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Effects of elevated CO_2 and temperature on phytoplankton community biomass, species composition and photosynthesis during an experimentally induced autumn bloom in the western English Channel

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Discrimination of nano- and picophytoplankton species, groups and size ranges

Over an annual cycle at an approximate monthly frequency, samples of seawater from Station L4 were gravity-filtered through a series of membrane filters ranging from 10 µm down to 0.2 µm and the filtrate was analysed by flow cytometry. The cell numbers for different groups of algae were recorded and compared with counts from unfiltered seawater to assess what percentage of cells passed through each of the filters. The 'percentage of cells remaining' values were then plotted against pore size of the filters in µm. For each algal group, a line was drawn from the point at which 50 % of cells remained on the Y axis to each of the algal group plot lines and, at the intersection a vertical line was drawn down to the x axis. The point at which the line crossed the X axis was recorded as being the median cell diameter for whichever algal group was being measured. The median summaries provide information about changes in algal group median cell diameter variability throughout the year, where there were enough cells to calculate a median cell diameter.

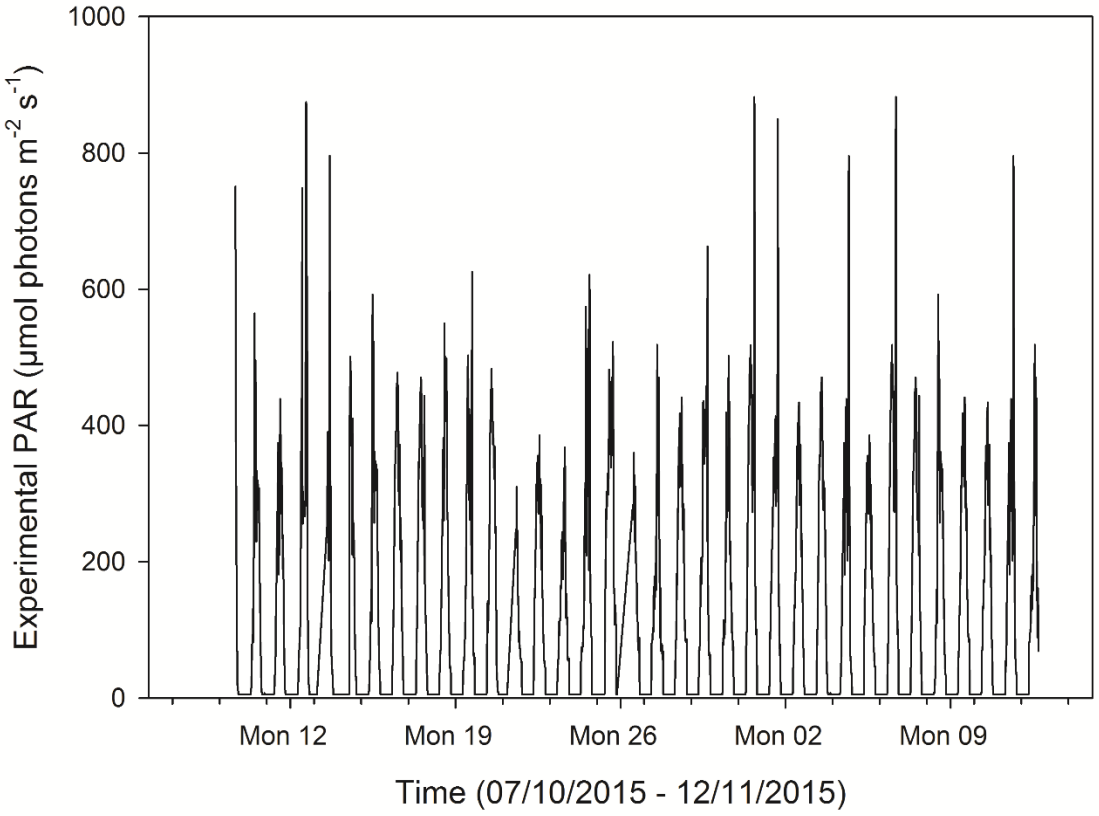

Fig. S1. Time course of PAR levels during the experiment. Neutral density spectrally corrected blue filters were used to provide ~50% irradiance in the incubation chambers, approximating PAR measured at 10 m depth at station L4 on the day of phytoplankton community sampling. Experimental PAR levels were measured with a scalar PAR radiometer located in one of the incubation chambers under the filter material (QSPL-2200, Biospherical Instruments Inc).

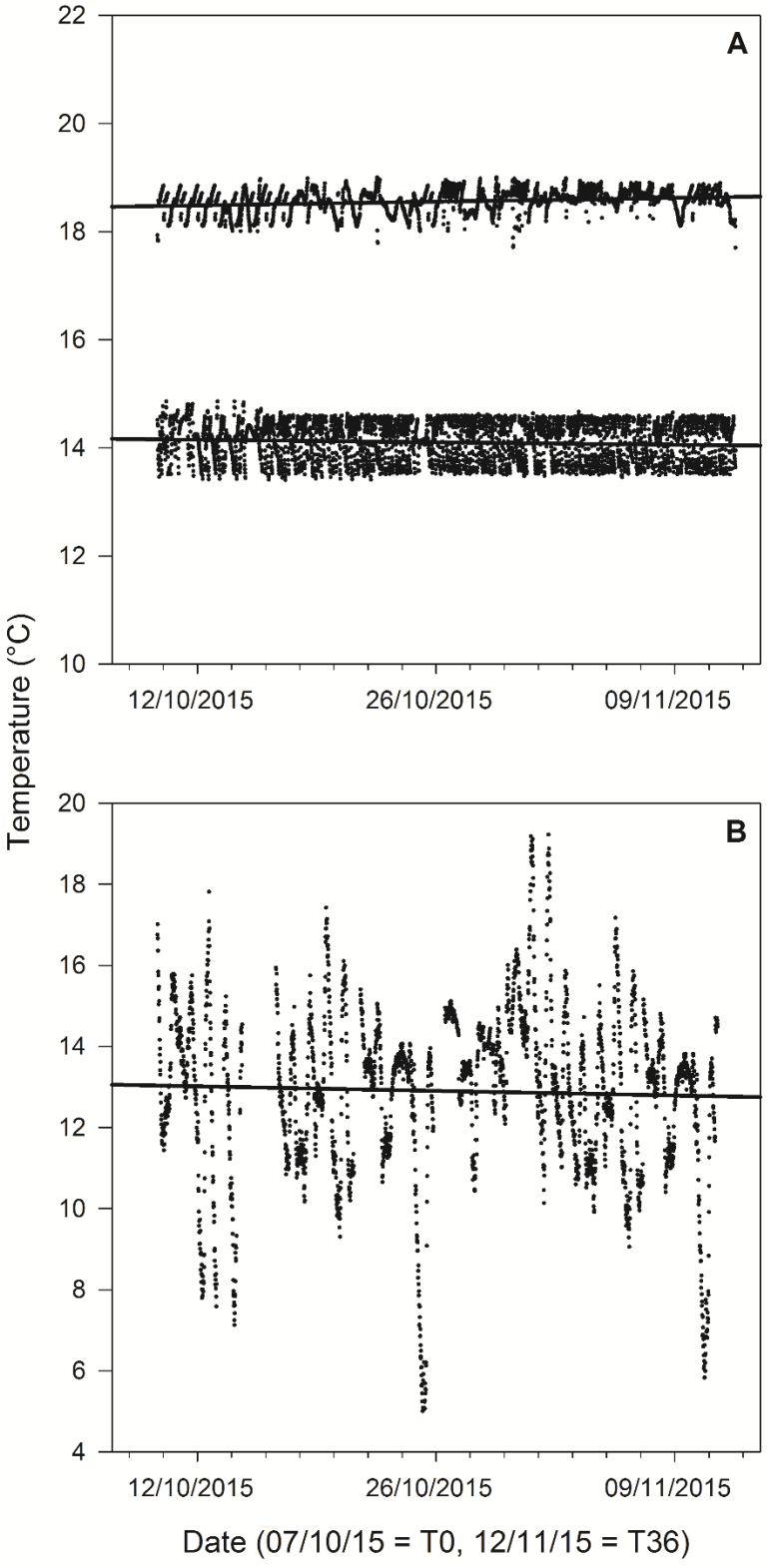

Fig. S2. Time course of temperature treatments throughout the experiment. Control and high CO_2 treatments maintained at a mean temperature of 14.1 °C (± 0.35 sd) and the high temperature and combination treatments maintained at a mean temperature of 18.6 °C (± 0.42 sd) (**A**). External ambient air temperature logged over the experimental period (**B**).

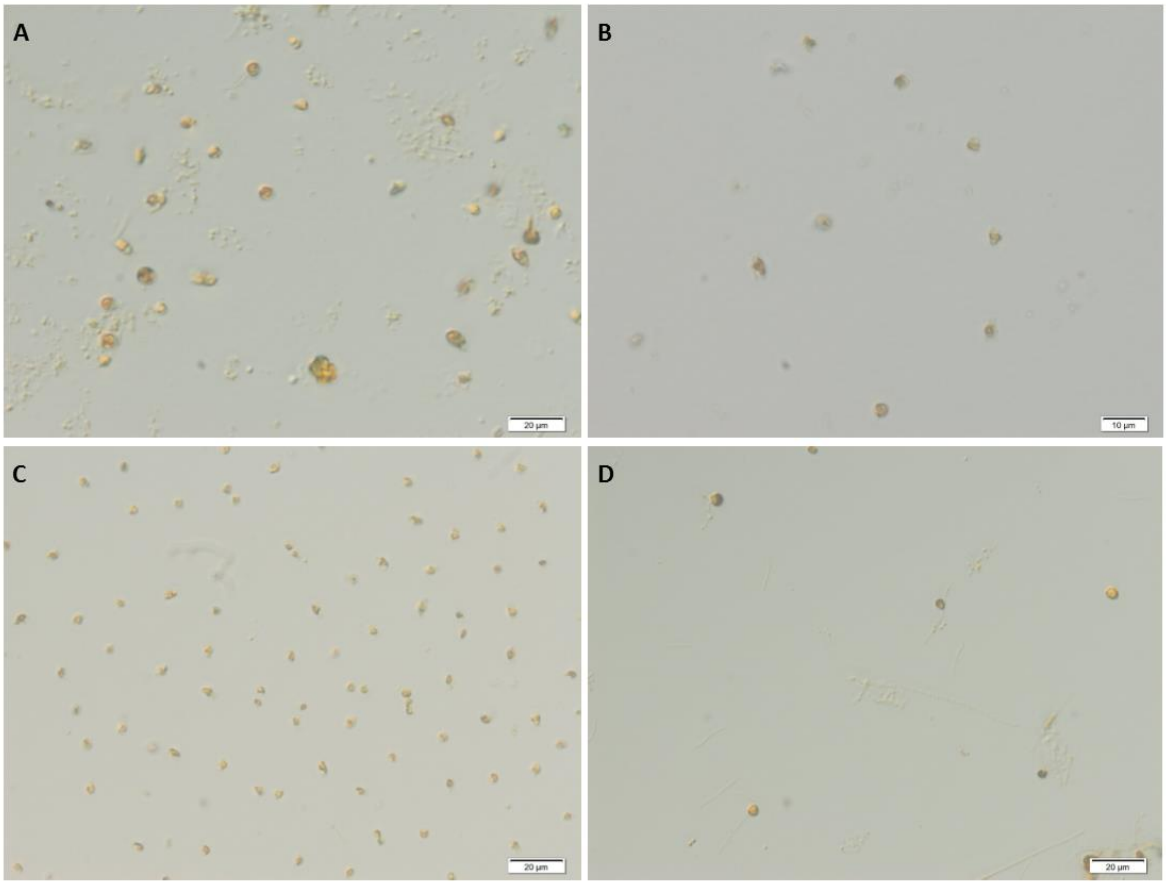

Fig. S3. Nanophytoplankton was the dominant group throughout the experiment between T10-T24 and remained dominant in the high temperature and high CO_2 treatments at T36. Large nano-flagellates observed in the control (**A**), smaller nano-flagellates observed in the high temperature and combination treatments (**B & D**) and *Phaeocystis* spp. observed in the high CO_2 treatment (**C**). Image magnification = x 300.

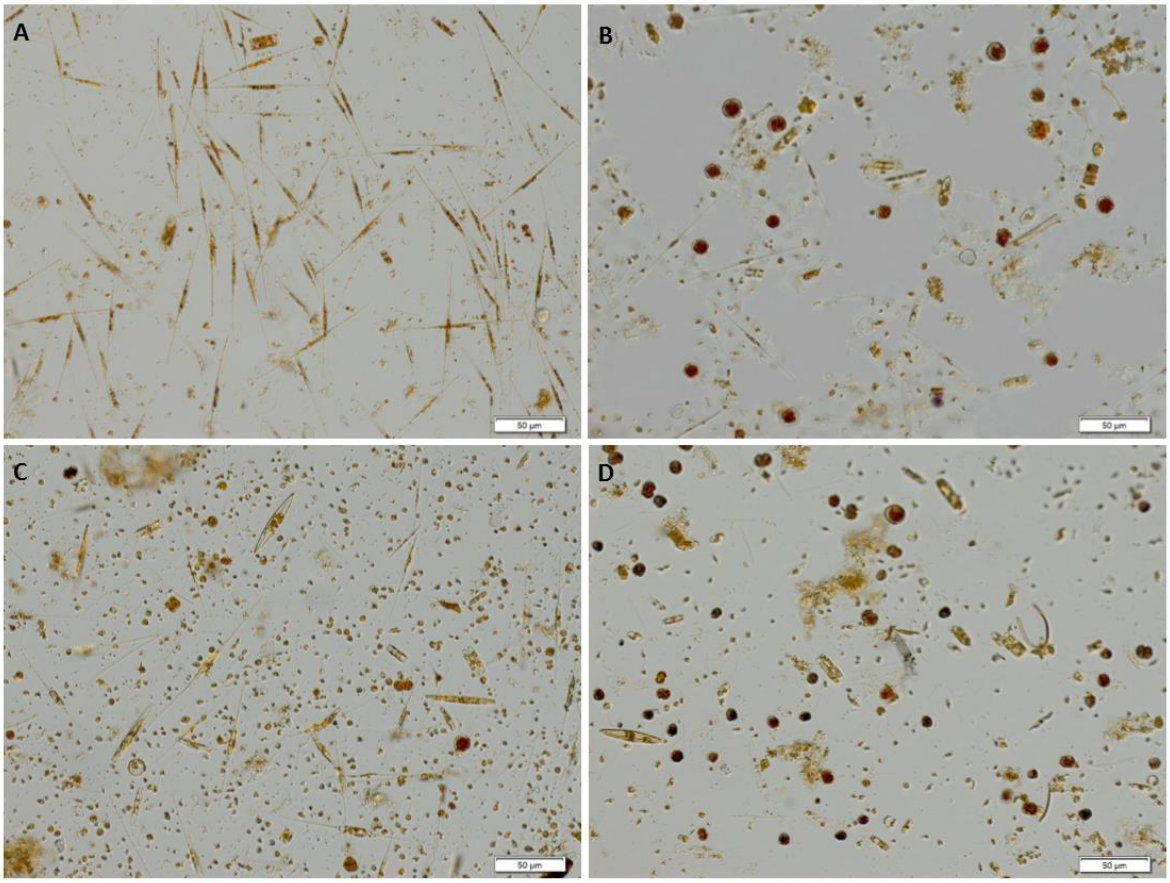

Fig. S4. The micro size fraction of the phytoplankton biomass at T36. Diatoms dominated the community in the control where *C. closterium* was numerically most abundant but *N. distans* dominated the biomass (**A**), diatoms and dinoflagellates (dominated by *N. distans*, *P. cordatum* and undet. *Gymnodiniales*) in the high temperature treatment (**B**), diatoms dominating in the high CO_2 treatment (*N. distans*, *T. subtilis* and *C. closterium*) (**C**) and diatoms and dinoflagellates (dominated by *N. distans* and *P. cordatum*) in the combination treatment (**D**).

Table S1. Measured and calculated seawater carbonate system values throughout the experiment in all treatments. Total alkalinity (TA) and dissolved inorganic carbon (DIC) were measured, with the remaining parameters (pCO_2, pH, HCO_3^-, CO_3^{2-}, and the saturation states for calcite (Ω Ca) and aragonite (Ω Ar)) calculated using the programme CO_2sys. Values presented are the mean of 4 replicates with standard deviations (sd).

Time	Treatment	Measured				Calculated											
		TA µmol kg^{-1}	(sd)	Total DIC µmol kg^{-1}	(sd)	pCO_2 µatm	(sd)	pH	(sd)	HCO_3^- µmol kg^{-1}	(sd)	CO_3^{2-} µmol kg^{-1}	(sd)	Ω Ca	(sd)	Ω Ar	(sd)
T0	All replicates	2339	2.50	2118	3.12	397	2.77	8.05	0.002	1943	3.63	160	0.60	3.80	0.01	2.44	0.01
T6	Control	2344	3.56	2118	1.64	389	8.20	8.05	0.008	1941	3.77	163	2.83	3.87	0.07	2.48	0.04
	High temp	2341	1.13	2082	3.80	392	7.84	8.05	0.007	1883	6.00	186	2.54	4.43	0.06	2.87	0.04
	High CO_2	2341	0.87	2177	1.92	550	8.20	7.92	0.006	2030	3.07	126	1.50	2.99	0.04	1.92	0.02
	Combination	2343	1.91	2152	1.23	569	9.05	7.91	0.006	1990	2.74	143	1.87	3.41	0.04	2.21	0.03
T10	Control	2342	1.48	2118	4.01	391	7.86	8.05	0.007	1941	5.86	162	2.26	3.85	0.05	2.47	0.03
	High temp	2342	2.18	2079	2.49	384	1.60	8.06	0.001	1878	2.62	188	0.36	4.49	0.01	2.91	0.01
	High CO_2	2341	3.83	2239	4.67	828	28.82	7.76	0.014	2116	5.87	91	2.76	2.16	0.07	1.38	0.04
	Combination	2341	1.83	2214	1.89	838	19.10	7.76	0.009	2080	3.33	106	2.10	2.53	0.05	1.64	0.03
T13	Control	2341	3.93	2118	2.31	393	11.50	8.05	0.012	1942	5.68	161	3.89	3.83	0.09	2.46	0.06
	High temp	2342	3.15	2084	1.18	394	6.81	8.05	0.007	1885	3.31	185	2.59	4.42	0.06	2.86	0.04
	High CO_2	2341	3.72	2238	2.89	826	36.39	7.76	0.018	2115	5.07	91	3.57	2.17	0.09	1.39	0.05
	Combination	2342	2.10	2212	3.51	823	27.86	7.77	0.013	2077	5.58	108	3.04	2.57	0.07	1.66	0.05
T20	Control	2339	2.47	2119	3.19	398	7.10	8.04	0.007	1944	4.66	159	2.13	3.79	0.05	2.43	0.03
	High temp	2342	1.34	2083	2.93	391	7.66	8.05	0.007	1883	5.21	186	2.58	4.44	0.06	2.88	0.04
	High CO_2	2341	4.60	2238	4.55	824	31.99	7.76	0.016	2115	5.85	91	3.14	2.17	0.07	1.39	0.05
	Combination	2341	1.71	2212	3.42	829	21.69	7.77	0.010	2077	4.86	107	2.28	2.55	0.05	1.65	0.04
T24	Control	2341	3.15	2115	2.77	388	11.36	8.05	0.011	1938	6.05	163	3.74	3.87	0.09	2.48	0.06
	High temp	2340	2.02	2080	0.46	389	3.23	8.05	0.003	1880	1.28	187	1.32	4.45	0.03	2.88	0.02
	High CO_2	2341	2.23	2242	2.70	847	28.38	7.75	0.014	2120	4.22	89	2.62	2.12	0.06	1.36	0.04
	Combination	2340	2.09	2217	1.85	864	7.45	7.75	0.004	2085	1.95	103	0.80	2.46	0.02	1.60	0.01
T27	Control	2342	1.08	2116	2.21	386	4.71	8.06	0.005	1938	3.36	163	1.44	3.89	0.03	2.49	0.02
	High temp	2342	2.83	2080	0.76	386	5.73	8.06	0.006	1879	2.71	188	2.26	4.48	0.05	2.90	0.03
	High CO_2	2341	1.56	2236	1.57	819	13.47	7.77	0.007	2114	2.24	92	1.33	2.18	0.03	1.40	0.02
	Combination	2341	1.89	2216	0.93	855	12.64	7.76	0.006	2083	1.72	104	1.38	2.49	0.03	1.61	0.02
T34	Control	2343	1.42	2117	3.64	387	5.56	8.06	0.005	1939	4.92	163	1.53	3.88	0.04	2.49	0.02
	High temp	2343	2.38	2083	3.74	390	9.54	8.05	0.009	1883	6.52	187	3.29	4.45	0.08	2.88	0.05
	High CO_2	2344	1.00	2239	2.62	817	12.26	7.77	0.006	2116	3.21	92	1.11	2.19	0.03	1.40	0.02
	Combination	2341	4.90	2214	3.33	840	25.69	7.76	0.013	2080	4.43	106	2.93	2.52	0.07	1.63	0.05
T36	Control	2341	0.98	2116	2.80	390	7.69	8.05	0.007	1940	4.86	162	2.36	3.85	0.06	2.47	0.04
	High temp	2343	2.60	2079	2.17	383	1.04	8.06	0.001	1877	1.90	189	0.53	4.50	0.01	2.92	0.01
	High CO_2	2342	2.61	2239	4.09	825	15.93	7.76	0.007	2116	4.66	91	1.39	2.17	0.03	1.39	0.02
	Combination	2342	1.33	2213	3.29	829	19.70	7.77	0.009	2079	4.65	107	2.09	2.55	0.05	1.65	0.03