

Article

Transcriptomic and Histopathological Effects of Bifenthrin to the Brain of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Table S1. Primer pair sequences used for qPCR validation.

Gene	Direction	Sequence	Amplicon Length	Efficiency (%)	Accession Number
<i>csf1</i>	Forward	CCTTCACCCAATGGAAGTCTGAGTC	78	90.8	XM_036947884.1
	Reverse	CGTCTGCCACGAATGAACCT			
<i>cbs</i>	Forward	GGATAGGATCAGTCTGCGTATGGTG	110	90.0	NM_001124686.1
	Reverse	GCAATACAGGTATCGGTCTGGCTC			
<i>ptafr</i>	Forward	CTTATCATTGTGGGGTCTTCGTC	96	88.4	XM_021584417.2
	Reverse	TACTAGCCCAGTCCCTTACCCAG			
<i>mtap</i>	Forward	CCATCATGCCAACCGACGT	78	93.7	NM_001160570.1
	Reverse	CTGCACACATCTCCTGGTCA			
<i>ef1α</i>	Forward	GAGAACCATTGAAAAGTTCGAGAAG	71	91.4	XM_021561980.1
	Reverse	GCACCCAGGCATACTTGAAAG			

Table S2. Mean bifenthrin concentration $\pm$ standard deviation (SD) in extract following a 24 hour (h), 7 day (d), and 14 d treatment. Mean percent recoveries are based on decachlorobiphenyl surrogate standards. Corrected mean bifenthrin concentrations in parentheses.

Nominal Bifenthrin Concentration (ng/L)	Mean 24 h (ng/L)	Mean 24 h Recovery (%)	Mean 7 d (ng/L)	Mean 7 d Recovery (%)	Mean 14 d (ng/L)	Mean 14 d Recovery (%)
0	< BRL	86.89 $\pm$ 2.70	< BRL	32.75 $\pm$ 18.31	< BRL	36.36 $\pm$ 10.55
15	11.36 $\pm$ 2.39 (12.82)	86.74 $\pm$ 4.16	5.50 $\pm$ 0.43 (8.78)	40.55 $\pm$ 6.49	3.50 $\pm$ 0.63 (6.26)	21.04 $\pm$ 0.65
30	19.75 $\pm$ 0.11 (21.68)	90.21 $\pm$ 0.61	22.58 $\pm$ 8.43 (30.66)	60.9 $\pm$ 17.73	7.10 $\pm$ 1.24 (12.88)	18.05 $\pm$ 4.37

BRL, Below reporting limit.

Table S3. Total number of raw and clean RNA sequencing reads.

Sample	Total Raw Reads	Total Clean Reads	Clean Reads Q20 (%)	Clean Reads Q30 (%)	GC Content (%)
Control 1	28526973	27648230	97.59	93.49	47.49
Control 2	25532536	24524205	97.76	93.93	47.00
Control 3	26102740	25419205	97.82	94.05	47.94
Control 4	28596217	27447141	97.73	93.85	47.47
15 ng/L bifenthrin 1	27416655	26453013	97.74	93.90	47.33
15 ng/L bifenthrin 2	29876619	29070074	97.55	93.42	47.72
15 ng/L bifenthrin 3	26466053	25427697	97.76	93.96	47.13
15 ng/L bifenthrin 4	28003792	27033403	97.59	93.55	47.43
30 ng/L bifenthrin 1	29355288	28305590	97.51	93.41	47.21
30 ng/L bifenthrin 2	22426419	21954219	97.56	93.46	47.61
30 ng/L bifenthrin 3	21877612	21212383	98.04	94.84	47.79
30 ng/L bifenthrin 4	22243920	21770438	98.10	95.03	47.61

Table S4. Top 10 gene ontology (GO) biological pathways (BP) and molecular functions (MF) impaired in juvenile rainbow trout exposed to 15 and 30 ng/L bifenthrin.

Treatment	GO Category	ID	Description	p-Value	FDR
15 ng/L	BP	GO:0006879	cellular iron ion homeostasis	2.50×10^{-7}	1.75×10^{-6}
		GO:0055072	iron ion homeostasis	2.50×10^{-7}	1.75×10^{-6}
		GO:0006873	cellular ion homeostasis	1.03×10^{-6}	1.91×10^{-6}
		GO:0006875	cellular metal ion homeostasis	1.03×10^{-6}	1.91×10^{-6}
		GO:0030003	cellular cation homeostasis	1.03×10^{-6}	1.91×10^{-6}
		GO:0055065	metal ion homeostasis	1.03×10^{-6}	1.91×10^{-6}
		GO:0055082	cellular chemical homeostasis	1.03×10^{-6}	1.91×10^{-6}
		GO:0050801	ion homeostasis	1.23×10^{-6}	1.91×10^{-6}
		GO:0055080	cation homeostasis	1.23×10^{-6}	1.91×10^{-6}
		GO:0048878	chemical homeostasis	1.46×10^{-6}	2.04×10^{-6}
	MF	GO:0008199	ferric iron binding	2.25×10^{-7}	2.48×10^{-6}
		GO:0005506	iron ion binding	4.16×10^{-4}	2.29×10^{-3}
		GO:0001071	nucleic acid binding transcription factor activity	9.10×10^{-3}	2.50×10^{-2}
		GO:0003700	sequence-specific DNA binding transcription factor activity	9.10×10^{-3}	2.50×10^{-2}
		GO:0005184	neuropeptide hormone activity	1.72×10^{-2}	3.79×10^{-2}
		GO:0051082	unfolded protein binding	4.31×10^{-2}	7.91×10^{-2}
		GO:0005179	hormone activity	8.95×10^{-2}	1.41×10^{-1}
		GO:0008270	zinc ion binding	1.17×10^{-1}	1.61×10^{-1}
		GO:0043565	sequence-specific DNA binding	2.09×10^{-1}	2.55×10^{-1}
		GO:0005102	receptor binding	3.07×10^{-1}	3.37×10^{-1}
30 ng/L	BP	GO:0006869	lipid transport	7.22×10^{-3}	3.95×10^{-1}
		GO:0010876	lipid localization	7.22×10^{-3}	3.95×10^{-1}
		GO:0071702	organic substance transport	9.79×10^{-3}	3.95×10^{-1}
		GO:0006457	protein folding	3.12×10^{-2}	5.30×10^{-1}
		GO:0009190	cyclic nucleotide biosynthetic process	3.32×10^{-2}	5.30×10^{-1}
		GO:0009187	cyclic nucleotide metabolic process	3.52×10^{-2}	5.30×10^{-1}
		GO:0006164	purine nucleotide biosynthetic process	4.59×10^{-2}	5.30×10^{-1}
		GO:0072522	purine-containing compound biosynthetic process	5.28×10^{-2}	5.30×10^{-1}
		GO:0006325	chromatin organization	7.29×10^{-2}	5.30×10^{-1}
		GO:0016571	histone methylation	9.76×10^{-2}	5.30×10^{-1}
	MF	GO:0005201	extracellular matrix structural constituent	1.42×10^{-5}	1.72×10^{-3}
		GO:0001071	nucleic acid binding transcription factor activity	4.24×10^{-3}	1.65×10^{-1}
		GO:0003700	sequence-specific DNA binding transcription factor activity	4.24×10^{-3}	1.65×10^{-1}
		GO:0005319	lipid transporter activity	7.07×10^{-3}	1.65×10^{-1}
		GO:0005507	copper ion binding	8.19×10^{-3}	1.65×10^{-1}
		GO:0009975	cyclase activity	8.19×10^{-3}	1.65×10^{-1}
		GO:0051082	unfolded protein binding	2.71×10^{-2}	4.68×10^{-1}
		GO:0016849	phosphorus-oxygen lyase activity	3.75×10^{-2}	5.54×10^{-1}

GO:0005198	structural molecule activity	4.12×10^{-2}	5.54×10^{-1}
GO:0004222	metalloendopeptidase activity	4.92×10^{-2}	5.92×10^{-1}

Table S5. Top 5 KEGG pathways impaired in juvenile rainbow trout exposed to 15 and 30 ng/L bifenthrin.

Treatment	KEGG ID	Description	p-Value	FDR
15 ng/L	otw04912	GnRH signaling pathway	4.06×10^{-3}	2.55×10^{-2}
	otw04371	Apelin signaling pathway	7.29×10^{-3}	2.55×10^{-2}
	otw04010	MAPK signaling pathway	3.12×10^{-2}	7.28×10^{-2}
	otw04620	Toll-like receptor signaling pathway	9.22×10^{-2}	1.61×10^{-1}
	otw04210	Apoptosis	1.37×10^{-1}	1.73×10^{-1}
30 ng/L	otw04512	ECM-receptor interaction	1.69×10^{-4}	7.08×10^{-3}
	otw04510	Focal adhesion	1.48×10^{-3}	3.11×10^{-2}
	otw04145	Phagosome	4.30×10^{-2}	5.40×10^{-1}
	otw04620	Toll-like receptor signaling pathway	8.22×10^{-2}	5.40×10^{-1}
	otw00650	Butanoate metabolism	9.29×10^{-2}	5.40×10^{-1}

Table S6. Top 20 diseases and functions predicted in Ingenuity Pathway Analysis in rainbow trout exposed to 15 and 30 ng/L bifenthrin.

Treatment	Category	Diseases or Functions Annotation	p-Value
15 ng/L	Cell-To-Cell Signaling and Interaction	Attachment of cells	6.86×10^{-6}
	Cellular Compromise	Disorganization of cytoskeleton	7.99×10^{-5}
	Organismal Injury and Abnormalities	Formation of scar tissue	8.99×10^{-5}
	Cancer	Proliferation of tumor cells	1.31×10^{-4}
	Cellular Development	Colony formation of phagocytes	1.31×10^{-4}
	Cellular Growth and Proliferation	Colony formation of phagocytes	1.31×10^{-4}
	Tumor Morphology	Proliferation of tumor cells	1.31×10^{-4}
	Cell Death and Survival	Apoptosis of macrophage precursor cells	1.76×10^{-4}
	Connective Tissue Development and Function	Survival of osteoclasts	1.76×10^{-4}
	Skeletal and Muscular System Development and Function	Survival of osteoclasts	1.76×10^{-4}
	Hematological Disease	Infection of myeloid cells	2.89×10^{-4}
	Infectious Diseases	Infection of myeloid cells	2.89×10^{-4}
	Hematological System Development and Function	Colony formation of phagocytes	3.47×10^{-4}
	Hematopoiesis	Colony formation of phagocytes	3.47×10^{-4}
	Lymphoid Tissue Structure and Development	Colony formation of phagocytes	3.47×10^{-4}
	Tissue Development	Colony formation of phagocytes	3.47×10^{-4}
	Cellular Function and Maintenance	Macropinocytosis	4.54×10^{-4}
	Amino Acid Metabolism	Deamination of L-lysine	7.92×10^{-4}
	Cardiovascular Disease	Familial thoracic aortic aneurysm type 10	7.92×10^{-4}
	Cell Cycle	Replication of epidermal cells	7.92×10^{-4}
30 ng/L	Cardiovascular Disease	Abnormal morphology of blood vessel	6.42×10^{-5}
	Cardiovascular System Development and Function	Abnormal morphology of blood vessel	6.42×10^{-5}
	Organismal Injury and Abnormalities	Abnormal morphology of blood vessel	6.42×10^{-5}

Tissue Morphology	Abnormal morphology of blood vessel	6.42×10^{-5}
Hematological Disease	Hereditary thrombophilia	7.02×10^{-5}
Hereditary Disorder	Hereditary thrombophilia	7.02×10^{-5}
Molecular Transport	Accumulation of cyclic GMP	1.42×10^{-4}
Nucleic Acid Metabolism	Accumulation of cyclic GMP	1.42×10^{-4}
Small Molecule Biochemistry	Accumulation of cyclic GMP	1.42×10^{-4}
Cell-To-Cell Signaling and Interaction	Adhesion of bacteria	1.63×10^{-4}
Reproductive System Development and Function	Regulation of reproductive system	2.11×10^{-4}
Cellular Movement	Cell movement of muscle cells	2.26×10^{-4}
Developmental Disorder	Growth failure or short stature	3.09×10^{-4}
Post-Translational Modification	Hydrolysis of protein fragment	4.19×10^{-4}
Protein Degradation	Hydrolysis of protein fragment	4.19×10^{-4}
Protein Synthesis	Hydrolysis of protein fragment	4.19×10^{-4}
Organismal Development	Abnormal morphology of limb	5.65×10^{-4}
Skeletal and Muscular Disorders	Abnormal morphology of limb	5.65×10^{-4}
Skeletal and Muscular System Development and Function	Abnormal morphology of limb	5.65×10^{-4}
Cell Death and Survival	Purpura fulminans	6.77×10^{-4}

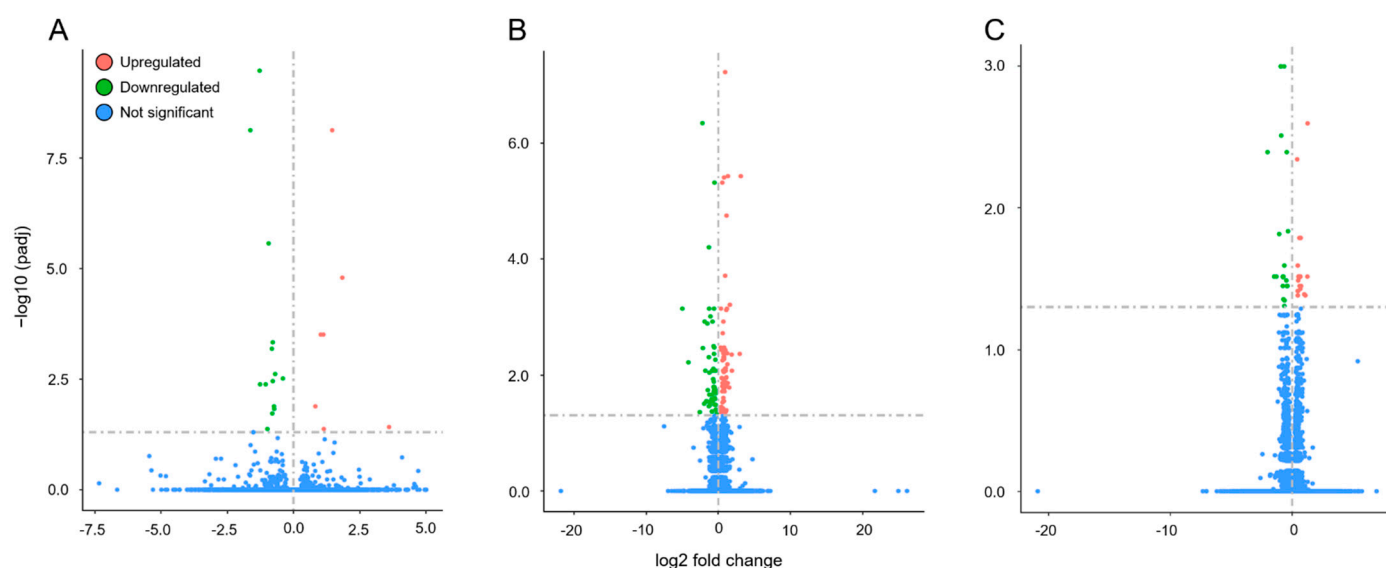


Figure S1. Volcano plots of differentially expressed genes (DEGs) between control and 15 ng/L (A), control and 30 ng/L (B), and 15 and 30 ng/L bifenthrin (C) treatment groups. Upregulated genes are depicted in red, downregulated genes depicted in green, and those not significantly different depicted in blue (FDR < 0.05).

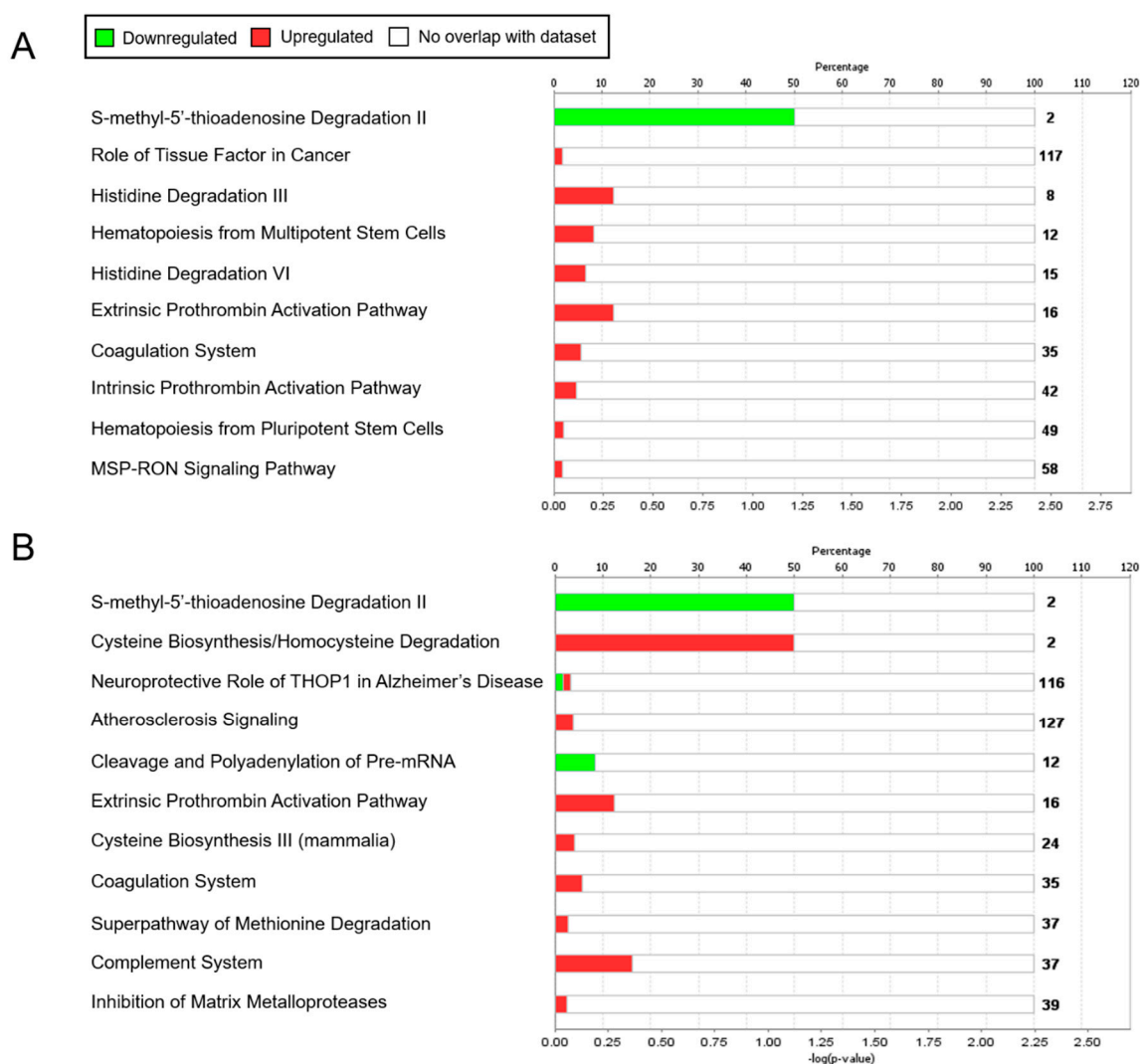


Figure S2. Top predicted Ingenuity Pathway Analysis (IPA) canonical pathways for juvenile rainbow trout treated with (A) 15 and (B) 20 ng/L bifenthrin.

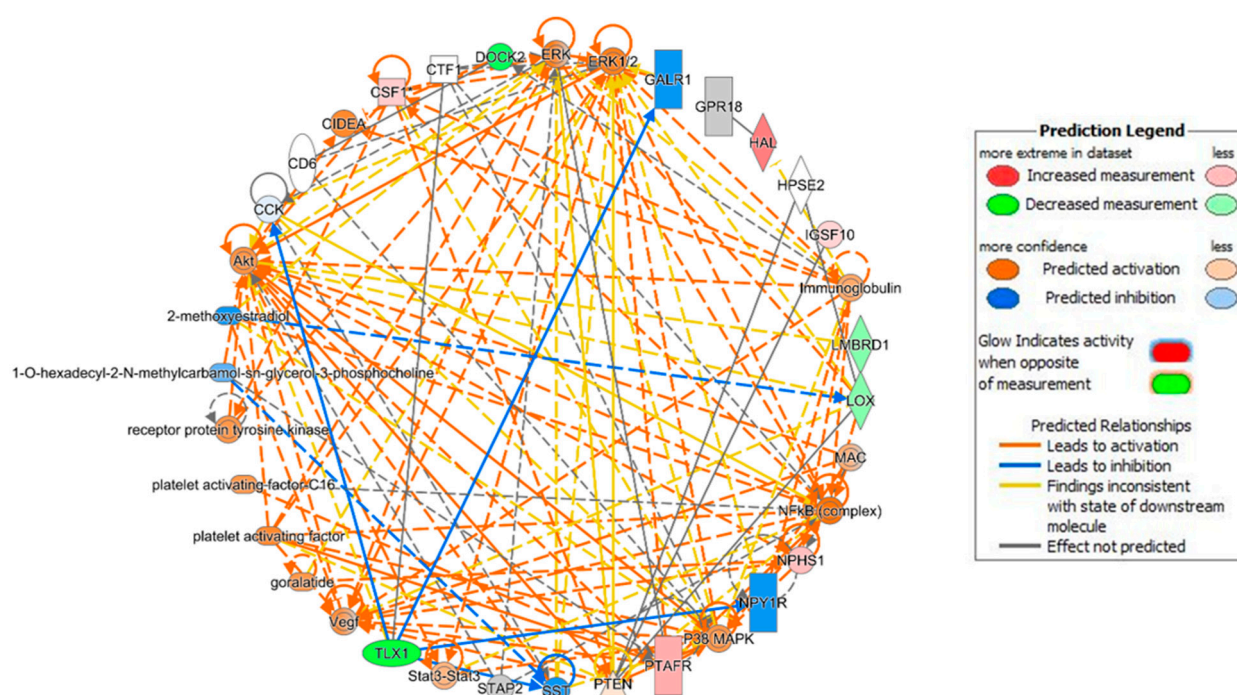


Figure S3. Top predicted Ingenuity Pathway Analysis (IPA) network involved in cell-to-cell signaling and interaction, cellular movement, and immune cell trafficking in juvenile rainbow trout exposed to 15 ng/L bifenthrin.

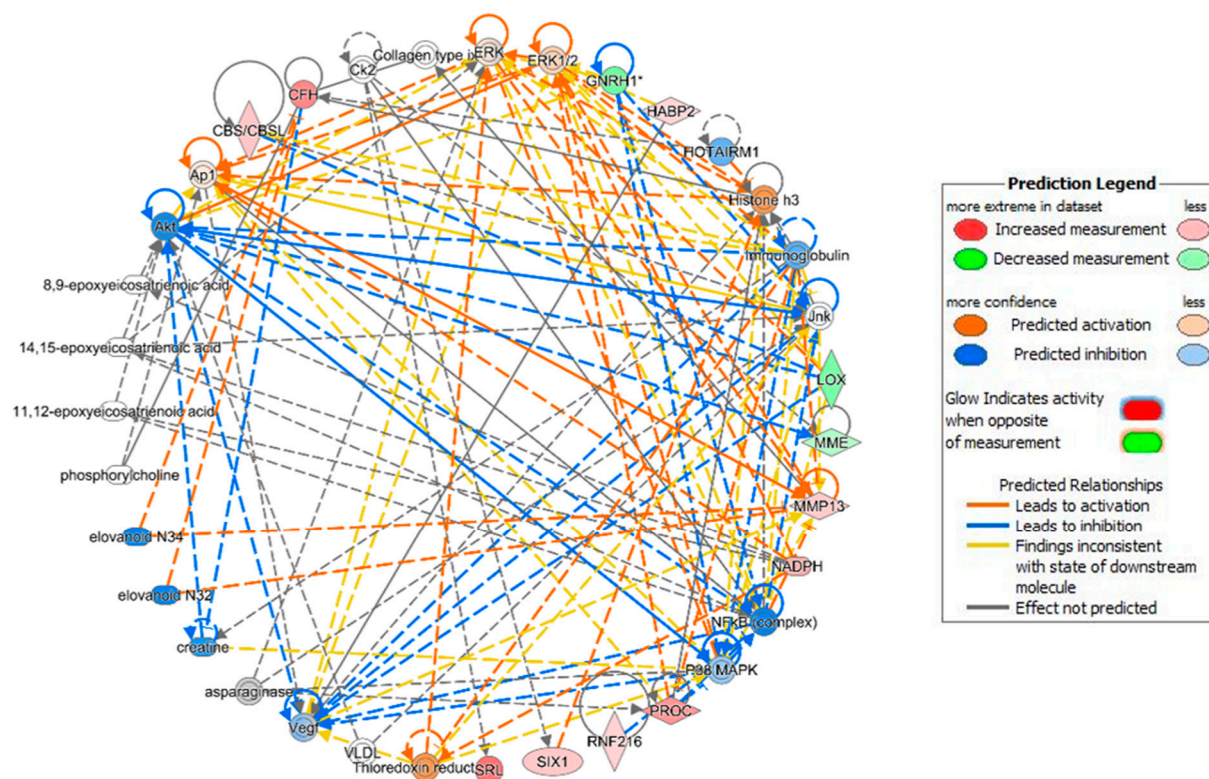


Figure S4. Top predicted Ingenuity Pathway Analysis (IPA) network involved in cell cycle, cell-to-cell signaling and interaction, and cellular compromise in juvenile rainbow trout exposed to 30 ng/L bifenthrin.

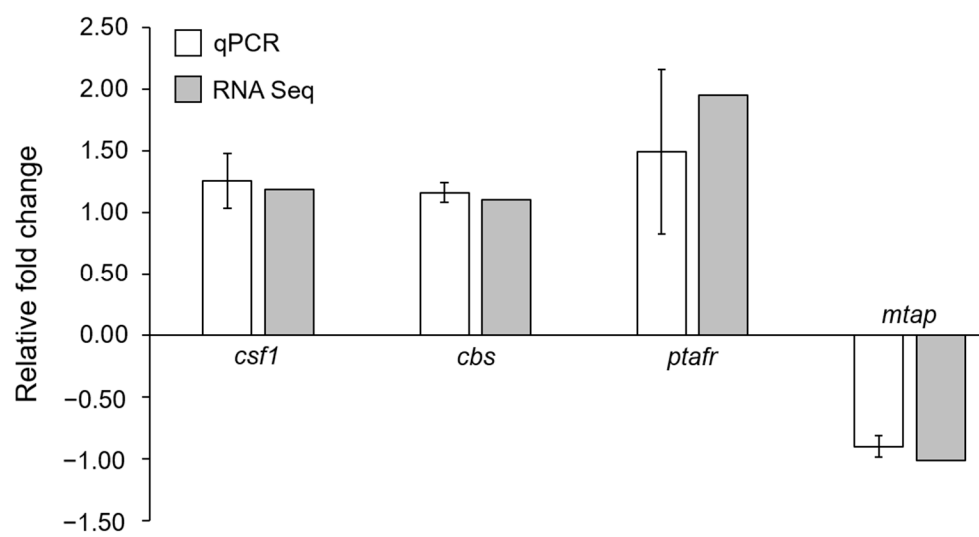


Figure S5. Comparison between differentially expressed genes determined by RNA sequencing and qPCR expression fold change patterns in the brains of bifenthrin-exposed rainbow trout (mean $\pm$ SD, One-way ANOVA, Tukey's post hoc ($p < 0.05$)).