Supplementary Information

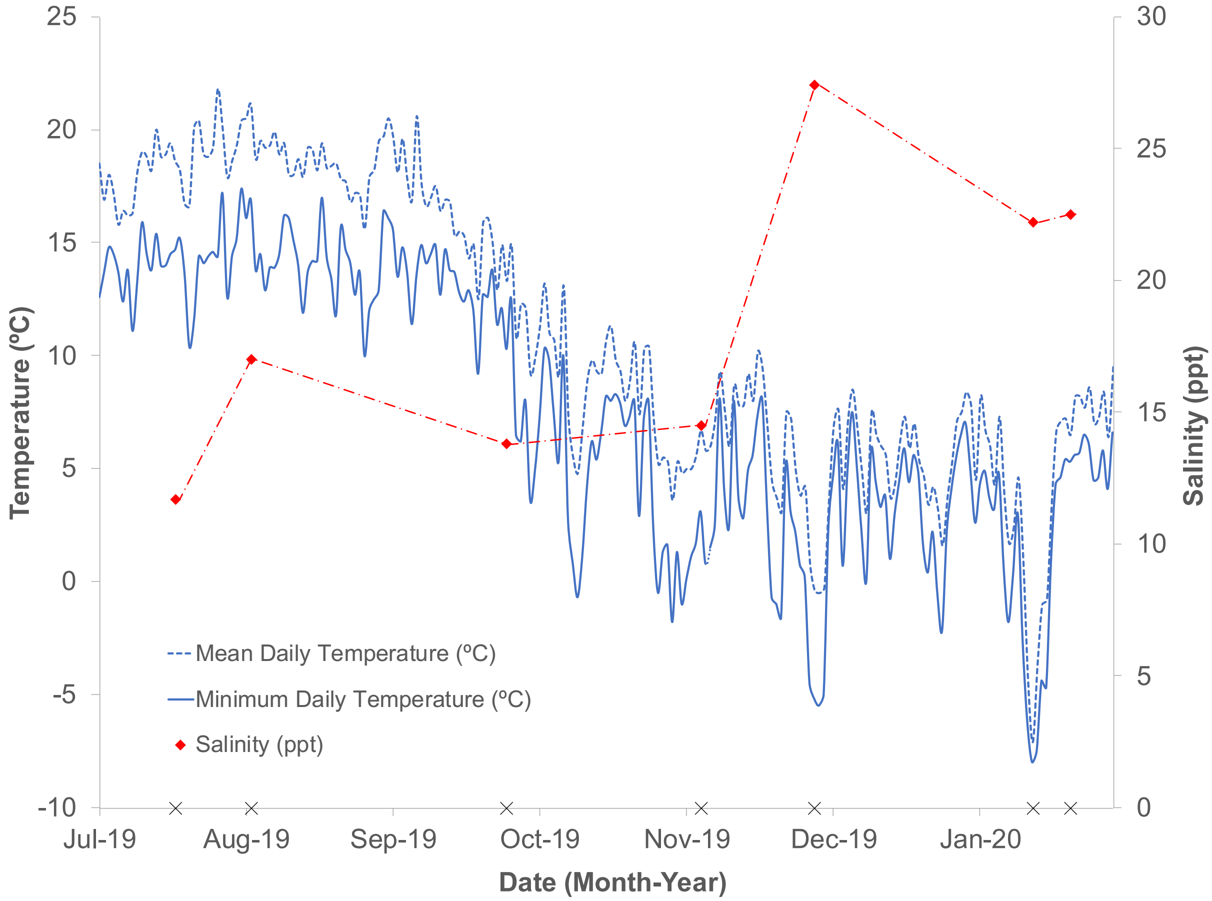

Figure S1. Temperature and salinity during the study period, July 2019- February 2020. Salinity (represented by the red dashed/dotted line) was measured at Tower Beach, Vancouver, BC at approximately 25-50 cm depth using a YSI probe during low tide. Mean daily temperatures (represented by the dotted line) and minimum daily temperatures (represented by the solid line) are from the weather station located at Vancouver International Airport (approximately 10 km SSE of Tower Beach; data retrieved from climate.weather.gc.ca). Crosses on the x-axis indicate dates when mussels were collected.

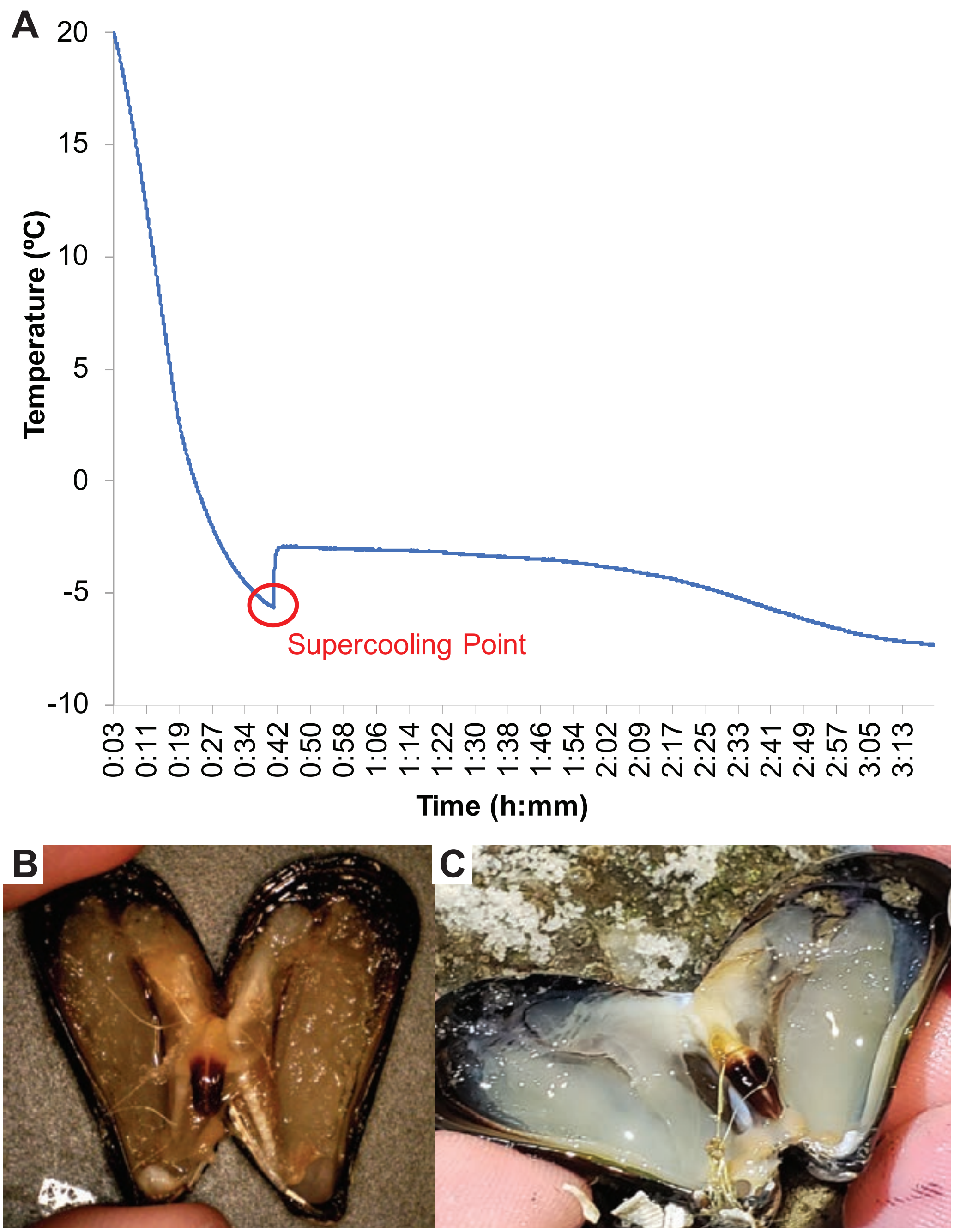

Figure S2. Evidence for freezing in *Mytilus trossulus*. A) An example of a mussel's temperature trace during an experimental exposure to -8 ºC. Mussels were frozen with a cooling rate of -1.5 ºC minute^{-1} using a refrigerated circulating bath. The supercooling point is the lowest temperature reached before the exothermic release of energy caused by ice formation. The x-axis represents the time after the freezing exposure began in hours:minutes. B, C) Dissected frozen mussels, *M. trossulus*, B) immediately after a lab cold exposure for 3 h at -8 ºC and C) at Tower Beach, Vancouver during a winter low tide (air temperature was approximately -8 ºC). Ice formation occurs in the mantle cavity; ice is most concentrated in the posterior region of the mantle cavity (ie. the upper portion of the mantle cavity).

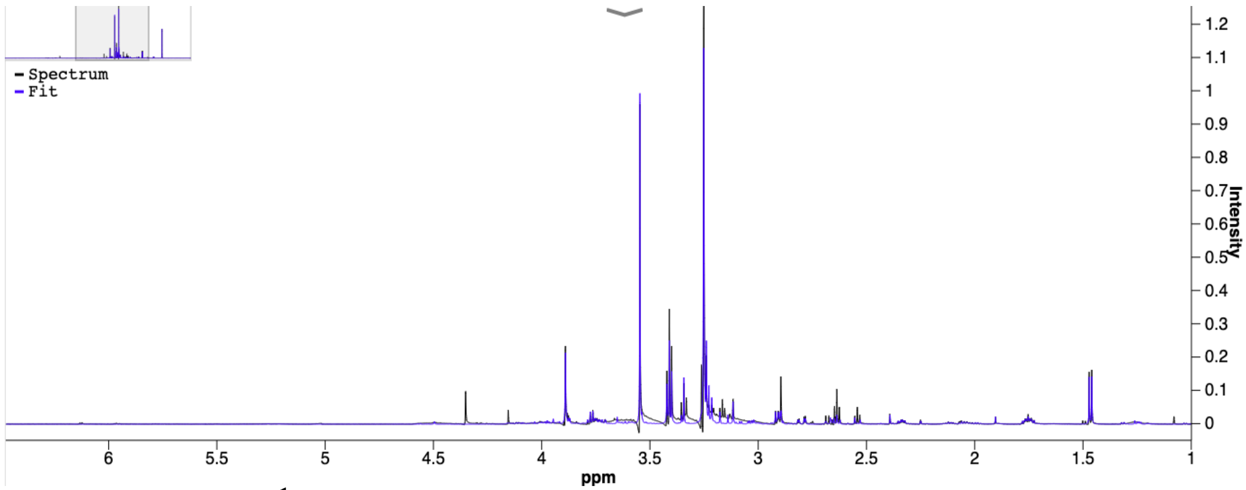

Figure S3. Example ^{1}H NMR spectrum of *M. trossulus* gill tissue. Visualized by http://bayesil.ca/. Blue lines represent fit by library compounds. Water region (~4.5 – 5.2 ppm) was deleted.

Table S1. ***M. trossulus* survival following 3h freeze exposures to a series of test temperatures.** This analysis was done considering only mussels collected on November 29, 2019 and January 22, 2020. Mussels were collected from Tower Beach, Vancouver, BC. Reported values are from a generalized linear model, bolded values indicate $p < 0.05$ (df=333). n= 12 mussels per test temperature/salinity in November; n=15 per group in January.

	Deviance	p value
Shell Length	2.70	**< 0.001**
Test Temperature	7.94	**< 0.001**
Time of Year (Number of Days Past July 1)	0.0080	0.8134
Salinity	24.0	**< 0.001**
Shore Level	2.73	**< 0.001**
Days Past July 1: Salinity	0.0263	0.668
Days Past July 1: Shore Level	0.0044	0.861
Salinity: Shore Level	0.0108	0.784
Days Past July 1: Salinity: Shore Level	0.0215	0.698

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Table S2. **Metabolites in *M. trossulus* gill tissue and their relative change in concentration with four predictor variables.** The four predictor variables considered are: salinity (change from 15 ppt to 30 ppt), time of year (change from August, 2019 to November, 2019), shore position (change from low intertidal to high intertidal group), and -6 ºC exposure (change after -6 ºC exposure). Reported values are from general linear models, one model per metabolite. *n*=4 or 5 mussel gills per group (see Methods for a detailed description of sample sizes). *Increased concentration*, **decreased concentration,** no significant change in concentration.

Predictor	Metabolite	t value	p value
Salinity	*Glycine*	*16.46*	*<0.001*
	β-Alanine	*9.59*	*<0.001*
	TMAO	*7.04*	*<0.001*
	Alanine	*6.72*	*<0.001*
	Malonate	*5.67*	*<0.001*
	Betaine	*5.44*	*<0.001*
	Taurine	*3.47*	*0.0015*
	Glutamate	*2.97*	*0.00537*
	Malate	0.623	0.537
	TMA	0.536	0.595
	Aspartate	0.111	0.912
	Lactate	-0.476	0.637
	Succinate	-0.543	0.591
	Guanidoacetate	-0.837	0.409
	Acetoacetate	**-2.18**	**0.0362**
	Acetate	**-2.21**	**0.0341**
	AMP	**-2.38**	**0.0233**
Time of year	*Betaine*	*10.35*	*<0.001*
	TMAO	*7.52*	*<0.001*
	Alanine	*7.005*	*<0.001*
	Glycine	*5.70*	*<0.001*
	Malonate	*4.92*	*0.0017*
	β-Alanine	*3.79*	*0.0068*
	Taurine	*3.12*	*0.0170*
	Glutamate	1.99	0.0873
	Lactate	1.08	0.315
	Malate	0.983	0.358
	Succinate	0.906	0.395
	AMP	-0.316	0.761
	Guanidoacetate	-2.14	0.0701
	Trimethylamine	**-2.99**	**0.0203**

	Acetate	**-3.33**	**0.0126**
	Aspartate	**-4.34**	**0.00339**
Shore position	*β-Alanine*	*2.87*	*0.00697*
	Glycine	1.42	0.164
	AMP	0.830	0.412
	Malate	0.553	0.584
	TMA	0.367	0.716
	Guanidoacetate	0.169	0.867
	Lactate	-0.270	0.789
	Alanine	-0.439	0.664
	Aspartate	-0.597	0.554
	Glutamate	-0.685	0.498
	Acetate	-0.844	0.404
	Succinate	-0.897	0.376
	TMAO	-1.23	0.227
	Taurine	-1.60	0.120
	Acetoacetate	**-2.22**	**0.0333**
	Betaine	**-2.64**	**0.0125**
	Malonate	**-2.99**	**0.00513**
-6 ºC exposure	*Aspartate*	*2.29*	*0.0283*
	TMAO	1.31	0.199
	Malonate	1.23	0.228
	Acetate	1.02	0.314
	Lactate	0.702	0.488
	Acetoacetate	0.509	0.614
	TMA	0.014	0.989
	Glycine	-0.035	0.972
	Guanidoacetate	-0.044	0.965
	Taurine	-0.413	0.682
	AMP	-0.800	0.430
	Betaine	-1.94	0.0607
	Malate	-2.01	0.0524
	β-Alanine	**-2.06**	**0.0473**
	Glutamate	**-3.45**	**0.00153**
	Succinate	**-4.30**	**<0.001**
	Alanine	**-4.74**	**<0.001**

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Table S3. **How salinity, cold exposure at -6 ºC for 3 h, and shore position affect the concentration of metabolites in *M. trossulus* gill tissue**. Reported values are *p* values from general linear models, one model per metabolite. Bolded values indicate $p<0.05$. The three-way interaction term was not statistically significant for any metabolite ($p>0.05$ in all cases). $n=4$ or 5 mussel gills per group (see Methods for a detailed description of sample sizes).

Metabolite	Salinity	Cold	Position	Salinity×Cold	Salinity×Position	Cold×Position
Taurine	0.102	0.822	0.763	0.968	0.889	0.960
TMAO	**0.006**	0.368	0.420	0.176	0.666	0.799
Betaine	**<0.001**	0.515	0.077	0.312	0.242	0.435
Alanine	**<0.001**	**0.003**	0.429	**<0.001**	0.330	0.356
Glycine	**<0.001**	0.707	**<0.001**	0.395	**0.045**	**<0.001**
Aspartate	0.772	0.906	0.606	0.625	0.585	0.377
Malonate	**0.0458**	0.640	0.808	0.425	0.331	0.317
Succinate	0.569	0.990	0.050	0.640	0.200	0.169
β-Alanine	**<0.001**	0.849	0.938	0.686	0.471	0.394
Glutamate	0.167	0.131	0.703	0.417	0.784	0.518
Guanidoacetate	0.194	0.102	0.851	0.092	0.798	0.768
Malate	0.919	0.471	0.620	0.944	0.990	0.954
Trimethylamine	0.442	0.146	0.549	0.218	0.821	0.749
Acetate	**0.012**	0.078	0.387	**0.044**	0.290	0.163
Lactate	0.221	0.243	0.882	0.104	0.629	0.498
AMP	0.695	0.183	0.084	0.148	0.135	0.264
Acetoacetate	0.159	0.678	0.207	0.739	0.316	0.249

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