

Supplementary Information

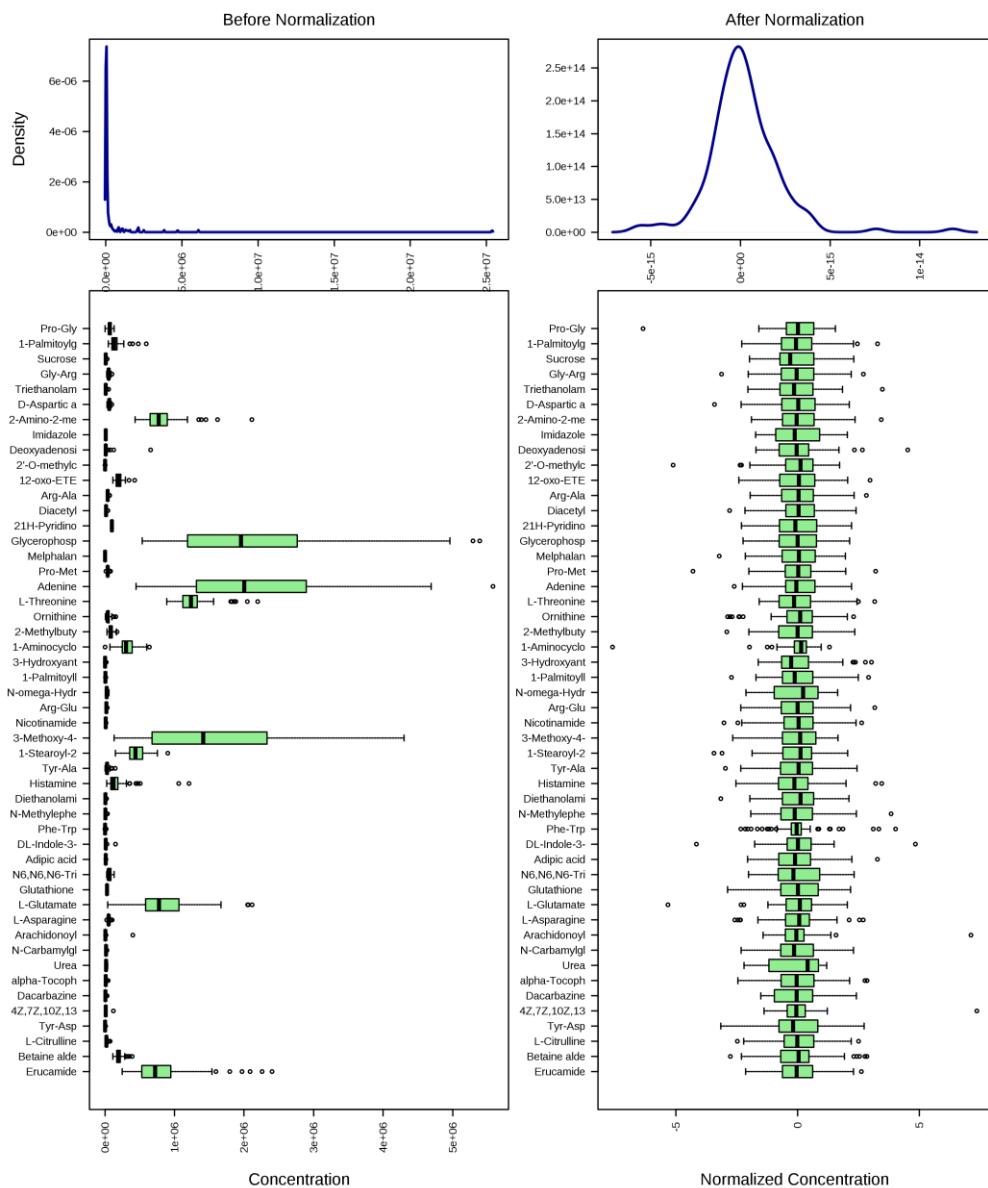


Figure S1. Data conversion in positive mode, showing a graphical summary of the data before and after the normalization procedure. The data showed the appearance of the characteristic “bell-shaped” distribution after conversion.

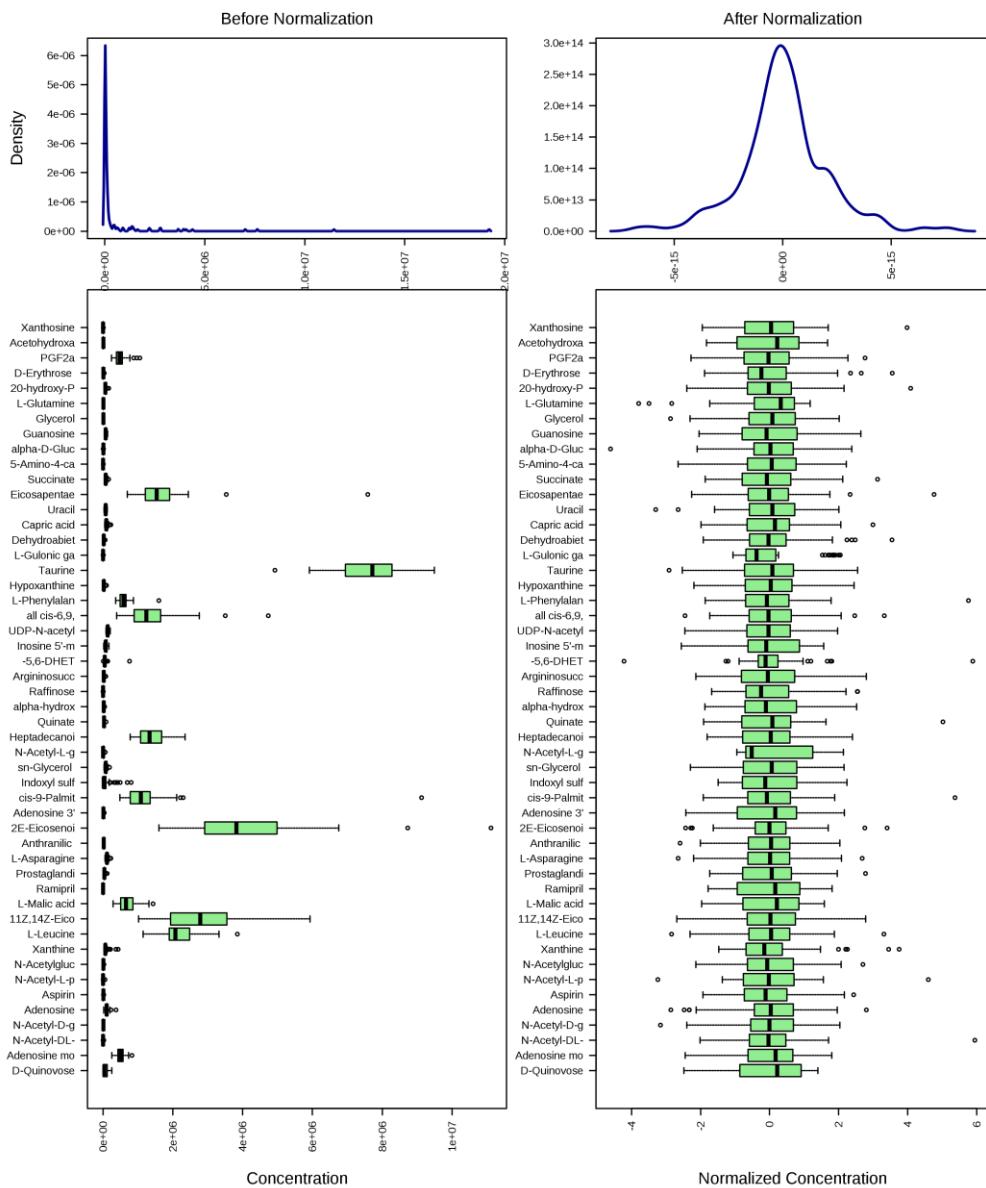


Figure S2. Data conversion in negative mode, showing a graphical summary of the data before and after the normalization procedure. The data showed the appearance of the characteristic “bell-shaped” distribution after conversion.

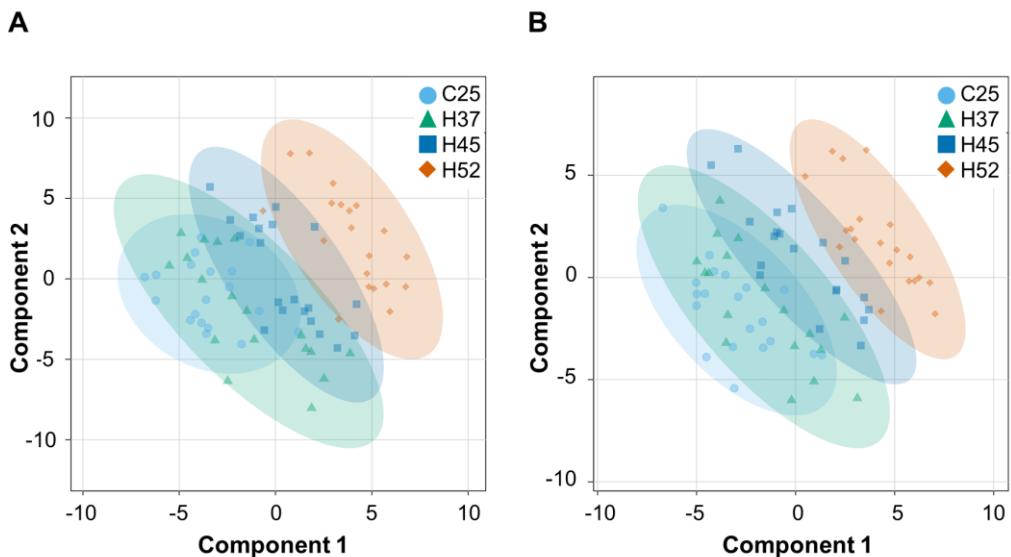


Figure S3. Effect of heat stress on the metabolic profile of muscle tissue from *Echinolittorina malaccana* in positive mode (A) and negative mode (B). The PLS-DA 2D score plot of data for the normalized concentrations of foot muscle metabolites found in *E. malaccana* sampled in all four groups (C25, H37, H45, H52), n = 18-19, ellipses correspond to a confidence interval of 95%.

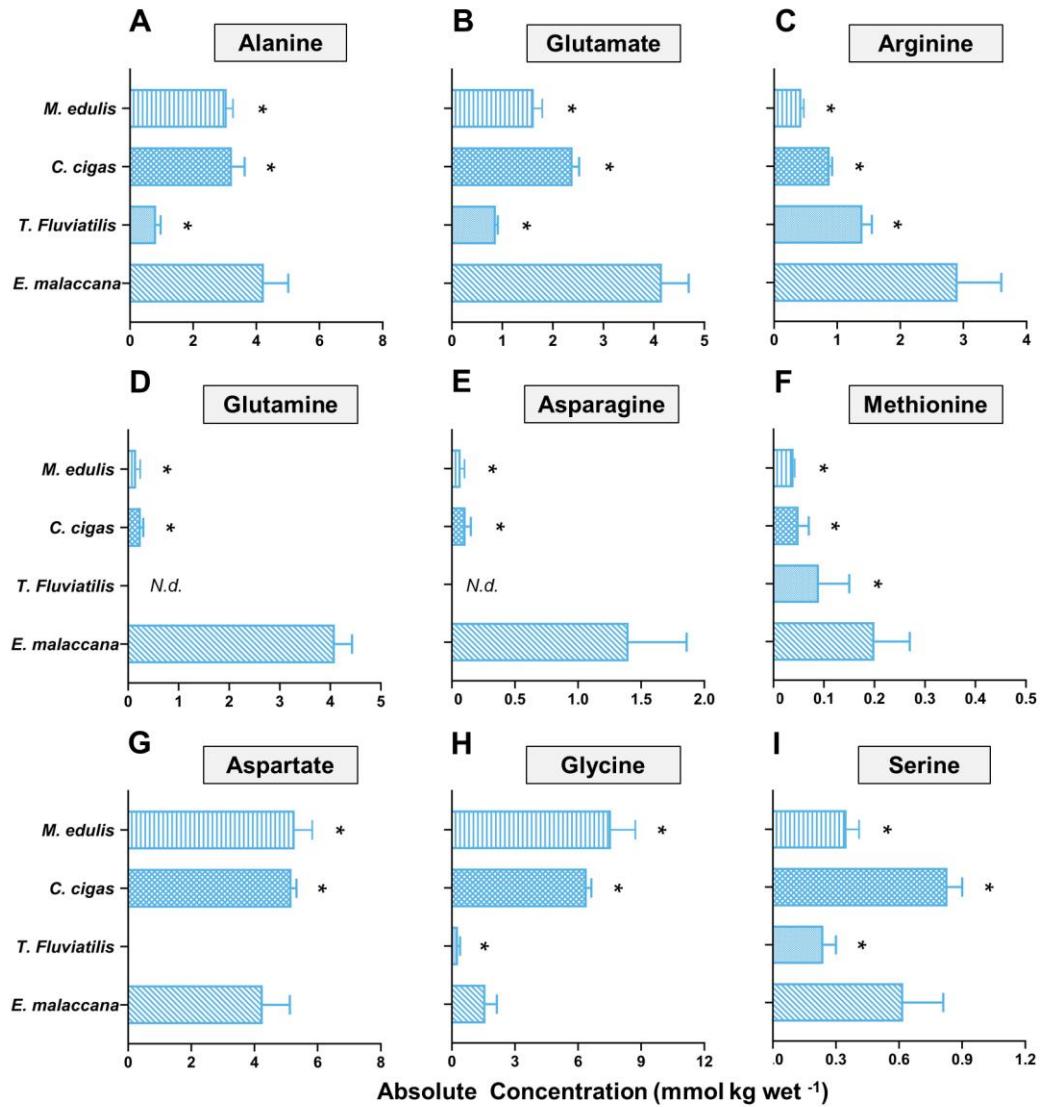


Figure S4. The concentrations of free amino acids (mmol kg wet weight⁻¹) under non-stressful conditions of *E. malaccana* compared to *Theodoxus fluviatilis*, *Mytilus edulis* and *Crassostrea gigas*. The concentrations were analyzed by One-way ANOVA. Significant differences between *E. malaccana* and other organisms are indicated by asterisk ($p < 0.05$). Data are shown as columns plots. The pictures of *T. fluviatilis*, *M. edulis* and *C. gigas* were obtained from previous studies (Haider et al., 2020; Wiesenthal et al., 2019).

Reference

- Haider, F., Falushynska, H. I., Timm, S. and Sokolova, I. M. (2020). Effects of hypoxia and reoxygenation on intermediary metabolite homeostasis of marine bivalves

Mytilus edulis and *Crassostrea gigas*. *Comp. Biochem. Phys. A* **242**, 110657-110672.

Wiesenthal, A. A., Muller, C., Harder, K. and Hildebrandt, J. P. (2019). Alanine, proline and urea are major organic osmolytes in the snail *Theodoxus fluviatilis* under hyperosmotic stress. *J. Exp. Biol.* **222**, 1-10.

Table S1. The activities of hexokinase (HK) and pyruvate Kinase (PK) of *E. malaccana*

		The activity of enzyme (U g wet weight ⁻¹)			
		25	37	45	52
HK	5.4896	5.27276	11.36704	7.863563	
	7.126419	6.326526	11.6865	7.427757	
PK	6.149138	6.046979	10.94965	7.195203	
	9.614135	6.540617	11.39232	15.46975	
	10.13383	7.835439	10.82403	10.40446	
	11.29303	3.61978	15.56496	12.40386	

Table S2. Concentrations of free amino acids in molluscs under non-stressful conditions.

Amino acids	<i>E. malaccana</i> (foot)	<i>T. fluviatilis</i> (foot)	<i>C. gigas</i> (gill)	<i>M. edulis</i> (gill)
Glu	4.16 ± 0.53	0.87 ± 0.04*	2.39 ± 0.13*	1.62 ± 0.17*
Ala	4.24 ± 0.76	0.83 ± 0.13*	3.23 ± 0.2*	3.06 ± 0.19*
Asp	4.26 ± 0.85	Nd	5.17 ± 0.15*	5.27 ± 0.55*
Gln	4.09 ± 0.33	Nd	0.25 ± 0.04*	0.16 ± 0.07*
Arg	2.91 ± 0.68	1.40 ± 0.14*	0.88 ± 0.03*	0.43 ± 0.03*
Gly	1.60 ± 0.53	0.29 ± 0.09*	6.41 ± 0.21*	7.56 ± 1.15*
Asn	1.40 ± 0.45	Nd	0.11 ± 0.03*	0.07 ± 0.02*
Thr	1.20 ± 0.30	0.21 ± 0.04*	0.19 ± 0.03*	0.13 ± 0.01*
Lys	1.06 ± 0.35	0.39 ± 0.12*	0.26 ± 0.03*	0.29 ± 0.01*
Ser	0.62 ± 0.18	0.24 ± 0.05*	0.83 ± 0.06*	0.35 ± 0.05*
Orn	0.51 ± 0.26	0.89 ± 0.38*	Nd	Nd
Met	0.20 ± 0.06	0.09 ± 0.05*	0.05 ± 0.01*	0.04 ± 0.001*
His	0.15 ± 0.05	0.22 ± 0.03*	0.22 ± 0.02*	0.24 ± 0.01*
Phe	0.13 ± 0.02	0.10 ± 0.01*	0.09 ± 0.007*	0.06 ± 0.003*
Tyr	0.08 ± 0.02	0.13 ± 0.02*	0.05 ± 0.003*	0.03 ± 0.002*
Pro	0.10 ± 0.03	0.03 ± 0.005*	0.48 ± 0.04*	0.13 ± 0.01*
Val	0.07 ± 0.009	0.20 ± 0.01*	0.14 ± 0.01*	0.22 ± 0.07*
Trp	0.018 ± 0.007	0.005 ± 0.001*	0.014 ± 0.001	0.02 ± 0.002

The concentrations of free amino acids (mmol kg wet weight⁻¹) under normal conditions of *E. malaccana* compared to *Theodoxus fluviatilis* sampled in fresh water or brackish water, *Mytilus edulis* and *Crassostrea gigas*. Bold fonts indicated that there was a significant difference between *E. malaccana* and other organisms, black – higher or lower than other organisms, red – higher than all others, green – lower than all others ($p < 0.05$). The data of *T. fluviatilis* were measured by Wiesenthal et al. (2019), and the data of *M. edulis* and *C. gigas* were measured by Haider et al. (2020).

Reference

- Haider, F., Falfushynska, H. I., Timm, S. and Sokolova, I. M.** (2020). Effects of hypoxia and reoxygenation on intermediary metabolite homeostasis of marine bivalves *Mytilus edulis* and *Crassostrea gigas*. *Comp. Biochem. Phys. A* **242**, 110657-110672.
- Wiesenthal, A. A., Muller, C., Harder, K. and Hildebrandt, J. P.** (2019). Alanine, proline and urea are major organic osmolytes in the snail *Theodoxus fluviatilis* under hyperosmotic stress. *J. Exp. Biol.* **222**, 1-10.