Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/2/7/e1501660/DC1

Supplementary Materials for

Long-term dynamics of adaptive evolution in a globally important phytoplankton species to ocean acidification

Lothar Schlüter, Kai T. Lohbeck, Joachim P. Gröger, Ulf Riebesell, Thorsten B. H. Reusch

Published 8 July 2016, *Sci. Adv.* **2**, e1501660 (2016)
DOI: 10.1126/sciadv.1501660

This PDF file includes:

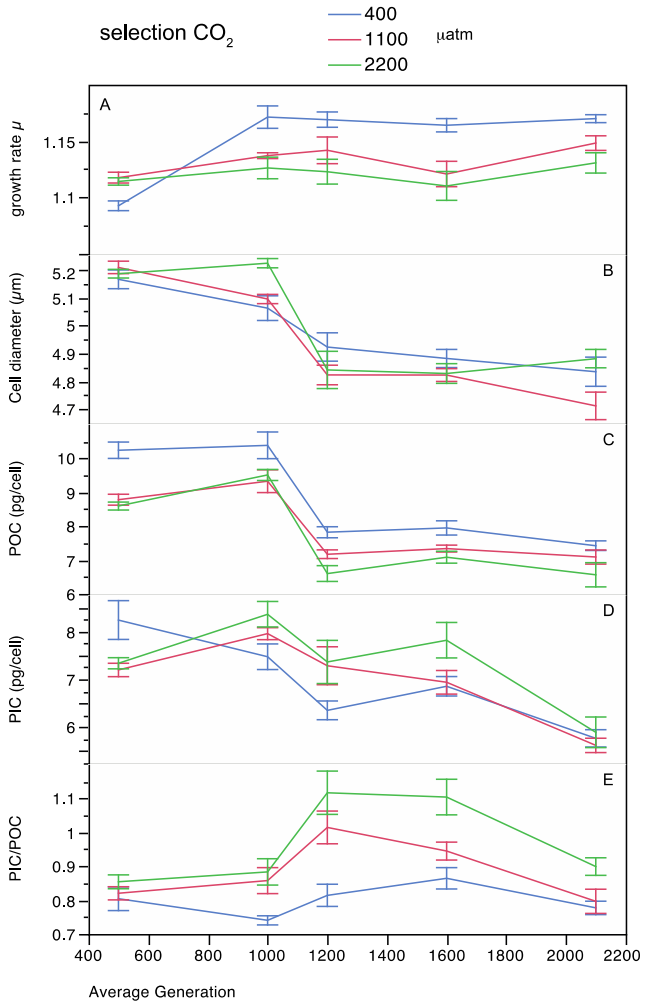

fig. S1. Correlated response in replicated *E. huxleyi* populations during 4 yrs of selection under three CO_2 levels simulating ocean acidification. Depicted is the response (mean±1SE, *n*=5) of medium- and high-CO_2 adapted vs. non-adapted (=control) populations in the ambient CO_2 condition (400 µatm) measured during assay experiments at 5 time points (x-axis, average generations), (**A**) exponential growth rate (**B**) cell diameter (**C**) particulate organic carbon per cell (POC cell^{-1}) (**D**) particulate inorganic carbon per cell (PIC cell^{-1}) (E) ratio of PIC:POC.

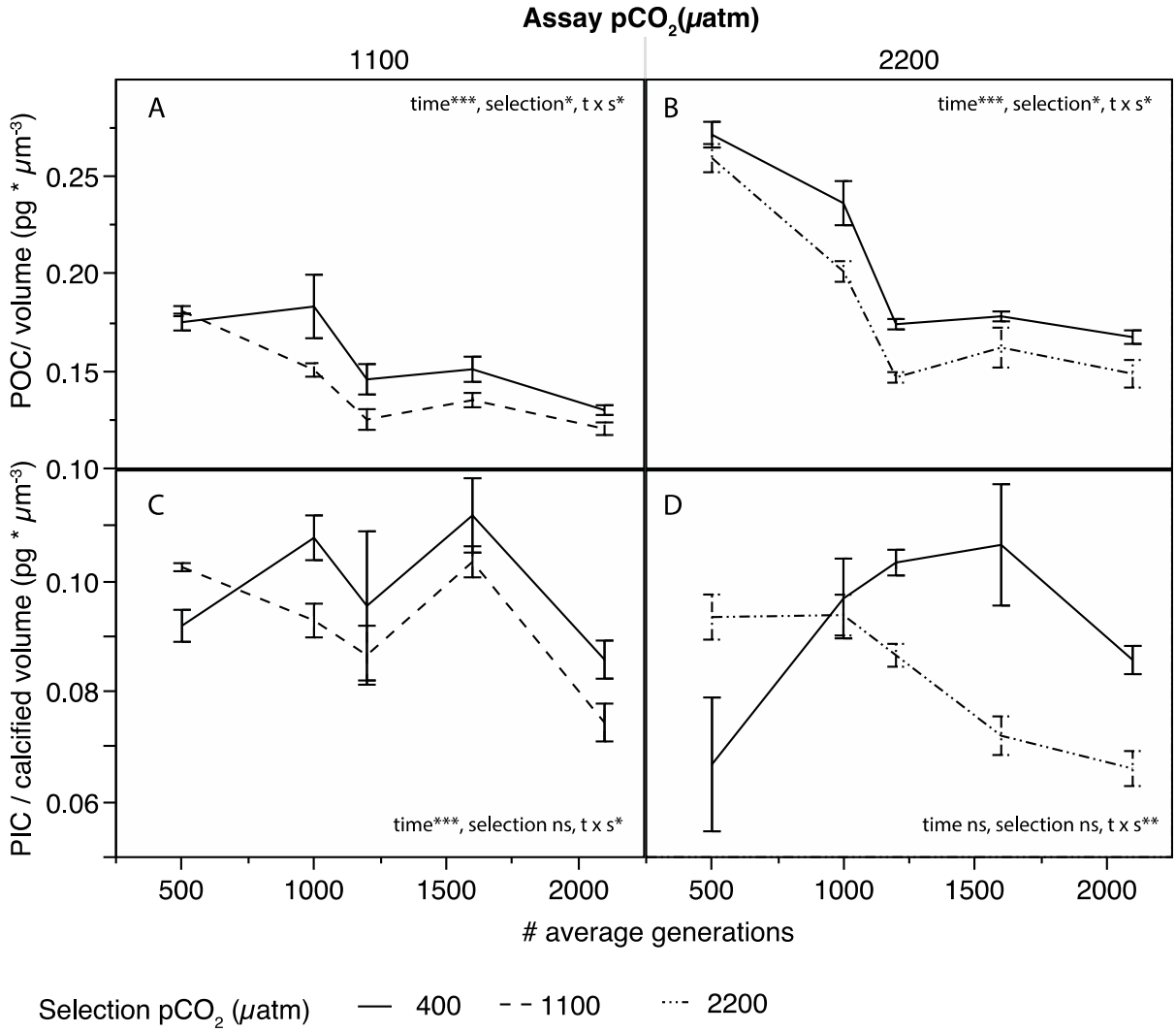

fig. S2. Adaptive response of *E. huxleyi* to selection under three CO_2 conditions, simulating ocean acidification. Depicted is the adaptive response measured during assay experiments at 5 time points (x-axis, average generations), always comparing medium- (left) and high-CO_2 adapted (right) vs. non-adapted populations of *E. huxleyi* in three different CO_2 environments, when assayed under elevated CO_2 (mean±SD, n=5). (**A**, **B**) POC standardized by cell volume (**C**; **D**) PIC standardized by cell volume Significant results of main and interaction effects are depicted with asterisks (*0.05≥*P*>0.01, **0.01≥*P*>0.001, ****P*<0.001). Complete repeated measures ANOVA results are given in Table S1.

table S1. Statistical analysis of the adaptive response in exponential growth rate and other cell traits of *E. huxleyi* to elevated CO_2 under two different CO_2-levels as repeated measures ANOVA (rmANOVA) with univariate and sphericity tests. *p*CO_2-selection is a between-subjects effect, CO_2 x time and time are within subject effects. For particulate inorganic carbon (PIC) additionally the correlated response was analyzed. Original data have been deposited in the World Data Center for Marine Environmental Sciences (WDC-MARE) (accession http://doi.pangaea.de/10.1594/PANGAEA.846062).

response/trait	Assay level	*p*CO_2 selection	time	CO_2 selection x time
growth rate	medium	$F_{1,8}$=43.92 p=0.0002	$F_{4,32}$=92.46 p<0.0001	$F_{4,32}$=2.941 p=0.0354
	high	$F_{1,8}$ = 53.72 p<0.0001	$F_{4,32}$=47.58 p<0.0001	$F_{4,32}$=1.991 ns
cell size	medium	$F_{1,8}$=0.762 ns	$F_{4,32}$=46.40 p<0.0001	$F_{4,32}$=11.44 p<0.0001
	high	$F_{1,8}$=0.0741 ns	$F_{4,32}$=61.83 p<0.0001	$F_{4,32}$=10.89 p<0.0001
particular organic carbon POC (pg/cell)	medium	$F_{1,7}$=2.590 P=0.0038	$F_{4,28}$=56.55 p<0.0001	$F_{4,28}$=4.68 p<0. 0051
	high	$F_{1,6}$=2.908 p=0.0058	$F_{4,24}$=70.76 p<0.0001	$F_{4,24}$=0.897 ns
particular inorganic carbon PIC (pg/cell)	medium	$F_{1,7}$=6.534 p=0.0378	$F_{4,28}$=17.749 p<0.0001	$F_{4,28}$=4.852 p=0.0042
	high	$F_{1,6}$=3.171 ns	$F_{4,24}$=5.345 p=0.0032	$F_{4,24}$=6.798 p=0.0008
PIC:POC ratio	medium	$F_{1,7}$=0.121 ns	$F_{4,28}$=1.000 p<0.0001	$F_{4,28}$=0,3378 ns
	high	$F_{1,6}$=0.500 ns	$F_{4,24}$=14.72 p<0.0001	$F_{4,24}$=6.356 p=0.0012
POC per cell volume	medium	$F_{1,7}$=6.471 p=0.0384	$F_{4,28}$=26.46 p<0.0001	$F_{4,28}$=3.444 p=0.0207
	high	$F_{1,6}$=12.42 p=0.0124	$F_{4,24}$=88.8577 p<0.0001	$F_{4,24}$=1.932 ns
PIC per cell volume	medium	$F_{1,7}$=4.736 p=0.066	$F_{4,28}$=14.08 p<0.0001	$F_{4,28}$=3.954 p=0.0115
	high	$F_{1,6}$=2.754 ns	$F_{4,24}$=2.349 ns	$F_{4,24}$=5.585 p=0.0025
PIC per cell correlated response	medium	$F_{1,7}$=0.311 ns	$F_{4,28}$=19.46 p<0.0001	$F_{4,28}$=3.797 p=0.0137
	high	$F_{1,6}$=3.091 ns	$F_{4,24}$=14.10 p<0.0001	$F_{4,24}$=2.460 p=0.0727

table S2. Statistical analysis of the reciprocal assay experiment after 4 yrs (average asexual generations = 2,100) to assess the adaptive and correlated response of *Emiliania huxleyi* to elevated CO_2. Evolutionary adaptation to two different CO_2-levels was analyzed as two separate 2x2 factorial ANOVA (see Fig. 2 for graphical depiction of treatment means). Medium OA treatment = 1,100 µatm pCO_2, high = 2,200 µatm pCO_2. Original data have been deposited in the World Data Center for Marine Environmental Sciences (WDC-MARE) (accession http://doi.pangaea.de/10.1594/PANGAEA.846062).

response/trait	OA treatment (sub-experiment)	CO_2 selection	Assay condition	CO_2 selection x assay condition
Daily growth rate µ	medium	$F_{1,16}$=1.37 ns	$F_{1,16}$=41.18 p<0.001	$F_{1,16}$=20.60 P<0.001
	high	$F_{1,16}$=0.3 ns	$F_{1,16}$=242.9 p<0.001	$F_{1,16}$=25.99 P<0.001
particular organic carbon POC (pg/cell)	medium	$F_{1,16}$=21.26 p<0.001	$F_{1,16}$=12.95 P=0.002	$F_{1,16}$=8.212 P=0.011
	high	$F_{1,16}$=18.18 p<0.001	$F_{1,16}$=57.69 p<0.001	$F_{1,16}$=1.802 ns
particular inorganic carbon PIC (pg/cell)	medium	$F_{1,16}$=9.862 p=0.0063	$F_{1,16}$=7.344 P=0.016	$F_{1,16}$=6.073 P=0.025
	high	$F_{1,16}$=8.322 P=0.011	$F_{1,16}$=38.96 p<0.001	$F_{1,16}$=11.88 P=0.0033
PIC:POC ratio	medium	$F_{1,16}$=0.150 ns	$F_{1,16}$=21.67 p<0.001	$F_{1,16}$=1.003 ns
	high	$F_{1,16}$=1.930 ns	$F_{1,16}$=299.2 p<0.001	$F_{1,16}$=20.20 P<0.001