridine	Liver	28	↘	40.1	0.014	1
	Ureidopropionic acid	Liver	6	↗	177.75	0.012	1
	Dihydrothymine	Liver	21	↗	79.04	0.031	1
Fatty acid metabolism	L-carnitine	Liver	4	↘	61.6	0.003	1
	L-acetylcarnitine	Liver	22	↗	68.5	0.000	1
	12-hydroxydodecanoic acid [a]	Liver	16	↘	34.5	0.016	2
Nicotinate and nicotinamide metabolism	L-glutamine[b]	Plasma	5	↗	33.1	0.042	1
	Nicotinamide riboside	Liver	31	↗	42.6	0.053	1
	Iminoaspartic acid[a]	Liver	24	↘	34.1	0.029	2

Annotation level 1: metabolite identity confirmed by injection of the corresponding analytical standard in the same analytical conditions.
Annotation level 2: putative annotation, based on the use of public databases.
[a] MS/MS spectrum confirmed with database.
[b] Belong to several metabolic pathways.

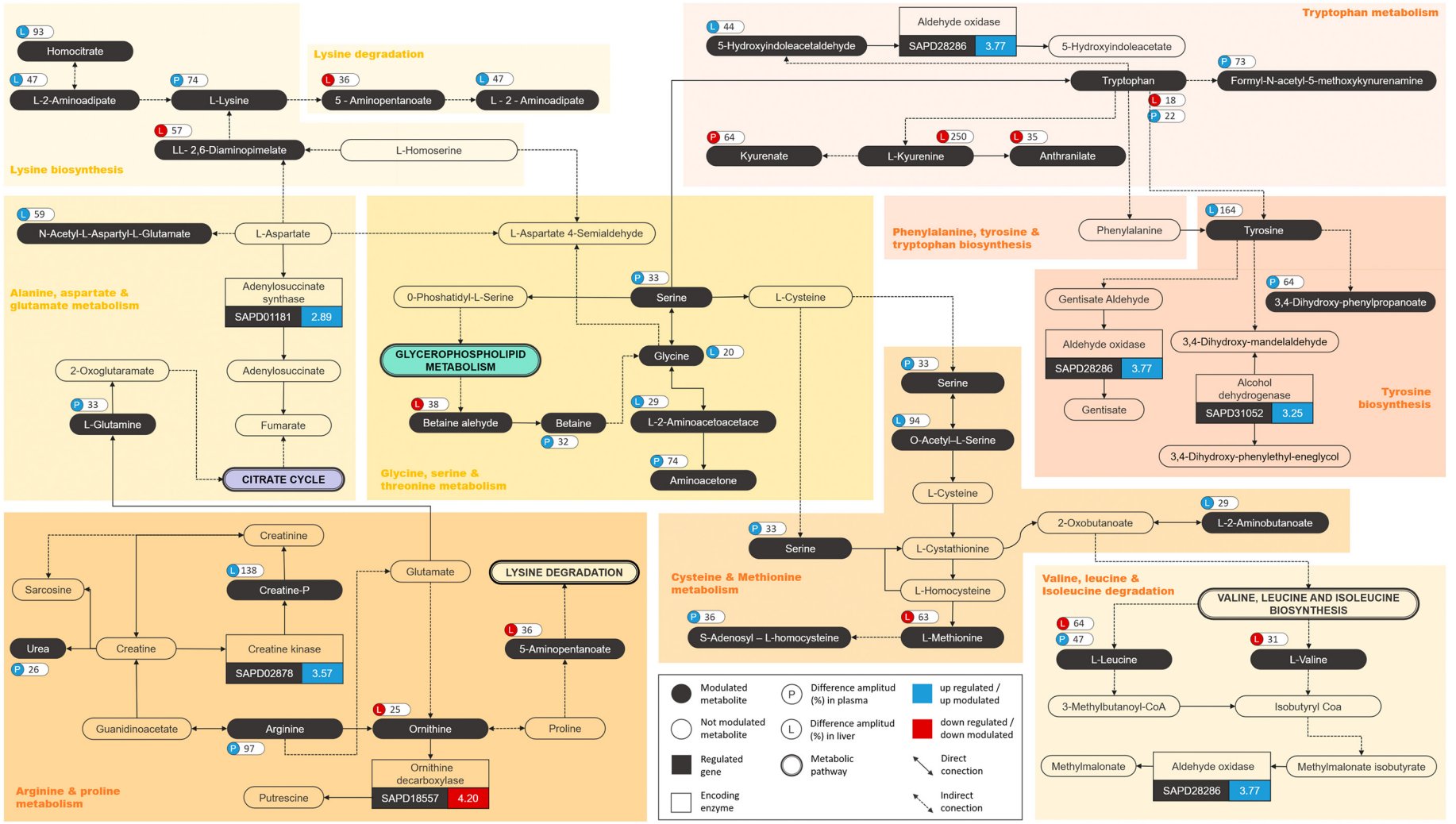

Fig. 3. Synthesized map of amino acid metabolism pathways affected after exposure of gilt-head bream to BP-4. Changes in metabolite and gene expression levels are mapped with values of difference amplitude (%) and FC indicated besides them, respectively. Enzymes involved in these pathways that were encoding by DEGs are also mapped.

type UV filter (Ziarrusta et al., 2018) and in other marine species exposed to other xenobiotics (Dumas et al., 2020a), which reinforces the annotation performed in the present work and our interpretation.

Metabolites involved in linoleic acid metabolism and certain unsaturated fatty acids such as docosahexaenoic acid, among others, are measured as biomarkers of oxidative stress *in vivo* (Niki, 2008). In the present work, and in agreement with other studies (Gómez-Canela et al., 2017; Huang et al., 2017; Ortiz-Villanueva et al., 2017; Ziarrusta et al., 2018), an impact in linoleic acid metabolism at both transcriptomic and metabolomic levels was observed (Tables S6 and S8). The genes encoding the enzyme cytochrome P450 were found to be up-regulated, and several signals were putatively annotated as final products of the transformation of linoleic acid (i.e.: 9,10-DHOME / 12,13-DHOME, 13-OxoODE / 9-OxoODE) (Niki, 2008), supporting the hypothesis of lipid peroxidation.

Additionally, methionine, a precursor for glutathione (or GSH, which plays vital role in detoxification processes, protecting cells from oxidative damage through its oxidation to form GSGG) (Caylak et al., 2008) was found to be down-modulated in liver, reinforcing the oxidative stress hypothesis. Methionine is also a precursor of L-carnitine (Ziarrusta et al., 2019) that, together with its esterified form (acetylcarnitine), act as intermediates in the β-oxidation of long-chain fatty acids to obtain energy, transporting them into the mitochondria (Gómez-Canela et al., 2017; Roszkowska et al., 2018; Simmons et al., 2015). Abnormalities in fatty acid metabolism were also observed by Stein and coworkers in a marine coral (*Pocillopora damicornis*) exposed to octocrylene for 7 days. They observed the disruption of 15 acylcarnitines, derived from the oxidation of L-carnitine (Stien et al., 2019), suggesting the oxidation of fatty acids as a specific mechanism of action of certain UV-filters. The modulation of both methionine and L-carnitine in our study highlights the aforementioned link between oxidative stress and energy disruption, and the impact of BP-4 exposure of both functions in gilt-head sea bream.

Finally, proteins in the cytoskeleton are one of the principal targets for the reactive oxygen species (ROS) that are produced under oxidative stress conditions (Karim et al., 2011; Zhu et al., 2018). In this context, after GO enrichment analysis, we could observe that some of the impacted GO terms within biological function were actin cytoskeleton reorganization (GO:0031532), skeletal muscle fiber development (GO:0048741), and cytoskeleton (GO:0005856) within cellular component. Alterations at transcriptomic level in xenobiotic biodegradation metabolism and oxidative phosphorylation metabolism were also detected (Fig. 1), two additional lines of evidence confirming oxidative stress occurring in fish exposed to BP-4.

4.3. DNA and RNA synthesis

BP-4 exposure altered purine and pyrimidine at both transcriptomic and metabolomic levels. Nine purine metabolites were impacted in plasma and liver, and the activity of 4 enzymes regulated by DEGs were disrupted (Fig. 4). Regarding pyrimidine metabolism, six metabolites were disrupted. In general terms, nucleosides (i.e.: inosine, guanosine, and adenine) present in liver and plasma showed

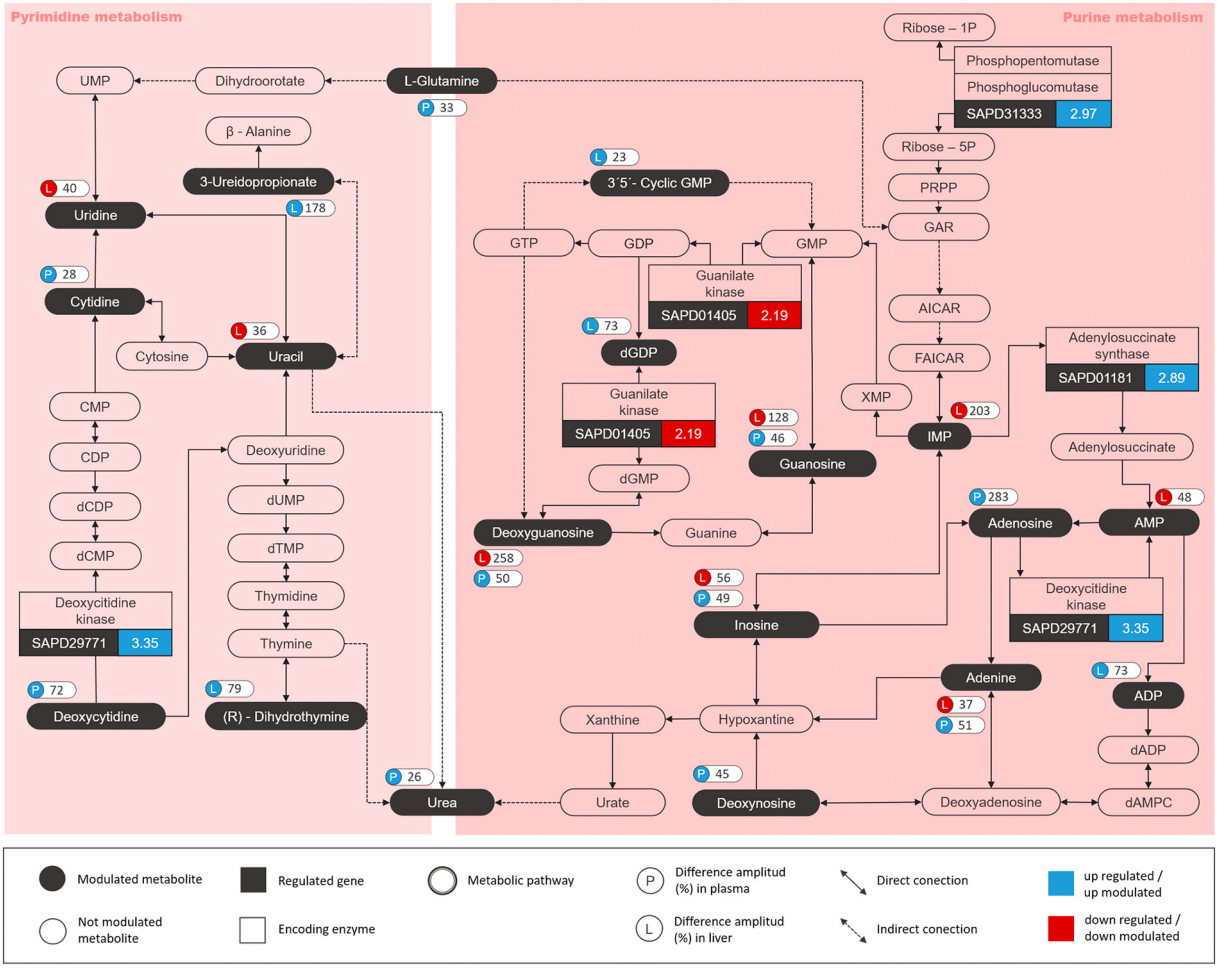

Fig. 4. Synthesized map of nucleotide metabolism pathways affected after exposure of gilt-head bream to BP-4. Changes in metabolites and gene expression levels are mapped with values of difference amplitude (%) and FC indicated besides them, respectively. Enzymes involved in these pathways that were encoding by DEGs are also mapped.

opposite modulation, being always down-modulated in liver and up-modulated in plasma. On the other hand, nucleotides that were only differentially modulated in liver, had a variable modulation: adenosine monophosphate and inosine-5'-monophosphate were down-modulated, and dGDP/ADP and cyclic GMP were up-modulated. Pyrimidine and purine metabolites are structural components of RNA and DNA. A deregulation of these molecules might contribute to limitations on products implicated in RNA and DNA turnover and repair (Dumas et al., 2020b). However, they not only act as building blocks of genetic molecules, but also as source of energy for cellular processes (ATP) and intracellular signaling (GTP), as well as cofactors of many other metabolic processes (Ren et al., 2018).

Kristal et al. (1999) hypothesized that, as a response to oxidative stress or energy disruption, purine catabolism (degradation) is activated to release antioxidant molecules such as uric acid, and to recover purine bases via the nucleotide salvage pathway, reducing energy consumption (Pedley and Benkovic, 2017). In this context, our results show that the gene encoding the enzyme deoxycytidine kinase -which catalyzes one of the initial steps in the nucleoside salvage pathway- was up-regulated (Table S6), suggesting the activation of this pathway to cope with the energy demand and oxidative stress. These results are in line with previous ecotoxicological studies that also reported activation of purine degradation and oxidative stress in fish after pollutant exposure (Elie et al., 2015; Ortiz-Villanueva et al., 2018; Sotto et al., 2017; Yoon et al., 2017).

4.4. Endocrine disrupting activity

Estrogenic activity of benzophenone type UV filters is known (Blüthgen et al., 2012; Kim et al., 2014; Kunz et al., 2006b; Lee et al., 2018; Zucchi et al., 2011) and related to their molecular structure (Du et al., 2019) resembling that of 17β-estradiol (Carve et al., 2021). In the present study, the endocrine disrupting activity of BP-4 exposure is evidenced at both transcriptomic and metabolomic levels. The clearest evidence of endocrine disruption can be observed through the impact in the steroid hormone biosynthesis pathway at transcriptomic level (Figs. 1 and S8). This pathway is proposed as a target for endocrine disrupting compounds affecting cytochrome P450 (CYP) enzymes and several steroid dehydrogenases and reductases, whose activity directly control steroid hormone synthesis (Acconcia and Marino, 2016; Sanderson, 2006). In this work, three DEGs encoding enzymes involved in the steroid hormone pathway (cytochrome P450 family, steroid dehydrogenase, and alcohol sulfotransferase) (Table S6) were up-regulated in the liver of gilt-head sea bream, suggesting the constant activation of this pathway, and proving the estrogenic activity of BP-4. Similar results were reported at transcriptomic level in zebrafish exposed to oxybenzone (Meng et al., 2020), whereas data from a metabolomic approach were not conclusive (Ziarrusta et al., 2018). Additionally, estrogenic disruption has been demonstrated to have an impact in thyroid hormone biosynthesis (Maharjan et al., 2005; Rolland, 2000), where L-tyrosine, down-modulated in our study, plays an essential role (Carvalho and Dupuy, 2017) (Table 2). Estrogenic response can be also determined by analysis of purine metabolites as potential bioindicators (Ekman et al., 2015), since previous works reported an estrogen-mediated response of purine metabolism (Ruggeri et al., 2008; Sreedevi and Chitra, 2014; Wan et al., 2011). This response was observed in our experiments, as we discussed in the previous section on DNA and RNA synthesis.

Among the different effects derived from exposure of gilt-head sea bream to endocrine disrupting chemicals, García-Hernández and coworkers have reported that even short-term occasional exposure can lead to reproductive failure due to alteration of the male-to female sex change process that naturally occurs in the live stage of gilt head sea bream. Long-term consequences are decrease of natural populations and hence increase of the fishery cost (García Hernández et al., 2020). The effects of BP-4 on endocrine systems have been previously assessed in fish (Kunz et al., 2006a; Kunz and Fent, 2006; Zucchi et al., 2011) but to the best of our knowledge, this is the first study that reports them *in vivo* and at environmentally realistic concentrations. Our metabolomic approach could be complemented by achieving an effective extraction and later ionization of less polar compounds such as steroid hormones. This was, however, out of the scope of this and other analytical protocols based on LC-HRMS (Ziarrusta et al., 2018) and further analyses using complementary analytical techniques (e.g., GC–MS) are required to detect these chemicals and their up and/or downregulations (Noppe et al., 2008). In any case, our multiomic approach highlights the advantages of combining different tools to identify different modes of action that otherwise could have been overlooked by using only one class of omic.

5. Conclusions

This study demonstrates that the UV filter BP-4 (sulisobenzone) is capable of inducing alterations in the metabolite concentrations and transcription of genes in gilt-head sea bream at environmentally relevant concentrations. After integration of metabolomics and transcriptomics results, the main impacted pathways were the metabolism of various amino acids, carbohydrate metabolism, pyrimidine and purine metabolic pathway, and lipid metabolism. These results reveal potential disruption of certain biological functions such as energy generating process, RNA and DNA synthesis, oxidative stress, and endocrine system, respectively. The metabolic approach used revealed alterations in metabolites with medium to high polarity, whereas those alterations in hydrophobic metabolites (e.g., lipids and hormones) were only detected by transcriptomic analysis. Additional screening analyses would be necessary to address this limitation and evaluate potential effects of BP-4 in gilt-head sea bream lipidome and/or hormonal system at the metabolomic level. In any case, this study shows that the integration of multi-omic data presents a clear advantage over the more conventionally used approaches based on single omics, which can oversee potential toxicological effects due to analytical limitations. Additionally, our work reveals that BP-4 has an impact pattern similar to other UV-filters, such as BP-3, or octocrylene, recently prohibited in certain areas such as Hawaii, U.S. Virgin Islands or the Republic of Palau among others, because of its potential impact on marine wildlife. Therefore, further research using integrative approaches is encouraged to increase the current knowledge on the toxicity mechanisms of organic UV-filters and other contaminants of emerging concern in aquatic organisms, one of the first basis towards the regulation of these chemicals in the environment.

CRediT authorship contribution statement

Nieves R. Colás-Ruiz: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Gaëlle Ramirez:** Formal analysis, Data curation. **Frédérique Courant:** Conceptualization, Methodology, Writing – review & editing. **Elena Gomez:** Writing – review & editing, Supervision. **Miriam Hampel:** Investigation, Resources, Supervision, Writing – review & editing, Funding acquisition. **Pablo A. Lara-Martín:** Writing – review & editing, Project administration, Funding acquisition.

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.2021.150080.

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