

Combined proteomic and gene expression analysis to determine effects of environmentally realistic microplastics concentrations on fish health

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- Supplementary Material -

Table S1. List of oligonucleotide primers used in the gene expression analysis. Genes were derived from the previous proteomic analysis or from literature.

gene/ LOC symbol	gene product	NCBI accession code	sense primer (5'-3')	antisense primer (5'-3')	fragment length [bp]
<i>abcb11b</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 11b	NM_001124656	CACGAAACAGGACACAAAGTCTC	CGTACTCAATGAAGGTGTCTGTG	197
<i>c3-3^a</i>	Complement component C3, isoform 3	XM_021568201	CGACCAGGGAAAGATGTTTGGA	AGTACAGCGCTAATTTTGGCTAC	168
<i>col1a1</i>	Collagen, type I, alpha-1 chain	NM_001124177	CCCCCTCGCCTCAAAGAGT	GTGCGATCGTCCTCGCCTTG	127
<i>ctrl</i>	Chymotrypsin-like protein	XM_021625136	CACGAAACAGGACACAAAGTCTC	CGTACTCAATGAAGGTGTCTGTG	127
<i>drp1</i>	Differentially regulated trout protein 1	NM_001124601	GATCGAAAGACTCCTGAAGAAAG	TTTCAGGCCCTCTCCAAAGCTA	94
<i>hamp^a</i>	Hepcidin antimicrobial peptide	XM_021595153	AGTGTTGCAGTTGCAGTGGTACTC	AAATGCTCCGGCAGACGC	164
<i>ighd</i> (LOC110485215)	Immunoglobulin delta, heavy chain (secreted form)	JQ034605	ACTTCTGCCTCATCAACTGCTC	CCCGCCTTCGTCATTCCATTTG	141
<i>ighm^a</i> (LOC100136770)	Immunoglobulin mu, heavy chain (membrane-bound form)	U04616	AAGCCTACAAGAGGGAGACCGA	GGCAGTTGCTCCAACGCCATA	151
<i>il1b^a</i>	Interleukin-1 beta	XM_036979104	GAGAGTGCTGTGGAAGAACATAT	GCAGCTCCATAGCCTCATTCAT	156
<i>krt13</i>	Keratin, type I cytoskeletal 13	XM_021624710	CCAGAGCCTCCAGCTTCAGTAT	TGTGAACCTTCGCCAGGTAGGT	120
<i>mhc2</i> (LOC110494409)	MHC class II antigen, alpha-1 chain	FR688130	CAGTGATTCAGATGGAGTGAATAT	CATAATATCCAGGGAAGGAAATCT	131
<i>mthfd1</i>	Methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1	XM_021600758	CTATCAGTTTCTCTTGCAGGGAG	GGTCATCCTCATCTGCCTGTAT	120
<i>otop3</i> (LOC110499463)	Otopetrin-3	XM_021576601	TGGCCTACAGACCTATTTGCTC	ATACGTGGCGCGGCCATCCT	184
<i>rpl7^b</i>	60S ribosomal protein L7	XM_021562213	TCAAACCTGAACAAGGCTTCCATC	TATTTACCCAGGGCCTTCTCGA	178
<i>tmem63b</i> (LOC110493827)	CSC1-like protein 2, Transmembrane protein 63b	XM_021568328	ATAACGGCTTCTGTTCTGTTA	CGCTGGAAGGAAAGGTAGTGC	103

^a Genes derived from a literature survey. ^b Reference gene, which also upregulated 2.5-fold in the proteomic analysis

Table S2. Overview of utilized linear models to analyse the microplastic concentration in fish, as well as the performance parameters, health parameters and gene expression, obtained from the exposure experiment.

dataset	method	dependent variable	time points	model effects
microplastic exposure	standard least-square (method: REML ^a)	MP concentration	n.a.	exposure level, pellet size, exposure level x Pellet size
start weight, end weight, food conversion ratio (FCR), specific growth rate (SGR), mortality	standard least-square	weight, FCR, SGR, mortality	n.a.	exposure level
blood glucose, hematocrit, hepatosomatic index	standard least-square (method: REML ^a)	blood glucose, hematocrit, hepatosomatic index	all	exposure level, sampling time, fish total length, exposure level x fish total length
TBARS Assay	standard least-square (method: REML ^a)	MDA concentration	week 1, week 4, week 17	exposure level, sampling time, fish total length, exposure level x fish total length
gene expression	standard least-square	RNA concentration	week 1, week 4, week 17	exposure level, sampling time, log(fish total length), exposure level x sampling time

^a restricted maximum likelihood (Tank[Fish ID] was added as a random effect).

Table S3. Overview of all computed contrasts for the analysis of the label-free proteomics quantification (LFQ).

Contrast			Given
low exposure	-	control	week 1
high exposure	-	control	
low exposure	-	high exposure	
low exposure	-	control	week 4
high exposure	-	control	
low exposure	-	high exposure	
low exposure	-	control	week 7
high exposure	-	control	
low exposure	-	high exposure	
week 1	-	week 4	control
week 1	-	week 7	
week 4	-	week 7	
week 1	-	week 4	low exposure
week 1	-	week 7	
week 4	-	week 7	
week 1	-	week 4	high exposure
week 1	-	week 7	
week 4	-	week 7	

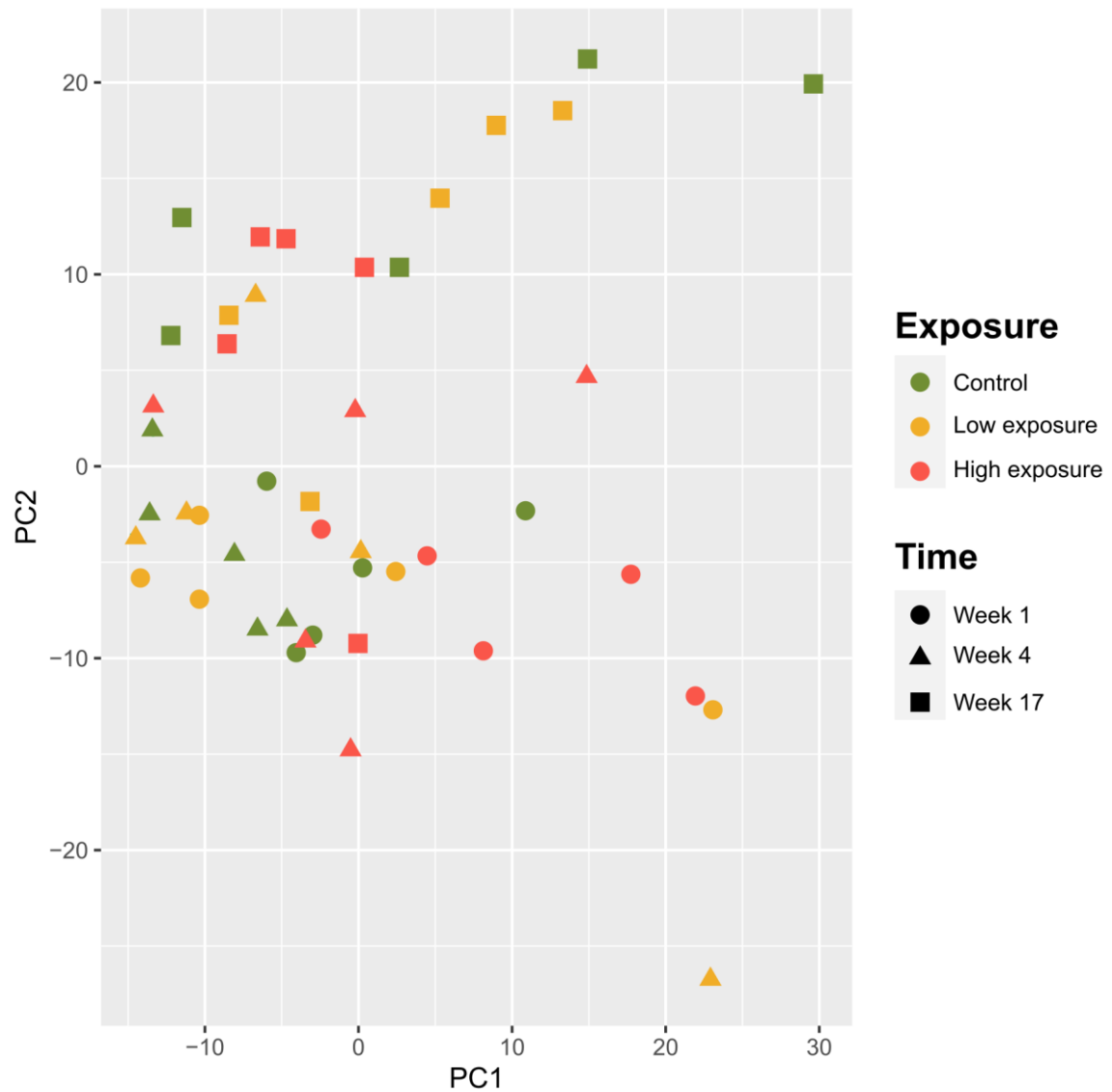


Fig. S1. Results of a principal component analysis (PCA) using transformed and normalized protein intensities. The first two principle components are shown. Each point represents one sample ($n = 5$ per treatment). The different colours indicate the exposure level, the symbols different time points in the experiment.

Table S4. Overview of the general functions of the identified proteins in the proteomic analysis (see Fig. 5 (B) in the text) and genes examined in the gene expression analysis (see Fig. 7 and 8 in the text).

protein name	protein function	protein/LOC symbol	UniProt accession
ATP-binding cassette, sub-family B (MDR/TAP), member 11b	bile salt export pump	abcb11b	A0A060Z4M4
Otopetrin-3	proton channel activity	otop3	A0A060XWM0
Differentially regulated trout protein 1	unknown	drtp1	Q9DFD5
Collagen alpha-1(I) chain	structural formation	col1a1	A0A060XQS9
Otopetrin-3 X1	proton channel activity	otop3	A0A060XWM0
MHC class II antigen, beta-chain	adaptive immune response/antigen presentation	mhc2	Q2XPB1
Collagen alpha-1(I) chain	structural formation	col1a1	A0A060XQS9
Differentially regulated trout protein 1	unknown	drtp1	Q9DFD5
Membrane-bound immunoglobulin delta heavy chain, isotype D	adaptive immune response/antigen binding	ighd	A0A060ZHD6
Keratin, type I cytoskeletal 13	structural molecule activity	krt13	A0A060WLW3
Chymotrypsin-like protease	serine-type peptidase activity	ctrl	A0A060X1S1
Mitochondrial dnaJ homolog subfamily A member 3	protein folding	dnaja3	A0A060ZJV2
Chymotrypsin-like protease	serine-type peptidase activity	ctrl	A0A060X1S1
CSC1-like protein 2	ion channel	tmem63b	A0A060VRU7
ATP-binding cassette, sub-family B (MDR/TAP), member 11b	bile salt export pump	abcb11b	A0A060Z4M4
Chymotrypsin-like protease	serine-type peptidase activity	ctrl	A0A060X1S1
Keratin, type I cuticular Ha3-I	structural molecule activity	krt33a	O76009
Plectin a	cytoskeletal protein binding	plec	A0A060W9D0
Calponin-2	actin-binding, Calmodulin-binding	cnn2	A0A060ZBW0
Fatty acid amide hydrolase	lipid degradation	faah	A0A060WL80
Microfibril-associated glycoprotein 4	cell adhesion	LOC110491216	A0A060Z1T2
Calcium-binding and coiled-coil domain-containing protein 2	metal ion binding	calcoco2	A0A060W3Z2
Translation initiation factor eIF-2B, subunit alpha	identical protein binding	eif2b1	A0A060XIN0
SEC61 translocon, subunit alpha	calcium channel activity	sec61a	A0A060XW28
Catechol-O-methyltransferase domain containing protein 1	Methyltransferase	comtd1	A0A060YXK5
ATP-citrate synthase	ATP binding	acly	A0A060YPA0
Mid1-interacting protein 1		mid1ip1	C1BGT4
Catechol-O-methyltransferase domain containing protein 1	methyltransferase	comtd1	A0A060YXK5
RAB GTPase-activating protein 1-like protein	GTPase activation	rabgap1l	A0A060Z4U9
Plexin D1	protein domain specific binding	plxnd1	A0A060X3U2
Complement component C3 (isoform 3)	innate immunity/opsonisation	c3-3	See Table S1
Immunoglobulin mu (heavy chain, membrane-bound form)	adaptive immunity/antigen binding	ighm	See Table S1
Hepcidin antimicrobial peptides	antimicrobial activity	hamp	See Table S1
60S ribosomal protein L7	RNA binding	rpl7	See Table S1

Table S5. Results of the linear models utilized to analyse individual effects on gene expression. n = number of observations. df = degrees of freedom. Asterisks indicate statistically significant differences ($p < 0.05$).

gene	n	df	model effect	F-value	p-value
<i>abcb11b</i>	31	6, 24		3.2753	0.0169*
			exposure level	1.2696	> 0.05
			sampling time	3.0424	> 0.05
			log(fish total length)	2.8391	> 0.05
			sampling time x exposure level	2.9555	> 0.05
<i>col1a1</i>	31	6, 24		3.0798	0.0222*
			exposure level	6.0119	0.0076*
			sampling time	1.3454	> 0.05
			log(fish total length)	0.6650	> 0.05
			sampling time x exposure level	0.8984	> 0.05
<i>ctrl</i>	31	6, 24		4.5394	0.0033*
			exposure level	1.4621	> 0.05
			sampling time	3.7395	> 0.05
			log(fish total length)	3.3125	> 0.05
			sampling time x exposure level	3.9598	0.0326*
<i>drp1</i>	31	6, 24		1.6120	> 0.05
<i>ighd</i>	31	6, 24		2.8762	0.0295*
			exposure level	5.1342	0.0139*
			sampling time	1.1121	> 0.05
			log(fish total length)	0.6952	> 0.05
			sampling time x exposure level	1.3161	> 0.05
<i>mhc2</i>	26	5, 20		1.1139	> 0.05
<i>rpl7</i>	31	6, 24		10.1064	< 0.0001*
			exposure level	28.046	< 0.0001*
			sampling time	0.2714	> 0.05
			log(fish total length)	0.1834	> 0.05
			sampling time x exposure level	0.0046	> 0.05
<i>tmem63b</i>	31	6, 24		4.4931	0.0035*
			exposure level	1.2617	> 0.05
			sampling time	4.6046	0.0422*
			log(fish total length)	4.6796	0.0407*
			sampling time x exposure level	4.4805	0.0222*
<i>c3-3</i>	31	6, 24		4.1745	0.0052*
			exposure level	3.9413	0.0331*
			sampling time	3.3181	> 0.05
			log(fish total length)	3.6714	> 0.05
			sampling time x exposure level	4.9804	0.0155*
<i>ighm</i>	31	6, 24		2.6638	0.0400*
			exposure level	2.8778	> 0.05
			sampling time	0.9781	> 0.05
			log(fish total length)	1.0300	> 0.05
			sampling time x exposure level	0.0572	> 0.05

Table S6. Example calculation of the percentage by weight of microplastics (MP) in the utilized fish feed. For a pellet size of 2 mm, a fish weight of 20 g and an amount of feed of 1.5 % of the body weight is assumed. For the pellet size of 4 mm, the fish weight is assumed to be 65 g and the feed amount is 1.8 % of the body weight.

pellet size	exposure level	MP particle weight per gram MP feed [g]	weight MP feed per fish [g]	total feed weight [g]	% MP by weight
2 mm	low exposure	5.0×10^{-4}	0.019	0.3	0.032
	high exposure		0.085	0.3	0.141
4 mm	low exposure	1.0×10^{-2}	0.019	1.17	0.017
	high exposure		0.085	1.17	0.076