Contrasting offspring responses to variation in salinity and temperature among populations of a coastal crab: A maladaptive ecological surprise?

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SUPPLEMENTARY TABLES AND FIGURES

Section 1. Material and methods: Bivariate analysis using MCMCglmm

We carried out additional analyses accounting for the covariation between survival and duration of development using multi-response models and then compared the output of bivariate models with those obtained from univariate models. For simplicity, and because bivariate models require large data sets, these models were based on a factorial combination of temperature, salinity, and female of origin (population was not included); also note that we restricted the analysis of developmental time to larvae hatched from embryos kept in seawater. Bivariate and univariate models were fitted using the package MCMCglmm, following Hadfield (2021). In all cases, the response variables were centred and standardised to variance = 1 as in Hadfield (2021).

For the bivariate model, we specified two random structures: first with covariance between survival and development (coded as *random=~us(trait):ffem*) and without covariance (*random=~idh(trait):ffem*) indicating yet that variances may depend on the response variable. In the code, the term "trait" refers to the type of response variable (there are two "traits": survival and duration of development) and *ffem* is the factor 'female of origin'. Likewise, we specified and tested similar structures for the variance term (coded respectively as *rcov=~us(trait):units vs rcov=~idh(trait):units*).

Because the variables were centred and standardised to variance=1, the priors were coded so as to partition the total variation in equal parts between the random and variance structures with zero covariance (giving diagonal matrices) between traits:

> *prior.multi<-list(R=list(V=diag(2)/2, n=2), G=list(G1=list(V=diag(2)/2, n=2)))*

For the univariate model, the random part considered that variation among larvae from different females may be contingent on temperature and salinity. Hence, the random term was coded as: *random=~idh(ftemp):ffem+idh(fsal):ffem*. Likewise, the prior considered also that variance was partitioned between components contingent on temperature and salinity. The code used for the prior (for four temperatures and two salinities) was as follows:

> *prior.uni<-list(R=list(V=diag(1),nu=0.002),G=list(G1=list(V=diag(4)*0.02,nu=5),*
> *G2=list(V=diag(2)*0.02, nu=3)))*

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References

Hadfield M (2021) MCMCglmm Course Notes located in the Vignettes of: https://cran.r-project.org/web/packages/MCMCglmm/index.html)

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Section 2. Results

Table S1. *Carcinus maenas.* Model selection for larval developmental duration (raw and logarithmic data) of zoea II (Z II) and zoea III (Z III) in response to population (P), embryonic salinity (E_S), larval temperature (L_T), larval salinity (L_S) and female of origin (F). Note that larval salinity 20 PSU is excluded from the analysis. F is a random factor, nested in the interaction $P \times E_S$. The remaining four factors are fixed and form a 4-way factorial design. Model selection on random terms was carried out through restricted maximum likelihood (REML) fitting. In all cases, the best-fitted model was without any random interaction. Fixed effects were tested after maximum likelihood (ML) fitting where the best model was 3-way factorial with only $P \times E_S \times L_S$ (+ $P \times L_T \times L_S$ for Z III raw data) interaction retained. Further 2-way interactions and additive terms with the temperature were tested.

Model selection:		Raw		Logarithmic	
		Z II	Z III	Z II	Z III
Random (REML)					
$F \times L_T \times L_S$		429.43	458.88	22.61	14.44
$F \times L_S$		408.20	429.34	-5.29	-15.51
$F \times L_T$		429.28	464.98	28.76	23.30
F		402.85	428.73	-5.11	-15.66
No random term		**400.98**	**426.73**	**-7.72**	**-18.10**
Fixed (ML)	**Term removed**				
4-way (full model)		410.56	457.64	-134.38	-148.94
	$P \times E_S \times L_T \times L_S$	401.28	456.49[a]	-143.89	-152.03
3-way factorial		401.28	456.49	-143.89	-152.03
	$E_S \times L_T \times L_S$	395.93	446.58	-145.67	-160.77
	$P \times L_T \times L_S$	392.94	**456.52**	-153.25	-156.21
	$P \times E_S \times L_T$	391.66	446.52	-152.98	-161.15
	$P \times E_S \times L_S$	**415.38**	458.75	-133.41	-150.78
3-way factorial	$E_S \times L_T \times L_S$; $P \times L_T \times L_S$; $P \times E_S \times L_T$	379.64	-	-162.56	-171.88
	$P \times E_S \times L_S$	394.01	-	-151.45	-169.10
2-way with L_T +$P \times E_S \times L_S$		379.64	-	-162.56	-171.88
	$L_T \times P$	374.58	-	-169.67	-177.47
	$L_T \times E_S$	**396.45**	-	-165.35	-176.58
	$L_T \times L_S$	379.51	-	-166.91	**-166.50**
Additive		-	-	**-175.79[b]**	-

Bold values = terms retained in the final model
a = interaction not significant and the term is removed (ANOVA, $p < 0.05$)
b = 2-way interactions of temperature when other factors are not retained, however temperature as a factor is important (ANOVA, $p<0.001$) in additive model (AICc = 12.36 with L_T removed), hence temperature is retained.

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Table S2. *Carcinus maenas*. Model selection for developmental duration (raw and logarithmic data) of all stages from $E_S = 32.5$ PSU and reared at $L_S = 32.5$ PSU in response to population (P), larval temperature (L_T) and female of origin (F). F is a random factor, nested in the interaction $P \times E_S$. The remaining two factors are fixed and form a 2-way factorial design. Model selection on random terms was carried out through restricted maximum likelihood (REML) fitting. Zoea II model kept F as a random part, while for other stages the best-fitted model was without any random interaction. Fixed effects were tested after maximum likelihood (ML) fitting. Model for raw zoea II data kept 2-way interaction, while all the other models were additive with both terms retained.

Model selection:		Raw				Logarithmic			
		Z II	Z III	Z IV	M	Z II	Z III	Z IV	M
Random (REML)									
F×L_T		98.45	133.06	149.23	149.96	-20.22	-20.83	-26.14	-45.58
F		**65.16**	110.82	130.47	130.30	**-55.32**	-49.42	-51.08	-72.82
No random term		65.99	**107.81**	**127.74**	**128.46**	-51.85	**-52.61**	**-54.45**	**-75.51**
Fixed (ML)	**Term removed**								
2-way (full model)		**49.53**	103.75	128.66	129.56	-120.16	-96.78	-99.08	-125.41
	P×L_T	51.18	99.70	122.72	121.15	-123.18	-103.59	-105.03	-131.34
Additive		-	99.70	122.72	121.15	-123.18	-103.59	-105.03	-131.34
	L_T	-	**177.87**	**210.50**	**240.68**	**-12.43**	**-16.15**	**-10.97**	**-6.19**
	P	-	**133.33**	**144.99**	**142.71**	**-92.82**	**-61.40**	**-77.89**	**-104.68**

Bold values = terms retained in the model.

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Table S3. *Carcinus maenas.* Model selection for individual dry mass (DM), carbon (C) and nitrogen (N) content (raw data, logarithmic data and instant growth) of megalopa in response to population (P), larval temperature (L_T), larval salinity (L_S) as fixed terms. Note that analysis is performed only for megalopae hatched at E_S = 32.5 PSU, while L_T = 15 °C and L_S = 20 PSU were excluded. Female is a random factor and was kept in the random structure of the model. Model selection of the fixed terms was carried out through maximum likelihood (ML) fitting.

Model selection:		Raw			Logarithmic			Instant growth		
		DW	N	C	DW	N	C	DW	N	C
Fixed (ML)	**Term removed**									
3-way (full model)		967.88	**267.84**	**659.62**	**-401.37**	**-433.72**	**-454.46**	**-1041.16**	**-1028.03**	**-1031.14**
	$P \times L_S \times L_T$	**969.32**[a]	271.46	669.48	-398.30	-428.98	-443.65	-1037.17	-1022.80	-1023.76

Bold values = terms retained in the final model.

a = 3-way interaction is not retained (ANOVA, not significant); 2-way interactions AICc checked with terms removed: $L_S \times L_T$=**972.11**; $P \times L_T$=**974.18**; $P \times L_S$=967.26; important 2-way interactions (bold) are kept in the model.

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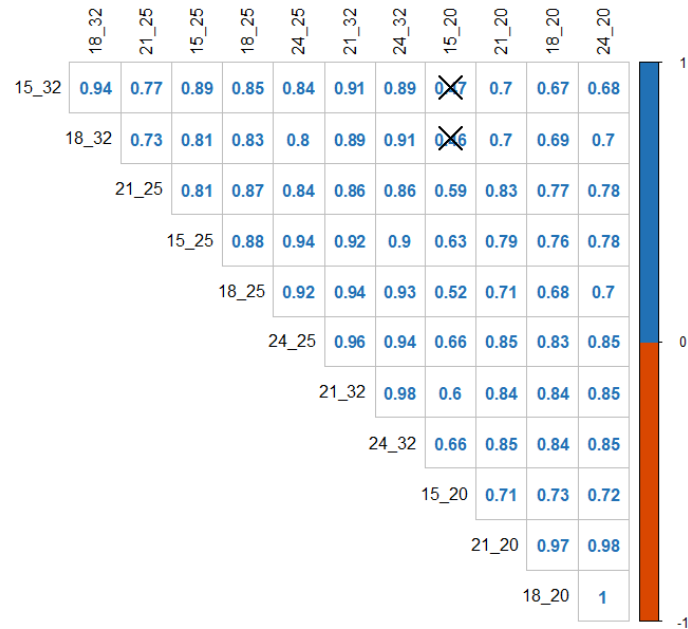
Table S4. *Carcinus maenas*. Model selection for dry mass (DW), carbon (C) and nitrogen (N) content (raw data, logarithmic data and instant growth) of North Sea population megalopa larvae in response to larval temperature (L_T) and larval salinity (L_S) as fixed factors. Female of origin is a random factor and was kept in the random structure of the model. Model selection on the fixed terms was carried out using maximum likelihood (ML) fitting.

Model selection:		Raw			Logarithmic			Instant growth		
		DW	N	C	DW	N	C	DW	N	C
Fixed (ML)	**Term removed**									
2-way (full model)		**804.52**	**226.73**	**555.73**	**-309.77**	**-343.34**	**-355.80**	**-854.69**	**-855.17**	**-846.31**
	$L_S{\times}L_T$	810.43	234.76	568.27	-300.45	-334.10	-341.76	-826.19	-822.50	-811.03

Bold values = terms retained in the final model.

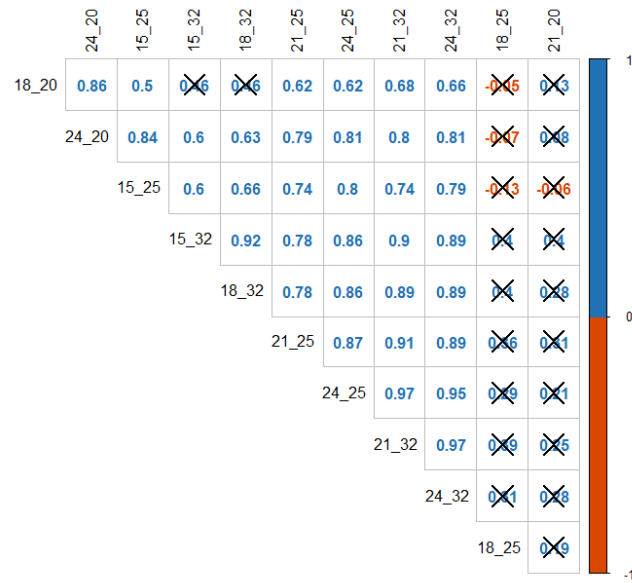Šargač Z, Giménez L, Harzsch S, Krieger J, Fjordside K, Torres G

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Table S5. Correlation among survival to zoea II for larvae hatched from different females at each factor combination (temperature and salinity). Non-significant correlations are crossed out. Notice that all correlations are positive, indicating that females producing larvae that have a high survival in a given combination treatment, also produce larvae with high survival in other combinations.

	18_32	21_25	15_25	18_25	24_25	21_32	24_32	15_20	21_20	18_20	24_20
15_32	0.94	0.77	0.89	0.85	0.84	0.91	0.89	~~0.7~~	0.7	0.67	0.68
18_32		0.73	0.81	0.83	0.8	0.89	0.91	~~0.6~~	0.7	0.69	0.7
21_25			0.81	0.87	0.84	0.86	0.86	0.59	0.83	0.77	0.78
15_25				0.88	0.94	0.92	0.9	0.63	0.79	0.76	0.78
18_25					0.92	0.94	0.93	0.52	0.71	0.68	0.7
24_25						0.96	0.94	0.66	0.85	0.83	0.85
21_32							0.98	0.6	0.84	0.84	0.85
24_32								0.66	0.85	0.84	0.85
15_20									0.71	0.73	0.72
21_20										0.97	0.98
18_20											1

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Table S6 Correlation among survival to megalopa for larvae hatched from different females at each factor combination (temperature and salinity). Non-significant correlations are crossed out. Notice that all significant correlations are positive, indicating that females producing larvae that have a high survival in a given combination treatment, also produce larvae with high survival in other combinations.

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Table S7. Credible interval 95% for the components of the random structure of multiple models for the effect of temperature and salinity on duration of development and survival from hatching to zoea II and to megalopa (separated by population). The models were factorial with temperature, salinity and female of origin as factors.

	Zoea II		Megalopa Helgoland		Megalopa Kerteminde	
	-95%CI	+95%CI	-95%CI	+95%CI	-95%CI	+95%CI
Develop. (D)	0.10	0.68	0.05	0.91	0.06	1.31
Cov (D,S)	-0.83	0.08	-0.73	0.68	-1.07	0.51
Survival (S)	0.28	1.98	0.12	2.41	0.13	2.30

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Table S8. Credible interval 95% for the components of the variance structure of multiple models for the effect of temperature and salinity on duration of development and survival from hatching to zoea II and to megalopa (separated by population). The models were factorial with temperature, salinity and female of origin as factors.

	Zoea II		Megalopa Helgoland		Megalopa Kerteminde	
	-95%CI	+95%CI	-95%CI	+95%CI	-95%CI	+95%CI
Develop. (D)	0.26	0.33	0.05	0.07	0.21	0.36
Cov (D,S)	-0.16	-0.08	-0.036	0.009	-0.19	-0.03
Survival (S)	0.27	0.36	0.22	0.32	0.42	0.68

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Table S9. Comparison of parameter estimates and output from credible interval 95% for bivariate and univariate models of the effect of temperature on duration of development to zoea II. Note that we did not include the parameter estimates of survival as our focus is on duration of development. Both analyses indicated an interactive effect of temperature and salinity on developmental time. In all cases but one (marked in bold and red) the outputs coincided on whether zero was in the credible interval.

Treatment combination	Parameter estimate		Zero in credible interval 95%	
Temperature-Salinity	Bivariate	Univariate	Bivariate	Univariate
15 C^o - 25‰	1.60	1.66	No	No
18 C^o - 25‰	-1.11	-1.19	No	No
21 C^o - 25‰	-1.75	-1.84	No	No
24 C^o - 25‰	-2.37	-2.45	No	No
15 C^o - 32‰	-0.70	-0.75	No	No
18 C^o - 32‰	0.04	0.11	Yes	Yes
21 C^o - 32‰	0.27	0.32	**Yes**	**No**
24 C^o - 32‰	0.73	0.79	No	No

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Table S10. Comparison of parameter estimates and output from credible interval 95% for bivariate and univariate models of the effect of temperature on duration of development to megalopa, for the Helgoland population. Note that we did not include the parameter estimates of survival as our focus is on duration of development. Both analyses indicated an interactive effect of temperature and salinity on developmental time. In all cases, the outputs coincided on whether zero was in the credible interval.

Treatment combination	Parameter estimate		Zero in credible interval 95%	
	Bivariate	Univariate	Bivariate	Univariate
15 C^o - 25‰	2.08	2.07	No	No
18 C^o - 25‰	-1.34	-1.36	No	No
21 C^o - 25‰	-2.56	-2.55	No	No
24 C^o - 25‰	-3.08	-3.08	No	No
15 C^o - 32‰	-1.01	-1.00	No	No
18 C^o - 32‰	0.17	0.19	Yes	Yes
21 C^o - 32‰	0.99	0.97	No	No
24 C^o - 32‰	0.98	0.96	No	No

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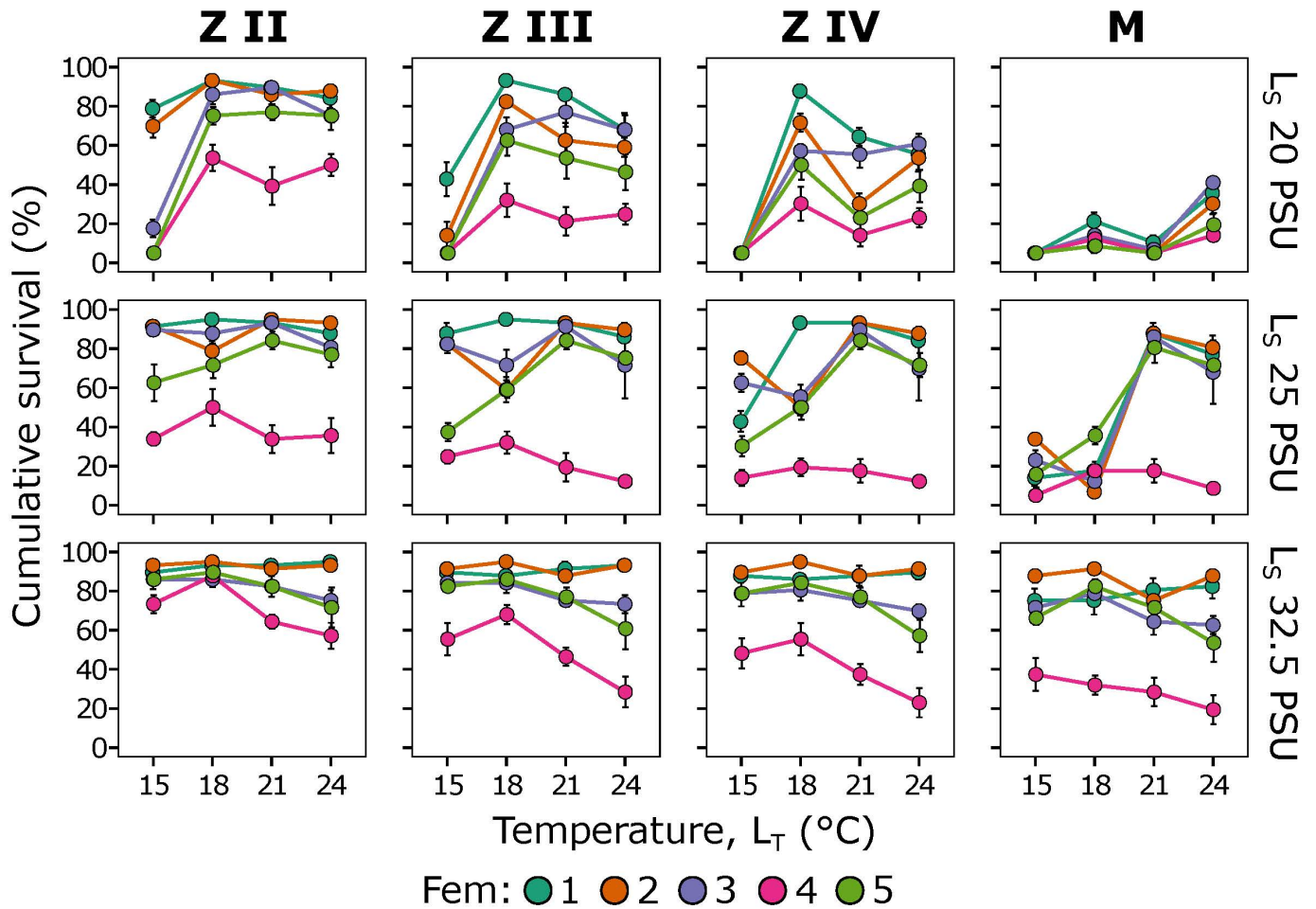
Table S11. Comparison of parameter estimates and output from credible interval 95% for bivariate and univariate models of the effect of temperature on duration of development to megalopa, for the Kerteminde population. Note that we did not include the parameter estimates of survival as our focus is on duration of development. In addition, treatment combinations considering T = 15 °C were not included because data on developmental time was not collected in sufficient amounts. Both analyses indicated an interactive effect of temperature and salinity on developmental time. In all cases, the outputs coincided on whether zero was in the credible interval.

Treatment combination	Parameter estimate		Zero in credible interval 95%	
	Bivariate	Univariate	Bivariate	Univariate
18 C^o - 25‰	1.42	1.44	No	No
21 C^o - 25‰	-1.40	-1.42	No	No
24 C^o - 25‰	-2.42	-2.43	No	No
18 C^o - 32‰	-0.78	-0.79	No	No
21 C^o - 32‰	0.82	0.76	No	No
24 C^o - 32‰	0.95	0.92	No	No

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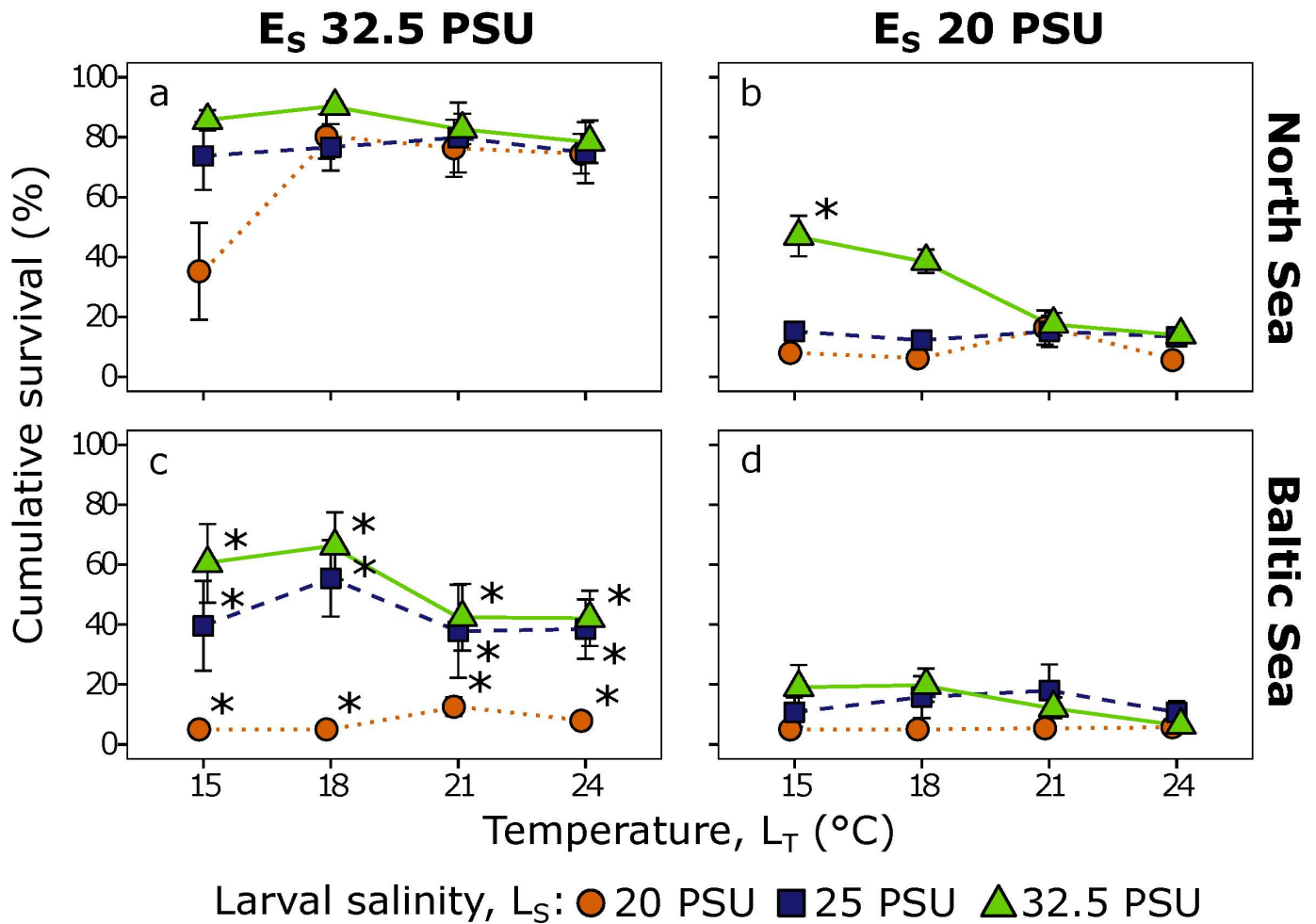

Figure S1. *Carcinus maenas*. Variation of larval survival among hatches from females from the North Sea population with embryonic salinity, E_S = 32.5 PSU. Symbols represent cumulative survival proportion to each larval stage (Z II-IV = zoea II-IV; M = megalopa) for each female (Fem) in different combinations of larval salinity, L_S (20 PSU = top panels, 25 PSU = middle panels, and 32.5 PSU = bottom panels) and temperature, L_T (15, 18, 21 and 24 °C). Data shown as mean values ± SE among replicates (n = 5) produced by each female (color-coded lines and symbols for larvae originating from Fem 1-5).

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E$_S$ 32.5 PSU   E$_S$ 20 PSU

Figure S2. *Carcinus maenas*. Average survival to zoea II. Comparison between populations (North Sea: a, b; Baltic Sea: c, d) hatching at different embryonic salinities (E$_S$) for twelve combinations of larval temperature (L$_T$) and salinity (L$_S$). Symbols represent each combination of factors per population. Data shown as mean values ± SE among larvae produced by different females (n = 5 or 3). Asterisks represent significant differences between populations for each combination of E$_S$ × L$_S$ × L$_T$.

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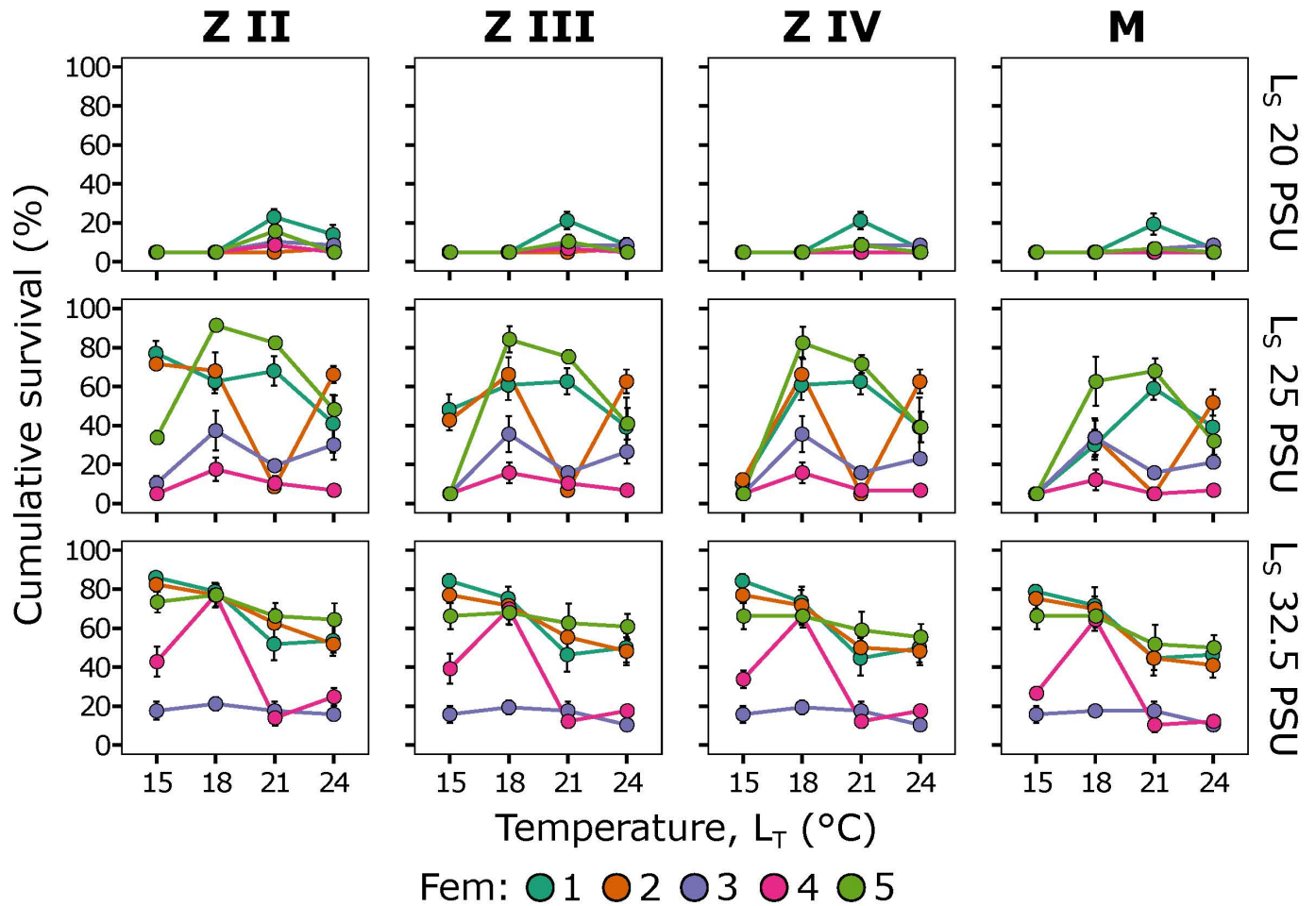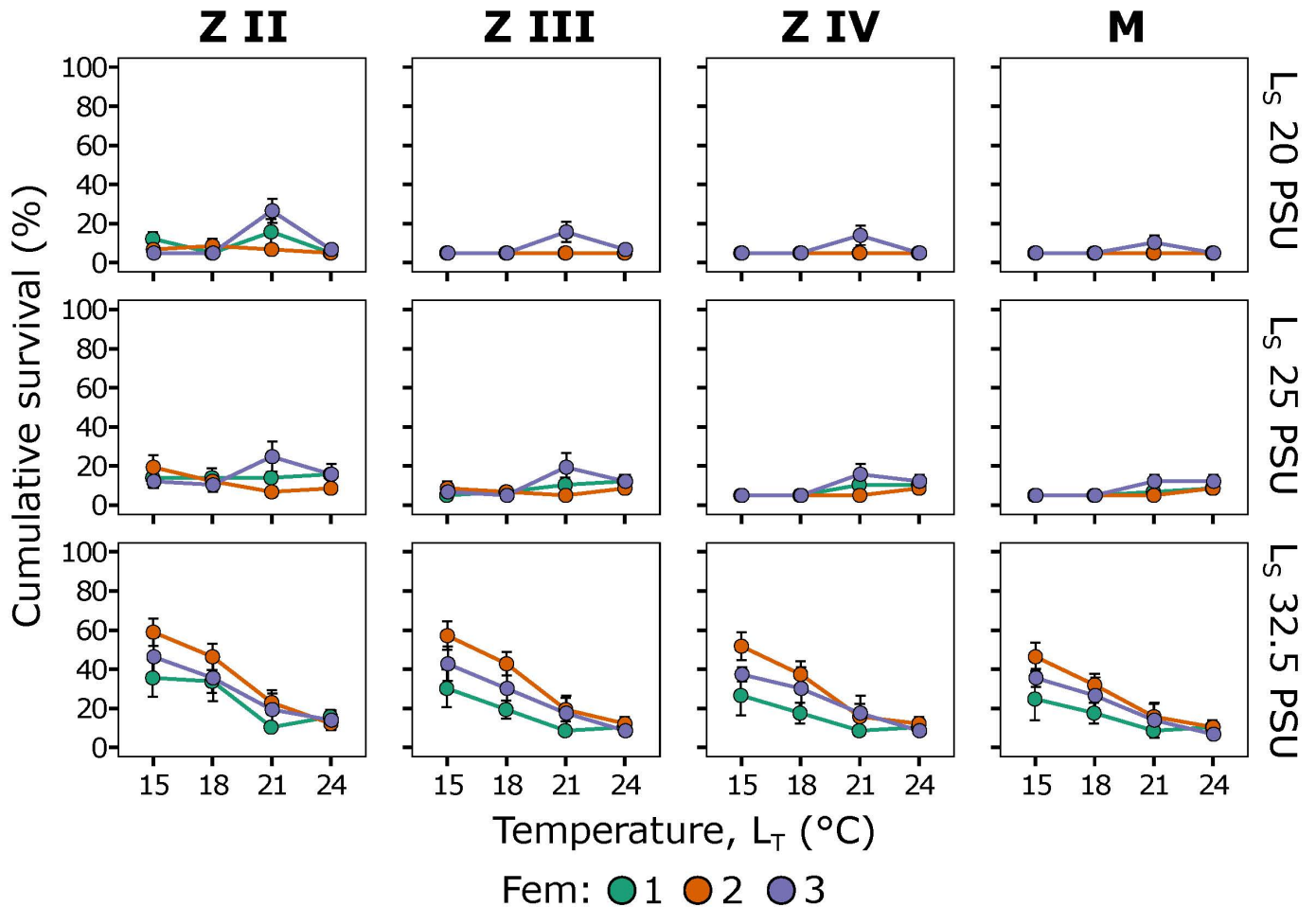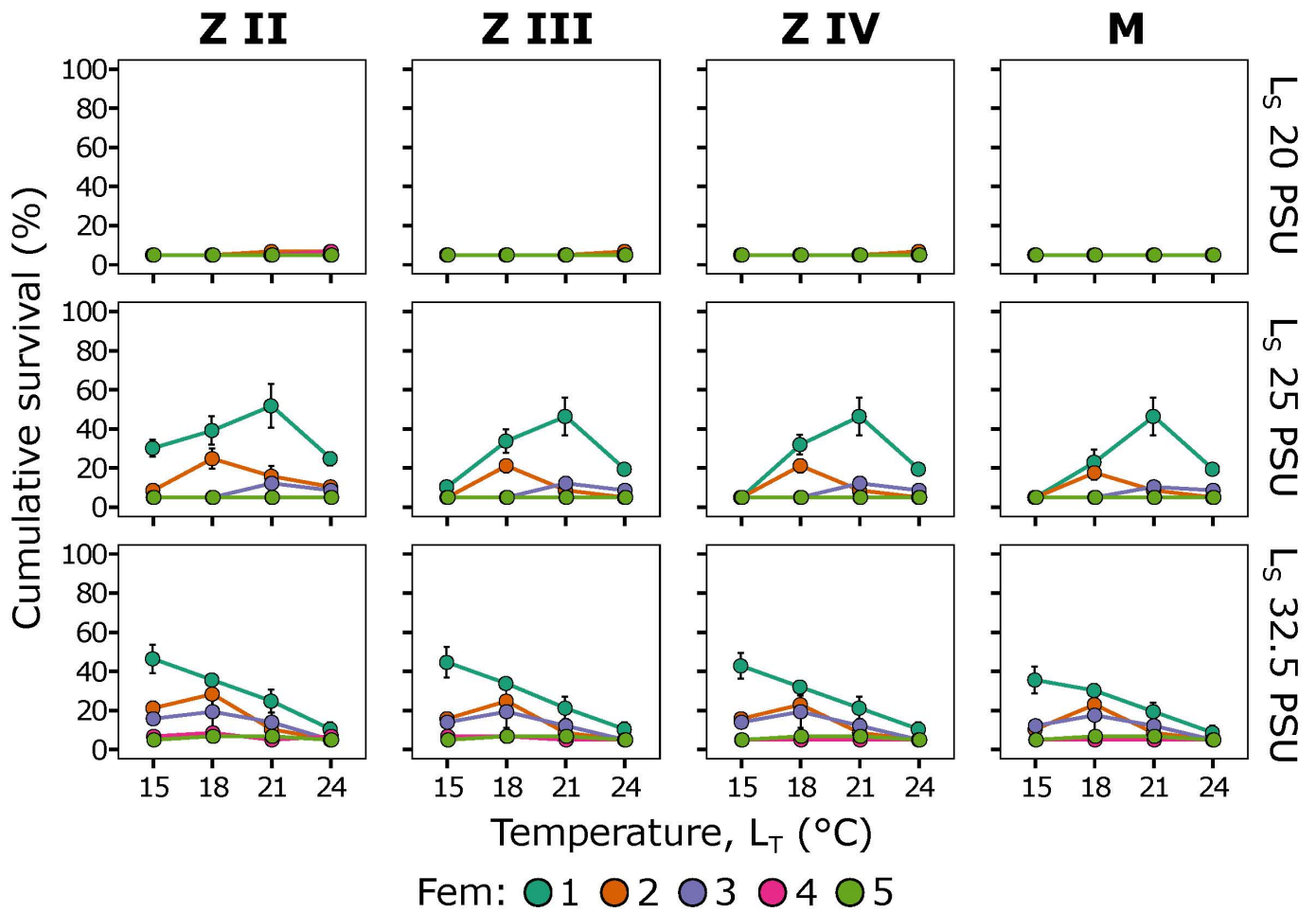

Figure S3. *Carcinus maenas*. Variation of larval survival among hatches from females from the Baltic Sea population with embryonic salinity, E_S = 32.5 PSU. Symbols represent cumulative survival proportion to each larval stage (Z II-IV = zoea II-IV; M = megalopa) for each female (Fem) in different combinations of larval salinity, L_S (20 PSU=top panels, 25 PSU = middle panels, and 32.5 PSU = bottom panels) and temperature, L_T (15, 18, 21 and 24 °C). Data shown as mean values ± SE among replicates (n = 5) produced by each female (color-coded lines and symbols for larvae originating from Fem 1-5).

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Figure S4. *Carcinus maenas*. Variation of larval survival among hatches from females from the North Sea population with embryonic salinity, E_S = 20 PSU. Symbols represent cumulative survival proportion to each larval stage (Z II-IV = zoea II-IV; M = megalopa) for each female (Fem) in different combinations of larval salinity, L_S (20 PSU = top panels, 25 PSU = middle panels, and 32.5 PSU = bottom panels) and temperature, L_T (15, 18, 21 and 24 °C). Data shown as mean values ± SE among replicates (n = 5) produced by each female (color-coded lines and symbols for larvae originating from Fem 1-3).

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Figure S5. *Carcinus maenas*. Variation of larval survival among hatches from females from the Baltic Sea population with embryonic salinity, E_S = 20 PSU. Symbols represent cumulative survival proportion to each larval stage (Z II-IV = zoea II-IV; M = megalopa) for each female (Fem) in different combinations of larval salinity, L_S (20 PSU = top panels, 25 PSU = middle panels, and 32.5 PSU = bottom panels) and temperature, L_T (15, 18, 21 and 24 °C). Data shown as mean values ± SE among replicates (n = 5) produced by each female (color-coded lines and symbols for larvae originating from Fem 1-5).

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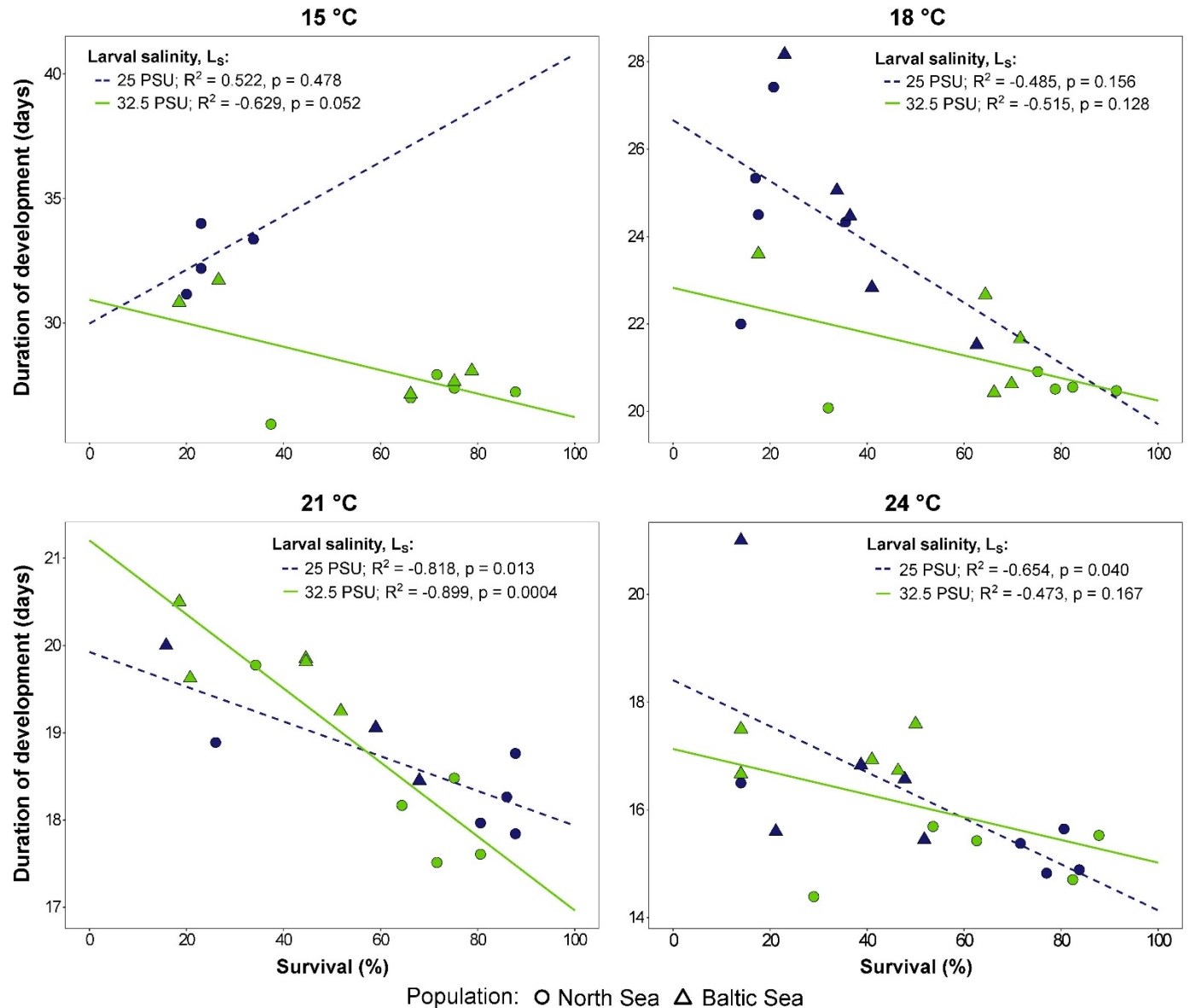

Figure S6. *Carcinus maenas*. Correlation of survival (%) and duration of development (days) for megalopae in different larval treatments. Note that L_S 20 PSU is excluded. Each symbol (circle = North Sea; triangle = Baltic Sea) represent female average for each combination of larval salinity (25 PSU = blue, 32.5 PSU = green) and temperature (15, 18, 21 and 24 °C). The determination coefficient (R^2) and p values were calculated for each treatment.

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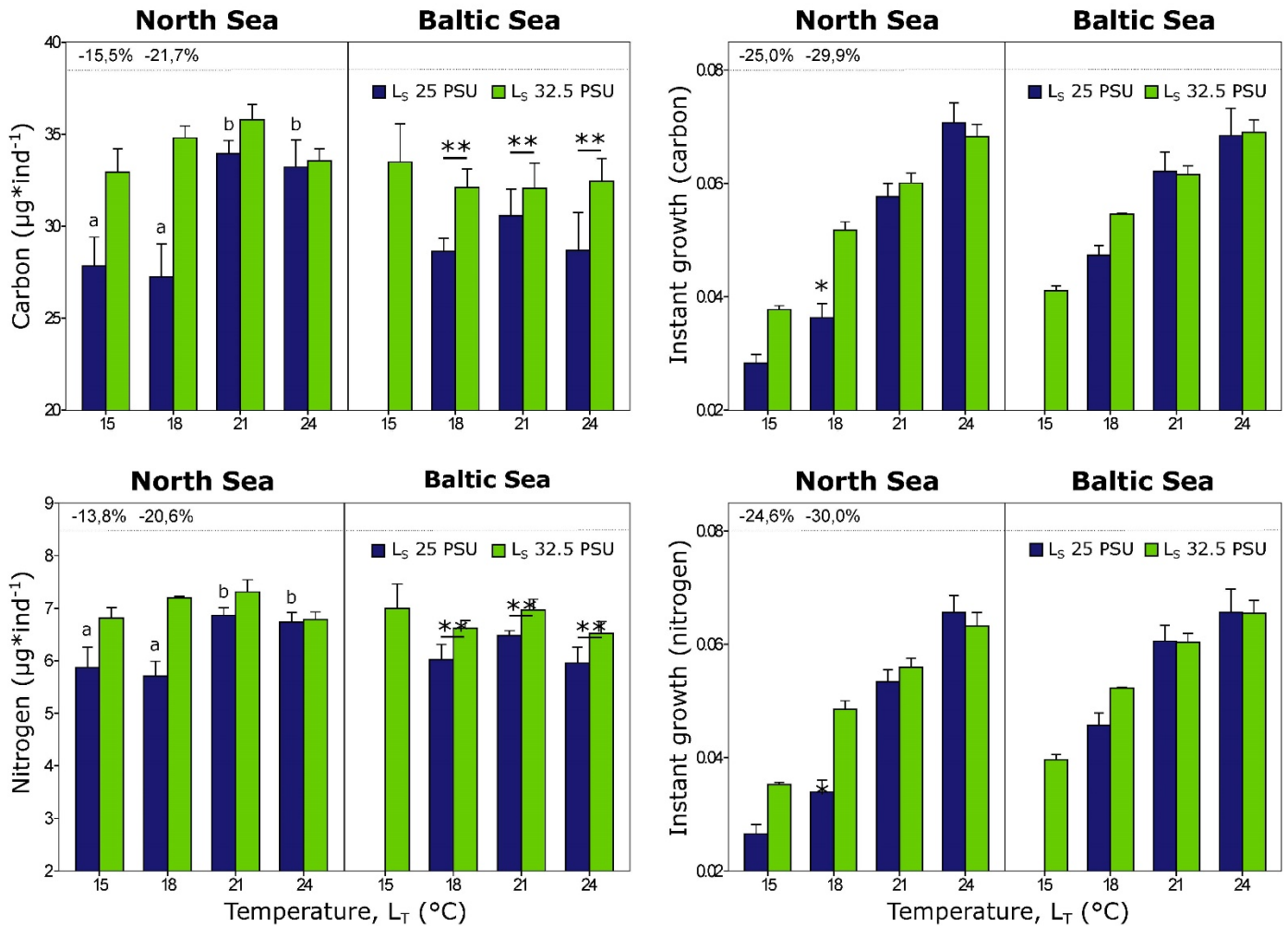

Figure S7. *Carcinus maenas*. Carbon (top panels) and nitrogen (bottom panels) content of megalopa and corresponding instantaneous growth rates from hatching to megalopa for two populations (North Sea *vs.* Baltic Sea) reared at two larval salinities (L_S: 32.5 PSU, green and 25 PSU, blue) and four temperatures (L_T: 15, 18, 21 and 24 °C). Data shown as average individual carbon or nitrogen content (µg*ind^{-1}) ± SE and average individual growth (µg*ind^{-1}day^{-1}) ± SE among larvae produced by different females (n = 5 for North Sea, n = 3 for Baltic Sea). Different letters represent significant difference for carbon or nitrogen content between temperature treatments (lowercased = L_S 25 PSU, capitalised = L_S 32.5 PSU). Percentage values on top of the panels represent significant differences between larval salinities in each population. * show significant difference in the instant growth rates between populations for the same treatment. ** show significant differences between larval salinities in the Baltic Sea population (additional three-way ANOVA analysis of larval salinity excluding 15 °C).

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Contrasting offspring responses to variation in salinity and temperature among coastal populations: a maladaptive ecological surprise?
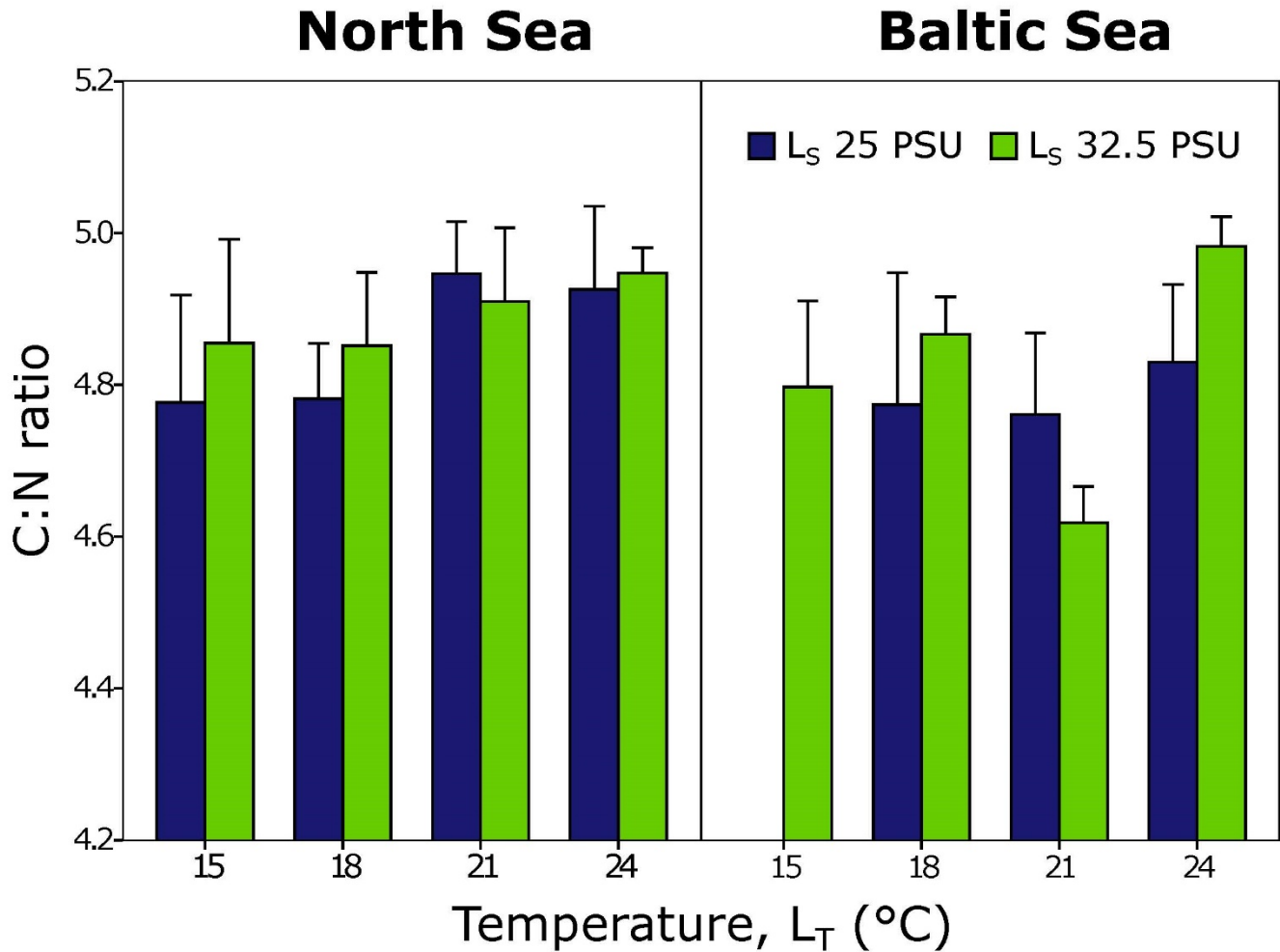

Figure S8. *Carcinus maenas*. Carbon/nitrogen (C:N) ratio for megalopa comparing two populations (left panel = North Sea, right panel = Baltic Sea) reared at two larval salinities, (L_S: 32.5 PSU, green and 25 PSU, blue) and four temperatures (L_T: 15, 18, 21 and 24 °C). Data shown as average C:N ratio ± SE among larvae produced by different females (n = 5 for North Sea, n = 3 for Baltic Sea).

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Contrasting offspring responses to variation in salinity and temperature among coastal populations: a maladaptive ecological surprise?

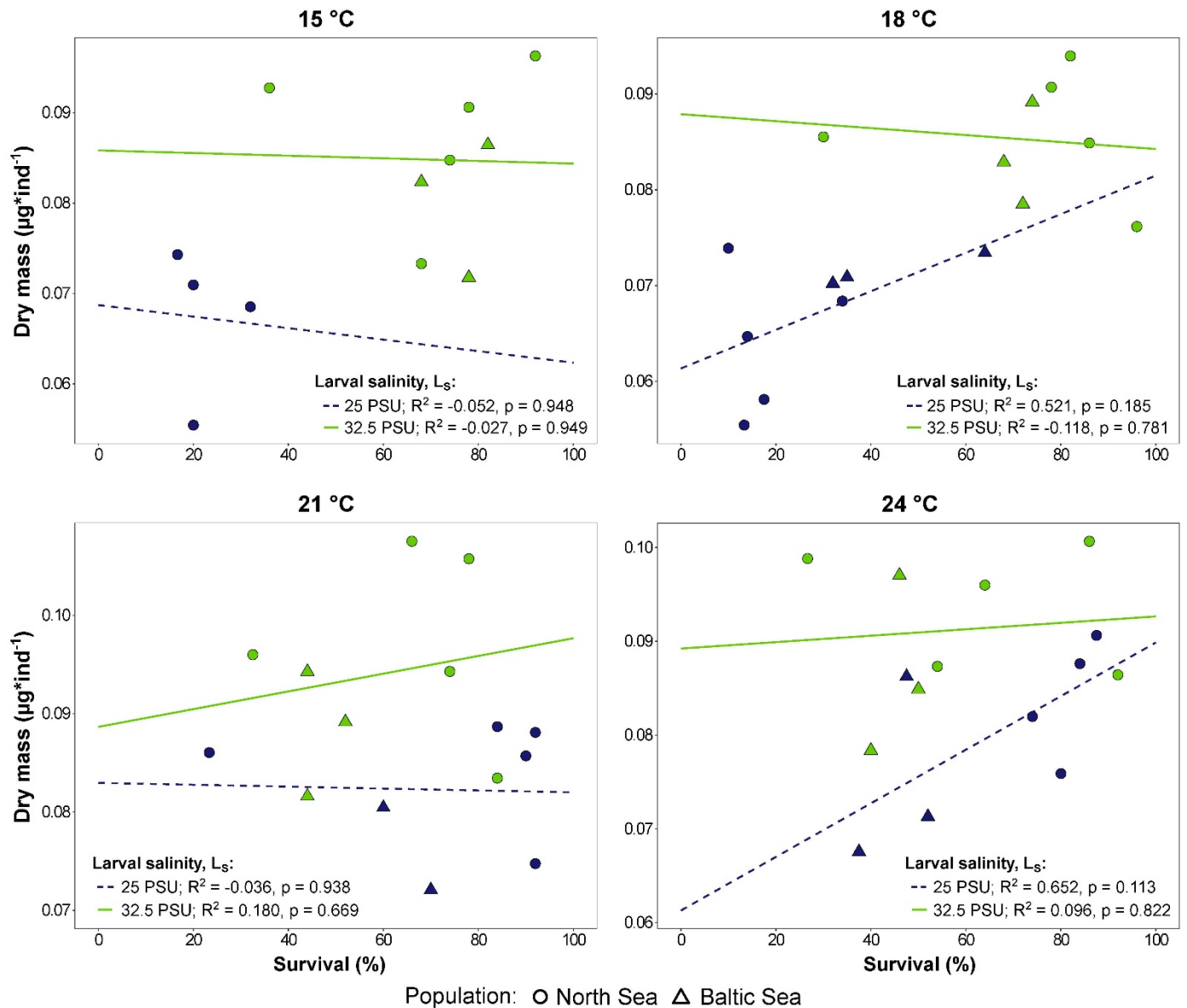**Figure S9.** *Carcinus maenas*. Correlation of survival (%) and dry mass (µg/ind) for megalopae in different larval treatments. Note that L_S 20 PSU is excluded. Each symbol (circle = North Sea; triangle = Baltic Sea) represent female average for each combination of larval salinity (25 PSU = blue, 32.5 PSU = green) and temperature (15, 18, 21 and 24 °C). The determination coefficient (R^2) and p values were calculated for each treatment.

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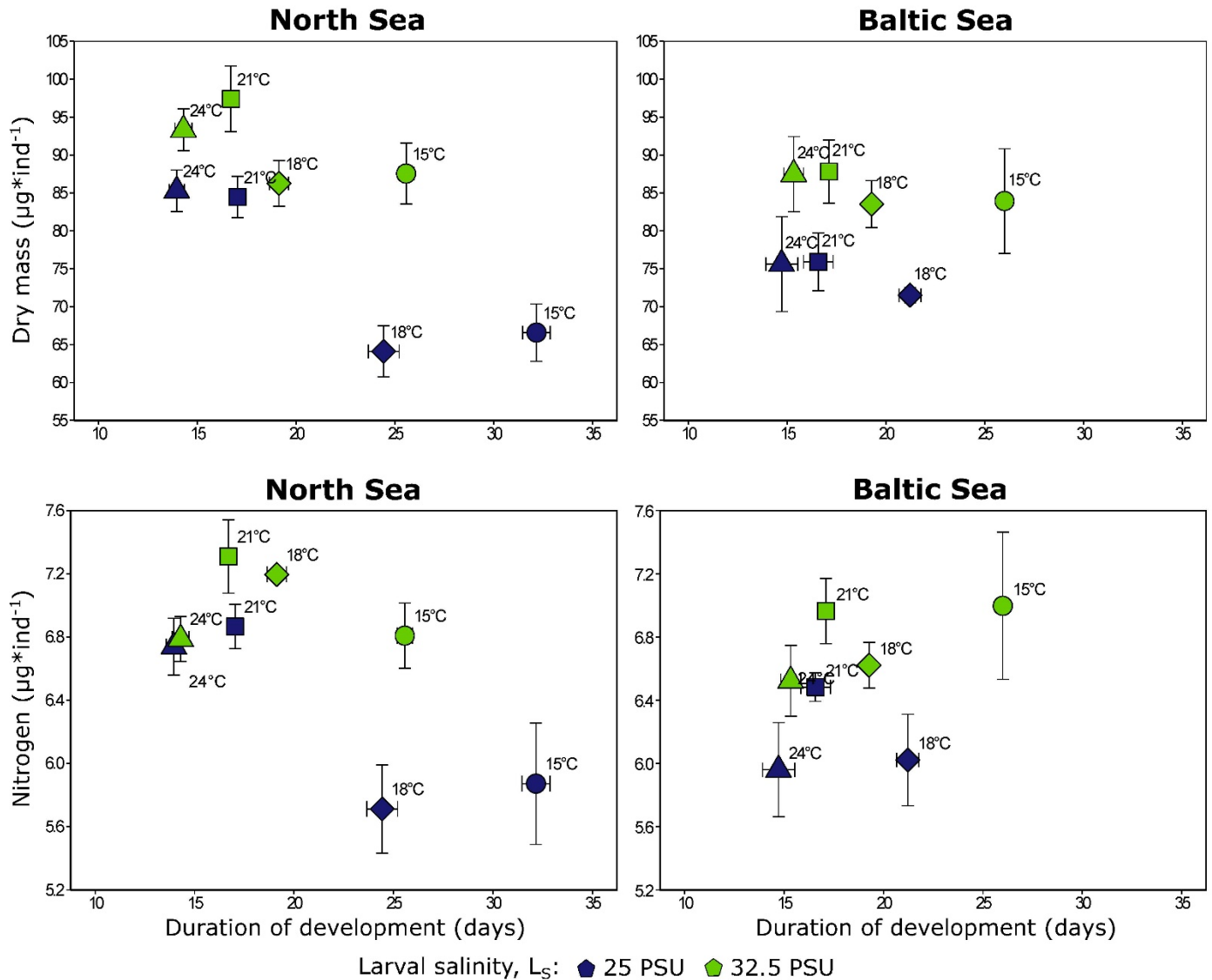

Figure S10. *Carcinus maenas*. Integrated responses of dry mass (top panels) and nitrogen (bottom panels) content and developmental time for megalopa presented for two populations (North Sea=left panels *vs* Baltic Sea = right panels) and different combination of larval temperatures, L_T (15 °C = circles, 18 °C = diamonds, 21 °C = squares, 24 °C = triangles) and larval salinities, L_S (25 PSU = blue, 32.5 PSU = green). Error bars represent ± SE among larvae produced by different females (n = 5 for North Sea, n = 3 for Baltic Sea).

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