Biogeosciences

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Supplement of

Decoupling salinity and carbonate chemistry: low calcium ion concentration rather than salinity limits calcification in Baltic Sea mussels

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Datasets

Dataset #1
Product name
BALTIC SEA PHYSICS ANALYSIS AND FORECAST
Product identifier
BALTICSEA_ANALYSIS_FORECAST_PHY_003_006
Link
https://resources.marine.copernicus.eu/?option=com_csw&task=results?option=com_csw&view=details&product_id=BALTICSEA_ANALYSIS_FORECAST_PHY_003_006   last accessed 29/08/2017
Short description
This Baltic Sea physical model product provides forecasts for the physical conditions in the Baltic Sea. The Baltic forecast is updated twice daily providing a new six days forecast with hourly data for sea level variations, ice concentration and thickness at the surface, and temperature, salinity and horizontal velocities for the 3D field. The product is produced by the 3D ocean model code HBM developed within the Baltic ocean community. The product grid has a resolution of 1 nautical mile in the horizontal, and up to 25 vertical depth levels. The area covers the Baltic Sea including the transition area towards the North Sea (i.e. the Danish Belts, the Kattegat and Skagerrak).
Spatial resolution
2 km x 2 km
Vertical coverage
From -400 to 0 (25 levels)
Temporal resolution
Hourly – instantaneous, daily – mean, monthly - mean
Update frequency
2 x daily
Production unit
BAL-DMI-COPENHAGEN-DK; BAL-BSH-HAMBURG-GE

Supplementary Tables

Table S1: Constituent chemicals added to solution to make calcium free artificial seawater (CFASW) after Kester, 1967. Mass of each chemical added was adjusted for the

Salt	Molecular weight	g/L at respective salinity			
		35	16	11	6
NaCl	58.44	23.9260	10.9376	7.5196	4.1016
Na$_2$SO$_4$	142.04	4.0080	1.8322	1.2597	0.6871
MgCL$_2$	95.21	2.37	1.0834	0.7449	0.4063
KCl	74.56	0.6770	0.3095	0.2128	0.1161
NaHCO$_3$	84.00	0.1960	0.0896	0.0616	0.0336
KBr	119.01	0.0980	0.0448	0.0308	0.0168
H$_3$BO$_3$	61.83	0.0260	0.0119	0.0082	0.0045
SrCl$_2$	158.51	0.0085	0.0039	0.0027	0.0015
NaF	41.99	0.0030	0.0014	0.0009	0.0005

experimental salinities in this study (16, 11 and 6). All stock solutions also contained 5 % filtered seawater from the collections site to ensure presence of trace elements.

Table S2: Mean experimental biomass densities and food availability over the duration of both experiments. Metrics presented include: aquarium volumes, mean animal no. per tank, mean mortality rates (no mortality was observed in the calcium experiment), ml of seawater per animal, body dry mass (BM) per animal, total BM per tank, BM L^{-1} seawater, mean no. of feeds day^{-1}, final phytoplankton concentration in experimental vessels after feeding, total no. of phytoplankton cells day^{-1} and the no. of cells mg^{-1} BM day^{-1}. Mean values are presented across treatments (n = 4 replicates) over the duration of both experiments. The two most important metrics, BM litre^{-1} and no. of phytoplankton cells mg^{-1} BM day^{-1} were not identical, but within the same order of magnitude for both experiments. For comparison, inter-treatment range values are given below experimental means within the bicarbonate ion manipulation experiment, illustrating BM litre^{-1} and phytoplankton cell no. mg^{-1} BM day^{-1} varied by a larger degree within the bicarbonate experiment than between both experiments, further supporting comparability between both experiments.

Experiment	Aquarium volume (ml)	no. animals per tank	mortality rate end of exp.	ml per animal	BM per animal (mg)	total BM per tank (mg)	**BM per litre (mg l^{-1})**	no. of feeds per day	Phytoplankton cell conc. in tanks (cells ml^{-1})	total no. of cells per day	**no. of cells mg^{-1} BM day^{-1}**
Calcium manipulation	50	2	N/A	25.0	0.329	0.658	**13.2**	1.0	10 000	5 x 10^5	**7.6 x 10^5**
Bicarbonate manipulation	2000	752 *(1440 - 400)*	53 % *(10 – 75 %)*	1.3 *(1.4 - 5)*	0.064 *(0.163 – 0.746)*	48.128 *(65.2 – 1074.2)*	**24.06** *(32.6 – 537.1)*	2.5	10 000	5 x 10^7	**4.9 x 10^5** *(4.7 x 10^4 – 7.7 x 10^5)*

Table S3: List of all R packages used for data analysis complete with versions, years, authors and intended use.

Package	Complete name	Version	Year	Author	Use
fitdistrplus	Fit distribution	1.0-14	2019	Marie Laure Delignette-Muller, Christophe Dutang (2015)	Fitting data distributions
ggplot2	Elegant graphics for data analysis	3.3.0	2016	Wickham H (2016)	Graphics
nlme	Linear and non-linar mixed effect models	3.1-147	2020	Pinheiro J, Bates D, Debroy S, Sarkar D, R Core Team (2020)	Fit and compare models
drc	Dose response analysis	3.0-1	2016	Ritz, C., Baty, F., Streibig, J. C., Gerhard, D. (2015)	Analysis of dose-response curves
ncdf4	Interface to Unidata netCDF files	1.17	2019	David Pierce (2010)	Read data from netCDF files
cmocean	cmocean	0.2	2019	Thyng, K., Richards, C. and Krylov, I.,	Colour maps for oceanography

Table S4: **Model parameters for the power relationship between shell length and CaCO$_3$ mass with statistical results; standard error, T value and p value (Fig. S3). These separate relationships were used to calculate calcification rates in the field from shell length measurements for each population.**

Parameter	Site	value	Std. Error	T-value	p-value
intercept	Usedom	0.171	0.032	5.36	**< 0.001**
	Ahrenshoop	0.050	0.012	4.29	**< 0.001**
	Kiel	0.036	0.004	8.17	**< 0.001**
power	Usedom	2.163	0.092	23.64	**< 0.001**
	Ahrenshoop	2.716	0.107	25.43	**< 0.001**
	Kiel	3.000	0.046	65.24	**< 0.001**

Table S5: Model comparisons using AIC for selection of the most parsimonious negative exponential decay model fit to laboratory calcification rates across experimental [HCO$_3^-$] in the bicarbonate ion manipulation experiment. Model names and equations are shown with fixed parameters allowing direct comparison between salinity treatments. The lowest AIC value (in bold) represents the model which best explains the experimental data and was chosen for statistical analysis.

Model	Equation	Fixed parameters	Estimated parameters	AIC
Von Bertalanffy	y~C$_{max}$*(1-e(-K*(x-s$_0$)))	s$_0$ = 380	C$_{max}$, K	**115.58**
Gompertz	y~C$_{max}$*(1+s$_0$*(e(-K*x)))$^{-1}$	s$_0$ = 380	C$_{max}$, K	116.49
Logistic	y~C$_{max}$*(1+s$_0$*(e(-K*x)))$^{-1}$	s$_0$ = 380	C$_{max}$, K	115.79
Negative exponential	y~C$_{max}$*(1-e(x/K))		C$_{max}$, K	121.82
Michaelis-Menten	y~C$_{max}$*x/(K+x)		C$_{max}$, K	122.21

Table S6: Statistical test results of all analyses graphically depicted in Figs 2 - 7. Pairwise comparisons (*post-hoc* tests) between treatments for statistically significant factors are listed on the right with significant *P*-values being shown in bold.

ANCOVA – laboratory calcification rates [Ca^{2+}] manipulation experiment (Fig. 5b)							
factor	*df*	*Sum Sq.*	*Mean Sq.*	*F-value*	*P-value*	*pair-wise comparisons*	*P-value*
Calcium	1	7160	7160	106.9	**< 0.001**	11-16	0.988
Salinity	2	110.9	55.5	0.83	0.442	6-16	0.559
Calcium:Salinity	2	452.4	226.2	3.38	**0.041**	6-11	0.47
Residuals	54	3616	67				
Negative exponential decay model – Laboratory calcification and $\Omega_{aragonite}$ (Fig. 6a)							
parameter	*estimate*	*Std. Error*	*T-value*	*P-value*			
C_{max}	31.2315	5.244	5.956	**< 0.001**			
K	0.374	0.1598	2.341	**0.027**			
Negative exponential decay model – Laboratory calcification and ESIR (Fig. 6b)							
parameter	*estimate*	*Std. Error*	*T-value*	*P-value*			
C_{max}	33.7696	5.9676	5.659	**< 0.001**			
K	0.2752	0.1117	2.463	**0.020**			
ANCOVA – Field calcification rates (Fig. 7)							
factor	*df*	*Sum Sq.*	*Mean Sq.*	*F-value*	*P-value*	*pair-wise comparisons*	*P-value*
time	1	952304	952304	517.7	**< 0.001**	Kie:Ahp	**< 0.001**
population	2	2096837	1048419	570.0	**< 0.001**	Use:Ahp	0.99
time:population	2	1769631	884815	491.0	**< 0.001**	Use:Kie	**< 0.001**
Residuals	12	30873	2573				
ANOVA – Mean salinity at field monitoring sites (Fig. 2)							
factor	*df*	*Sum Sq.*	*Mean Sq.*	*F-value*	*P-value*	*pair-wise comparisons*	*P-value*
site	2	1248	624.2	38518	**< 0.001**	Ahp-Kie	**< 0.001**
residuals	17253	279.6	0.02			Ahp-Use	**< 0.001**
						Kie-Use	**< 0.001**
ANOVA – Mean [HCO$_3^-$] at field monitoring sites (Fig. 3c)							
factor	*df*	*Sum Sq.*	*Mean Sq.*	*F-value*	*P-value*	*pair-wise comparisons*	*P-value*

factor	df	Sum Sq.	Mean Sq.	F-value	P-value	pair-wise comparisons	P-value
site	2	414716	207358	38.80	**< 0.001**	Ahp-Kie	**< 0.001**
residuals	55	293930	5344			Ahp-Use	0.722
						Kie-Use	**< 0.001**

ANOVA – Mean pH at field monitoring sites (Fig. 3a)

factor	df	Sum Sq.	Mean Sq.	F-value	P-value		
site	2	0.092	0.046	1.217	0.304		
residuals	55	2.070	0.038				

ANOVA – Salinity-A_T relationship across all field monitoring sites (Fig. 4)

factor	df	Sum Sq.	Mean Sq.	F-value	P-value		
salinity	1	571206	571206	86.6	**< 0.001**		
residuals	56	369521	6599				

ANOVA – Mean $\Omega_{aragonite}$ at field monitoring sites (Fig. 3e)

factor	df	Sum Sq.	Mean Sq.	F-value	P-value	pair-wise comparisons	P-value
site	2	1.89	0.94	7.22	**0.002**	Ahp-Kie	**0.044**
residuals	55	7.18	0.13			Ahp-Use	**0.002**
						Kie-Use	0.654

ANOVA – Mean ESIR at field monitoring sites (Fig. 3g)

factor	df	Sum Sq.	Mean Sq.	F-value	P-value	pair-wise comparisons	P-value
site	2	3.98	1.99	10.88	**< 0.001**	Ahp-Kie	**0.020**
residuals	55	10.07	0.18			Ahp-Use	0.363
						Kie-Use	**< 0.001**

ANOVA - Mean Chl-a values at field monitoring sites (Fig. 3i)

factor	df	Sum Sq.	Mean Sq.	F-value	P-value	pair-wise comparisons	P-value
site	2	11.41	5.7	13.8	**< 0.001**	Ahp-Kie	**< 0.001**
residuals	77	31.82	0.41			Ahp-Use	**< 0.001**
						Kie-Use	0.35

Table S7: Model parameters and R^2 values for the linear relationships: Salinity-A_T and $\Omega_{aragonite}$-ESIR. Also shown are the parameter estimates and residual sum of squares (RSS) for the negative exponential decay model fit to laboratory calcification rates in both experiments and substrate inhibitor ratio (SIR) not including $[Ca^{2+}]$.

Linear model – Salinity-A_T (Fig. 4)					
factor	*estimate*	*Std. Error*	*T-value*	*P-value*	*R^2*
intercept	1679.9	32.4	51.9	**<0.001**	0.6
slope	22.13	2.4	9.3	**<0.001**	
Linear model – Field $\Omega_{aragonite}$-ESIR relationship (Fig. S8)					
factor	*estimate*	*Std. Error*	*T-value*	*P-value*	*R^2*
intercept	0.337	0.049	6.89	**<0.001**	0.805
slope	0.781	0.051	15.39	**<0.001**	
Negative exponential decay model – Laboratory calcification and SIR (Fig. S5)					
parameter	*estimate*	*Std. Error*	*T-value*	*P-value*	*RSS*
C_{max}	22.66	2.75	8.23	**<0.001**	4631
K	0.02	0.01	1.45	0.157	

Table S8: Parameters for the linear model fit to $[Ca^{2+}]$ and calcification rates in the calcium ion manipulation experiment and the negative exponential decay model fit to $[HCO_3^-]$ and calcification rates in the bicarbonate manipulation experiment. Significant *p*-values are shown in bold and graphical representations of these models are depicted in Fig. 5.

Linear model parameters – $[Ca^{2+}]$-calcification					
salinity	*treatment*	*value*	*Std. Error*	*T-value*	*P-value*
6	intercept	7.21	3.84	1.88	0.065
11		2.42	5.44	-0.88	0.382
16		-2.69	2.33	1.66	0.102
6	slope	1.67	1.67	3.9	**< 0.001**
11		5.56	2.33	-1.78	0.081
16		2.37	2.37	2.57	**0.013**
Negative exponential decay model parameters - $[HCO_3^-]$-calcification					
salinity	*treatment*	*value*	*Std. Error*	*T-value*	*P-value*
6	C_{max}	4.68	2.03	2.31	**< 0.001**
11		34.00	2.07	16.39	**< 0.001**
16		35.25	1.78	19.79	**< 0.001**
6	*K*	0.007	0.008	0.843	**< 0.001**
11		0.007	0.001	5.966	**< 0.001**
16		0.011	0.002	6.106	**< 0.001**

Table S9: Parameters for negative exponential decay models fit to calcification rates (µg CaCO$_3$ d^{-1}) and [Ca^{2+}] and [HCO$_3^-$] predictors across both laboratory experiments. Parameters with significant estimates have *P*-values in bold.

| Negative exponential model – [Ca^{2+}] – calcification (Fig. S4b)				
parameter	*value*	*Std. Error*	*T-value*	*P-value*
C_{max}	47.29	30.18	1.57	0.128
K	5.10	4.89	1.04	0.306
Negative exponential model – [HCO$_3^-$] – calcification (Fig. S4a)				
parameter	*value*	*Std. Error*	*T-value*	*P-value*
C_{max}	25.00	6.49	3.85	**< 0.001**
K	740.38	618.65	1.20	0.241

Table S10: **Parameters and statistical results for the linear model fit to field calcification over time during the first 7 months. The linear slopes of these models express the calcification rates for each of the 3 populations (Table 1).**

| Linear model - Field calcification rates over first 7 months.					
salinity	*parameter*	*value*	*Std. Error*	*T-value*	*P-value*
Usedom	intercept	-495.0	7657.9	-0.07	0.953
Ahrenshoop		-457.5	7657.9	-0.08	0.938
Kiel		-116675.0	5414.9	-15.24	**< 0.001**
Usedom	slope	18.6	53.2	-0.33	0.761
Ahrenshoop		36.4	53.2	0.97	0.405
Kiel		2202.9	37.6	40.70	**< 0.001**

Table S11: Statistical results for the Kruskal-Wallis test comparing temperatures (°C) at each site during the monitoring period.

| Kruskal-Wallis test – Mean temperatures at field monitoring sites (Fig. S7)				
Kruskal-Wallis chi squared	*df*	*P-value*	*Dunn test*	*P-value*
122.73	2	<0.001	Ahp-Kie	**< 0.001**
			Ahp-Use	**0.042**
			Kie-Use	**< 0.001**

Supplementary Figures
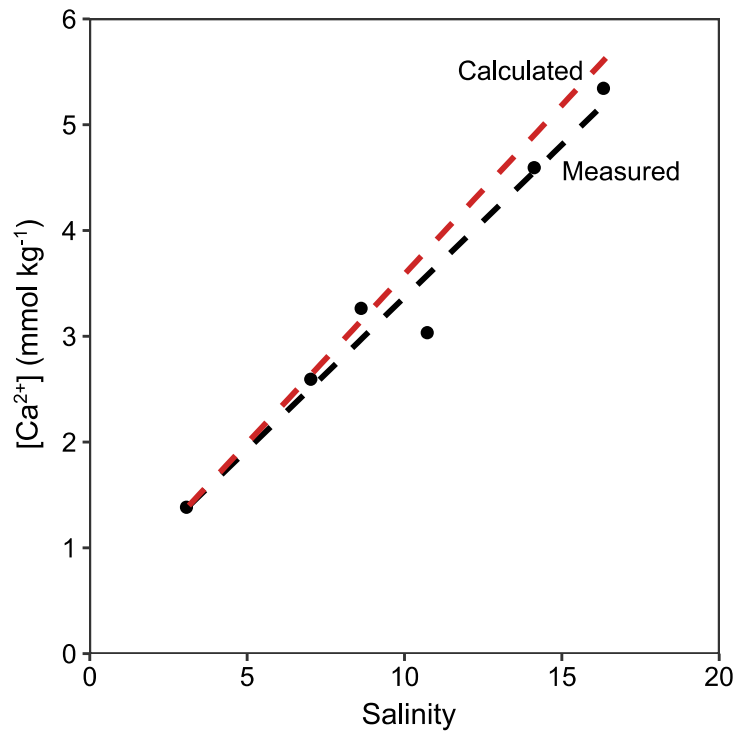
Figure S1: Comparison of the linear relationship between salinity and [Ca^{2+}] in the Southwest Baltic Sea from calculated [Ca^{2+}] values (red dashed line) and measured [Ca^{2+}] values (blue dashed line). Methodologies for calculated and measured [Ca^{2+}] values are given in the text (Sections 2.1 and .5). Samples for the lowest salinity (3.15) were taken from the Achterwasser in Usedom (54° 0' 5'' N, 14° 2' 47'' E).
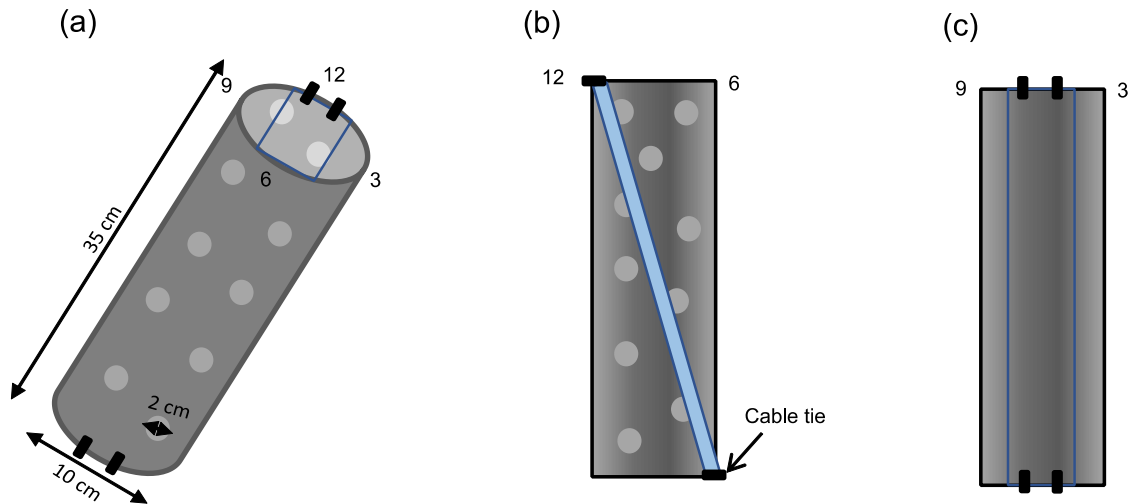
Figure S2: Settlement structures deployed at all three sites (Kiel, Ahrenshoop and Usedom) in March 2016 (a). A cross section of the settlement structures from 12 o'clock to 6 o'clock showing the orientation of the mesh net inside the cylinder (b). Cross section of the settlement structures showing the width of the mesh net inside the cylinder (c). A total of twenty x 2 cm diameter holes were drilled into each cylinder and a 0.2 cm pore size cotton/nylon spat sock was diagonally positioned across the inside of the cylinders using cable ties. Numbers at the top of each figure represent the orientation of the cross sections in panels b and c.
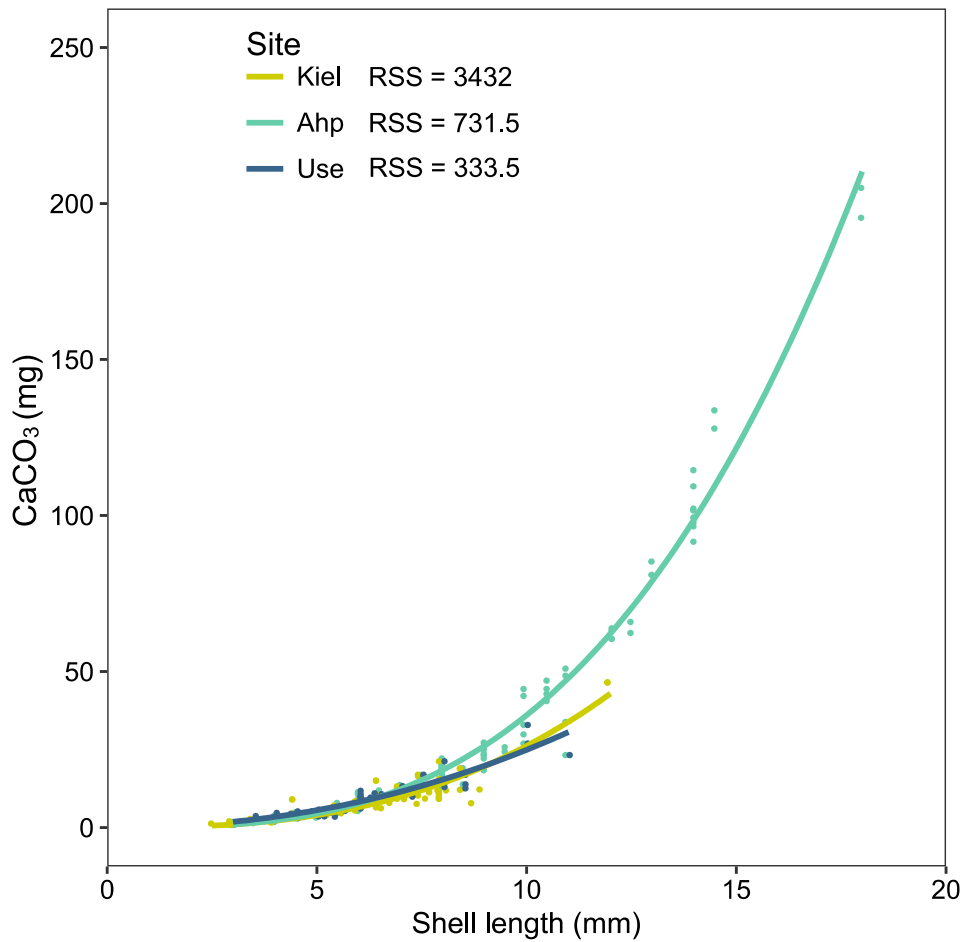
Figure S3: SL-CaCO₃ mass (mg) relationships specific to each of the three study populations: Kiel, Ahrenshoop and Usedom (data from Sanders et al., 2018). This was used to calculate initial CaCO₃ mass in laboratory experiments and field calcification at the three monitoring sites in this study. Power model parameters are given in Table S4 with the insets listing the residual sum of squares (RSS) for each population.
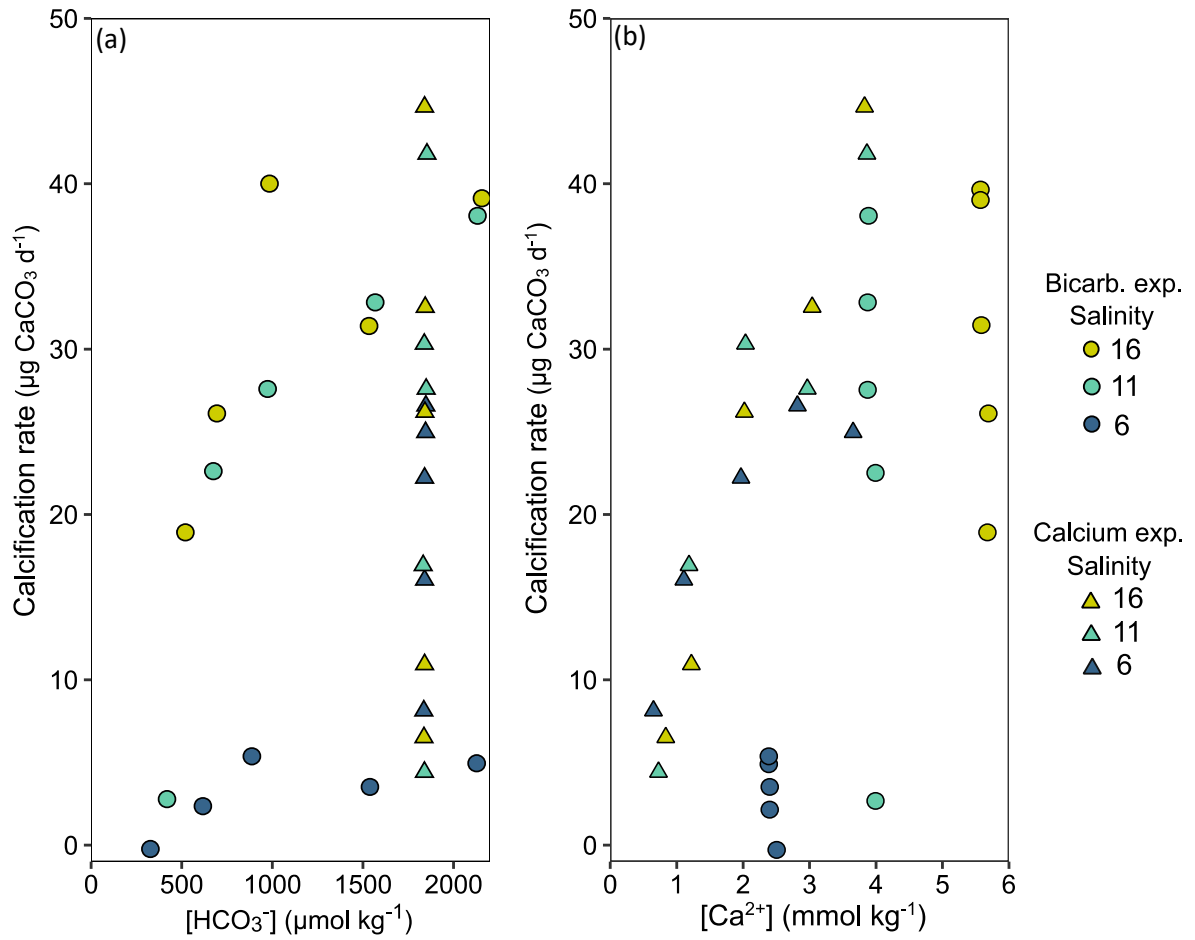
Figure S4: Calcification rates at all salinities from both laboratory experiments plotted across all [HCO₃⁻] (a) and all [Ca²⁺] (b). No significant relationship was found for either variable.
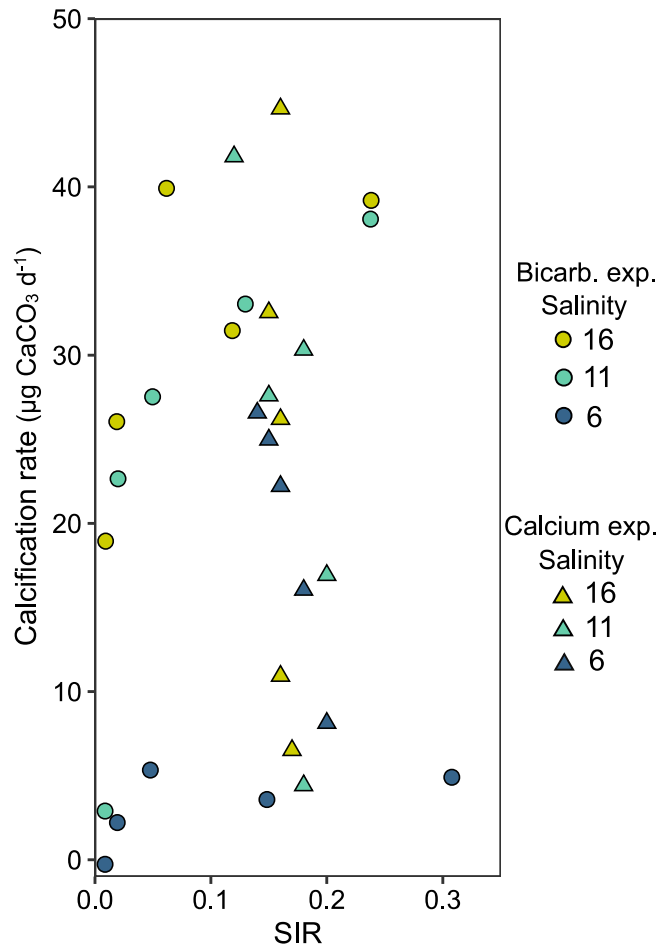
Figure S5. Calcification rates at all salinities from both laboratory experiments plotted across substrate inhibitor ratio (SIR). This measure excludes the effect of [Ca^{2+}] and no statistically significant model could be fit (Table S7).
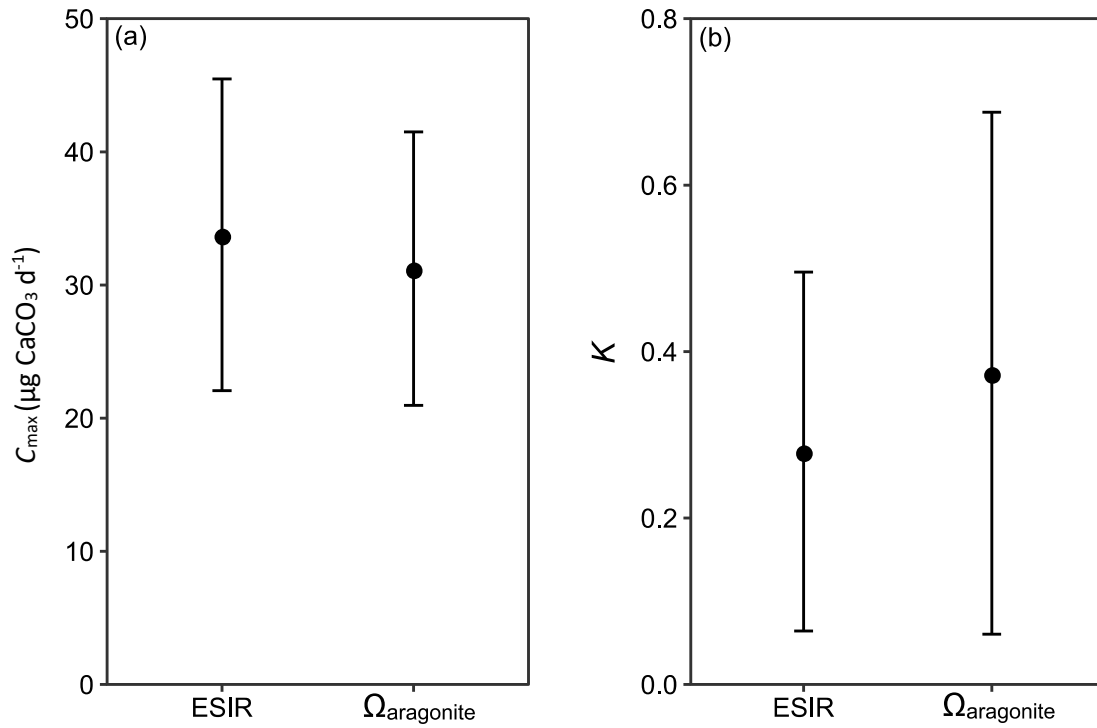
Figure S6: Graphical comparisons of parameters C_{max} (µg CaCO$_3$ d^{-1}) and K in the negative exponential decay model depicted in Fig. 6. Values are shown ± 95 % confidence intervals (CI). Overlaps in the CI indicates the two parameters in both models are not significantly different.
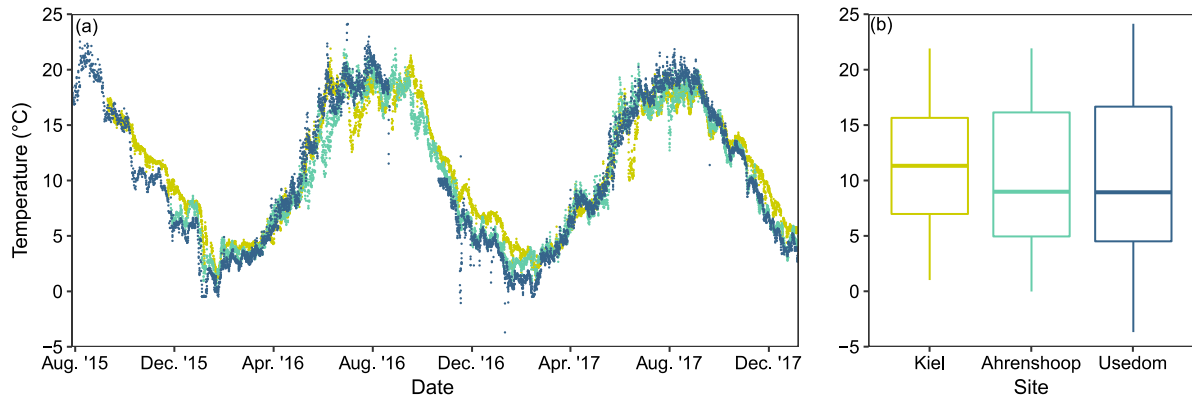
Figure S7: Temperature (°C) data from deployed CTD's at the three monitoring sites from Aug. 2015 - Dec. 2017 (a). Box pots are shown on the right depicting median temperatures and interquartile ranges (b).
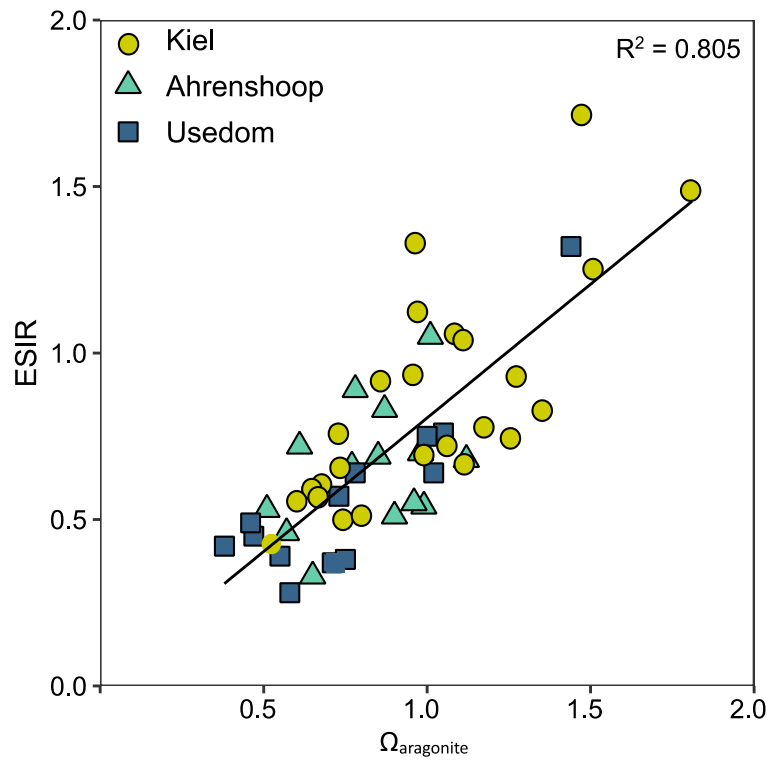
Figure S8: Field extended substrate inhibitor ratio (ESIR) plotted against aragonite saturation state ($\Omega_{aragonite}$) over the monitoring period at all three Baltic Sea sites. Parameters for the linear model are given in Table S7.
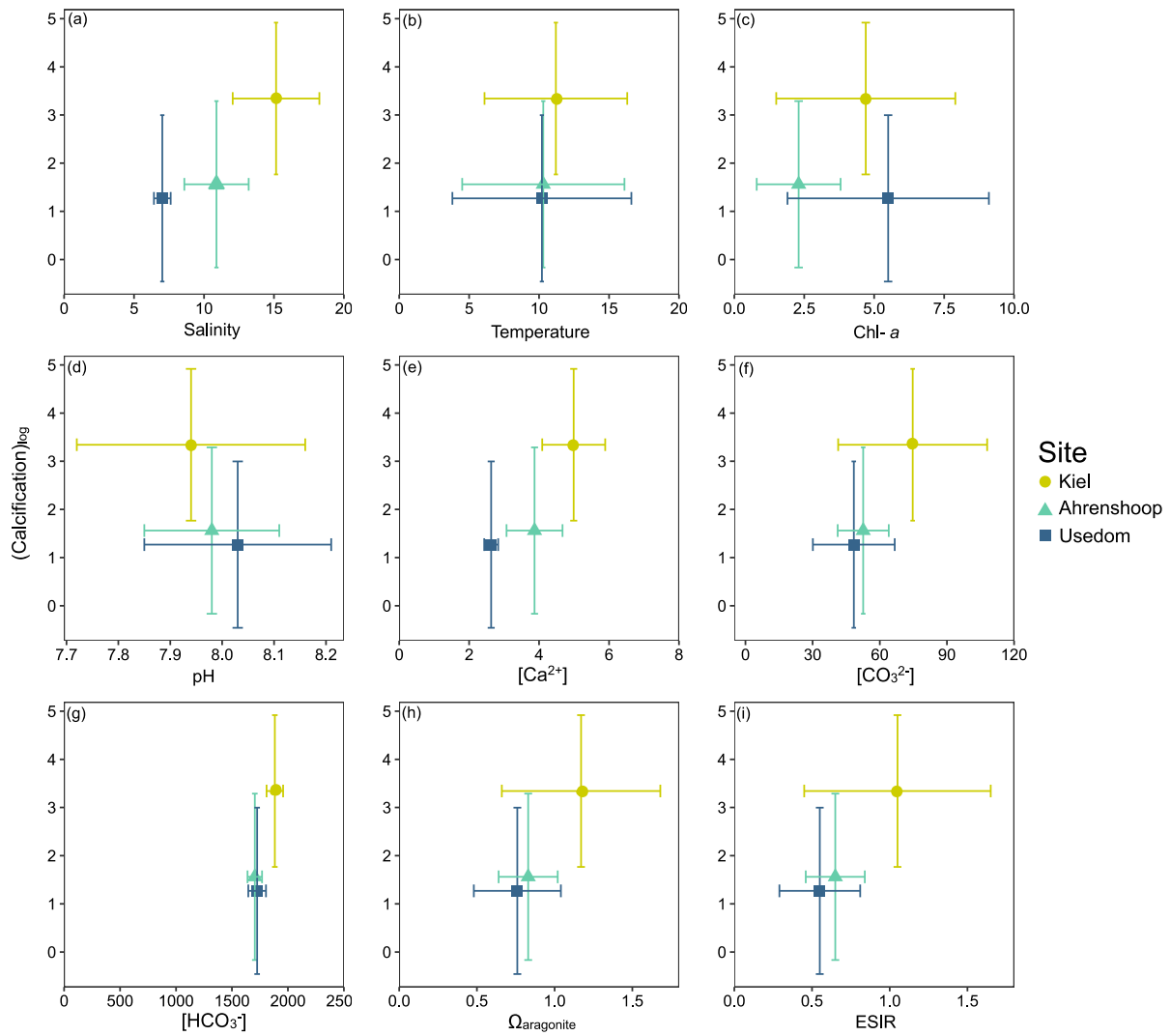
Figure S9: Log transformed field calcification rates (y-axes) plotted against multiple environmental parameters; Salinity (a), Temperature (°C) (b), chl-*a* (µg L⁻¹) (c), pH_total (d), [Ca²⁺] (mmol kg⁻¹) (e), [CO₃²⁻] (µmol kg⁻¹) (f), [HCO₃⁻] (µmol kg⁻¹) (g), Ω_aragonite (h), ESIR ([Ca²⁺][HCO₃⁻] / [H⁺]) (i). Values are shown ± standard deviation.
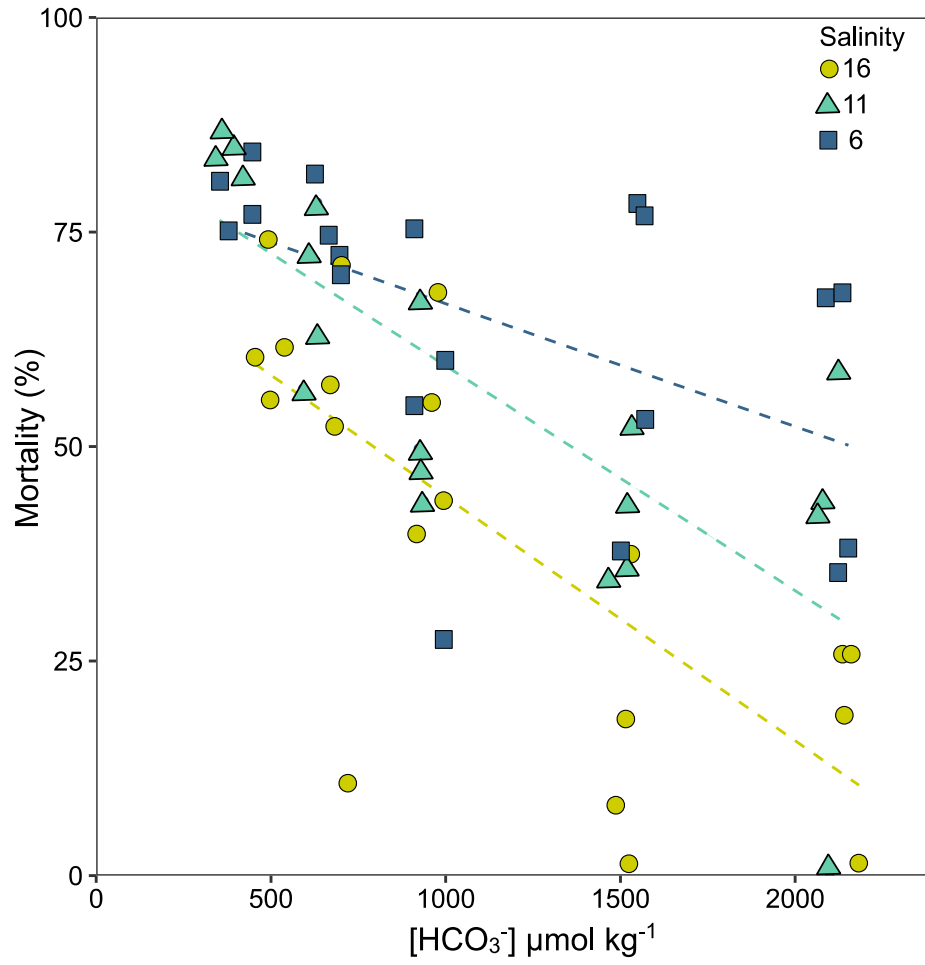
Figure S10: Total mortality rate in each treatment (n = 4 replicates) at the end of the bicarbonate ion manipulation experiment from an initial number of 1600 animals per 2L aquaria.

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