Supplementary material for Holding, J. *et al.* Experimentally determined temperature thresholds for Arctic planktonic community metabolism.

<u>Data sets</u>

Table S1. Experimentally measured values for all parameters in open-sea community experiment for each replicate (A, B, or pooled replicates) of all experimental treatments for each day of the experiment. Values are reported as available. Parameters include: actual temperature (ºC) phosphate (µM), silicate (µM), NO_3+NO_2 (µM), chlorophyll *a* (µg L^{-1}), and the metabolic rates (NCP, CR, GPP) reported as both volumetric (µmol O_2 L^{-1} day^{-1}) and chlorophyll standardized (µmol O_2 µg Chl a^{-1} day^{-1}) rates. All metabolic rate values are also accompanied by standard error calculated by propagation of error.

Experiment Day	Temperature Treatment	Replicate	Actual Temperature (ºC)	Phosphate (µM)	Silicate (µM)	NO_3 + NO_2 (µM)	Chlorophyll *a* (µg L^{-1})	NCP (µmol O_2 L^{-1} day^{-1})	NCP (µmol O_2 µgChla^{-1} day^{-1})	Std Err	CR (µmol O_2 L^{-1} day^{-1})	CR (µmol O_2 µgChla^{-1} day^{-1})	Std Err	GPP (µmol O_2 L^{-1} day^{-1})	GPP (µmol O_2 µgChla^{-1} day^{-1})	Std Err	GPP/CR
0		A		0.137	0.188	0.152	1.163	4.414	3.795	11.168	0.202	0.174	8.235	4.616	3.969	13.876	22.815
0		B		0.107	0.206		0.841	-9.308	-11.069	5.686	14.153	16.831	5.565	4.845	5.762	7.956	0.342
3	1	A	1.31	0.111	0.197	0.065	0.785	-1.681	-2.142	1.070	1.564	1.992	0.661				
3	2.5	A	2.78	0.112	0.225	0.014	0.754	3.215	4.263	3.657	1.551	2.057	1.266	4.766	6.320	3.869	3.072
3	4	A	3.92	0.121	0.208	0.003	0.812	0.719	0.886	1.641	0.869	1.070	2.156	1.589	1.956	2.709	1.828
3	5.5	A	6.11	0.153	0.198		0.754	-3.700	-4.906	1.317	2.085	2.765	1.372				
3	7	A	7.31	0.120	0.207	0.117	0.904	-2.608	-2.884	0.456	2.638	2.917	0.650	0.030	0.033	0.794	0.011
3	8.5	A	8.44	0.119	0.158	0.864	0.945	-2.584	-2.735	0.741	1.791	1.896	1.194				
3	10	A	11.26	0.185	0.157	0.050	1.009	-3.279	-3.248	1.303	4.967	4.921	1.315	1.688	1.673	1.851	0.340
4	1	A	1.43	0.096	0.193	0.564	0.665	5.682	8.544	2.146							
4	1	B	1.43	0.097	0.179	0.158	0.638	-1.921	-3.013	1.392							
4	2.5	A	2.53	0.097	0.214	0.336	0.699	-4.196	-6.001	6.822	2.660	3.804	7.500				
4	2.5	B	2.53	0.098	0.103	0.098	0.672	4.848	7.216	3.956							
4	4	A	4.21	0.102	0.251	0.247	0.802	0.420	0.523	1.061	1.349	1.682	1.123	1.768	2.205	1.545	1.311
4	4	B	4.21	0.096	0.193	0.040	0.734	-1.918	-2.615	0.898	3.634	4.954	1.255	1.716	2.339	1.543	0.472
5	5.5	A	5.62	0.088	0.190	0.279	0.555	-3.193	-5.755	3.170	3.729	6.721	2.543	0.536	0.966	4.064	0.144
5	5.5	B	5.62	0.089	0.155	0.202	0.589	-0.095	-0.162	2.148	3.113	5.281	2.152	3.017	5.119	3.040	0.969
5	7	A	7.52	0.092	0.190	0.326	0.541	-2.712	-5.012	2.485	4.409	8.150	1.849	1.698	3.138	3.098	0.385
5	7	B	7.52	0.094	0.157	0.053	0.884	-1.967	-2.226	0.812	2.450	2.772	0.908	0.483	0.546	1.218	0.197
5	8.5	A	8.67	0.089	0.198	0.111	0.510	-1.586	-3.110	1.783	4.045	7.932	1.599	2.459	4.823	2.395	0.608
5	8.5	B	8.67	0.095	0.152	0.019	0.500	-0.807	-1.617	0.598	2.033	4.071	0.705	1.226	2.454	0.925	0.603
5	10	A	10.72	0.083	0.219	0.127	0.730	0.418	0.573	0.884							
5	10	B	10.72	0.117	0.177	0.009	0.703	-0.393	-0.559	0.970							
8	1	A	2.51	0.097	0.152	0.680	0.341	-0.653	-1.912	2.138	1.357	3.976	1.980	0.705	2.064	2.914	0.519
8	1	B	2.51	0.096	0.141	0.023	0.806	-3.837	-4.758	1.529	2.747	3.407	1.387				
8	2.5	A	2.53	0.107	0.267	0.390	0.441	-1.610	-3.651	1.244	2.689	6.101	1.292	1.080	2.449	1.793	0.401
8	2.5	B	2.53	0.110	0.145	0.085	0.346	-2.348	-6.789	0.922	3.424	9.899	1.334	1.075	3.109	1.622	0.314
8	4	A	4.09	0.116	0.243	0.302	0.436	-1.449	-3.320	1.504							
8	4	B	4.09	0.120	0.206	0.042	0.382	-2.977	-7.789	1.798	2.464	6.448	1.835				
9	5.5	A	5.90	0.098	0.219	0.396	0.877	-3.731	-4.254	1.475	7.351	8.382	0.926	3.620	4.128	1.742	0.492
9	5.5	B	5.90	0.085	0.201		0.647	-6.200	-9.587	0.788	6.614	10.228	1.279	0.415	0.641	1.502	0.063
9	7	A	8.04	0.090	0.195	0.088	0.481	-6.230	-12.945	2.378	5.654	11.747	1.920				
9	7	B	8.04	0.105	0.204	0.094	0.400	-4.649	-11.616	1.313	2.808	7.017	1.170				
9	8.5	A	8.48	0.125	0.182	0.386	0.673	-7.466	-11.087	2.437	10.395	15.436	1.725	2.929	4.350	2.986	0.282
9	8.5	B	8.48	0.085	0.285	0.016	0.414	-2.422	-5.855	1.689	4.294	10.379	1.861	1.872	4.524	2.514	0.436
9	10	A	9.99	0.120	0.192	0.167	0.409	-4.603	-11.247	1.916	3.791	9.262	2.105				
9	10	B	9.99	0.098	0.183	0.135	0.463	-2.798	-6.039	1.168	3.170	6.842	1.230	0.372	0.804	1.696	0.117
15	1	POOLED	1.13				1.240	-3.253	-2.624	2.438	1.944	1.568	2.952				
15	2.5	POOLED	2.69				0.902	1.309	1.452	3.283	2.906	3.223	2.360	4.215	4.675	4.043	1.451
15	4	POOLED	4.38				0.826	3.530	4.271	2.892							
15	5.5	POOLED	5.44				0.735	-6.249	-8.500	3.188	9.323	12.682	2.428	3.075	4.182	4.008	0.330
15	7	POOLED	8.17				0.587	-5.056	-8.617	5.338	6.085	10.371	4.100	1.030	1.755	6.730	0.169
15	8.5	POOLED	8.44				0.168	-2.022	-12.043	1.558	2.280	13.575	1.508	0.257	1.533	2.169	0.113
15	10	POOLED	9.81				0.092	0.238	2.575	1.843	0.645	6.975	3.973	0.883	9.550	4.379	1.369

Table S2. Experimentally measured values for all parameters in fjord community experiment for each replicate (A or B) of all experimental treatments for each day of the experiment. Values are reported as available. Parameters include: actual temperature (ºC) phosphate (µM), silicate (µM), NO_3+NO_2 (µM), chlorophyll *a* (µg L^{-1}) and the metabolic rates (NCP, CR, GPP) reported as both volumetric (µmol O_2 L^{-1} day^{-1}) and chlorophyll standardized (µmol O_2 µg Chl a^{-1} day^{-1}) rates. All metabolic rate values are also accompanied by standard error calculated by propagation of error.

Experiment Day	Temperature Treatment	Replicate	Actual Temperature (ºC)	Phosphate (µM)	Silicate (µM)	NO_3 + NO_2 (µM)	Chlorophyll *a* (µg L^{-1})	NCP (µmol O_2 L^{-1} day^{-1})	NCP (µmol O_2 µgChla^{-1} day^{-1})	Std Err	CR (µmol O_2 L^{-1} day^{-1})	CR (µmol O_2 µgChla^{-1} day^{-1})	Std Err	GPP (µmol O_2 L^{-1} day^{-1})	GPP (µmol O_2 µgChla^{-1} day^{-1})	Std Err	GPP/CR
0				0.118	0.399	0.092	0.208	-0.382	-1.835	1.091	1.791	8.611	1.391	1.409	6.776	1.768	0.787
0				0.126	0.423	0.041	0.211	-1.086	-5.139	1.664							
4	1	A	1.101	0.069	0.446	0.390	0.436	14.478	33.182	2.818							
5	1	B	1.143	0.056	0.429		0.580	-5.331	-9.195	1.836	0.984	1.696	1.358				
4	2.5	A	3.033	0.058	0.416		0.481	-7.211	-14.982	2.209	7.241	15.044	2.212	0.030	0.062	3.126	0.004
5	2.5	B	2.876	0.054	0.408	0.354	0.598	0.089	0.149	1.502	3.653	6.113	3.601	3.743	6.262	3.902	1.024
4	4	A	4.066	0.055	0.413	0.277	0.531	14.193	26.747	1.530							
5	4	B	4.166	0.086	0.396		0.660	0.037	0.056	1.660	5.626	8.523	1.444	5.662	8.579	2.200	1.007
5	5.5	A	5.399	0.042	0.402	0.209	0.899	4.444	4.942	3.038							
5	5.5	B	5.488	0.044	0.392	0.011	0.873	1.492	1.710	2.976	2.817	3.228	1.801	4.309	4.938	3.478	1.530
4	8.5	A	8.511	0.048	0.417	0.082	0.687	0.282	0.410	2.336	1.273	1.853	2.275	1.555	2.264	3.261	1.221
5	8.5	B	8.528	0.057	0.402		0.744	1.767	2.374	2.992							
4	10	A	10.059	0.058	0.386		0.535	5.392	10.077	2.339	0.194	0.363	2.505	5.587	10.441	3.427	28.750
5	10	B	9.847	0.057	0.405	0.095	0.553	4.094	7.403	3.103	0.328	0.592	1.782	4.421	7.995	3.578	13.500
8	1	A	1.101	0.036	0.352	0.649	1.424	0.230	0.161	1.609							
8	1	B	1.101	0.021	0.315		1.840	1.728	0.939	2.205	2.206	1.199	3.015	3.934	2.139	3.735	1.783
8	2.5	A	2.762	0.026	0.346		1.904	0.248	0.130	2.820	6.431	3.377	3.752	6.678	3.507	4.694	1.039
8	2.5	B	2.762	0.025	0.323		1.766	0.635	0.359	2.315	7.241	4.099	1.241	7.875	4.459	2.627	1.088
8	4	A	3.942	0.026	0.349		1.960	-3.383	-1.726	1.667	4.578	2.335	2.324	1.195	0.610	2.860	0.261
8	4	B	3.942				1.956	-2.574	-1.316	3.115	5.598	2.862	2.360	3.024	1.546	3.908	0.540
8	5.5	A	5.513	0.034	0.346		2.757	-3.713	-1.347	2.246	6.077	2.205	1.733	2.364	0.858	2.837	0.389
8	5.5	B	5.513	0.027	0.369		1.805	-0.744	-0.412	1.643	6.745	3.736	1.996	6.001	3.324	2.586	0.890
8	8.5	A	8.147	0.036	0.358		2.321	-21.668	-9.336	1.877	22.349	9.629	1.994	0.682	0.294	2.738	0.030
8	8.5	B	8.147	0.078	0.356		2.389	-1.348	-0.564	1.252	3.432	1.436	0.948	2.084	0.872	1.571	0.607
8	10	A	9.893	0.055	0.373	0.069	1.450	-2.281	-1.573	1.556	5.964	4.113	4.302	3.683	2.540	4.575	0.618
8	10	B	9.893	0.060	0.372		1.224	-2.652	-2.167	1.387	2.883	2.356	1.720	0.231	0.189	2.210	0.080

Analysis of nutrient and heterotrophic dynamics

In order to determine the possible role of nutrients in regulating metabolic responses to temperature we have elaborated here the dynamics of nitrate-nitrate, phosphorous, and silicate concentrations for each experiment.

Open- ocean experiment

We find that there are no trends in phosphate, silicate, or nitrate-nitrate concentrations over time (Fig. S1; $p= 0.053, 0.44, 0.79$), nor do nutrient concentrations show a relationship with temperature (Fig. S2; $p= 0.27, 0.99, 0.40$ respectively). Even when possible outliers are removed nitrate-nitrite concentrations still do not show any relationship with temperature ($p= 0.10$).

Fjord experiment

Phosphate and silicate concentrations decreased over time at a rate of 0.009 µmol L^{-1} day^{-1} ($p< 0.0001$) and 0.01 µmol L^{-1} day^{-1} ($p< 0.0001$) respectively, however nitrate-nitrite concentrations showed no such decline ($p=0.18$) (Fig. S3). Meanwhile, nitrate-nitrate concentrations decrease with increasing temperature (0.05 µmol L^{-1} $°C^{-1}$; $p=0.006$) and phosphate and silicate concentrations had no relationship with temperature ($p= 0.19$; $p=0.85$ respectively; Fig. S4). However, the negative relationship of nitrate-nitrate with temperature is based only a few viable data points ($n=9$).

Analysis of the relationship of metabolic rates with other drivers: nutrients and heterotrophic components

Open- ocean experiment

We investigated the possible effects of nitrate-nitrite, phosphate, and silicate concentrations on metabolic rates (NCP, CR, GPP; Fig. S5) in the open ocean experiment. We find no significant relationships of NCP, CR, or GPP with nitrate-nitrate ($p=0.94, 0.66, 0.87$), phosphate ($p=0.43, 0.94. 0.72$), or silicate ($p= 0.59, 0.94, 0.40$).

As for the heterotrophic components, we investigated the possible relationship between bacterial abundance and production on the metabolic rates of NCP, CR, and GPP (Fig. S6). We find that in the open ocean community, NCP is negatively related to both abundance and production ($p=0.03$, $R^2=0.13$; $p= 0.001$, $R^2=0.24$ respectively) however CR is only positively related to bacterial production ($p=0.005$, $R^2= 0.22$) and we find no relationship of CR to bacterial abundance ($p=0.09$).

Fjord experiment

We have also investigated the possible effects of nitrate-nitrite, phosphate, and silicate concentrations on metabolic rates (NCP, CR, GPP; Fig. S7) in the fjord community experiment. Again, we find no significant relationships of NCP, CR, or GPP with nitrate-

nitrate (p=0.35, 0.77, 0.88), phosphate (p=0.57, 0.23, 0.26), and silicate (p=0.11, 0.19, 0.26).

As for the heterotrophic components of the fjord community, we investigated the possible relationship between bacterial abundance and production on the metabolic rates of NCP, CR, and GPP (Fig. S8). We find that in the fjord community, both NCP and CR are not related to abundance or production (NCP: p=0.16, 0.83 respectively; CR: p=0.15, 0.68).

Analysis of metabolic rates standardized by bacterial abundance

Open- ocean community

Finally, as there was some relationship of metabolic rates to bacterial abundance in the open- ocean community, we also analyzed the CR and NCP vales standardized by bacterial abundance instead of chlorophyll *a* values. Metabolic rates should be standardized to biomass to determine their real relationship with temperature as the metabolic theory of ecology refers to rates per unit biomass (Brown et al., 2004). Community respiration has both heterotrophic and autotrophic components and is difficult to select a parameter that encompasses both. For our main analysis we have used chlorophyll *a* as a proxy for biomass as it was able to reveal a strong relationship of metabolism with temperature as expected by the metabolic theory. When standardizing for bacterial abundance we find in the open-ocean community, standardizing metabolic rates per unit biomass of bacteria eradicate any previous metabolic relationship with temperature (p=0.12, 0.47*;* Figs. S9).

Fjord community

Although there was no relationship of metabolic rates to the heterotrophic component in the Fjord experiment, nor any relationship of volumetric metabolic rates to temperature, we tested standardizing Fjord community metabolic rates to bacterial abundance and found that again there was no relationship of metabolism with temperature (NCP: p=0.50, CR: p= 0.71; Fig. S10).

Fig. S1. Nutrient concentrations over-time for the open-water community. Blue markers represent phosphate concentrations, green markers represent NO_3^- +NO_2^- concentrations and red markers represent silicate concentrations over the course of the experiment.

Fig. S2. Relationship of nutrient concentration with temperature for the open-sea community. Blue markers represent phosphate concentrations, green markers represent NO_3^- +NO_2^-concentrations and red markers represent silicate concentrations over the course of the experiment.

Fig. S3. Nutrient concentrations over-time for the fjord community. Blue markers represent phosphate concentrations, green markers represent NO_3^- +NO_2^- concentrations and red markers represent silicate concentrations over the course of the experiment. Red line represents the significant decreasing trend in silicate concentrations over time ($p< 0.0001$; $R^2=0.58$) and blue line represents significant decreasing trend phosphate concentrations over time ($p<0.0001$; $R^2=0.62$).

Fig. S4. Relationship of nutrient concentration with temperature for the fjord community Blue markers represent phosphate concentrations, green markers represent NO_3^- +NO_2^- concentrations and red markers represent silicate concentrations over the course of the experiment. Green line represents negative relationship between NO_3^- +NO_2^- concentrations and temperature ($p=0.006$; $R^2=0.69$).

Fig. S5. Plots of nutrient concentrations of nitrate (left column), phosphate (middle column), and silicate (right column) concentrations against NCP (top row), CR (middle row) and GPP (bottom row) rates throughout the experiment with the open ocean community.

Fig. S6. Open ocean community: plots of the relationships of bacterial abundance (left column) and production (right column) to NCP (bottom row) and CR (top row) throughout the experiment. Black lines represent significant relationships of NCP and bacterial abundance (bottom left; $p=0.03$; $R^2=0.13$) , NCP and bacterial production (bottom right; $p=0.001$; $R^2=0.24$) and CR and bacterial production (top right; $p=0.005$, $R^2=0.22$).

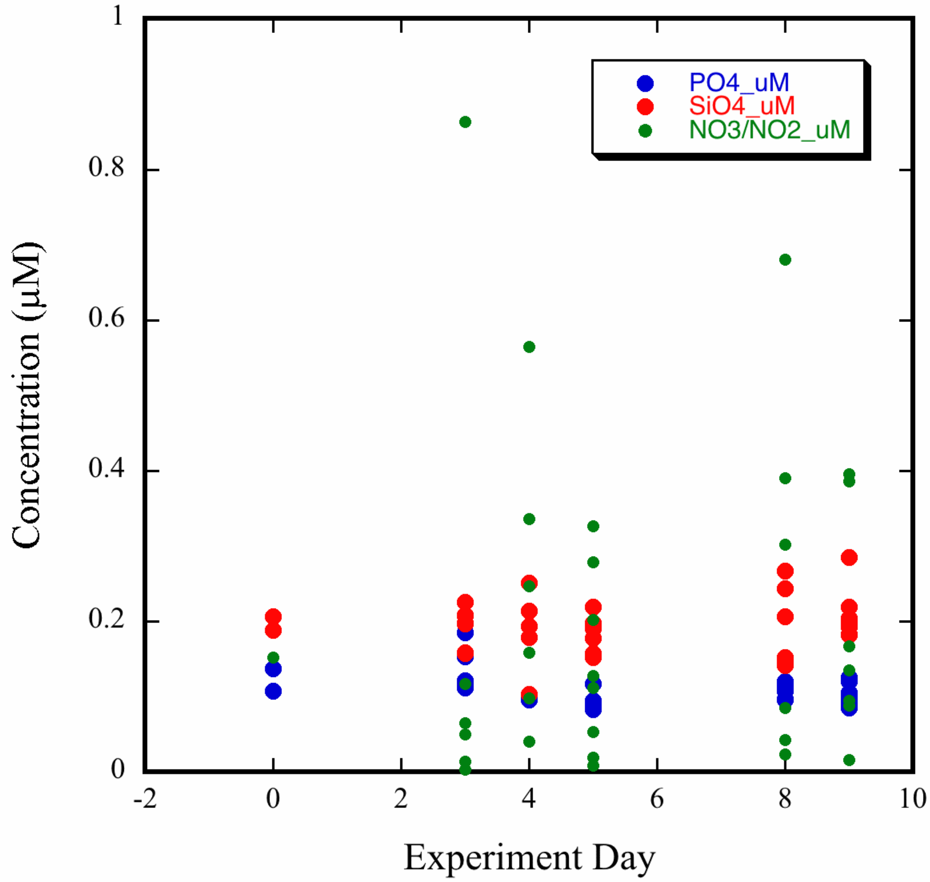
Fig. S7. Fjord community: plots of nutrient concentrations of nitrate (left column), phosphate (middle column), and silicate (right column) concentrations against NCP (top row), CR (middle row) and GPP (bottom row) rates throughout the experiment.

Fig. S8. Fjord community. Plots of the relationships of bacterial abundance (left column) and production (right column) to NCP (bottom row) and CR (top row).

Fig. S9. Open ocean community: metabolic rates NCP (bottom) and CR (top) standardized per unit bacteria (μmol O_2 (10^9 cell)$^{-1}$ day^{-1}) plotted against experimental temperatures along the entire experiment.

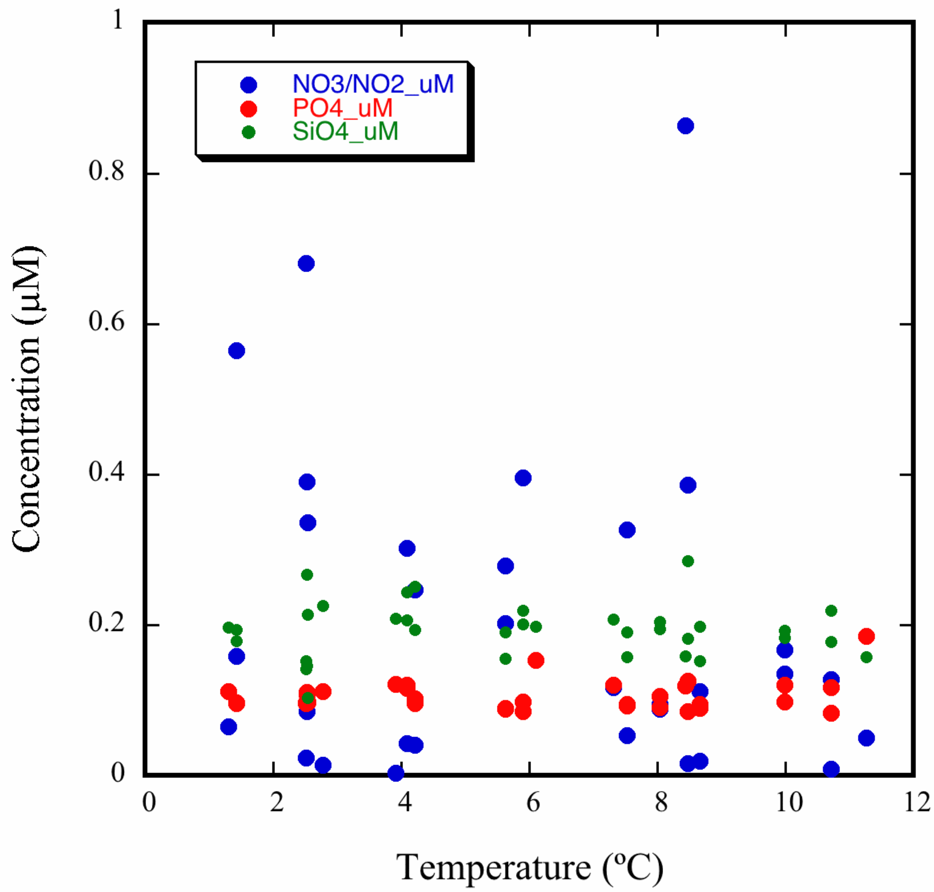
Fig S10. Fjord community: metabolic rates NCP (bottom) and CR (top) standardized per unit bacteria (µmol O_2 (10^9 cell)$^{-1}$ day^{-1}) plotted against experiment temperatures along the entire experiment.

Fig. S1.



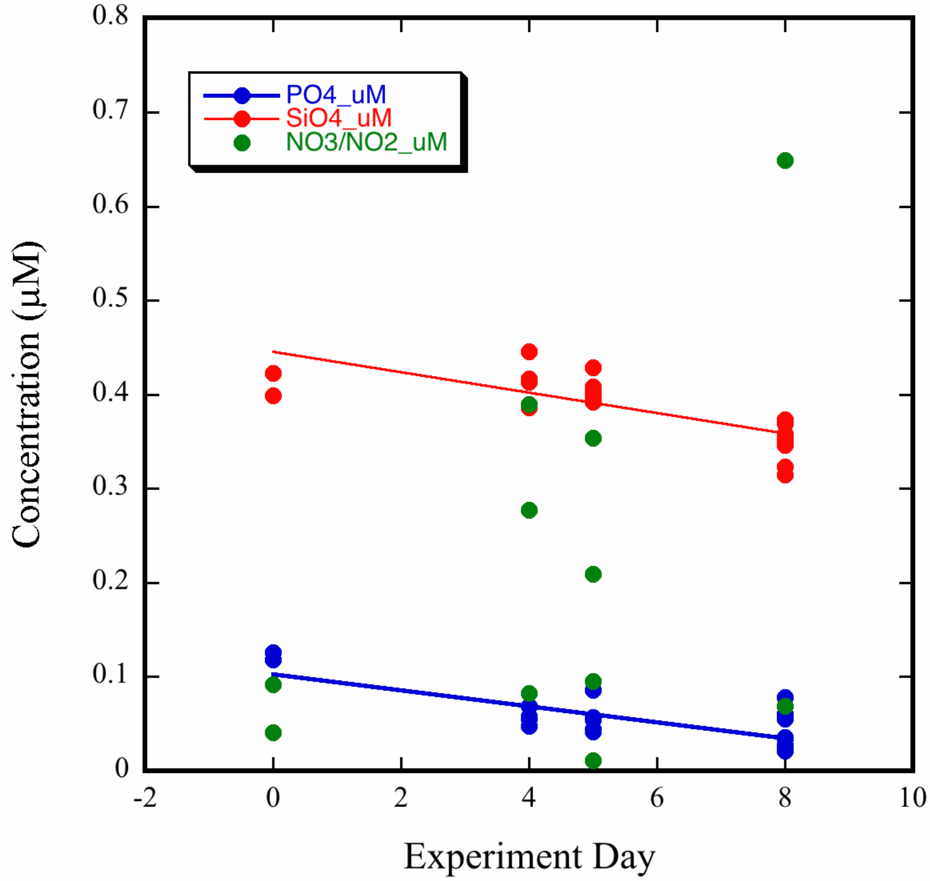
PO4_uM
SiO4_uM
NO3/NO2_uM
Concentration (µM)
Experiment Day
1
0.8
0.6
0.4
0.2
0
-2
0
2
4
6
8
10

Fig. 2S.



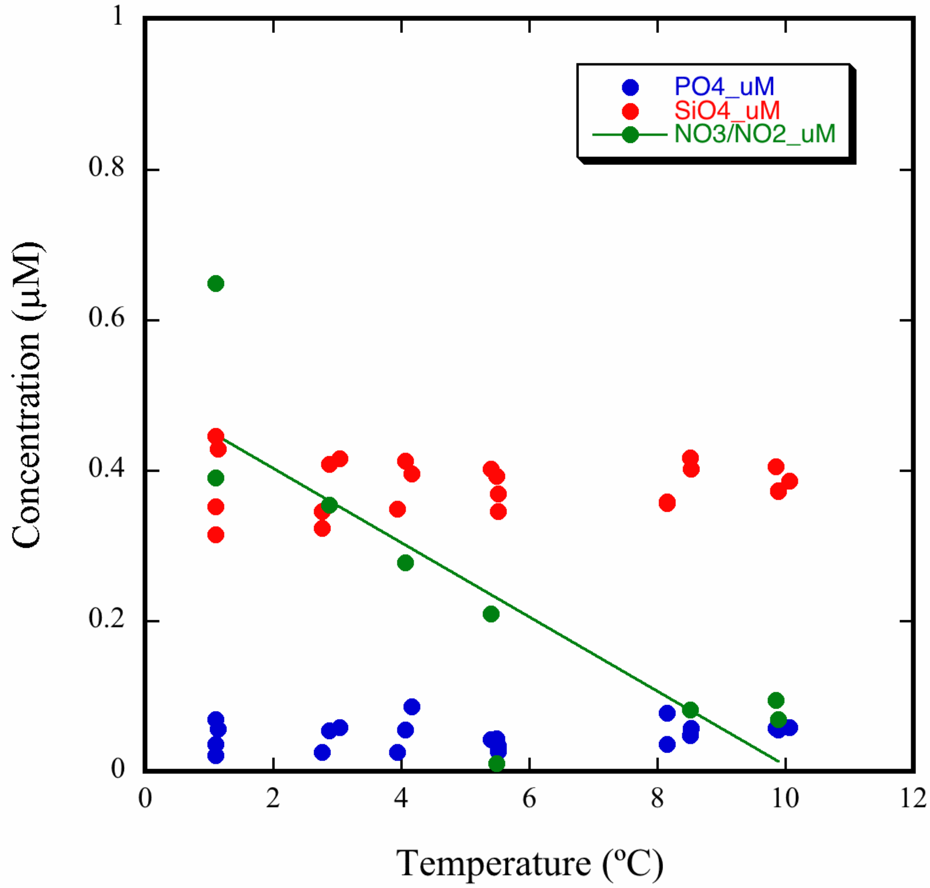
NO3/NO2_uM
PO4_uM
SiO4_uM
Concentration (µM)
Temperature (ºC)
0
0.2
0.4
0.6
0.8
1
0
2
4
6
8
10
12

Fig. 3S.



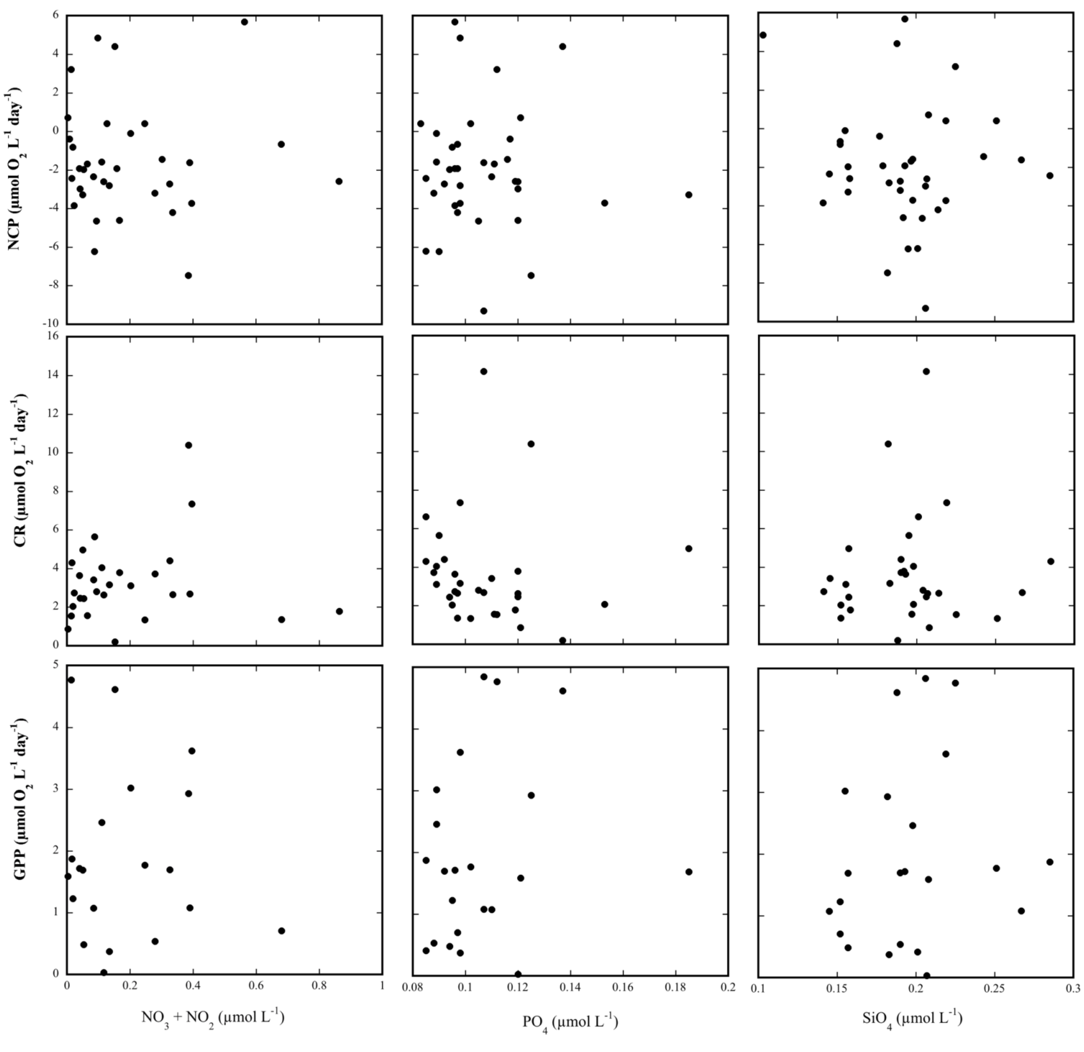
PO4_uM
SiO4_uM
NO3/NO2_uM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-2
0
2
4
6
8
10
Concentration (µM)
Experiment Day

Fig. 4S.



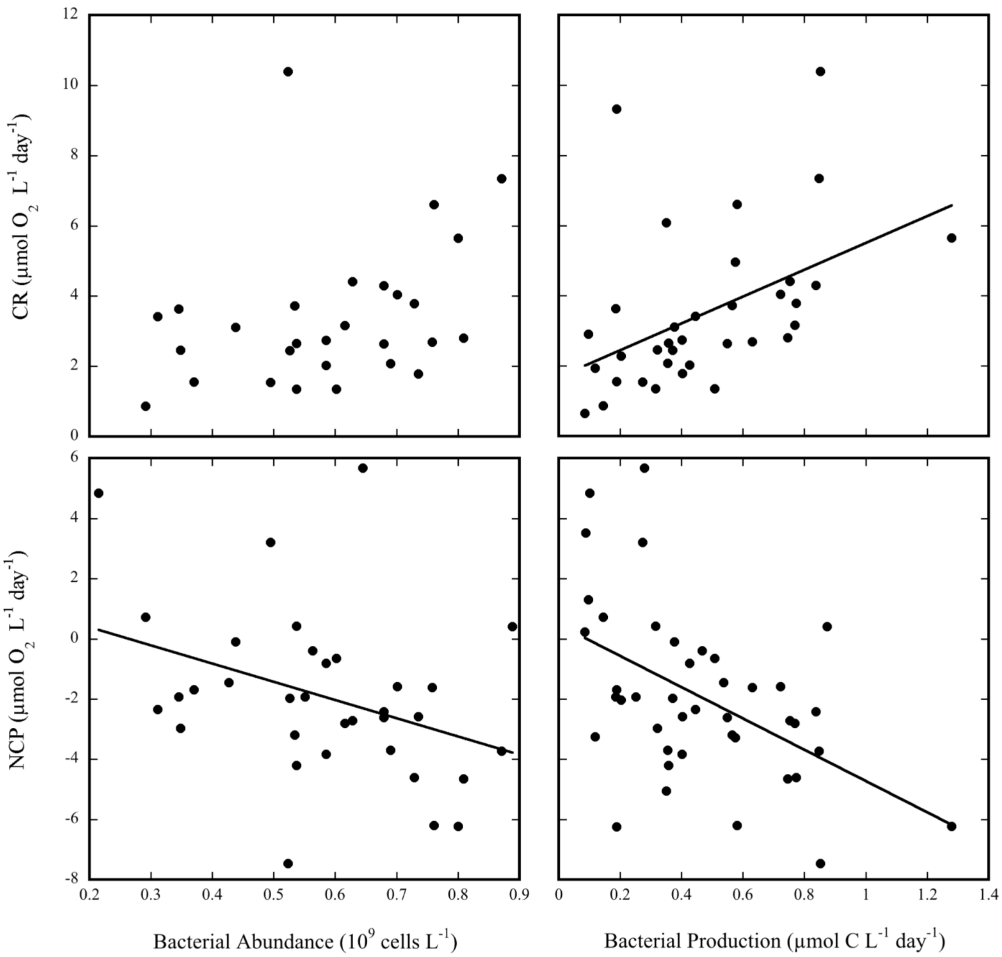
PO4_uM
SiO4_uM
NO3/NO2_uM
Concentration (µM)
Temperature (ºC)
0
0.2
0.4
0.6
0.8
1
0
2
4
6
8
10
12

Fig. 5S.



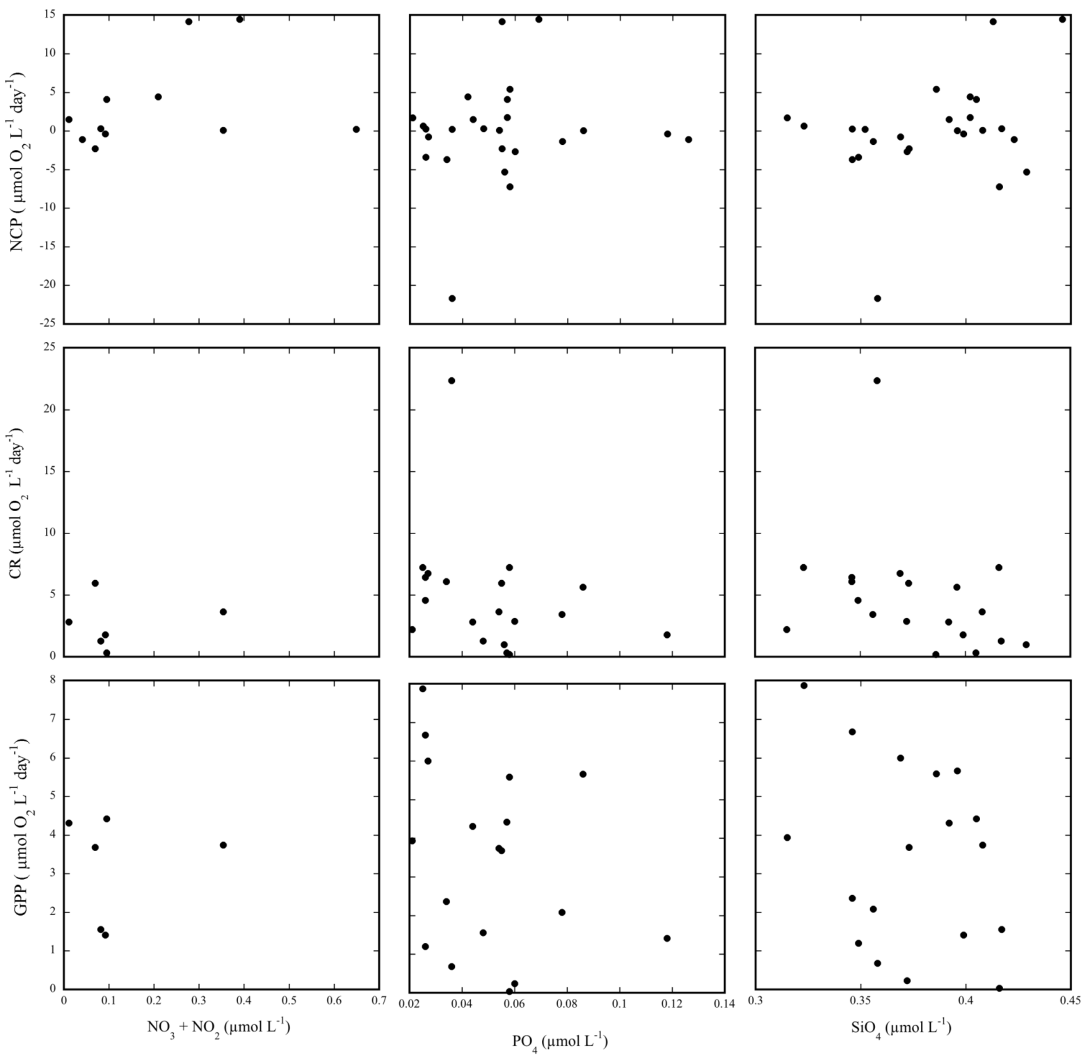
NCP (µmol O2 L-1 day-1)
CR (µmol O2 L-1 day-1)
GPP (µmol O2 L-1 day-1)
NO3 + NO2 (µmol L-1)
PO4 (µmol L-1)
SiO4 (µmol L-1)

Fig. S6.



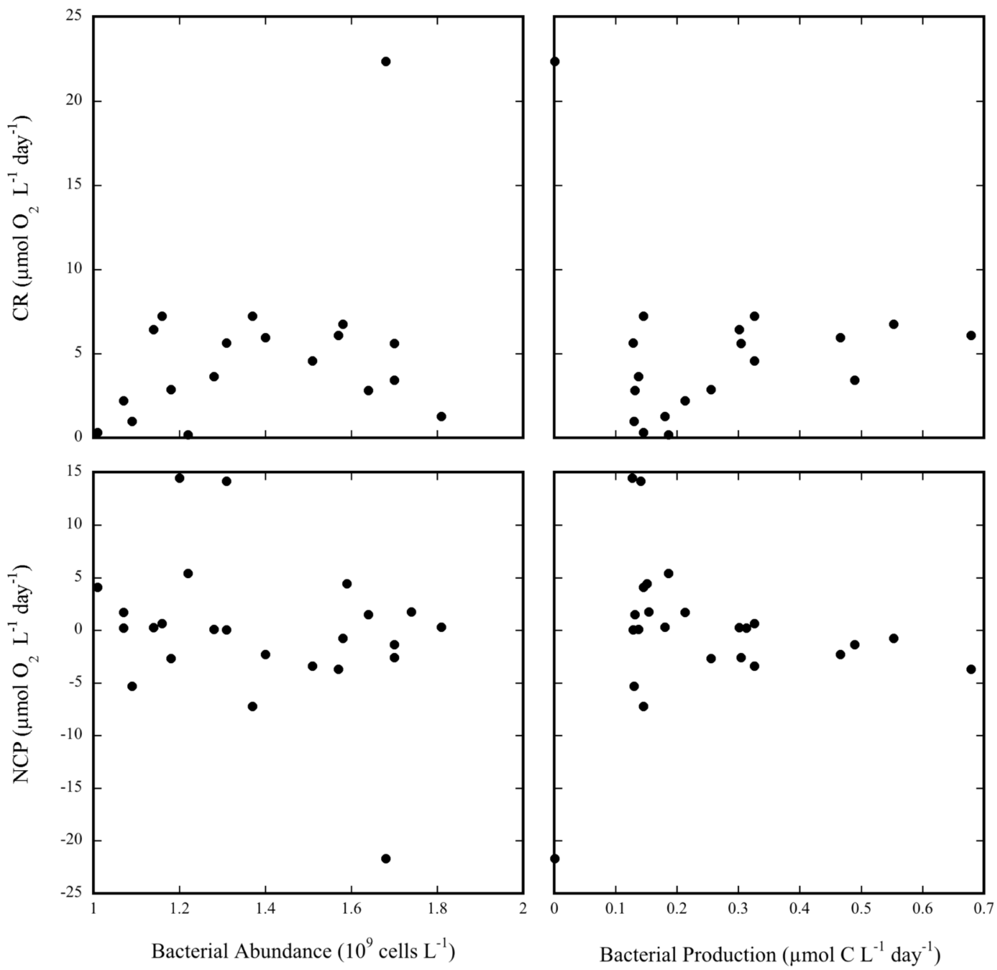
CR (µmol O2 L-1 day-1)
NCP (µmol O2 L-1 day-1)
Bacterial Abundance (10^9 cells L-1)
Bacterial Production (µmol C L-1 day-1)

Fig. S7.



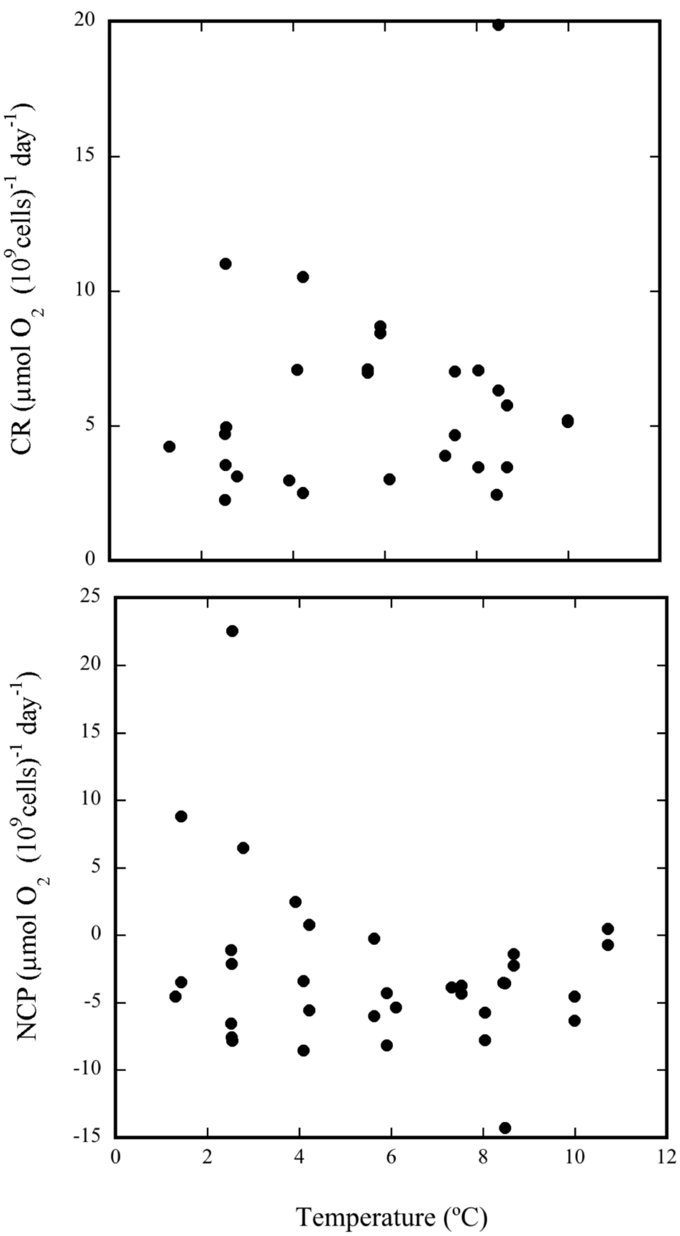
NCP (µmol O_2 L^{-1} day^{-1})
CR (µmol O_2 L^{-1} day^{-1})
GPP (µmol O_2 L^{-1} day^{-1})
NO_3 + NO_2 (µmol L^{-1})
PO_4 (µmol L^{-1})
SiO_4 (µmol L^{-1})

Fig. S8.



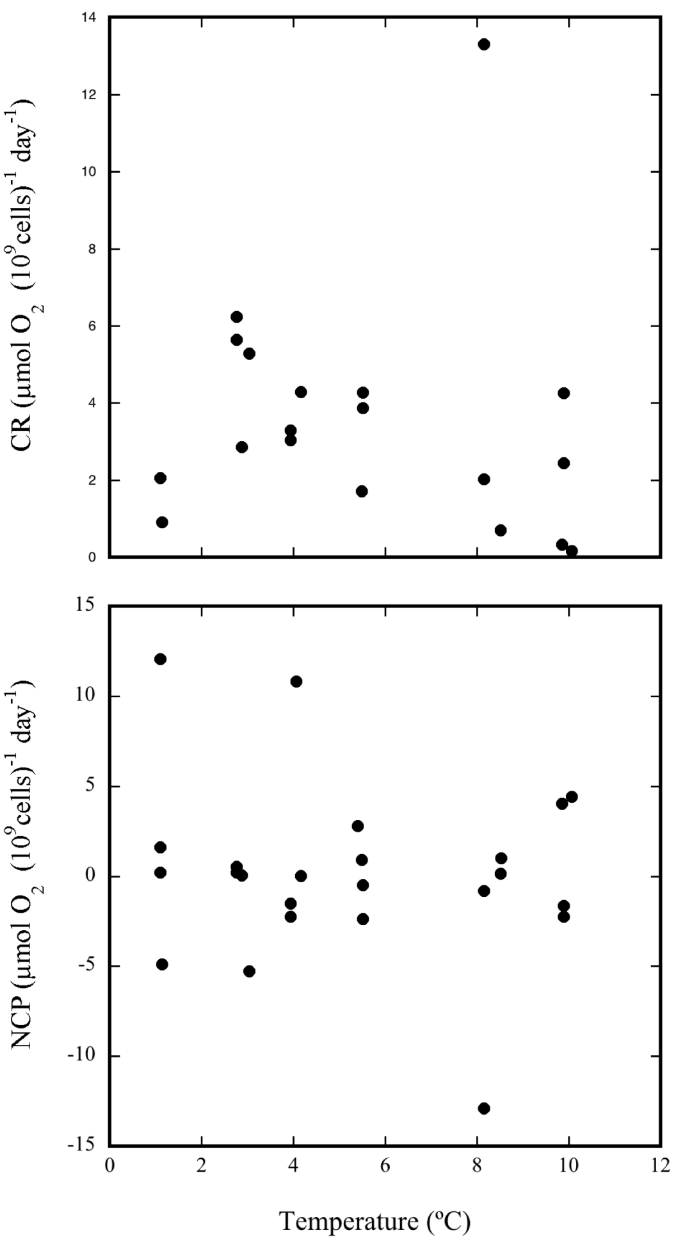
CR (µmol O2 L-1 day-1)
NCP (µmol O2 L-1 day-1)
Bacterial Abundance (10^9 cells L-1)
Bacterial Production (µmol C L-1 day-1)
25
20
15
10
5
0
-5
-10
-15
-20
-25
1
1.2
1.4
1.6
1.8
2
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Fig. S9.



CR (µmol O2 (10^9 cells)^-1 day^-1)
NCP (µmol O2 (10^9 cells)^-1 day^-1)
Temperature (ºC)
0
5
10
15
20
25
-5
-10
-15
0
2
4
6
8
10
12

Fig. S10.


CR (µmol O2 (10^9 cells)^-1 day^-1)
NCP (µmol O2 (10^9 cells)^-1 day^-1)
Temperature (ºC)
14
12
10
8
6
4
2
0
15
10
5
0
-5
-10
-15
0
2
4
6
8
10
12