Electronic Supplements

Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen

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Supplement 1. Statistics

1.1 Temperature × salinity experiment

Table S1. Results of the repeated two-way ANOVA for effects of temperature and salinity on the increase of size (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
size %[a]	Time	4	97.469	< 0.001*
	Temperature	3	5.354	0.0016*
	Salinity	1	5.593	0.0193*
	Temperature × Salinity	3	15.257	< 0.001*

[a] $\log_{10}$ transformation

Table S2. Results of the repeated two-way ANOVA for effects of temperature and salinity on the photosynthetic efficiency F_v/F_m (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
F_v/F_m %[a]	Time	4	5.136	< 0.001*
	Temperature	3	0.732	0.53425
	Salinity	1	5.136	0.44404
	Temperature × Salinity	3	0.339	0.79708

[a] ranked data-set (transformation)

Table S3. Results of the generalized linear model (GLM) for effects of temperature and salinity compared to initial measurements on chlorophyll *a* (Chl *a*), accessory pigment pool (Acc), xanthophyll pool (VAZ = violaxanthin, antheraxanthin, zeaxanthin), accessory pigments : chlorophyll *a* ratio (Acc:Chl*a*) and the de-epoxidation state of the xanthophyll cycle (DPS) of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	χ^2 value	*p* value
	Within-subjects effects + initial			
Chl *a*	Temperature	3	8.8685	0.0311*
	Salinity	1	1.9622	0.1613
	Temperature × Salinity	3	1.5917	0.6613
Acc	Temperature	3	7.0210	0.0712
	Salinity	1	1.7522	0.1856
	Temperature × Salinity	3	2.3428	0.5044
VAZ	Temperature	3	3.8095	0.2828
	Salinity	1	3.5260	0.0604
	Temperature × Salinity	3	5.0887	0.1654
Acc:Chl*a*[a]	Temperature	3	13.8878	0.0031*
	Salinity	1	0.8609	0.3535
	Temperature × Salinity	3	2.4050	0.4927
DPS	Temperature	3	3.0970	0.3769
	Salinity	1	1.4584	0.2272
	Temperature × Salinity	3	2.2026	0.5314

[a] ranked data-set (transformation)

Table S4. Results of the generalized linear model (GLM) and one-way ANOVA for effects of temperature and salinity compared to initial measurements on the C:N ratio, total C and total N content of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
C:N	Temperature	ANOVA	4	13.00	< 0.001*
	Salinity	ANOVA	2	13.88	< 0.001*
	Within-subjects effects + initial				
C:N	Temperature	GLM	3	15.3288	0.0016*
	Salinity		1	1.3186	0.2509
	Temperature × Salinity		3	2.5378	0.4685
	Between-subjects effects + initial				
Total C	Temperature	ANOVA	4	0.987	0.429
	Salinity	ANOVA	2	1.637	0.210
	Within-subjects effects + initial				
Total C	Temperature	GLM	3	0.74839	0.8618
	Salinity		1	0.03853	0.8444
	Temperature × Salinity		3	0.64861	0.8852
	Between-subjects effects + initial				
Total N	Temperature	ANOVA	4	6.325	0.0047*
	Salinity	ANOVA	2	18.86	<0.001*
	Within-subjects effects + initial				
Total N	Temperature	GLM	3	49.449	< 0.001*
	Salinity		1	2.894	0.0889
	Temperature × Salinity		3	1.876	0.5985

Table S5. Results of the one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and salinity compared to initial measurements on mannitol of *Saccharina latissima* from Spitsbergen. Effects were also tested independently by temperature and salinity. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Mannitol	Temperature	Kruskal-Wallis	4	24.496	< 0.001* [x]
	Salinity	Kruskal-Wallis	2	21.285	< 0.001* [x]
	Within-subjects effects + initial				
Mannitol_4°C	Salinity	Kruskal-Wallis	2	8.985	0.011* [x]
Mannitol_6°C	Salinity	ANOVA	2	7.222	0.006*
Mannitol_8°C	Salinity	ANOVA	2	2.574	0.109
Mannitol_10°C	Salinity	ANOVA	2	8.272	0.004*
Mannitol_low	Temperature	ANOVA	4	6.051	< 0.001 *
Mannitol_control	Temperature	ANOVA	4	2.309	0.086

[x] Dunn-Bonferroni test: reject H_0 if $p \leq alpha/2$, alpha = 0.05

Table S6. Results of the generalized linear model (GLM) or one-way ANOVA for effects of temperature and salinity compared to initial measurements on phlorotannins of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Phlorotannins[a]	Temperature	ANOVA	4	9.46	< 0.001*
	Salinity	ANOVA	2	37.03	< 0.001*
	Within-subjects effects + initial				
Phlorotannins[a]	Temperature	GLM	3	4.0690	0.2541
	Salinity		1	30.2870	< 0.001*
	Temperature × Salinity		4	3.8938	0.2732

[a] $\log_{10}$ transformation

1.2 Temperature × nutrients experiment

Table S7. Results of the repeated two-way ANOVA for effects of temperature and nutrients on the increase of size (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
size %[a]	Time	4	61.678	< 0.001*
	Temperature	3	17.340	< 0.001*
	Nutrients	1	2.705	0.102
	Temperature × Nutrients	3	0.230	0.875

[a] $\log_{10}$ transformation

Table S8. Results of the repeated two-way ANOVA for effects of temperature and nutrients on the photosynthetic efficiency F_v/F_m (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
F_v/F_m %[a]	Time	4	5.457	< 0.001*
	Temperature	3	43.486	< 0.001*
	Nutrients	1	0.760	0.38473
	Temperature × Nutrients	3	3.000	0.03255*

[a] $\log_{10}$ transformation

Table S9. Results of the generalized linear model (GLM) for effects of temperature and nutrients compared to initial measurements on chlorophyll *a* (Chl *a*), accessory pigment pool (Acc), xanthophyll pool (VAZ = violaxanthin, antheraxanthin, zeaxanthin), accessory pigments : chlorophyll *a* ratio (Acc:Chl*a*) and the de-epoxidation state of the xanthophyll cycle (DPS) of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	χ^2 value	*p* value
	Within-subjects effects + initial			
Chl *a*	Temperature	3	0.3954	0.9412
	Nutrients	1	0.9617	0.3268
	Temperature × Nutrients	3	3.5522	0.3141
Acc	Temperature	3	0.2557	0.9681
	Nutrients	1	1.9907	0.1583
	Temperature × Nutrients	3	3.6266	0.3047
VAZ	Temperature	3	1.5933	0.6609
	Nutrients	1	0.2625	0.6084
	Temperature × Nutrients	3	4.4743	0.2146
Acc:Chl*a*	Temperature	3	1.9796	0.5767
	Nutrients	1	2.8690	0.0903
	Temperature × Nutrients	3	3.4242	0.3307
DPS[a]	Temperature	3	15.7690	0.0013*
	Nutrients	1	0.15322	0.6955
	Temperature × Nutrients	3	4.0870	0.2522

[a] reciprocal (transformation)

Table S10. Results of the generalized linear model (GLM) and one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on the C:N ratio, total C and total N content of *Saccharina latissima* from Spitsbergen. Effects in C:N ratio were also tested independently by temperature and nutrients. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
C:N	Temperature	Kruskal-Wallis	4	9.4872	0.0501
	Nutrients	Kruskal-Wallis	2	2.4036	0.3007
	Within-subjects effects + initial				
C:N_4°C	Nutrients	Kruskal-Wallis	2	1.1429	0.5647
C:N _6°C	Nutrients	Kruskal-Wallis	2	1.1429	0.5647
C:N _8°C	Nutrients	Kruskal-Wallis	2	0.8571	0.6514
C:N _10°C	Nutrients	Kruskal-Wallis	2	0.2857	0.8669
C:N _SW	Temperature	Kruskal-Wallis	4	2.4	0.6626
C:N _SW+N+P	Temperature	Kruskal-Wallis	4	4.8	0.3084
	Between-subjects effects + initial				
Total C	Temperature	ANOVA	4	1.613	0.1980
	Nutrients	ANOVA	2	0.529	0.5940
	Within-subjects effects + initial				
Total C	Temperature	GLM	3	8.0698	0.0446*
	Nutrients		1	1.7119	0.1907
	Temperature × Nutrients		3	8.2049	0.0419*
	Between-subjects effects + initial				
Total N	Temperature	ANOVA	4	2.677	0.0516
	Nutrients	ANOVA	2	1.049	0.3630
	Within-subjects effects + initial				
Total N	Temperature	GLM	3	3.2482	0.0562
	Nutrients		1	1.1118	0.2917
	Temperature × Nutrients		3	2.6964	0.4409

Table S11. Results of the one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on mannitol of *Saccharina latissima* from Spitsbergen. Effects were also tested independently by temperature and nutrients. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Mannitol[a]	Temperature	Kruskal-Wallis	4	30.556	< 0.001* [x]
	Nutrients	Kruskal-Wallis	2	0.071	0.9652
	Within-subjects effects + initial				
Mannitol_4°C	Nutrients	ANOVA	2	0.219	0.806
Mannitol_6°C[a]	Nutrients	ANOVA	2	1.515	0.252
Mannitol_8°C	Nutrients	ANOVA	2	3.154	0.0829
Mannitol_10°C	Nutrients	Kruskal-Wallis	2	4.456	0.1077
Mannitol_SW	Temperature	ANOVA	4	0.649	0.633
Mannitol_SW+N+P	Temperature	ANOVA	4	7.733	< 0.001*

[a] reciprocal (transformation)

[x] Dunn-Bonferroni test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S12. Results of the generalized linear model (GLM) or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on phlorotannins of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	χ^2 value	*p* value
	Between-subjects effects + initial				
Phlorotannins[a]	Temperature	Kruskal-Wallis	4	19.527	< 0.001* [x]
	Nutrients	Kruskal-Wallis	2	15.248	< 0.001* [x]
	Within-subjects effects + initial				
Phlorotannins[b]	Temperature	GLM	3	10.3303	0.01596*
	Nutrients		1	4.5437	0.03304*
	Temperature × Nutrients		4	10.2102	0.01686*

[a] $\log_{10}$ transformation

[b] ranked data-set (transformation)

[x] Dunn-Bonferroni test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Supplement 2. Physiological and biochemical extra data

Table S13: Maximum quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* as absolute values on t_0, t_6 and t_{14} during the 2-factor treatment at 4 temperatures (4, 6, 8, 10°C) and (a) salinity treatments (low, control) or (b) nutrient treatments (SW: sea water , SW+N+P: sea water + nitrate + phosphate). t_0 was the start of the experiment (initial), t_6 was after the temperature acclimation and t_{14} at the end of the experiment. Values are means ± SD (n = 4).

	Treatments		F_v/F_m		
			t_0	t_6	t_{14}
a) Temperature × Salinity	4°C	low	0.697 (± 0.012)	0.697 (± 0.012)	0.708 (± 0.009)
		control	0.692 (± 0.007)	0.692 (± 0.007)	0.685 (± 0.010)
	6°C	low	0.682 (± 0.013)	0.682 (± 0.013)	0.684 (± 0.009)
		control	0.688 (± 0.007)	0.688 (± 0.007)	0.694 (± 0.008)
	8°C	low[x]	0.679 (± 0.008)	0.679 (± 0.008)	0.669 (± 0.003)
		control[x]	0.682 (± 0.002)	0.682 (± 0.002)	0.679 (± 0.006)
	10°C	low[x]	0.691 (± 0.007)	0.691 (± 0.007)	0.689 (± 0.004)
		control[x]	0.671 (± 0.015)	0.671 (± 0.015)	0.683 (± 0.004)
b) Temperature × Nutrients	4°C	SW	0.662 (± 0.011)	0.662 (± 0.011)	0.673 (± 0.008)
		SW+N+P	0.654 (± 0.009)	0.654 (± 0.009)	0.653 (± 0.044)
	6°C	SW	0.671 (± 0.003)	0.671 (± 0.003)	0.657 (± 0.020)
		SW+N+P	0.676 (± 0.010)	0.676 (± 0.010)	0.680 (± 0.008)
	8°C	SW	0.680 (± 0.009)	0.680 (± 0.009)	0.684 (± 0.011)
		SW+N+P	0.664 (± 0.011)	0.664 (± 0.011)	0.679 (± 0.011)
	10°C	SW	0.679 (± 0.013)	0.679 (± 0.013)	0.668 (± 0.018)
		SW+N+P	0.672 (± 0.011)	0.672 (± 0.011)	0.687 (± 0.016)

[x] marks diatom contamination.

Fig. S1: Total C content in *Saccharina latissima* after 1 wk of 2-factor treatment at 4 temperatures (4, 6, 8, 10°C) and (a) salinity treatments (low, control) or (b) nutrient treatments (SW: sea water , SW+N+P: sea water + nitrate + phosphate). The boxplots represent the median (50th percentile), the interquartile range (25th to the 75th percentile) and the minimum and maximum values of the data sets. Significant differences are marked with different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (n = 4; 2-way ANOVA with post-hoc Tukey test; $p < 0.05$).