

Supplementary Materials: High-Resolution Magic Angle Spinning (HRMAS) NMR Identifies Oxidative Stress and Impairment of Energy Metabolism by Zearalenone in Embryonic Stages of Zebrafish (*Danio rerio*), Olive Flounder (*Paralichthys olivaceus*) and Yellowtail Snapper (*Ocyurus chrysurus*)

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Table S1. Concentration of metabolites measured in embryos of Zebrafish (*Danio rerio*), Olive Flounder (*Paralichthys olivaceus*) and Yellowtail Snapper (*Ocyurus chrysurus*) exposed to 1 parts-per-million (ppm) zearalenone ("Exposed"), compared to embryos exposed to solvent vehicle, i.e., 0.0001% ethanol, only ("Control"), as measured by high-resolution magic angle spinning NMR (HRMAS NMR). Given are average concentrations (\pm one standard deviation), N = 6; except Flounder control, N = 3. Values indicated by "--" were not detected or able to be resolved. Concentration was measured based on reference signal of TSP.

Concentration (mM)

Metabolite	Zebrafish		Flounder		Snapper	
	Control	Exposed	Control	Exposed	Control	Exposed
αKg	0.093 \pm 0.004	0.094 \pm 0.014	0.008 \pm 0.002	0.004 \pm 0.003	0.018 \pm 0.001	0.018 \pm 0.001
ADP	0.18 \pm 0.006	0.441 \pm 0.048	0.021 \pm 0.002	0.075 \pm 0.024	0.011 \pm 0.001	0.018 \pm 0.003
ATP	0.309 \pm 0.007	0.153 \pm 0.022	0.091 \pm 0.008	0.046 \pm 0.011	0.037 \pm 0.005	0.021 \pm 0.004
Ace	1.332 \pm 0.023	1.33 \pm 0.039	---	---	0.015 \pm 0.001	0.015 \pm 0.002
Ala	1.826 \pm 0.043	1.799 \pm 0.030	0.078 \pm 0.012	0.063 \pm 0.011	0.02 \pm 0.003	0.018 \pm 0.002
Ans	0.151 \pm 0.003	0.149 \pm 0.019	---	---	---	---
Asn	0.806 \pm 0.059	0.755 \pm 0.085	0.128 \pm 0.021	0.118 \pm 0.018	0.02 \pm 0.002	0.018 \pm 0.003
Asp	0.279 \pm 0.009	0.288 \pm 0.010	0.116 \pm 0.032	0.116 \pm 0.023	0.018 \pm 0.001	0.018 \pm 0.003
Carn	0.12 \pm 0.008	0.101 \pm 0.002	---	---	---	---
Carno	0.779 \pm 0.036	0.646 \pm 0.033	0.119 \pm 0.01	0.047 \pm 0.02	---	---
Cho	0.356 \pm 0.034	0.223 \pm 0.009	0.082 \pm 0.009	0.017 \pm 0.005	0.006 \pm 0.001	0.004 \pm <0.001
Cit	0.249 \pm 0.017	0.229 \pm 0.025	0.015 \pm 0.006	0.013 \pm 0.003	0.012 \pm <0.001	0.013 \pm 0.004
CRN	0.043 \pm 0.003	0.037 \pm 0.006	0.004 \pm 0.002	0.005 \pm 0.001	0.004 \pm 0.001	0.001 \pm 0.001
Glc	1.018 \pm 0.021	1.688 \pm 0.138	0.213 \pm 0.033	0.389 \pm 0.036	0.029 \pm 0.005	0.048 \pm 0.004
G1P	0.401 \pm 0.011	0.29 \pm 0.006	0.356 \pm 0.041	0.127 \pm 0.048	0.026 \pm 0.003	0.008 \pm 0.002
G6P	0.832 \pm 0.028	1.454 \pm 0.033	0.216 \pm 0.012	0.336 \pm 0.02	0.009 \pm 0.002	0.018 \pm 0.004
Glu	1.478 \pm 0.040	1.528 \pm 0.063	0.193 \pm 0.02	0.199 \pm 0.014	0.015 \pm 0.004	0.033 \pm 0.005
Gln	1.391 \pm 0.043	1.425 \pm 0.051	0.658 \pm 0.078	0.623 \pm 0.056	0.005 \pm 0.002	0.021 \pm 0.008
GSH	0.506 \pm 0.009	0.229 \pm 0.028	0.171 \pm 0.017	0.056 \pm 0.033	0.013 \pm 0.001	0.006 \pm 0.001
Gly	1.268 \pm 0.008	1.055 \pm 0.115	0.197 \pm 0.043	0.045 \pm 0.014	0.031 \pm 0.005	0.018 \pm 0.002

Lac	2.803 ±0.009	3.213 ±0.076	0.069 ±0.009	0.14 ±0.026	0.012 ±0.001	0.016 ±0.001
Lys	1.283 ±0.023	1.239 ±0.083	0.216 ±0.024	0.081 ±0.024	0.005 ±0.001	0.005 ±0.001
Mal	0.198 ±0.006	0.174 ±0.031	0.015 ±0.006	0.009 ±0.002	0.013 ±0.003	0.014 ±0.002
NAA	1.005 ±0.014	1.142 ±0.138	0.158 ±0.035	0.129 ±0.026	0.013 ±0.001	0.014 ±0.001
AcLys	1.189 ±0.058	1.205 ±0.150	0.229 ±0.03	0.251 ±0.019	---	---
NADH	0.53 ±0.032	0.354 ±0.032	0.214 ±0.012	0.095 ±0.036	0.015 ±0.001	0.004 ±0.001
PChol	0.08 ±0.006	0.08 ±0.005	---	---	---	---
Phe	0.373 ±0.013	0.379 ±0.033	0.044 ±0.024	0.066 ±0.009	0.017 ±0.002	0.029 ±0.005
Pyr	0.95 ±0.009	0.623 ±0.012	0.164 ±0.029	0.038 ±0.015	0.02 ±0.001	0.014 ±0.003
Succ	0.21 ±0.005	0.238 ±0.030	0.042 ±0.016	0.038 ±0.012	0.01 ±0.003	0.012 ±0.002
Tau	0.712 ±0.026	0.701 ±0.035	0.072 ±0.007	0.013 ±0.004	0.009 ±0.001	0.016 ±0.006
TMAO	1.698 ±0.022	1.476 ±0.161	---	---	0.005± <0.001	0.003 ±0.001
Trp	0.276 ±0.005	0.285 ±0.027	0.164 ±0.02	0.068 ±0.033	0.011 ±0.002	0.015 ±0.001
Tyr	0.991 ±0.031	0.966 ±0.043	0.202 ±0.026	0.202 ±0.013	0.023 ±0.004	0.034 ±0.005
mIns	0.62 ±0.024	0.644 ±0.098	---	---	0.026 ±0.001	0.026 ±0.002
GPC	0.634 ±0.040	0.497 ±0.041	---	---	0.005 ±0.001	0.003 ±0.001

Abbreviations: Pyruvate (Pyr), lactate (Lac), glucose-6-phosphate (G6P), glucose-1-phosphate (G1P), glucose (Glc), adenosine diphosphate (ADP), adenosine triphosphate (ATP), reduced nicotinamide adenine dinucleotide (NADH), α-ketoglutarate (α-KG), citrate (Cit), malate (Mal), succinate (Succ), alanine (Ala), asparagine (Asn), aspartate (Asp), glutamate (Glu), glutamine (Gln), glycine (Gly), lysine (Lys), phenylalanine (Phe), tryptophan (Trp), tyrosine (Tyr), taurine (Tau), choline (Cho), glycerophosphorylcholine (GPC), O-phosphocholine (PChol), acetate (Ace), carnitine (Carn), reduced glutathione (GSH), carnosine (Carno), N-acetylaspartate (NAA), anserine (Ans), acetyllysine (AcLys), myo-inositol (mIns) and trimethylamine-N-oxide (TMAO).

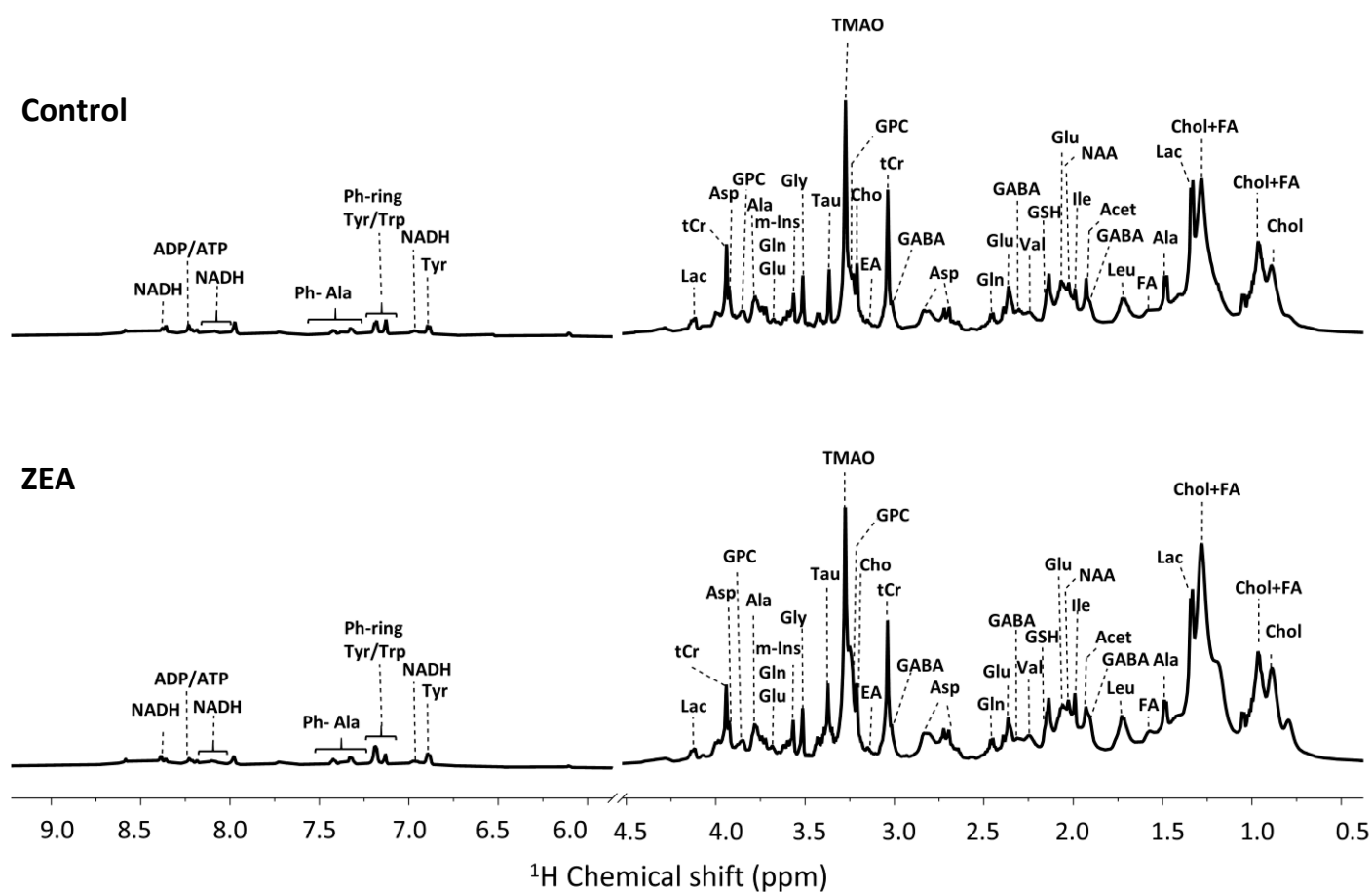


Figure S1. Proton (¹H) HRMAS NMR spectra of 72-hour post-fertilization (hpf) embryos of zebrafish exposed to 1 ppm zearalenone (ZEA) for 24 h, and control embryos (“Control”) exposed to solvent vehicle, 0.001% ethanol, only for 24 h. Relevant signals used to identify and quantify metabolites are given. Solvent peak between 4.5 and 6 ppm removed from spectrum. For quantitation, and list of abbreviations, see Table S1.

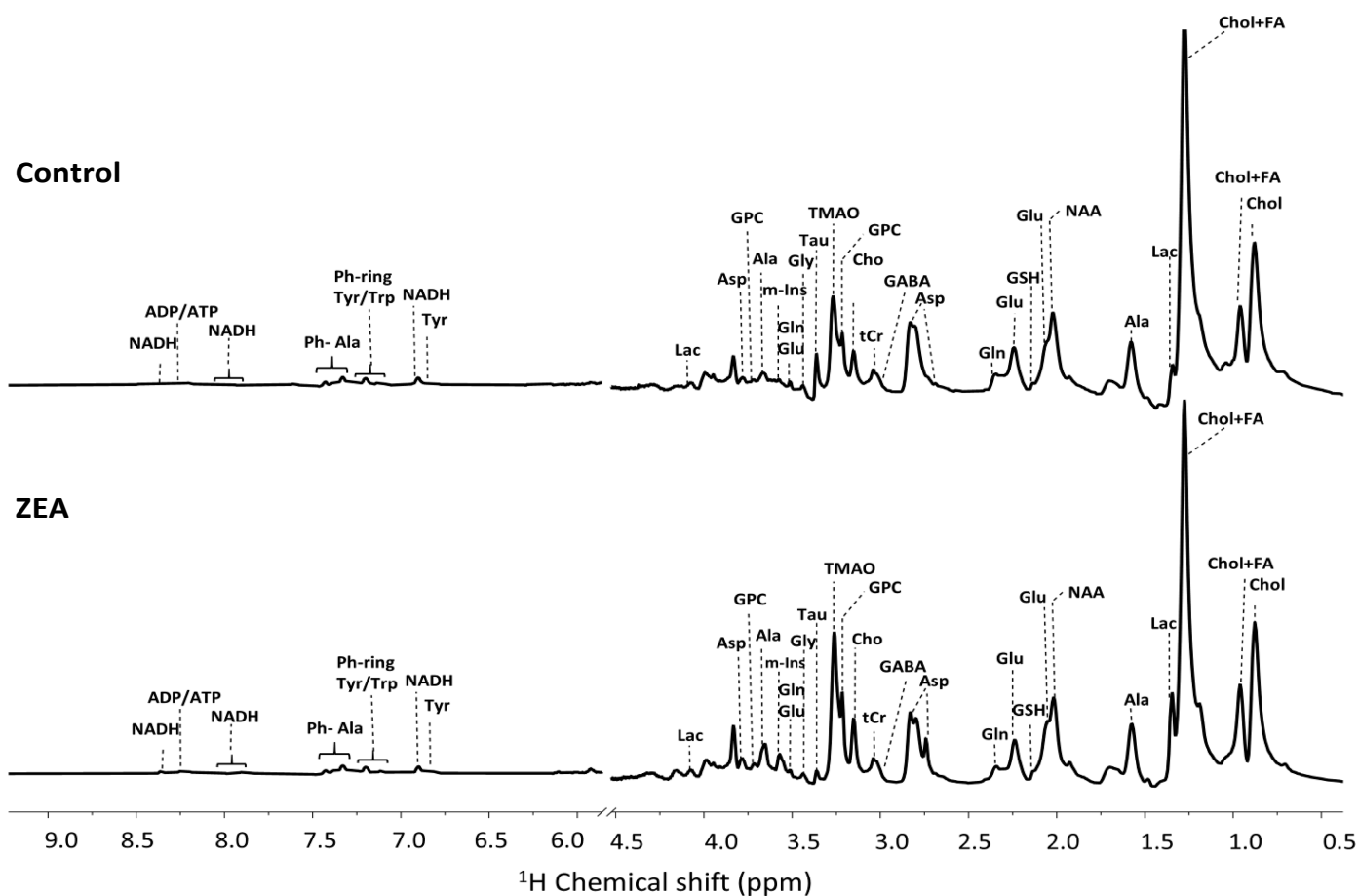


Figure S2. Proton (^1H) HRMAS NMR spectra of 72-hour post-fertilization (hpf) embryos of Oliver Flounder exposed to 1 ppm zearalenone (ZEA) for 24 h, and control embryos (“Control”) exposed to solvent vehicle, 0.001% ethanol, only for 24 h. Relevant signals used to identify and quantify metabolites are given. Solvent peak between 4.5 and 6 ppm removed from spectrum. For quantitation, and list of abbreviations, see Table S1.

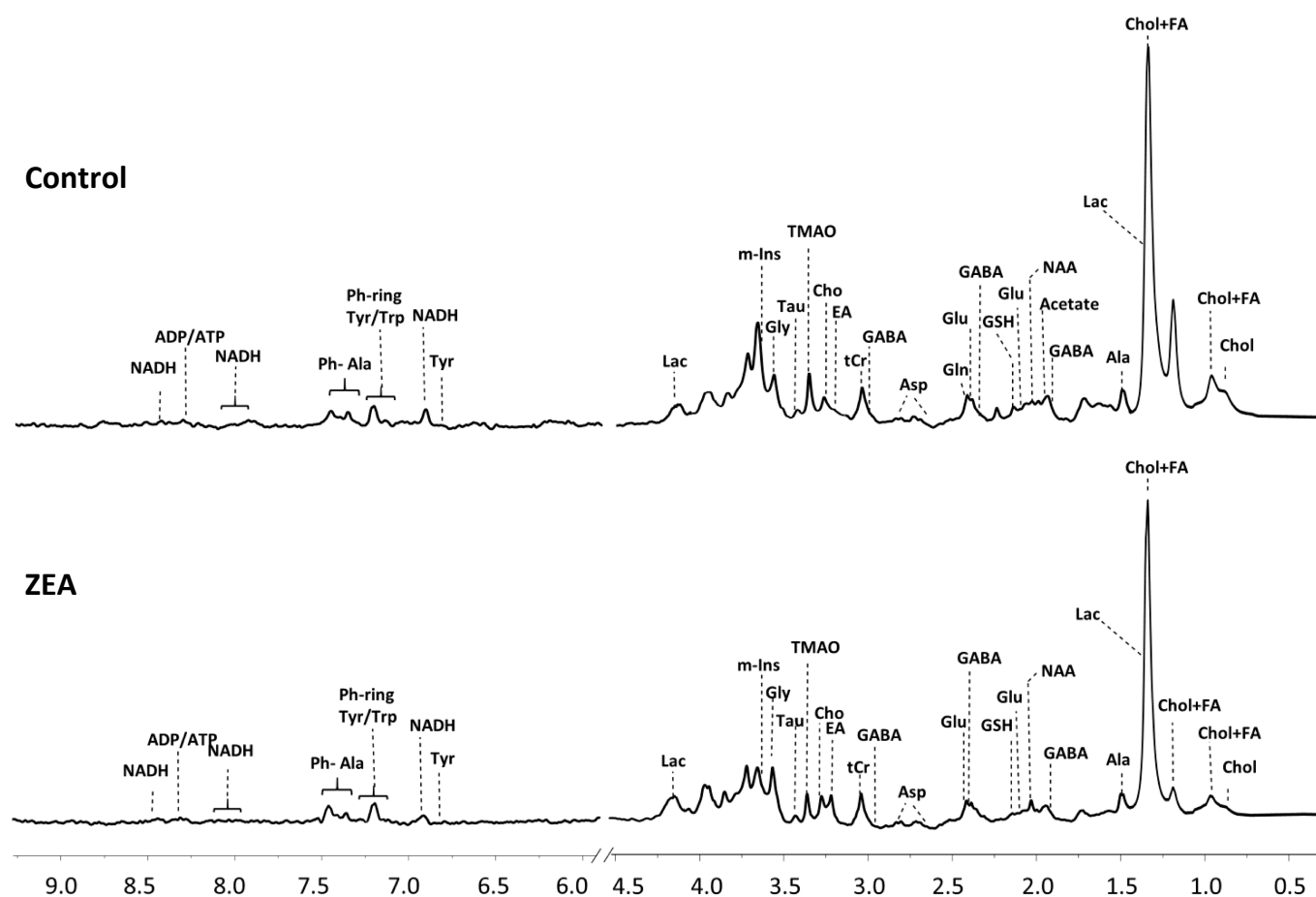


Figure S3. Proton (^1H) HRMAS NMR spectra of 72-hour post-fertilization (hpf) embryos of Yellowtail Snapper exposed to 1 ppm zearalenone (ZEA) for 24 h, and control embryos (“Control”) exposed to solvent vehicle, 0.001% ethanol, only for 24 h. Relevant signals used to identify and quantify metabolites are given. Solvent peak between 4.5 and 6 ppm removed from spectrum. For quantitation, and list of abbreviations, see Table S1.