

Ocean warming has greater and more consistent negative effects than ocean acidification on the growth and health of subtropical macroalgae

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Marine climate change system set-up

Two 60 L flow-through reservoirs were injected with CO₂ and regulated to pH of 7.8 and 7.6 respectively using a pH millivolt controller (Tunze 7075/2), pH probe (Tunze pH electrode 7070.10), and solenoid valve (Tunze 7074.110) connected to food grade CO₂ gas cylinders. The pH of the reservoirs was adjusted in relation to the pH measured in the containers used to rear the algae described in the main text. A third flow-through reservoir was not manipulated to provide ambient pH seawater. All reservoirs were bubbled with air with powerheads and airstones to aid mixing and maintain dissolved oxygen levels (DO > 90%). To obtain the three temperature treatments (23 °C, 26 °C and 28 °C) the seawater from each header tank flowed into three separate 11 L reservoirs, heated with 200 W aquarium heaters (Jager). The heaters were connected to temperature controllers (Tunze) with temperature sensors placed in rearing containers without experimental algae within the system. Treatment water was constantly circulated through irrigation tubes and back into the sub-header tanks to maintain a constant pH and temperature across the system. The conditioned water was delivered to the rearing containers using dripper valves (Pope) inserted into the irrigation tubes.

Tables

Table S1. Experimental conditions during the algal growth experiment. pH NIST scale (pH_{NIST}), pH total scale (pH_{T}), Total alkalinity (A_{T} ; $\mu\text{mol}/\text{kgSW}$), pCO_2 values (μatm) and calcite saturation states (ΩCa) for each seawater pH/temperature treatment were calculated from the CO2SYS Excel Macro (Pierrot *et al.*, 2006). Data are means and (SE).

Temperature Treatment	pH Treatment	pH_{NIST} , n=75	pH_{T} , n= 75	A_{T} , n=15	pCO_2 , n=15	ΩCa , n=15
23 °C	8.1	8.09 (-0.004)	7.98 (-0.004)	2346.96 (-4.16)	485.69 (-12.15)	4.42 (-0.08)
	7.8	7.80 (-0.003)	7.69 (-0.003)	2346.96 (-4.16)	1040.66 (-18.3)	2.51 (-0.04)
	7.6	7.61 (-0.003)	7.50 (-0.003)	2346.96 (-4.16)	1701.46 (-29.39)	1.67 (-0.03)
26 °C	8.1	8.08 (-0.003)	7.97 (-0.003)	2346.96 (-4.16)	503.75 (-12.71)	4.73 (-0.09)
	7.8	7.78 (-0.002)	7.67 (-0.002)	2346.96 (-4.16)	1098.29 (-15.09)	2.69 (-0.03)
	7.6	7.61 (-0.006)	7.50 (-0.006)	2346.96 (-4.16)	1719.8 (-41.6)	1.87 (-0.04)
28 °C	8.1	8.06 (-0.004)	7.95 (-0.004)	2346.96 (-4.16)	530.74 (-15.45)	4.9 (-0.09)
	7.8	7.77 (-0.003)	7.66 (-0.003)	2346.96 (-4.16)	1134.59 (-17.07)	2.85 (-0.04)
	7.6	7.58 (-0.004)	7.47 (-0.003)	2346.96 (-4.16)	1825.87 (-23.34)	1.87 (-0.04)

Table S2a. Results from linear mixed models contrasting the effects of pH and temperature treatments on the percent carbon (% C) for *S. linearifolium*, *D. pulchra*, and *L. decussata* (3 pH x 3 temperature treatments with 3 replicates each). Temperature and pH were fixed factorial factors and individual thallus was a random factor. Significant treatment effects ($p \leq 0.05$) are in bold font. Non-parametric tests with Wilcox rank sum post hoc tests were run for *A. anceps* and *C. officinalis* as transformations did not improve heterogeneity.

Source	<i>Sargassum linearifolium</i>			<i>Delisea pulchra</i>			<i>Laurencia decussata</i>		
	df	F	P	df	F	P	Den df	F	P
pH	2,30	0.563	0.57	2,23	1.947	0.16	2,22	0.671	0.52
Temperature	2,30	11.800	0.0002	2,23	3.217	0.06	2,22	2.358	0.12
pH x Temperature	4,30	1.959	0.13	4,23	1.405	0.26	4,22	1.384	0.27

Table S2b: Kruskal-Wallis Tests examining the effects of pH and temperature treatments on the percent carbon (% C) for *A. anceps* and *C. officinalis*

Test	<i>Amphiroa anceps</i>			<i>Corallina officinalis</i>		
	χ^2	df	p	χ^2	df	p
%C vs pH	1.6719	2	0.43	1.8248	2	0.40
%C vs Temp	19.627	2	<0.01	4.3156	2	0.12

Table S3. Analyses for the percent nitrogen (% N) of each algal species in the pH and temperature treatments (3 pH x 3 temperature treatments with 5 replicates each). For *A. anceps* and *C. officinalis*, growth was analysed with analyses of variance with temperature and pH as fixed factorial factors. For *S. linearifolium*, *D. pulchra* and *L. decussata*, growth was analysed with linear mixed models with temperature and pH as fixed factorial factors and individual thallus as a random factor. Significant treatment effects ($p \leq 0.05$) are in bold font.

	<i>Sargassum linearifolium</i>			<i>Delisea pulchra</i>			<i>Laurencia decussata</i>		
Source	df	F	P	df	F	P	df	F	P
pH	2,30	0.346	0.71	23	1.254	0.30	22	2.09	0.15
Temperature	2,30	2.948	0.07	23	0.540	0.59	22	2.24	0.13
pHx Temp	4,30	0.464	0.76	23	0.242	0.91	22	1.90	0.15

	<i>Amphiroa anceps</i>				<i>Corallina officinalis</i>			
Source	df	MS	F	P	df	SS	F	P
pH	2	0.062	0.1082	0.90	2	0.129	0.11	0.89
Temperature	2	1.9096	3.3268	0.047	2	4.483	3.98	0.03
pHx Temp	4	0.0448	0.0781	0.988	4	0.164	0.07	0.99
Residual	36	0.5740			34	19.147		

Table S4. Analyses of variance for the effects of pH and temperature on absolute growth (g) of coralline algae *C. officinalis* and *A. anceps* (3 pH x 3 temperature treatments with 5 replicates each). Significant treatment effects ($p \leq 0.05$) are in bold font.

Source	df	<i>Corallina officinalis</i>			<i>Amphiroa anceps</i>		
		MS	F	P	MS	F	P
pH	2	1.56×10^{-4}	4.34	0.02	4.98×10^{-6}	0.34	0.72
Temperature	2	$6.33E \times 10^{-4}$	17.64	<0.01	3.64×10^{-4}	25.77	<0.01
pH x Temperature	4	1.76×10^{-4}	4.91	<0.01	1.43×10^{-6}	0.10	0.10
Residual	36	3.59×10^{-5}			1.48×10^{-5}		

Figures

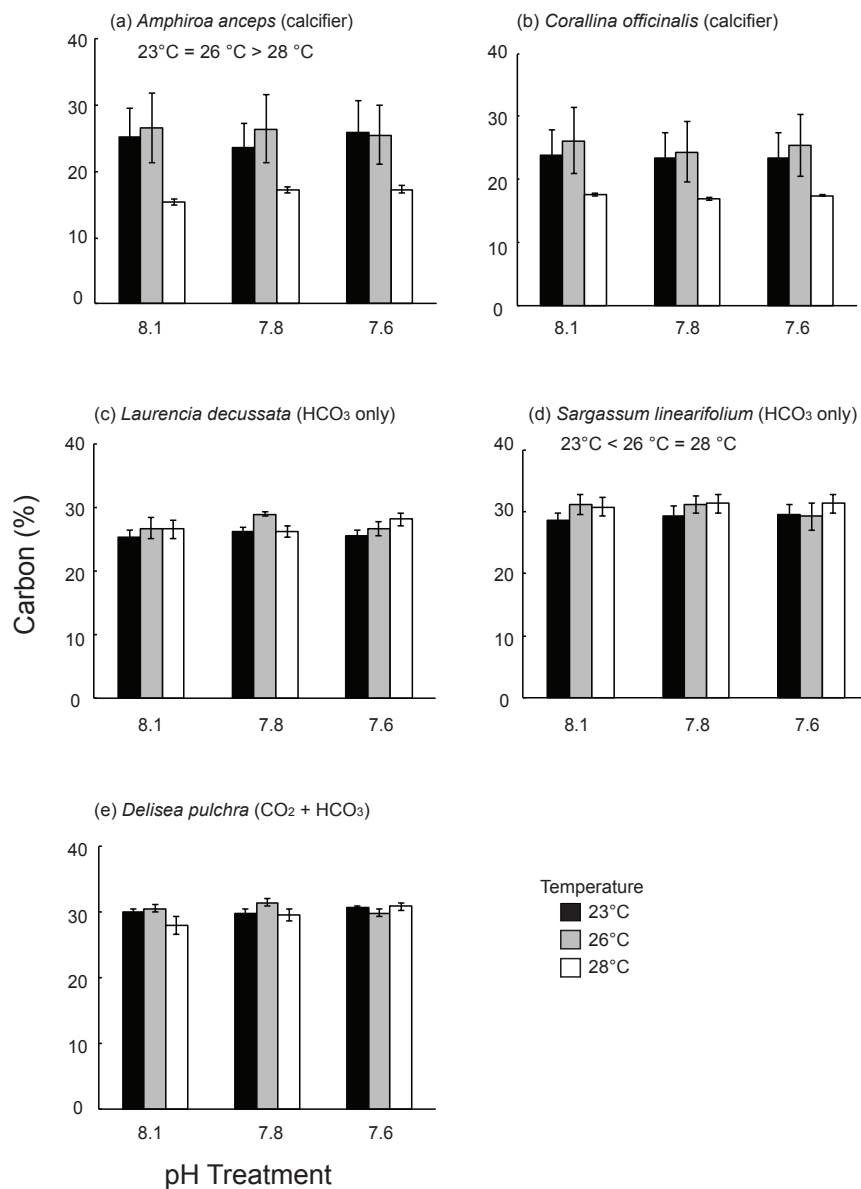


Figure S1: Percent carbon of all algal species (except *Ulva* sp.) in each of the nine combinations of pH (8.1, 7.8 and 7.6) and temperature (23 °C, 26 °C and 28 °C) after two weeks (*A. anceps*, after 9 days). Data are means \pm SE, n=4-5.

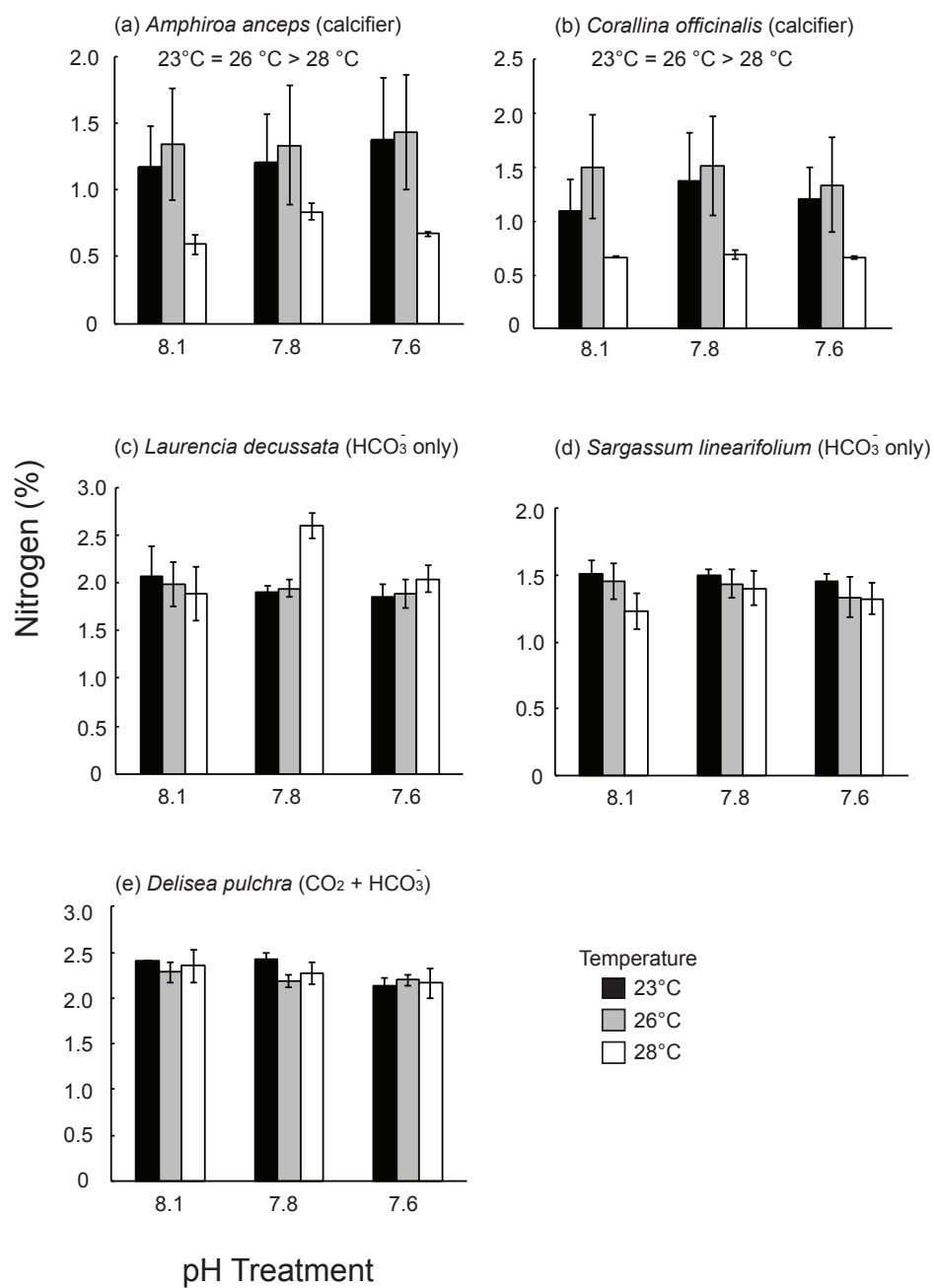


Figure S2: Percent nitrogen of all algal species (except *Ulva* sp.) in each of the nine combinations of pH (8.1, 7.8 and 7.6) and temperature (23 °C, 26 °C and 28 °C) after two weeks (*A. anceps*, after 9 days). Data are means \pm SE, n=4-5.

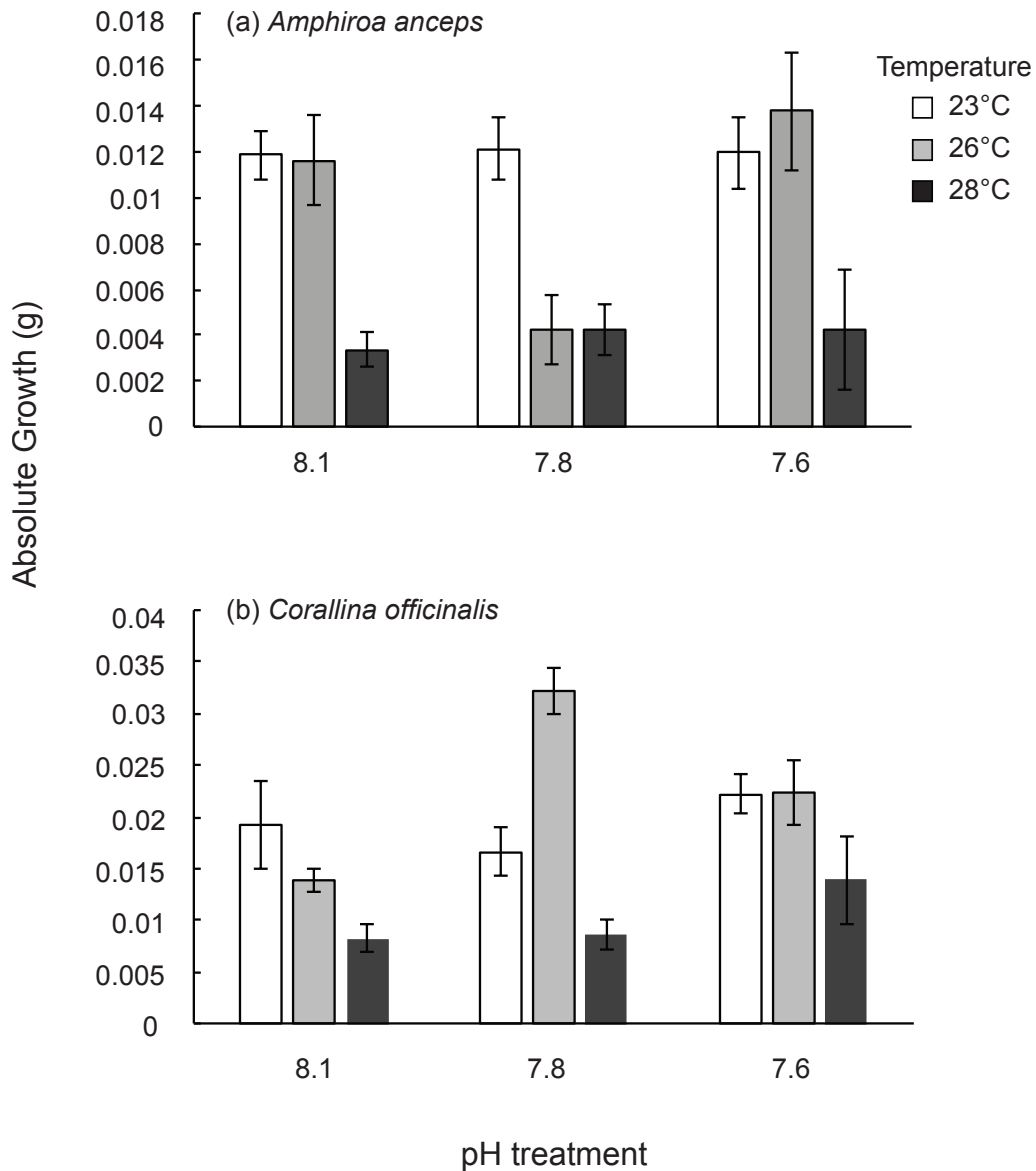


Figure S3: Absolute growth (g) of (a) *Corallina officinalis* and (b) *Amphiroa anceps* in each of the nine combinations of pH (8.1, 7.8 and 7.6) and temperature (23 °C, 26 °C and 28 °C) treatments over nine days for *A. anceps* and 15 days for *C. officinalis*. Data are means \pm SE, n=5.