Supporting Information for "Synergistic impacts of nanopollutants (nZnO) and hypoxia on bioenergetics and metabolic homeostasis in a marine bivalve *Mytilus edulis*"

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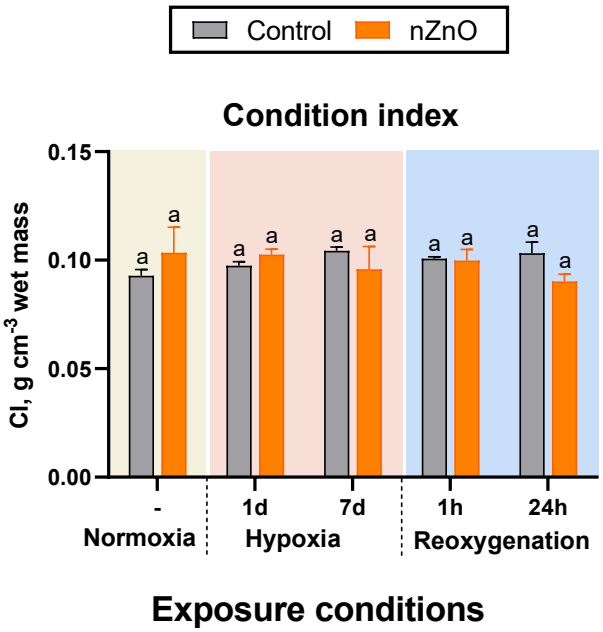
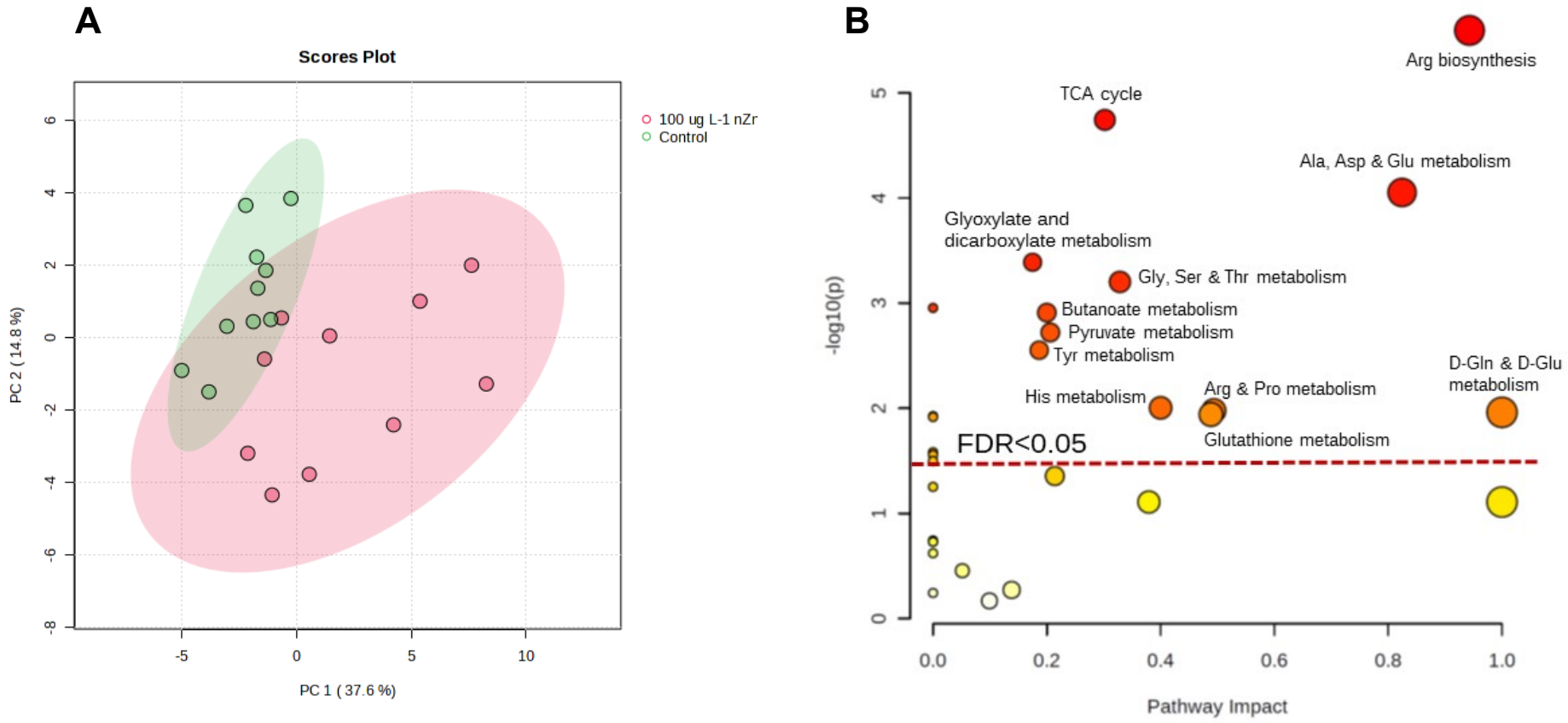
Supplementary Figure 1. Effects of hypoxia and nZnO exposure on condition index of *M. edulis*.

If the columns share a letter, the respective values are not significantly different (P>0.05). N=3



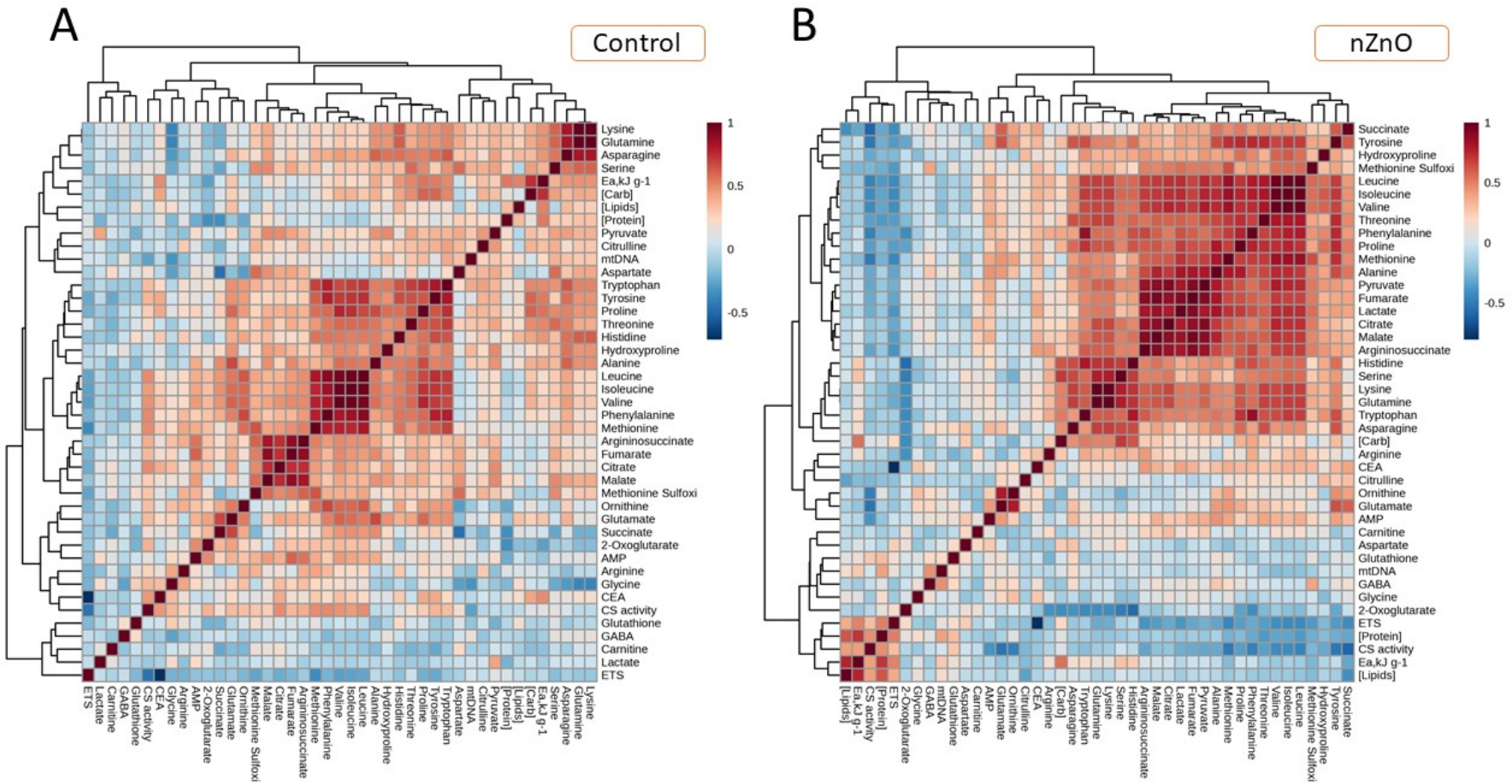
Supplementary Figure 2. The comparison of the metabolite profiles of control and nZnO-exposed mussels under normoxic conditions.

A - PCA -based biplot of metabolite profiles in the plane of two first principal components; green – control, red – nZnO-exposure, B – results of the pathway enrichment analysis of metabolite profiles in control and nZnO-exposed mussels. Red broken line indicates the FDR cut-off of less than 0.05. The pathways that are significantly different between the two groups and have impact >0 is labeled.



Supplementary Figure 3. Pearson correlations of metabolite concentrations and bioenergetics parameters in the whole body tissues of control (A) and nZnO-exposed (B) mussels across all oxygen treatment groups.

Abbreviations: Ea – total energy content of the body; [Carb], [Lipids], [Protein] – total body content of carbohydrates, lipids and proteins, respectively; CEA – Cellular Energy Allocation; CS – citrate synthase; GABA - γ-aminobutyric acid; ETS – electron transport system activity.



Supplementary Table 1. Mortality of the blue mussel *M. edulis* exposed to different combinations of dissolved oxygen (DO) and ZnO nanoparticles (nZnO).

nZnO treatment	DO treatment	Total number of mussels	Number of dead mussels	Mortality of mussels, %
Control	Normoxia	40	0	0
Control	1 d hypoxia	40	0	0
Control	7 d hypoxia	150	42	28
Control	1 h reoxygenation following 7 d hypoxia	40	0	0
Control	24 h reoxygenation following 7 d hypoxia	40	0	0
nZnO	Normoxia	40	0	0
nZnO	1 d hypoxia	40	0	0
nZnO	7 d hypoxia	150	94	63
nZnO	1 h reoxygenation following 7 d hypoxia	18	0	0
nZnO	24 h reoxygenation following 7 d hypoxia	18	2	11

Supplementary Table 2. Concentrations of free amino acids (μg g^{-1} wet tissue) in the whole soft tissue of the blue mussel *M. edulis* exposed to different combinations of dissolved oxygen (DO) and ZnO nanoparticles (nZnO). DO treatment: normoxia, 1 d hypoxia, 7 d hypoxia, 1 h reoxygenation following 7 d hypoxia and 24 h reoxygenation following 7 d hypoxia. nZnO treatment: control and 100 μg l^{-1} nZnO.

Data are shown as mean ± SE (N=8-10). Different letters indicate significant differences among DO treatments within the same nZnO treatment (P<0.05), and asterisks indicate significant differences between two nZnO treatments within fixed DO level (P<0.05).

FAAs	FAAs abbreviations	Groups	Normoxia	1 d hypoxia	7 d hypoxia	1 h reoxygenation	24 h reoxygenation
Asparagine	**Asn**	Control	8.14 ± 1.30^{ab}	13.84 ± 1.93^{a}	6.75 ± 1.52^{b}	7.89 ± 1.28^{ab}	9.55 ± 1.88^{ab}
		nZnO	8.32 ± 1.31^{ab}	11.69 ± 1.22^{a}	8.70 ± 1.43^{ab}	6.31 ± 1.79^{b}	8.20 ± 1.81^{ab}
Aspartate	**Asp**	Control	190.12 ± 14.26^{a}	157.39 ± 12.11^{ab}	131.62 ± 9.30^{b}	151.72 ± 9.46^{ab}	293.01 ± 18.27^{c}
		nZnO	165.14 ± 25.89	124.26 ± 9.41*	137.35 ± 17.22	103.20 ± 9.76*	118.80 ± 15.86*
Alanine	**Ala**	Control	74.47 ± 7.04^{a}	129.41 ± 11.40^{b}	95.73 ± 9.23^{ab}	108.15 ± 5.03^{ab}	82.33 ± 16.56^{a}
		nZnO	80.88 ± 10.95^{a}	179.26 ± 30.68^{b}	109.90 ± 8.04^{ab}	117.68 ± 15.18^{ab}	120.89 ± 17.03^{ab}
Threonine	**Thr**	Control	9.09 ± 1.90	12.56 ± 1.81	11.36 ± 2.01	12.69 ± 2.18	14.54 ± 2.89
		nZnO	13.71 ± 1.89^{a}	25.00 ± 3.28^{b*}	18.75 ± 3.02^{ab*}	12.46 ± 1.81^{a}	12.98 ± 2.66^{a}
Methionine Sulfoxide	**MetO**	Control	1.37 ± 0.15^{a}	1.53 ± 0.10^{a}	1.68 ± 0.16^{a}	1.65 ± 0.17^{a}	3.06 ± 0.42^{b}
		nZnO	2.73 ± 0.21*	3.84 ± 0.40*	2.99 ± 0.31*	3.03 ± 0.46*	2.54 ± 0.50
Proline	**Pro**	Control	11.3 ± 1.88	15.50 ± 2.34	17.48 ± 2.64	17.90 ± 2.45	16.37 ± 2.69
		nZnO	18.34 ± 4.82^{a}	41.54 ± 6.91^{b*}	27.43 ± 4.15^{ab*}	20.07 ± 2.42^{ab}	23.05 ± 4.80^{ab}
Histidine	**His**	Control	13.60 ± 1.81	18.55 ± 2.17	13.89 ± 2.06	15.25 ± 2.35	15.87 ± 1.90
		nZnO	23.87 ± 3.07^{ab*}	27.29 ± 1.31^{a*}	18.24 ± 1.71^{ac}	14.09 ± 3.06^{c}	14.55 ± 1.98^{bc}
AMP	**AMP**	Control	120.85 ± 6.00	134.65 ± 6.24	145.11 ± 6.69	139.89 ± 6.66	142.53 ± 10.32
		nZnO	126.75 ± 9.77	171.88 ± 18.46	145.01 ± 5.40	140.46 ± 13.13	143.98 ± 12.88
Arginine	**Arg**	Control	117.76 ± 13.86	136.31 ± 17.36	141.51 ± 13.79	147.59 ± 23.14	173.41 ± 8.66
		nZnO	208.95 ± 15.38^{a*}	208.62 ± 15.40^{a*}	183.88 ± 21.99^{ab}	122.21 ± 18.14^{b}	131.84 ± 13.58^{b*}
Tyrosine	**Tyr**	Control	6.09 ± 0.56	10.22 ± 1.47	9.23 ± 1.32	10.84 ± 1.94	8.92 ± 1.70
		nZnO	6.93 ± 1.35^{a}	19.66 ± 1.35^{b*}	15.72 ± 1.76^{bc*}	10.40 ± 1.48^{ac}	10.91 ± 1.70^{ac}
Tryptophan	**Trp**	Control	3.49 ± 0.50	5.05 ± 0.83	5.16 ± 0.72	6.07 ± 0.93	6.24 ± 0.79
		nZnO	6.41 ± 1.37^{a*}	16.51 ± 1.66^{b*}	11.14 ± 1.71^{b*}	5.13 ± 0.56^{a}	5.48 ± 0.77^{a}
Lactate	**Lactate**	Control	8.26 ± 0.91	9.88 ± 0.74	11.63 ± 1.18	11.18 ± 0.75	10.26 ± 1.23
		nZnO	14.26 ± 1.22^{a*}	42.85 ± 8.97^{b*}	18.73 ± 2.40^{a*}	17.31 ± 1.40^{a*}	13.87 ± 2.15^{a}
Gamma-aminobutyric acid	**GABA**	Control	0.28 ± 0.04	0.35 ± 0.03	0.36 ± 0.04	0.41 ± 0.04	0.51 ± 0.13
		nZnO	0.34 ± 0.06^{a}	0.58 ± 0.13^{ab}	0.54 ± 0.04^{b*}	0.77 ± 0.16^{b*}	0.46 ± 0.06^{ab}
Carnitine	**Carnithine**	Control	14.17 ± 0.99	12.94 ± 1.15	13.54 ± 1.13	13.26 ± 1.47	16.65 ± 1.51
		nZnO	16.18 ± 1.74^{a}	12.95 ± 1.35^{ab}	13.6 ± 0.8^{ab}	13.04 ± 1.49^{ab}	9.78 ± 0.86^{b*}

Citrulline	**Citrulline**	Control	0.47 ± 0.08	0.38 ± 0.07	0.32 ± 0.04	0.44 ± 0.06	0.55 ± 0.05
		nZnO	0.80 ± 0.13*	1.16 ± 0.22*	1.24 ± 0.28*	0.93 ± 0.12*	0.66 ± 0.12
Fumarate	**Fumarate**	Control	0.33 ± 0.03^{a}	0.44 ± 0.05^{a}	0.43 ± 0.09^{a}	0.84 ± 0.06^{b}	0.44 ± 0.05^{a}
		nZnO	0.58 ± 0.04*	0.72 ± 0.11*	0.64 ± 0.09	0.45 ± 0.08*	0.46 ± 0.09
Pyruvate	**Pyruvate**	Control	3.05 ± 0.54	3.10 ± 0.22	2.04 ± 0.18	3.18 ± 0.35	3.43 ± 0.53
		nZnO	2.69 ± 0.21^{ab}	4.23 ± 0.56^{a*}	2.64 ± 0.21^{ab*}	2.45 ± 0.45^{b}	2.06 ± 0.33^{b*}
2-oxoglutarate	**2-oxoglutarate**	Control	647.94 ± 78.24^{a}	1087.90 ± 47.45^{b}	1170.00 ± 66.44^{b}	1242.92 ± 59.65^{b}	1237.06 ± 28.50^{b}
		nZnO	1016.03 ± 70.82*	1048.58 ± 31.83	1053.39 ± 56.97	1194.81 ± 49.78	1086.5 ± 43.59*
4-hydroxyproline	**4-hydroxyproline**	Control	0.90 ± 0.16	1.32 ± 0.21	0.92 ± 0.10	1.26 ± 0.18	1.14 ± 0.26
		nZnO	2.29 ± 0.57^{ab*}	2.87 ± 0.71^{a*}	2.37 ± 0.27^{a*}	1.04 ± 0.17^{b}	1.18 ± 0.27^{b}
Serine	**Ser**	Control	20.18 ± 2.97^{ab}	16.97 ± 3.44^{ac}	3.46 ± 0.81^{d}	9.96 ± 1.73^{c}	32.78 ± 5.55^{b}
		nZnO	36.18 ± 6.76^{a*}	40.66 ± 6.84^{a*}	19.62 ± 3.39^{a*}	8.12 ± 2.24^{b}	6.55 ± 1.69^{b*}
Glycine	**Gly**	Control	213.53 ± 15.32^{ab}	176.88 ± 11.95^{a}	231.51 ± 13.89^{ab}	276.49 ± 22.59^{b}	206.30 ± 12.29^{ab}
		nZnO	130.55 ± 9.52^{a*}	175.14 ± 21.20^{ab}	173.54 ± 13.56^{ab*}	191.36 ± 24.84^{ab*}	214.01 ± 15.98^{b}
Glutamine	**Gln**	Control	23.06 ± 3.48^{a}	32.74 ± 5.04^{a}	7.26 ± 1.24^{b}	8.47 ± 1.61^{b}	29.03 ± 5.07^{a}
		nZnO	33.62 ± 6.74^{a}	48.23 ± 6.64^{a}	11.98 ± 3.12^{b}	9.43 ± 2.95^{b}	12.66 ± 2.93^{b*}
Glutamate	**Glu**	Control	88.54 ± 8.37^{a}	125.68 ± 8.64^{bc}	118.83 ± 6.31^{bc}	148.55 ± 7.93^{c}	102.77 ± 5.73^{ab}
		nZnO	78.03 ± 6.36^{a}	130.03 ± 5.10^{b}	144.93 ± 6.64^{b*}	128.73 ± 9.01^{b}	137.81 ± 10.59^{b*}
Lysine	**Lys**	Control	22.76 ± 3.47^{a}	32.09 ± 4.98^{a}	7.19 ± 1.22^{b}	8.30 ± 1.60^{b}	28.85 ± 5.11^{a}
		nZnO	33.24 ± 6.80^{a}	46.86 ± 6.62^{a}	11.73 ± 3.08^{b}	9.13 ± 2.87^{b}	12.34 ± 2.86^{b*}
Valine	**Val**	Control	7.80 ± 0.81^{a}	12.74 ± 0.76^{b}	15.54 ± 1.05^{b}	15.14 ± 1.49^{b}	13.18 ± 1.82^{b}
		nZnO	13.87 ± 2.86^{a}	30.94 ± 3.82^{b*}	19.96 ± 1.54^{bc*}	14.17 ± 1.21^{ac}	12.81 ± 1.07^{ac}
Methionine	**Met**	Control	3.87 ± 0.60^{a}	6.56 ± 0.54^{b}	8.79 ± 0.99^{b}	8.55 ± 1.17^{b}	9.54 ± 1.31^{b}
		nZnO	4.79 ± 0.73^{a}	16.05 ± 2.06^{b*}	10.11 ± 1.08^{bc}	8.33 ± 1.35^{ac}	8.74 ± 0.90^{c}
Isoleucine	**Ile**	Control	9.34 ± 1.00^{a}	16.04 ± 1.11^{b}	21.43 ± 1.86^{b}	20.43 ± 2.35^{b}	17.02 ± 2.39^{b}
		nZnO	18.8 ± 4.09^{a}	45.87 ± 5.51^{b*}	27.33 ± 2.71^{bc*}	17.36 ± 1.57^{ac}	15.78 ± 1.49^{ac}
Leucine	**Leu**	Control	7.89 ± 0.76^{a}	14.04 ± 0.93^{b}	18.52 ± 1.44^{b}	16.74 ± 2.00^{b}	15.15 ± 1.92^{b}
		nZnO	17.07 ± 3.05^{a*}	38.58 ± 4.16^{b*}	24.25 ± 1.86^{b*}	14.22 ± 0.98^{a}	14.18 ± 1.25^{a}
Phenylalanine	**Phe**	Control	6.81 ± 0.73^{a}	12.72 ± 1.22^{b}	16.72 ± 1.18^{b}	17.34 ± 2.72^{b}	14.51 ± 1.72^{b}
		nZnO	16.58 ± 2.18^{a*}	44.29 ± 5.30^{b*}	33.25 ± 2.99^{b*}	15.38 ± 1.12^{a}	16.92 ± 1.70^{a}
Malate	**Malate**	Control	2086.42 ± 158.43^{ab}	2105.13 ± 170.98^{ab}	1475.03 ± 168.49^{a}	2706.72 ± 251.06^{b}	3088.58 ± 717.41^{b}
		nZnO	3826.54 ± 498.77^{a*}	3641.69 ± 465.68^{a*}	2717.25 ± 349.45^{ab*}	2087.64 ± 324.45^{b}	2288.93 ± 449.08^{ab}
(iso)citrate	**Citrate**	Control	3.14 ± 0.46^{ab}	3.37 ± 0.39^{a}	2.00 ± 0.33^{b}	10.71 ± 1.18^{c}	9.47 ± 1.60^{c}
		nZnO	8.37 ± 1.61^{ab*}	13.85 ± 3.07^{a*}	6.68 ± 1.85^{ac*}	3.19 ± 0.55^{c*}	3.49 ± 0.83^{bc*}
Succinate	**Succinate**	Control	7.02 ± 2.40^{a}	94.27 ± 10.68^{b}	95.36 ± 17.6^{b}	113.2 ± 15.02^{b}	13.24 ± 4.21^{a}
		nZnO	26.72 ± 6.93^{a*}	180.06 ± 25.99^{bc*}	257.85 ± 35.02^{c*}	123.67 ± 26.96^{b}	94.27 ± 18.7^{b*}
Ornithine	**Ornithine**	Control	10.62 ± 1.26^{a}	16.65 ± 1.44^{ab}	21.02 ± 2.02^{b}	20.65 ± 2.21^{b}	12.69 ± 1.69^{a}
		nZnO	10.37 ± 1.01^{a}	22.75 ± 1.46^{b*}	28.18 ± 2.57^{b*}	24.91 ± 2.95^{b}	29.52 ± 1.40^{b*}
Argininosuccinate	**Argininosuccinate**	Control	11.28 ± 1.83^{a}	16.03 ± 2.20^{ab}	13.57 ± 2.21^{a}	26.54 ± 2.85^{b}	27.50 ± 5.24^{b}
		nZnO	24.73 ± 2.20^{a*}	25.49 ± 4.47^{a*}	23.98 ± 3.78^{a*}	10.23 ± 1.36^{b*}	10.92 ± 1.83^{b*}
Total free amino acids	**Total FAAs**	Control	837.85 ± 35.49^{a}	945.29 ± 52.25^{ab}	882.00 ± 16.06^{ab}	1008.06 ± 50.47^{ab}	1089.37 ± 71.78^{b}
		nZnO	915.28 ± 52.91^{a}	1270.48 ± 78.51^{b*}	1007.80 ± 41.02^{a*}	827.79 ± 55.63^{a*}	898.51 ± 45.29^{a*}

Supplementary Table 3. Loadings of different metabolites on the first three principal components (PCs) in control and nZnO-exposed mussels exposed to different oxygen regimes. Loadings with the absolute values >0.2 are shown in bold.

	Control mussels			nZnO-exposed mussels		
Metabolite	PC1	PC2	PC3	PC1	PC2	PC3
Asparagine	0.188	**-0.204**	**0.204**	-0.154	0.001	**-0.253**
Aspartate	0.080	**-0.317**	**-0.227**	0.017	-0.094	-0.071
Serine	0.153	**-0.291**	-0.025	-0.165	**-0.266**	-0.192
Alanine	**0.208**	-0.002	0.120	**-0.216**	0.179	0.108
Glycine	0.031	**0.272**	**-0.223**	0.008	0.169	0.147
Glutamine	0.157	**-0.309**	0.155	**-0.201**	-0.187	-0.118
Threonine	**0.206**	-0.069	0.107	**-0.215**	0.040	-0.111
Methionine Sulfoxide	0.155	-0.125	**-0.268**	-0.150	0.072	0.149
Glutamate	0.178	0.194	0.082	-0.062	**0.438**	-0.187
Proline	**0.231**	0.019	0.117	**-0.219**	0.073	-0.110
Lysine	0.158	**-0.309**	0.150	**-0.201**	-0.189	-0.115
Histidine	0.195	-0.055	0.175	-0.180	-0.171	**-0.234**
AMP	0.135	0.098	**-0.241**	-0.100	0.184	0.024
Arginine	0.073	0.133	-0.136	-0.075	**-0.213**	-0.069
Valine	**0.249**	0.151	0.037	**-0.237**	0.021	0.034
Methionine	**0.224**	0.098	-0.055	**-0.213**	0.192	0.051
Tyrosine	**0.222**	0.031	0.196	-0.190	**0.209**	**-0.269**
Isoleucine	**0.244**	0.162	0.053	**-0.240**	0.007	-0.004
Leucine	**0.243**	0.161	0.057	**-0.238**	-0.004	0.000
Phenylalanine	**0.228**	0.174	0.062	**-0.215**	0.010	-0.134
Tryptophan	**0.237**	-0.025	0.146	-0.197	-0.105	-0.177
Malate	0.187	-0.126	**-0.277**	**-0.217**	-0.073	**0.224**
Citrate	0.169	-0.016	**-0.314**	**-0.210**	-0.091	**0.249**
Succinate	0.092	**0.304**	0.095	-0.127	**0.279**	-0.054

Lactate	0.002	-0.048	-0.100	**-0.209**	0.023	**0.254**
GABA	-0.008	-0.097	0.042	-0.022	**0.205**	0.081
Carnitine	-0.020	-0.089	**-0.222**	-0.032	-0.188	**0.212**
Citrulline	0.115	-0.152	-0.040	-0.031	0.057	-0.180
Ornithine	0.149	**0.262**	0.106	-0.029	**0.415**	-0.094
Glutathione	0.008	-0.008	-0.107	0.007	0.107	0.020
Fumarate	0.189	-0.016	**-0.308**	**-0.210**	0.012	**0.286**
Pyruvate	0.141	-0.192	-0.078	**-0.218**	-0.015	**0.248**
Argininosuccinate	0.188	0.035	**-0.328**	**-0.212**	-0.091	0.197
2-oxoglutarate	0.081	0.193	-0.166	0.079	0.127	**0.332**
4-hydroxyproline	0.185	-0.085	0.074	-0.139	-0.056	-0.030

Supplementary Table 4. Two-way ANOVA analysis of the bioenergetics and metabolites biomarkers in the whole soft tissue of the blue mussels *M. edulis* exposed to different combinations of dissolved oxygen (DO) and ZnO nanoparticles (nZnO). DO: normoxia, 1 d hypoxia, 7 d hypoxia, 1 h reoxygenation following 7 d hypoxia and 24 h reoxygenation following 7 d hypoxia. nZnO treatment: control and 100 μg l^{-1} nZnO.

MS: mean square; P: p-value. Degrees of freedom (d.f.) for the error were as follows: 85 for protein; 88 for lipid; 70 for ETS, Ea, CEA, and CS; and 87 for all other studied traits. Significant effects are highlighted in bold.

Source		MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
	df	Protein			Lipid			Carbohydrate			ETS			Ea			CEA			CS		
nZnO	1	418.913	21.015	**<0.001**	51.765	3.954	0.050	3.804	6.247	**0.014**	26055.283	15.764	**<0.001**	0.042	5.501	**0.022**	**0.030**	27.329	**<0.001**	7515.157	8.267	**0.005**
DO	4	129.717	6.507	**<0.001**	55.051	4.205	**0.004**	5.278	8.667	**<0.001**	2581.336	1.562	0.194	0.040	5.212	**0.001**	**0.001**	0.807	0.525	8555.767	9.412	**<0.001**
nZnO * DO	4	30.725	1.541	0.198	40.854	3.120	**0.019**	1.746	2.868	**0.028**	1732.765	1.048	0.389	0.001	0.083	0.987	**0.001**	0.811	0.522	11491.501	12.641	**<0.001**
	df	Asparagine			Aspartate			Serine			Alanine			Glycine			Glutamine			Threonine		
nZnO	1	0.202	0.628	0.430	115.653	34.900	**<0.001**	1.199	3.198	0.077	0.835	6.124	**0.015**	1.400	20.949	**<0.001**	0.004	0.083	0.774	2.493	10.264	**0.002**
DO	4	1.457	4.541	**0.002**	25.498	7.694	**<0.001**	6.430	17.145	**<0.001**	1.210	8.871	**<0.001**	0.317	4.740	**0.002**	1.173	26.880	**<0.001**	0.710	2.922	**0.026**
nZnO * DO	4	0.348	1.084	0.369	27.675	8.351	**<0.001**	8.193	21.846	**<0.001**	0.141	1.031	0.396	0.247	3.691	**0.008**	0.185	4.248	**0.003**	0.582	2.397	0.056
	df	Methionine Sulfoxide			Glutamate			Proline			Lysine			Histidine			AMP			Arginine		
nZnO	1	5.978	47.924	**<0.001**	1193.648	2.196	0.142	4.901	15.904	**<0.001**	0.002	0.039	0.844	5.542	8.036	**0.006**	0.046	0.937	0.336	18626.470	6.848	**0.010**
DO	4	0.275	2.206	0.075	9400.496	17.294	**<0.001**	1.394	4.523	**0.002**	1.106	26.538	**<0.001**	3.279	4.755	**0.002**	0.113	2.316	0.064	3951.827	1.453	0.224
nZnO * DO	4	0.823	6.602	**<0.001**	2573.099	4.734	**0.002**	0.530	1.719	0.153	0.177	4.246	**0.003**	1.921	2.786	**0.031**	0.043	0.885	0.477	16431.387	6.041	**<0.001**
	df	Valine			Methionine			Tyrosine			Isoleucine			Leucine			Phenylalanine			Tryptophan		
nZnO	1	2.192	17.620	**<0.001**	1.236	7.067	**0.009**	2.406	13.675	**<0.001**	2.527	17.123	**<0.001**	3.036	27.917	**<0.001**	8.204	70.795	**<0.001**	5.381	26.705	**<0.001**
DO	4	1.635	13.142	**<0.001**	2.688	15.372	**<0.001**	1.912	10.867	**<0.001**	2.143	14.521	**<0.001**	1.888	17.365	**<0.001**	2.343	20.215	**<0.001**	1.472	7.306	**<0.001**
nZnO * DO	4	0.617	4.957	**0.001**	0.674	3.851	**0.006**	0.479	2.724	**0.034**	1.045	7.081	**<0.001**	1.073	9.865	**<0.001**	1.316	11.359	**<0.001**	1.641	8.143	**<0.001**
	df	Malate			Citrate			Succinate			Lactate			GABA			Carnitine			Citrulline		
nZnO	1	1.111	5.745	**0.019**	0.567	1.644	0.203	8.309	42.061	**<0.001**	9.222	75.114	**<0.001**	1.267	11.949	**0.001**	0.004	2.674	0.106	13.101	53.228	**<0.001**

DO	4	0.456	2.359	0.060	1.484	4.307	**0.003**	8.251	41.765	**<0.001**	1.049	8.546	**<0.001**	0.621	5.862	**<0.001**	0.002	1.418	0.235	0.017	0.069	0.991
nZnO * DO	4	1.043	5.391	**0.001**	7.370	21.385	**<0.001**	0.819	4.147	**0.004**	0.854	6.957	**<0.001**	0.075	0.706	0.590	0.006	3.844	**0.006**	0.909	3.694	**0.008**
	df	Ornithine			Fumarate			Pyruvate			Argininosuccinate			2-oxoglutarate			4-hydroxyproline			Total FAAs		
nZnO	1	1122.906	32.412	**<0.001**	0.471	2.799	0.098	0.111	0.733	0.394	0.001	0.006	0.940	2.203	0.276	0.600	5.430	18.778	**<0.001**	0.013	0.464	0.498
DO	4	597.265	17.240	**<0.001**	0.360	2.141	0.083	0.572	3.778	**0.007**	0.114	0.565	0.689	124.608	15.633	**<0.001**	0.941	3.253	**0.016**	0.143	5.292	**0.001**
nZnO * DO	4	182.179	5.258	**0.001**	1.455	8.653	**<0.001**	0.613	4.048	**0.005**	3.709	18.437	**<0.001**	65.317	8.194	**<0.001**	1.299	4.492	**0.002**	0.219	8.084	**<0.001**