Phytoplankton cell counts at 3 pCO2 levels and 2 temperatures before and after 12 month conditioning off New Zealand South Island, near Taiaroa Head, 2011 (Plankton acclimation project)

Website: https://www.bco-dmo.org/dataset/515271
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Project
» Experimental studies to understand and evaluate acclimation of marine plankton assemblages to increased CO2 and temperature (Plankton acclimation)

Contributors	Affiliation	Role
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Dataset Description

The study included a short-term two-week temperature/pCO2 factorial matrix incubation experiment using a natural, mixed diatom assemblage, the isolation of clonal cultures from each treatment and conditioning of the clones to the pCO2 and temperature combinations from which they were isolated for 1 year. Finally, the conditioned clones were recombined into artificial communities and allowed to compete, followed by a comparison of final community structure with that observed in the original two-week natural community experiment.

For this dataset, cell abundances of Cylindrotheca fusiformis, Coscinodiscus sp., Thalassiosira sp., Pseudonitzschia delicatissima, Navicula sp and Chaetoceros criophilus assessed after the initial natural community experiment and the 12-month conditioned artificial community incubations at low, medium and high CO2 concentrations and temperatures of 14°C and 19°C. Values given include the three replicate sample counts, the mean of counts and standard deviations on the three replicate bottles.

Experimental Design Chart

Relevant References:

These data are published as Supplemental Table 2 in Avery O. Tatters, Michael Y. Roleda, Astrid Schnetzer,

Feixue Fu, Catriona L. Hurd, Philip W. Boyd, David A. Caron, Alle A. Y. Lie, Linn J. Hoffmann and David A. Hutchins. Research article: Short- and long-term conditioning of a temperate marine diatom community to acidification and warming. Phil. Tras. Roy. Soc. B. 368 (1627) doi: 10.1098/rstb.2012.0437

Methods & Sampling

Experimental design

An overview of the experimental design is depicted in figure 1, and followed the general protocols for recently published dinoflagellate community experiments [21]. Sequentially, the study included a short-term two-week temperature/pCO2 factorial matrix incubation experiment using a natural, mixed diatom assemblage, the isolation of clonal cultures from each treatment and conditioning of the clones to the pCO2 and temperature combinations from which they were isolated for 1 year. Finally, the conditioned clones were recombined into artificial communities and allowed to compete, followed by a comparison of final community structure with that observed in the original two-week natural community experiment.

Short-term natural community incubation experiment

A mixed diatom assemblage that consisted primarily of Cylindrotheca fusiformis Reimann and Lewin 1964, Coscinodiscus spp., Thalassiosira spp., Pseudo-nitzschia delicatissima (Cleve) Heiden 1928, Navicula sp. and Chaetoceros criophilus (Castracene) sensu Hust 1886 was collected off the city of Dunedin on the South Island of New Zealand in January of 2011. The water was collected approximately 3 km offshore from Tairoa Head at the mouth of Otago Harbour halfway to Munida (45, 45.098 S 170, 48.68 E). The ambient sea surface temperature was 14.88C.

Seawater was collected for both the initial incubations and the short-term experimental dilution water. All water was combined into an approximately 500 l container and subsampled after filtering through 80 mm mesh to remove large zooplankton. Volumes (800 ml) were added to triplicate polycarbonate bottles and spiked with an f/50 nutrient derivative (10 mm NaNO3 2, 0.8 mm NaH2PO4 32, 10 mm Na2SiO3 and f/50 vitamin and trace metal concentrations [27,28]) to promote diatom growth. The bottles were incubated on a 12 L : 12 D cycle under 140 mE of cool white fluorescent illumination in free-standing laboratory incubators at 14 or 198C. The temperatures (ambient and þ58C) were selected based on predicted sea surface warming from the IPCC [29]. Triplicate sterilized 1 l polycarbonate bottles were gently bubbled at each temperature using commercially prepared air/ CO2 mixtures (Alphagaz, Air Liquide) at three concentrations also based on IPCC scenarios (approx. 210 matm ¼ pre-industrial pCO2; approx. 370 matm ¼ current pCO2; and approx. 560 matm ¼ future, year 2050 projected pCO2) [29]. Cellular abundances in an unbubbled control treatment did not significantly deviate from results of the current pCO2-bubbled treatment (data not shown). This methodology has been used for other CO2 experiments [21,30,31], including previous diatom studies [10,16,20].

The six pCO2/temperature treatments were maintained in active growth using semicontinuous culture methods [21]. Each bottle was diluted to the original time-zero in vivo chlorophyll a fluorescence value every 2 days with nutrient-amended 0.2 mmfiltered seawater. Aliquots were removed initially and after one and two weeks for examination of carbonate system parameters and community structure using microscopic cell counts.

Establishment of clonal cultures

Two to four individual cells from the six dominant diatom species were isolated from each of the short-term incubation bottles at the termination of the experiment. Inverted light microscopy was used to make taxonomic determinations based on morphological characteristics to make sure the isolates for each cell line were from the same species [32]. These monospecific clones were propagated in 24-well plates prior to being transferred to tissue culture flasks for long-term maintenance under pCO2 and temperature conditions identical to those from which they were isolated. A set of the culture isolates were transported under controlled temperature conditions to the University of Southern California in Los Angeles, CA, USA, where conditioning of the isolates and the 12-month community recombination experiments presented here were carried out. The culture isolates were maintained unreplicated for the first few weeks until they were verified to be established and growing well, at which time they were transferred into triplicate cultures for long-term maintenance; initial growth rates were obtained from these original unreplicated cell lines. These cultures were then maintained for a period of 1 year in exponential growth phase using the same recipe of autoclave-sterilized enriched seawater growth medium, and with other environmental variables such as light, pCO2 bubbling, temperature etc., maintained as in the two-week natural community experiment. Semicontinuous weekly dilutions were performed based on specific growth rates within each bottle, calculated as in [21]. The approximate number of generations during this time period was: C. fusiformis (185-212), Coscinodiscus sp. (169-229), Thalassiosira sp. (179-200), P. delicatissima (178-221), Navicula sp. (188-212) and C. criophilus (194-236).

Artificial community competition experiments
After the 12-month pCO2/temperature conditioning period, the cultures were recombined into artificial communities in the same relative proportions and abundance as in the original natural assemblage collected from Otago Harbour. The incubations of these artificial communities were performed under experimental conditions, duration and dilution frequencies identical to those of the original short-term natural community experiment.

Cell counts and growth rates
Samples for cell counts were obtained at the time of collection, before and after dilution and upon termination of the natural and artificial community incubations to determine abundances of each species. Cell-specific growth rates for each clonal culture were determined in individual culture flasks at the beginning of the 1 year conditioning period and in triplicate replicates after approximately 10 months of conditioning. These were calculated from samples taken 3 days apart using the growth rate equation m ¼ ln(Nt/No)/t12t0 (where N is the number of cells at time t1 and t0 (in days)) and represent a long-term steady-exponential state of growth. Algal cells were collected in 30 ml borosilicate glass scintillation vials, preserved with acidified Lugol's solution and enumerated using an Accu-Scope v. 3032 inverted microscope using the Utermo¨hl method [33].

Carbonate system characterization
Samples for carbonate system parameter analysis were taken at the time of the natural sample collection and at the termination of the short- and long-term experiments. Spectrophotometric pH for the initial community incubations was measured after [34] as described in [35] using a UV-vis spectrophotometer (Ocean Optics USB4000). For samples from the 12-month community incubations, spectrophotometric pH was determined using a Shimadzu 1800UV spectrophotometer according to a similar method [36]. Temperature was monitored using standard laboratory incubator thermometers and salinity by conductivity with an interchangeable probe using an Orion 5-star plus pH meter. For pH measurements, temperature and salinity values for the initial experiment were 23.68C and 35, respectively. For the conditioned experiment, the temperature was 258C and salinity 35. Dissolved inorganic carbon was analysed using a CM5230 CO2 coulometer (UIC) [37]. Experimental pCO2 was calculated using CO2SYS software [22] with dissociation constants from Dickson & Millero [38] using the combined data of [39,40] and KSO4 from [41] (table 1).

Data Processing Description

Statistics
Multivariate analyses were conducted using the PRIMER v6 statistics package [42] with the PERMANOVA add-on [43]. Bray-Curtis similarities were computed following square-root transformation of final cell abundances (cells m21) for all six diatom species from replicate bottles. PERMANOVA was used to test for significant differences among and within predefined groups in response to differing pCO2 competition levels and differing temperature. Data from the original natural community experiment and from the artificial community competition trials 12 months later were analysed. Pseudo-F values of 1 are typical of a large overlap among sample groups that are being compared (confirmation of H0 hypothesis), whereas pseudo-F values greater than 1 indicate little or no overlap between the compared groups [43]. Observed interactions between pCO2 and temperature were interrogated using PERMANOVA as well as pairwise comparisons (one-way ANOSIM [38]). R-values close to zero were indicative of no difference among groups, whereas R-values close to 1 meant that dissimilarities among groups were larger than any dissimilarity within groups [42].

We used a two-way crossed design for the ANOSIM routine to examine the comparative effects of differing pCO2 competition levels and differing temperature on algal assemblages. This approach tests the average effect of pCO2 levels during competition removing differences in temperature and the average effect of temperature levels removing differences in competition pCO2 [42]. Cell abundance information for these analyses was taken from the final time points of our initial natural community experiment and the artificial community competition trial after 12 months.

Differences between specific growth rates after 10 months of conditioning in addition to cell abundances from the original natural community and the final artificial community experiments under the four temperature and pCO2 combinations were tested using one-way ANOVA using Microsoft EXCEL 2013.

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Data Files

File
phyto_abund.csv(Comma Separated Values (.csv), 10.39 KB) MD5:5d8b705f3325b722b52c430cfa23f5e7 Primary data file for dataset ID 515271

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Parameters

Parameter	Description	Units
species	species name	unitless
temp_init	temperature before conditioning period	degrees Celsius
pCO2_level	relative level of pCO2	unitless
sample	sample name: A, B, C, average, or standard deviation of the 3 samples	unitless
incubation_init	initial period of incubation	months
pCO2_init	initial partial pressure of carbon dioxide	micro-atmospheres
abundance_init	abundance of cells before conditioning period	cells/milliliter
incubation_cond	duration of pCO2/temperaure conditioning period of incubation	months
temp_cond	temperature during conditioning period	degrees Celsius
pCO2_cond	partial pressure of carbon dioxide during conditioning period	micro-atmospheres
abundance_cond	abundance of cells after conditioning period	cells/milliliter

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Instruments

Dataset-specific Instrument Name	CO2 coulometer
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	Dissolved inorganic carbon was analysed using a CM5230 CO2 coulometer (UIC)
Generic Instrument Description	A CO2 coulometer semi-automatically controls the sample handling and extraction of CO2 from seawater samples. Samples are acidified and the CO2 gas is bubbled into a titration cell where CO2 is converted to hydroxyethylcarbonic acid which is then automatically titrated with a coulometrically-generated base to a colorimetric endpoint.

Dataset-specific Instrument Name	Conductivity Meter
Generic Instrument Name	Conductivity Meter
Dataset-specific Description	Orion 5-star plus pH meter with an interchangeable probe for conductivity measurements to calculate salinity in conjunction with a standard laboratory incubator thermometer.
Generic Instrument Description	Conductivity Meter - An electrical conductivity meter (EC meter) measures the electrical conductivity in a solution. Commonly used in hydroponics, aquaculture and freshwater systems to monitor the amount of nutrients, salts or impurities in the water.

Dataset-specific Instrument Name	spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	UV-vis spectrophotometer: Ocean Optics USB4000
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Shimadzu 1800UV
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

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Deployments

Hutchins_2011

Website	https://www.bco-dmo.org/deployment/515350
Platform	USC
Start Date	2011-01-01
End Date	2011-01-31
Description	Collections were made off the city of Dunedin on the South Island of New Zealand in January of 2011. The water was collected approximately 3 km offshore from Taiaroa Head at the mouth of Otago Harbour halfway to Munida. http://marineregions.org/gazetteer.php?p=details&id=24560

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Project Information

Experimental studies to understand and evaluate acclimation of marine plankton assemblages to increased CO2 and temperature (Plankton acclimation)

Coverage: Bloom sample retrieved from near-shore water near Venice Beach, CA

Progressing ocean acidification and increasing sea surface temperature may significantly impact marine plankton community structure and community-level processes. Yet, our ability to predict specific responses is still limited because of the tremendous taxonomic complexity of microbial assemblages and the limitations of the methodological and experimental tools presently available to test specific hypotheses. Research to study community level effects due to a changing CO2/temperature regime often involve short-term field incubations that subject organisms to simulated 'greenhouse' conditions. A central question for understanding global climate change is whether the trends and patterns that are observed in communities during short-term manipulations can be extrapolated to the responses of fully acclimated plankton communities over decadal or longer timescales.

The specific objectives of this research program are: 1) to examine how protistan communities restructure in response to increased seawater CO2 concentrations and temperature in semi-continuous field incubation experiments, and 2) to evaluate if the dominant algal species that are isolated from either ambient or increased CO2 and temperature treatments in field experiments will re-establish dominance under the same conditions in acclimated laboratory culture competition studies. Changes in community structure of natural protistan assemblages in our experimental treatments will be followed using image-based methods (flow cytometry, FlowCAM and microscopy) in combination with state-of the art molecular tools (DNA fingerprinting). Molecular approaches have begun to reveal an incredible high diversity for marine microbes and stimulate debate in regard to the ubiquitous presence of a microbial 'Rare Biosphere' that is, the presence of a huge number of species that are present at extremely small percentages of the total abundance of microbes, among a much smaller percentage of dominant ones. Little is known about the ecological significance of these rare species, and the investigators hypothesize that change in CO2 and temperature will select for some of these members that are inconspicuous under ambient conditions.

The unique aspect of this experimental approach is the combined use of field incubations that encompass entire natural microbial assemblages, with a series of laboratory culture competition trials that focus on the same groups of algae after extended acclimation, to evaluate the validity of short-term experiments that examine changing CO2 and temperature. First, field incubation experiments will be conducted to characterize changes in protistan community structure under ambient and future CO2/temperature regimes. Second, clonal algal strains will be isolated from dominant taxa in present day and greenhouse treatments, and cultivated for extended periods under their 'preferred' CO2/temperature conditions. Finally, mixtures of these acclimated strains will be competed against each other, to re-examine their responses to ambient and greenhouse conditions and compare them to the responses observed in the unacclimated field incubation experiments.

Two graduate students will make this project the focus of their Ph.D. research at USC, and undergraduate students will be involved in the field and laboratory work. Results from this research will be incorporated in lesson plans on microbial diversity and global climate change. Dissemination of data and results is planned on a project website. The PIs in this project also participate in an on-going, innovative, NSF-funded program (Centers for Ocean Science Education Excellence; COSEE-West) which focuses on personal involvement of faculty in a custom framework to allow an effective connection with K-12 teachers, thus improving math and science education in disadvantaged parts of Southern California.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0962309

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