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Using Global Climate Models to Inform Multi-Driver Biological Ocean Experiments

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Running Title: Ocean multi-driver conditions

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Abstract:

This century, widespread shifts in temperature and CO₂-driven acidification are projected for the surface ocean. Determining how ocean biota will respond to these changes requires scaling multiple drivers together because biological responses to one driver often depend on the conditions of the others. However, ocean temperature and carbonate chemistry vary across regions, seasons, and emissions scenarios, creating more combinations than laboratory experiments can test. While best-practice literature recommends designing experiments around climate projections, translating complex model output into a tractable set of realistic multi-driver treatments requires simplifying substantial spatial, seasonal, and scenario-dependent variation. To address this, we synthesized surface-ocean model projections into relevant multi-driver scalings of temperature and the full carbonate system across three decades (1850s, 2000s, and 2090s). These scalings use public data from the Coupled Model Intercomparison Project Phase 6 (CMIP6) which we group into 14 open-ocean biomes with annual and monthly mean conditions. The biome means reveal that most projected temperature changes are smaller than spatial and seasonal variation, whereas possible future carbonate system changes exceed present day variability. We then compared these projections with 2,456 treatments reported in 196 phytoplankton studies spanning the last two decades. We find that the majority of treatments (57%) fall within the modeled condition space,

but many studies examine conditions unrealistic for the past or future open ocean. Finally, we applied North Atlantic conditions in batch cultures of two eukaryotic phytoplankton taxa. The achieved carbonate-system conditions fell within modeled biome variability, and the temperature increase matched projected warming. Together, this framework provides regional and emissions-scenario context for selecting tractable multi-driver treatments and comparing conditions across experiments.

Keywords: phytoplankton, climate change, experimental evolution, ocean acidification, multi-driver

1 | Introduction

Anthropogenic CO₂ emissions are altering the physical and chemical environments of surface ocean biota (IPCC, 2022). Over this century, both sea surface temperature and total dissolved inorganic carbon (DIC) are projected to increase across large areas of the ocean (Kwiatkowski et al., 2020; IPCC, 2022). The absorption of CO₂ by the ocean also lowers seawater pH and shifts the relative proportions of CO₂, HCO₃⁻, and CO₃²⁻ through a process known as ocean acidification (Doney et al., 2009). Most surface ocean biota will therefore be exposed, either directly or indirectly, to changes in multiple aspects of their environment (Kroeker et al., 2013; Jorda et al., 2020). These physical and chemical conditions altered by climate change are referred to as environmental ‘drivers’ rather than ‘stressors’ because their biological effects do not always have a consistent direction. Individual drivers can enhance, suppress, or have little effect on growth and physiology (Boyd et al., 2018). Understanding biological responses to these drivers and their consequences for marine ecosystems represents a major challenge for biological oceanography (van Moorsel et al., 2023).

Global change is altering multiple environmental drivers simultaneously, requiring an understanding of how organisms respond to their combined effects (Boyd et al., 2015). Many

marine organisms, for example, will experience warming and acidification together. Because the biological effect of either driver can depend on the other, responses to their combined changes cannot always be predicted from responses to each driver in isolation (Crain et al., 2008; Kroeker et al., 2013; Seifert et al., 2020; Meunier et al., 2025). While single driver experiments are useful for isolating biological mechanisms, experiments that vary multiple drivers together are needed to characterize biological responses to combinations of conditions organisms may experience in the ocean (Boyd et al., 2018). The inferences drawn from these experiments, however, depend on whether the tested combinations reflect how environmental conditions will vary and covary in nature (Boyd et al., 2015).

Designing experiments to simulate future ocean change requires selecting combinations of conditions that represent a baseline and a plausible future state. Earth system models (ESMs) provide spatially resolved projections of how ocean conditions may change under different emissions scenarios (O'Neill et al., 2016; Kwiatkowski et al., 2020). Ocean conditions vary across geographic regions and seasons, while projected changes depend on the projection period and greenhouse gas emissions scenario considered (Bates et al., 1998; Gregor and Gruber, 2021; Bates and Johnson, 2023). Accounting for these spatial, seasonal, temporal, and scenario-dependent differences yields more combinations of conditions than laboratory experiments can feasibly test, particularly when carbonate chemistry must be manipulated and maintained (LaRoche et al., 2010). Selecting which conditions to test is therefore important for maximizing what can be learned about biological responses in future oceans.

One approach for organizing spatial variability in marine conditions is to divide the ocean into regional subsets, commonly referred to as provinces or biomes (Longhurst, 2007). Biomes reduce the complexity of spatially continuous datasets, such as ESM outputs, by grouping areas of the

ocean according to shared physical or biological characteristics (Vichi et al., 2011). This, in turn, reduces variability in environmental conditions within each biome and provides clearer regional estimates. When productivity is a characteristic used to define biomes, they also distinguish broad ecological regimes, such as low-productivity subtropical gyres and more productive seasonally stratified regions (Fay and McKinley, 2014). A biome-based framework can therefore reduce spatially continuous ESM output to a smaller set of regional temperature and carbonate chemistry estimates for experimental design.

Phytoplankton are a particularly important group for applying this biome-based approach to multi-driver experiments. These diverse, single-celled photosynthetic organisms account for around half of global net primary production and form the base of pelagic food webs that support ocean ecosystems (Behrenfeld et al., 2006; Finkel et al., 2010). Through the biological carbon pump, the export of a portion of phytoplankton production from the surface to the ocean interior allows the ocean to store more carbon than it would through physical processes alone, thus lowering atmospheric CO₂ (Kwon et al., 2009; Henson et al., 2022). In contrast to terrestrial ecosystems, where most primary production is performed by long-lived plants, phytoplankton have short generation times and large, genetically diverse populations (Reusch and Boyd, 2013). Experimental evolution studies have demonstrated that marine phytoplankton can adapt to environmental change on timescales relevant to anthropogenic climate change (Reusch and Boyd, 2013). Over the next century, the collective physiological and evolutionary responses of phytoplankton to multiple climate change drivers will influence rates of carbon fixation and energy transfer through marine food webs, with potential consequences for carbon export and long-term storage (Henson et al., 2022).

Phytoplankton growth is strongly influenced by temperature and CO₂ availability, making these drivers important to consider together (Edwards et al., 2016; Paul and Bach, 2020; Henson et al., 2021). Responses to shifts in temperature and dissolved inorganic carbon can also be nonlinear, making the selection of multi-driver conditions critical for drawing inferences about biological responses in future oceans (Hennon et al., 2019; Barton et al., 2020). For example, in an experimental evolution study, populations of *Gephyrocapsa huxleyi* (*G. huxleyi*) adapted simultaneously to elevated temperature and *p*CO₂ showed a greater increase in relative fitness than populations adapted to either driver alone (Schlüter et al., 2014). A meta-analysis similarly found that temperature and *p*CO₂ interacted in their effects on phytoplankton growth: across taxa, elevated *p*CO₂ generally reduced the positive effect of warming on growth, although coccolithophores such as *G. huxleyi* showed the opposite response (Seifert et al., 2020). These nonlinear and taxon-specific responses make model-informed scaling of temperature and carbonate chemistry essential for obtaining meaningful estimates of future phytoplankton responses.

In this study, we group modeled spatially and temporally continuous drivers from the Coupled Model Intercomparison Project Phase 6 (CMIP6) into paired temperature-carbonate system conditions (i.e., scalings) for 14 open ocean biomes. The resulting dataset contains past, present, and future surface condition scalings for these drivers at annual and seasonal resolution. We compare treatments used in published phytoplankton studies with the modeled condition space and use phytoplankton culture experiments to demonstrate how conditions for a selected region, projection period, and emissions scenario can be translated into laboratory treatments. Finally, we discuss considerations for scaling temperature and carbonate chemistry in phytoplankton experiments and evaluate sources of variability and uncertainty associated with these drivers.

2 | Materials and Methods

2.1 ESM and observational data product selection and processing

We compiled model projections from multiple ESMs to assess future temperature and carbonate system conditions (see Supp. Table 1, John et al., 2018; Danabasoglu, 2019b, a; Neil Cameron Swart et al., 2019b, a; Neil C. Swart et al., 2019; Danabasoglu et al., 2020; Dunne et al., 2020; Christian et al., 2022). Our analysis of ESMs was limited to Coupled Model Intercomparison Project Phase 6 (CMIP6) ScenarioMIP model projections that were available on the ‘LEAP Pangeo’ server (Access date: May 8, 2025). We focused on ocean surface conditions given data availability (see section 2.2 for discussion of this constraint). Surface conditions were extracted from the shallowest grid cell of each ocean model; the depth of this cell varied among models (range 0-2 to 0-10 meters, Supp. Table 1). To determine which pair of carbonate parameters to use in calculating the whole carbonate system, we searched all available models for output of every parameter. We chose surface ocean pH and $p\text{CO}_2$ to constrain the rest of the carbonate system, as this yielded the greatest number of models ($n = 5$) for inclusion in our analysis (Supp. Table 1). In addition to these parameters, we retained surface ocean temperature and salinity, which were available in all selected models. We chose to focus on data from two contrasting emissions scenarios, SSP2-4.5 (i.e. ‘Middle of the Road’) and SSP5-8.5 (i.e. ‘Fossil-fueled Development’, O’Neill et al., 2016). Model output was obtained at a monthly resolution to assess seasonality in the analysis (Supp. Table 1). With xESMF, a Python tool for re-gridding geospatial data, all ensemble members were re-gridded using bilinear interpolation from their natural grids to a 1x1 degree latitude-longitude projection for consistent comparison.

For each model grid cell, the full carbonate system parameters were calculated using total scale pH and $p\text{CO}_2$ via a python implementation in the ‘*CO2SYS*’ family of software (*PyCO2SyS*,

Humphreys et al., 2022). Surface temperature and salinity were set in carbonate system calculations using ESM model data from each member. As concentrations were not provided for total borate, calcium, fluoride, and sulfate, these contributions to alkalinity were calculated based on assumed correlation with salinity within *PyCO2SYS*. A full list of parameters used to calculate carbonate system speciation across this study can be found in Supplementary Table 2. As the number of ensemble members varied among models, we averaged ensemble members within each model before calculating a grand mean to ensure equal weight in final averages. Finally, CMIP6 model output was subset to three separate decades for comparison: pre-industrial (1850 through 1859), a modern era baseline (2000 through 2009), and the end of the century (2090 through 2099). We refer to these decades as the 1850s, 2000s, and 2090s for simplicity throughout.

Lastly, we included inorganic carbon data from the Bermuda Atlantic Time Series (BATS) bottle data to compare to our laboratory treatments and condition projections (Bates and Johnson, 2023; Johnson et al., 2026). We calculated the full carbonate system using alkalinity and DIC with *PyCO2SYS* using the same constants as the CMIP6 projections (Supp. Table 2). Reported salinity, temperature, and pressure were used in the calculation. Data were subset to surface values (< 5 meters), averaged by month, and then by decade (1990s, 2000s, 2010s) to obtain annual means while minimizing temporal sampling bias.

2.2 Biome based spatiotemporal averages

To better constrain spatial variability and examine latitudinal patterns, we examined temperature and carbonate system surface conditions over defined global ocean biomes (Fay and McKinley, 2014). The boundaries of these biomes were calculated as previously described in Fay and McKinley using spatial patterns of sea ice, sea surface temperature, productivity, and stratification. Briefly, biomes are grouped based on basin (e.g., Atlantic) as well as limits in environmental

factors that define their type. These include equatorial (EQU), subtropical permanently-stratified (STPS), subtropical seasonally-stratified (STSS), and subpolar seasonally-stratified (SPSS). Temperature helps separate all biomes latitudinally while stratification and productivity help separate more seasonally productive upwelling regions (STSS) from the low productivity gyres (STPS). Thus, resulting biomes represent distinct open-ocean regions in their physical characteristics but also their biogeochemistry.

We chose to use ‘mean biomes’ based on climatology from 1998 to 2010 for our analysis. We choose not to spatially change these biomes when assessing 2090s values even though these boundaries will shift. Keeping fixed biomes allows users of the dataset to directly compare how parameters will shift in a present-day region of interest, which is critical for the utility of this exercise. Using mean boundaries based on modern climatology, we took the grand mean and standard deviation of monthly resolution CMIP6 output across time (120 months), model (5 models), and area (variable across biome, see Fay and McKinley, 2014). Thus, the standard deviations presented here consider variability from all of these sources. Means were weighted based on spatial area represented by each 1x1 degree grid cell. For seasonal means, we averaged all variables by month over each decade instead of annually.

There are three limitations to applying to the spatial constraints on biome conditions presented here. First, all of these model data only represent surface (<10 meters) conditions and do not examine variability with ocean depth. The fraction of primary production that occurs in the very surface (< 10 meters) of the water column where these values are applicable is highly dependent on light and nutrient conditions (Richardson and Bendtsen, 2019). While resolving driver values through the entire photic zone is currently outside the scope of this study, future work may attempt to better constrain future driver conditions for phytoplankton at depth. Second, we did not examine

values in biomes that featured dominant seasonal ice cover (Figure 1). Sea ice-covered regions are likely to experience some of the most dramatic and unique changes in future climate scenarios, but ESMs estimate these regions less robustly than the open ocean. Thus, we do not include them in the present study but recommend that future work could do so. Lastly, it is important to note that coastal regions are removed from these biomes, and the resulting values likely do not reflect coastal dynamics. Both temperature and carbonate system dynamics are more variable in coastal regions and would not be well characterized by this model synthesis. Similarly, although outside the approach of this paper, we encourage elucidation of more localized driver-scaling for specific coastal ecosystems (McElhany and Shallin Busch, 2013).

2.3 Compilation and screening of literature data from OA-ICC phytoplankton experiments

In order to compare modeled conditions to culture treatments found in the literature, we used a curated database from the Ocean Acidification International Coordination Centre (OA-ICC, Yang et al., 2016, 2024). For clarity, we refer to driver values from biomes as “conditions” and those values applied to a phytoplankton culture as a “treatment”. The OA-ICC maintains a database of studies related to ocean acidification that are available via the PANGAEA data library. Using the OA-ICC online portal (accessed August 28th 2025), we selected studies that met the following criteria: 1) the targeted biological group was phytoplankton, 2) the species was found in pelagic rather than benthic environments, and 3) the study was a laboratory-based experiment, not a field observation or field experiment. These criteria yielded 213 studies for consideration. Full details on the curation and screening of these lab treatments can be found in the supplemental material (Supp. Methods 1.1, Supp. Figure 1). Briefly, we extracted all carbonate chemistry and temperature values used in each study as well as experimental metadata to subset each study into defined treatments. We did not consider error (i.e., standard deviation of replicates) within a

treatment as many studies did not report individual replicates and the values we accessed were already averages of replicates. As a final screen, we only assessed studies in which all three inorganic carbon species (CO_2 , HCO_3^- , CO_3^{2-}) were reported which removed six studies. Overall, 92% (196 studies) were retained after screening (Supp. Methods 1.1).

2.4 Comparing OA-ICC treatments to biome conditions

We used a three-step procedure to compare literature treatments with modeled ocean conditions. First, we identified treatments that met our criterion for biome relevance (see below). Second, we assigned each biome-relevant treatment to its nearest reference condition across biomes, periods, and scenarios. Third, we classified treatments outside the biome-relevant condition space as high inorganic carbon, low inorganic carbon, or misaligned.

We defined biome relevance using 56 reference conditions, each representing the annual mean for one of 14 biomes in one of four temporal and scenario categories: the pre-industrial 1850s, the modern 2000s, the 2090s under SSP2-4.5, and the 2090s under SSP5-8.5. We evaluated temperature and four carbonate system variables: $[\text{CO}_2]$, $[\text{HCO}_3^-]$, $[\text{CO}_3^{2-}]$, and pH. These five variables produced 10 unique pairwise comparisons. For each comparison, we used the R package *concaveman* to construct a bounded region encompassing the approximate two-standard-deviation limits of the 56 reference conditions (Park and Oh, 2012; Supp. Figure 2). A treatment was considered biome relevant when it fell within the bounded region in all 10 pairwise comparisons (Supp. Figure 2 and 3).

For each biome-relevant treatment, we identified its nearest reference condition across biomes, periods, and scenarios using standardized Euclidean distance. We calculated the distance between each treatment and all 56 annual reference conditions across the five variables:

234

$$d_i = \min_b \sqrt{\sum_{k=1}^5 \left(\frac{t_{ik} - c_{bk}}{\sigma_k} \right)^2}$$

235 where t is the treatment's value for five variables ($k = [\text{CO}_2]$, $[\text{HCO}_3^-]$, $[\text{CO}_3^{2-}]$, pH, and
 236 temperature), " c " are the biome condition means for each of the 56 biome conditions (b) and
 237 variables (k), and σ is the standard deviation of biome means for each variable.

238 Treatments outside the biome-relevant condition space were classified according to the direction
 239 and alignment of their carbonate chemistry. A treatment was classified as high inorganic carbon if
 240 $[\text{CO}_2]$ exceeded $59.6 \mu\text{mol kg}^{-1}$, $[\text{HCO}_3^-]$ exceeded $2305 \mu\text{mol kg}^{-1}$, or pH was below 7.56,
 241 provided that none of these variables fell beyond the biome-relevant condition space in the
 242 opposite direction. A treatment was classified as low inorganic carbon if $[\text{CO}_2]$ was below 6.0
 243 $\mu\text{mol kg}^{-1}$, $[\text{HCO}_3^-]$ was below $1535 \mu\text{mol kg}^{-1}$, or pH exceeded 8.31, provided that none of these
 244 variables fell beyond the condition space in the opposite direction. We excluded $[\text{CO}_3^{2-}]$ from this
 245 directional classification, because its concentration can decrease at very low pCO_2 . Treatments
 246 outside the biome-relevant condition space that met neither the high nor low definition were
 247 classified as misaligned (Supp. Figure 4).

248 2.5 Phytoplankton culture data from preliminary acclimation experiments

249 We implemented our biome conditions in a lab environment with two representative eukaryotic
 250 taxa: *Gephyrocapsa huxleyi* (CCMP 371) and *Thalassiosira pseudonana* (CCMP 1335) to
 251 illustrate the translation of these conditions into treatments. Both the carbonate system and
 252 temperatures used were aimed at modern and future North Atlantic biome values. Full details on
 253 media preparation, culturing, harvesting, and laboratory measurements of the carbonate system
 254 (pH and DIC) can be found in Supplementary Methods (Sections S1.1 to S1.4). Briefly, we grew

phytoplankton in batch culture in shaking incubators with controlled temperature, light, and $p\text{CO}_2$. In order to maintain stable carbonate system conditions, we experimentally determined a cell density threshold at which the culture's inorganic carbon would be minimally perturbed (+0.1 pH) while yielding usable biomass for analysis. Cultures were pre-acclimated with at least four transfers before harvesting to avoid initial shock caused by changes in conditions. For final data, DIC was monitored at the beginning and end of the incubation with pH measured throughout. *PyCO2Sys* was used to constrain the carbonate system at the harvest time using DIC and total scale pH. Carbonate system values from the last time-point were compared to biome averages ($n = 33$ for baseline condition, $n = 37$ for future condition). The standard deviation between individual flask replicates was calculated to compare to biome variability.

Data and code to recreate all results from this study are publicly available and archived (Holm, 2026a, b).

3 | Results

Using CMIP6 output, we generated a dataset of mean annual and monthly scalings for temperature and the carbonate system. Here, we describe how these conditions vary across biomes, periods, and emissions scenarios. We show how the dataset can support the design of multi-driver experiments, comparisons across studies, and identification of gaps in literature treatments compared to projected ocean conditions.

3.1 Future change in temperature across biomes

Across the surface ocean, annual mean temperature increased by 1.7 ± 1.13 °C under the moderate emissions scenario (SSP2-4.5) and by 3.6 ± 1.8 °C under the high emission (SSP5-8.5) scenarios by the 2090s. These global changes in sea surface temperature are consistent with previous model compilation efforts (Kwiatkowski et al., 2020). Resolving the projections by biome, however,

revealed a wider range of warming. Across the 14 biomes warming ranged from 1.26 to 2.75 °C under SSP2-4.5 and from 2.85 to 5.35 °C of warming under for SSP5-8.5 (Figure 2G) across all biomes. The North Pacific SPSS biome exhibited the greatest average warming under both scenarios, while the North Atlantic SPSS and Southern Ocean SPSS biomes exhibited the least warming under the moderate and high emission scenarios respectively. However, the distributions of projected warming overlapped substantially across nearly all biomes (Figure 2G).

Despite broadly similar magnitudes of projected warming, absolute temperatures varied substantially both among and within biomes under both modern and future conditions. In the 2000s, annual mean biome temperatures range from 4.0 ± 2.6 in the Southern Ocean SPSS biome to $30.0 \pm 0.76^\circ\text{C}$ in the West Pacific EQU biome (Figure 2A). The same biomes defined the thermal extremes under both end-of-century scenarios, with temperatures ranging from 5.5 ± 2.8 to 31.7 ± 0.90 °C under SSP2-4.5 and from 6.8 ± 2.9 to 33.5 ± 1.12 °C under SSP5-8.5.

Within most biomes, temperature also exhibited pronounced seasonality relative to the magnitude of projected warming. We quantified seasonal amplitude as the difference between the minimum and maximum monthly mean temperature for each biome. In the modern ocean (2000s), equatorial biomes had the smallest seasonal amplitudes, ranging from 0.5 to 3.0 °C, while subtropical seasonal stratified (STSS) biomes had the largest seasonal amplitudes, ranging from 4.3 to 8.5 °C. Projected warming therefore did not shift temperatures entirely beyond their modern seasonal ranges in most biomes (Supp. Figure 5). Even under SSP5-8.5, the seasonal temperature range projected for the 2090s overlapped with the 2000s range in 64% of biomes.

3.2 Future change in the carbonate system across biomes

In the CMIP6 ensemble, surface ocean $p\text{CO}_2$ increases substantially by the 2090s, with all biomes converging toward ~ 600 ppm under the moderate emissions scenario SSP2-4.5 and ~ 1035 ppm for under the high emission scenarios SSP5-8.5 (Figure 2). Mean pH also shifted entirely outside present-day ranges under both scenarios by the end of the century (Figure 2E). Thus, in contrast to temperature, $p\text{CO}_2$ and pH show less spatial variation in the present-day ocean and are projected to undergo a large, relatively uniform shift across biomes by the end of the century (Figure 2). However, despite relatively consistent shifts in $p\text{CO}_2$ and pH, carbonate system speciation varies substantially across biomes due in part to differences in temperature and salinity (Figure 2B-D). By the 2090s, aqueous CO_2 concentrations are projected to range among biomes from 14.7 ± 0.6 to $30.2 \pm 2.8 \mu\text{mol kg}^{-1}$ under the moderate emissions scenario and from 24.2 ± 0.5 to $50.2 \pm 4.7 \mu\text{mol kg}^{-1}$ under the high emission scenarios (Figure 2B). Concentrations of HCO_3^- and CO_3^{2-} similarly vary substantially across biomes. Thus, even where changes in pH are similar, surface ocean biota will experience distinct chemical environments depending on their location.

Present day seasonal amplitudes in aqueous CO_2 reflect temperature seasonality: equatorial and subtropical biomes have the smallest amplitudes ($< 0.5 \mu\text{mol kg}^{-1}$), while subpolar seasonally stratified biomes having the largest. Unlike temperature, however, this seasonal variability is small relative to projected end-of-century shifts in carbonate system parameters. For all carbonate system parameters, projected changes between 2000s and 2090s exceeded seasonal variability, with no overlap between the seasonal ranges of the two periods in any biomes under either emissions scenario (Supp. Figure 5).

3.3 Comparing model projections with literature treatments

Having established the present day and projected future conditions across ocean biomes, we compared the resulting biome-specific ranges with treatments used in published laboratory

experiments on phytoplankton. The final dataset from the OA-ICC consisted of 2,456 unique treatments from 196 studies spanning the past two decades (Supp. Figure 6). The literature was dominated by studies conducted in bottles or other small culturing vessels (96%) and by studies of single species rather than co-cultures or entire communities (95%). Haptophytes were the most frequently represented taxonomic group, appearing in 93 studies, and *Gephyrocapsa huxleyi* alone was examined in 66 studies, making up 31% of the studies

A treatment was considered biome relevant when it fell within the approximately two-standard-deviation envelope in all 10 pairwise comparisons among temperature and the four carbonate system variables (Section 2.4; Supp. Figure 2). Of the 2,456 unique treatments, 1402 (57%) met our criterion biome relevance. Most studies (162 studies, 82.7%) included at least one biome relevant treatment (Supp. Figure 8A). All treatments were biome relevant in 38.8% of studies, while no treatments were biome relevant in 17.3% of studies. The remaining 43.9% of studies included both biome relevant and non-biome relevant treatments. Overall, treatments in the literature clustered near the multi-driver conditions described here, but their distributions had long tails extending toward inorganic carbon values substantially above and below the biome-specific ranges (Figure 2).

Given the wide spatial variability in temperature, almost all experimental treatments fell within the thermal range represented by at least one biome. Consequently, differences in biome relevance arose primarily from whether the inorganic carbon conditions of each treatment aligned with those of the biomes (Supp. Figure 7, Supp. Figure 8). For each biome-relevant treatment, we identified its nearest reference condition across biomes, periods, and scenarios using standardized Euclidean distance (Section 2.4).

Just over half of the studies ($n = 110$; 54%) included both a treatment assigned to a baseline reference condition (1850s or 2000s) and one assigned to a projected future condition (2090s). Only 30 studies included treatments assigned to both baseline periods: preindustrial conditions in the 1850s and present-day conditions in the 2000s. A greater proportion of studies included at least one treatment assigned to a future condition (76%) than one assigned to a baseline condition (62%). This difference may partly reflect the greater spread of projected end-of-century conditions and the overlap between present-day and SSP2-4.5 conditions. Among studies with treatments assigned to future conditions, slightly more included a treatment assigned to SSP5-8.5 than to SSP2-4.5, although most included treatments assigned to both scenarios (Supp. Figure 8C).

To examine patterns not captured by the discrete assignment of treatments to reference conditions, we analyzed the continuous distributions of inorganic carbon species concentrations (Supp. Figure 7). Treatments clustered densely near the present-day baseline aqueous CO_2 concentration of approximately $13 \mu\text{mol kg}^{-1}$, but there was limited evidence of clustering around common future values for any of the three inorganic carbon species (Supp. Figure 7B-C). A secondary peak in aqueous CO_2 concentration occurred near $31 \mu\text{mol kg}^{-1}$, approximately midway between the projected 2090s values under the two emissions scenarios. Because this peak lies approximately midway between the scenario-specific reference conditions, small differences in temperature or the other carbonate-system parameters could place otherwise similar treatments closer to either scenario, contributing to the relatively even assignment between SSP2-4.5 and SSP5-8.5 (Supp. Figure 8C).

We next classified treatments outside the biome-relevant condition space according to their direction and alignment with the biome condition space (Supp. Figures 3 and 8D). Similar proportions of studies contained at least one treatment classified as “low inorganic carbon” (32%)

or “high inorganic carbon” (31%). In addition, 37% of studies contained a misaligned treatment: its individual parameter values were not necessarily outside the ranges represented by the biomes, but their combination did not correspond to any biome condition. For example, some treatments had HCO_3^- concentrations that were too high relative to their CO_2 concentrations to represent any biome.

3.4 Applying biome conditions in laboratory cultures

We applied temperature and carbonate system conditions for the North Atlantic in batch culture experiments with two phytoplankton taxa, *Gephyrocapsa huxleyi* and *Thalassiosira pseudonana*. The baseline and future treatments represented present-day and projected end-of-century temperature and carbonate system conditions for the North Atlantic. Mean carbonate system conditions achieved in both treatments fell within two standard deviations of the North Atlantic subtropical seasonally stratified biome (NA-STSS) annual means for 2000s baseline and SSP5-8.5 2090s projection (Figure 4C). Variation among culture replicates ($n = 33$ for the baseline treatment; $n = 37$ for the future treatment) fell within the range of annual biome variability for all three inorganic carbon species and pH. The baseline treatment had slightly higher aqueous CO_2 and HCO_3^- concentrations and lower pH than the NA-STSS 2000s mean because the cultures were supplied with ambient headspace air and atmospheric $p\text{CO}_2$ has increased since the baseline period (Supp. Figure 10). The absolute temperatures used in the treatments fell between the NA-STSS and NA-STPS biome means. However, the temperature increase from the baseline to the future treatment was consistent with the warming projected under SSP5-8.5 (Figure 4D).

Because phytoplankton growth alters carbonate chemistry, we measured changes in culture pH with increasing cell density and used these relationships to establish harvest thresholds that provided sufficient biomass for downstream analyses while limiting changes from the target

carbonate system conditions (Figure 4A; Supp. Figure 9). We also tested whether shaking speed or headspace $p\text{CO}_2$ altered this relationship, as gas exchange with the headspace could replenish aqueous CO_2 . Neither factor had a substantial effect within the ranges tested (Supp. Figure 9). A decrease of approximately 0.1 pH unit was used to define the harvest-density threshold in each condition. For *G. huxleyi*, this criterion corresponded to 1.02×10^5 cells mL^{-1} , within the same order of magnitude as the previously proposed threshold of $0.5\text{--}0.6 \times 10^5$ cells mL^{-1} for batch cultures (LaRoche et al., 2010). Together, these results indicate that, under the batch-culture conditions tested, CO_2 resupply from the headspace did not prevent carbonate-system perturbation as cell density increased. Maintaining dilute cell densities was therefore the most reliable way to limit this perturbation.

4 | Discussion

Temperature and the carbonate system present different challenges when selecting conditions to represent future surface oceans. Projected changes in $p\text{CO}_2$ and pH differed most strongly between emissions scenarios, while carbonate species also varied across biomes. In contrast, temperature varied substantially across biomes and seasons. Our comparison with the OA-ICC literature showed that 57% of experimental treatments fell within two standard deviations of the modeled condition space. We then used conditions for a selected biome, period, and emissions scenario to define laboratory treatments and tested how phytoplankton growth altered the carbonate system. By organizing CMIP6 output into annual and monthly conditions for open ocean biomes, the resulting framework provides a common environmental context for selecting treatments for broad regions and future scenarios and for comparing conditions across experiments.

4.1 Experimental considerations for the carbonate system in ocean multi-driver experiments

412 Projected changes in $p\text{CO}_2$ and pH showed relatively little spatial variation due to the influence of
413 atmospheric $p\text{CO}_2$ on the surface ocean (McKinley et al., 2020), and the magnitude of these
414 changes depended strongly on the emissions scenario. Carbonate speciation, in contrast, varied
415 spatially within each period and emissions scenario (Figure 2B–D). Much of this spatial variation
416 reflects the effects of temperature on CO_2 solubility and carbonate equilibria (Barry et al., 2010).
417 This is evident in Figure 2B, where aqueous CO_2 concentrations varied spatially despite relatively
418 similar $p\text{CO}_2$ within each period and emissions scenario. Conditions intended to represent a
419 particular region therefore need to account for the temperature dependence of carbonate speciation,
420 even when temperature is not being manipulated. Because projected future carbonate system
421 conditions differed most strongly between emissions scenarios, uncertainty about future emissions
422 makes it difficult to select a single representative future condition. Including conditions from more
423 than one scenario can capture more of this range and test whether biological responses depend on
424 the magnitude of projected change. The ocean carbonate system also varies seasonally, with the
425 largest shifts generally occurring at high latitudes because of seasonal changes in temperature and
426 biological productivity (Fay and McKinley, 2017). However, projected future changes in the
427 carbonate system were larger than present-day seasonal amplitudes (Gallego et al., 2018, Supp.
428 Figure 6). In the CMIP6 model data, the ranges of monthly mean conditions projected for the
429 2090s did not overlap with the 2000s ranges for any carbonate system parameter or biome under
430 either emissions scenario. Projected changes therefore exceeded present-day seasonal variability,
431 at least at the scale of the biomes. Annual means may therefore provide reasonable targets for
432 experiments comparing broad present-day and end-of-century conditions, while monthly means
433 remain relevant for experiments focused on a particular season or seasonal variability.

There are also practical limits to using specific monthly carbonate system conditions as experimental targets. Ocean models have difficulty representing the timing and magnitude of carbonate system seasonality, particularly at high latitudes (Fay and McKinley, 2021; Bushinsky et al., 2026, *in press* at JAMES). A monthly condition derived from an ESM, such as a July biome mean, may therefore be less reliable than an annual mean. Differences among monthly conditions may also be smaller than the experimental and analytical variation of a culture system. The standard deviation of dissolved CO₂ and HCO₃⁻, which included spatial, seasonal, and inter-model variability, was similar in magnitude to the combined experimental and analytical variation in our laboratory conditions (Figure 4C–D; Supp. Figure 11). Similar levels of variation have been reported in other batch culture studies (e.g. Hoppe et al., 2011; Kremp et al., 2012; Kottmeier et al., 2014; Hennon et al., 2019). Although chemostats, mesocosms, and some batch culture systems may achieve greater precision, annual pCO₂ conditions may be more achievable targets for batch culture experiments comparing broad present-day and end-of-century conditions.

4.2 Experimental considerations for temperature in ocean multi-driver experiments

While inorganic carbon follows a more globally constrained trajectory because of the influence of atmospheric pCO₂, temperatures remains highly spatially variable (McKinley et al., 2020). Annual mean biome temperatures span a present-day 26°C-degree range, compared with only 1.26 to 5.35°C of possible warming. Projected warming was also smaller than present-day seasonal variation in most biomes. Even under SSP5-8.5, the seasonal temperature ranges of the 2000s and 2090s overlapped in 64% of biomes (Supp. Figure 6). Non-overlap occurred mainly in equatorial biomes, where projected warming exceeded the narrow present-day seasonal temperature range (Supp. Figure 6). The season being represented should therefore be considered when selecting even static temperature treatments. Seasonal temperature variation is relatively straightforward to

incorporate because temperature can be measured accurately, controlled with standard laboratory incubators, and, unlike carbonate chemistry, is not altered by phytoplankton growth.

Selecting temperature treatments relative to phytoplankton thermal optima represents another established approach to condition setting because temperature response curves have been characterize for many phytoplankton taxa (Edwards et al., 2016). This approach is not necessarily inconsistent with selecting treatments from environmental conditions because phytoplankton often inhabit regions where annual temperatures are at or just below their growth optima (Thomas et al., 2012). However, the biological response to projected warming will depend on whether the change moves a taxon toward or beyond its thermal optimum (Barton et al., 2020; Leles and Levine, 2023). For scenario based multi-driver experiments, regional projections describe expected changes in temperature and pCO₂ from a regional baseline, while thermal response curves place the warming in relation to taxon-specific optima.

4.3 Comparing experimental treatments with modeled ocean conditions

Comparing treatments from the OA-ICC literature with the range of modeled biome conditions shows how the dataset can place past experiments in geographic, temporal, and emissions-scenario context. Carbonate system treatments in the literature were relatively evenly split within and outside the modeled biome ranges, with some extending beyond the 1850s–2090s reference conditions (Supp. Figure 9A). Many studies also included treatments near projected future conditions, including a cluster near an aqueous CO₂ concentration of ~31 μmol kg⁻¹ (Supp. Figure 8). At 25°C and salinity 35, this concentration corresponds to approximately 1095 ppm pCO₂ and may reflect attempts to represent high emissions projections from Phase 5 of the Coupled Model Intercomparison Project (CMIP5) (Kwiatkowski et al., 2020). Treatments assigned to future conditions showed a slight preference for SSP5-8.5 over SSP2-4.5 and also occurred between the

scenario-specific estimates. The overall spread of treatments was similar to previous summaries of the OA-ICC dataset (Yang et al., 2016, 2024), but our analysis adds spatial and temporal context for temperature and the carbonate system. By summarizing conditions for both scenarios, the biome means provides reference points for experiments spanning a broader range of plausible future carbonate system change.

Temperature treatments were concentrated between 10 and 20°C, consistent with clusters at 15 and 20°C reported by Collins et al. (2022). This pattern may partly reflect the phytoplankton taxa most commonly used in laboratory studies (including this study), many of which have thermal optima within this range (reference needed). Treatments at or above 30°C were uncommon despite annual mean temperatures near this range in some equatorial biomes (Supp. Figure 3). Treatments below 10°C were also less common, although large subpolar biomes have present and projected future annual means below this temperature. Thus, although almost all individual temperature treatments fell within the range represented by at least one biome, coverage was uneven across the environmental temperature range. This uneven coverage may reflect the underrepresentation of equatorial and polar strains in the literature. Expanding multi-driver experiments to include taxa and conditions from the warm and cold ends of the environmental temperature range would improve our ability to characterize future biological responses across ocean regions.

Experimental treatments are selected for different reasons; some studies aim to represent plausible present and future conditions and use additional treatments between these endpoints to resolve biological responses within the expected range (Barry et al., 2010). Others deliberately extend treatments beyond projected ocean conditions to characterize the shape of the biological response across a broader environmental range (Collins et al., 2022; Thomas and Ranjan, 2024). Both approaches are useful but answer different questions. Whether the goal is to represent a specific

biome or examine a broader multi-driver response, comparing treatments with present and projected ocean conditions provides context for interpreting the results.

Many metaanalyses, however, group studies based on the change in a single carbonate system parameter such as pH or $p\text{CO}_2$, (Kroeker et al., 2013; Seifert et al., 2020). In our dataset, treatments with a total pH of 7.6-7.8 (i.e. 2090s, SSP5-8.5) had aqueous CO_2 concentrations ranging from 4 to $132 \mu\text{mol kg}^{-1}$, showing that similar pH values did not necessarily represent similar inorganic carbon conditions. Applying the biome-relevance criterion narrowed this range to $18\text{-}56 \mu\text{mol kg}^{-1} \text{CO}_2$ range. This narrowing is expected because the criterion considers multiple carbonate-system variables, but it illustrates how treatments selected by a single parameter can differ substantially in the remaining carbonate chemistry. Meta-analyses based on a single carbonate-system parameter should therefore consider whether variation in the other parameters contributes to differences among treatments.

4.4 Applying a biome-based framework to multi-driver phytoplankton experiments

Our laboratory experiments illustrate how the biome-based framework can be used to select temperature and carbonate system conditions for multi-driver experiments. For the North Atlantic STSS biome, we selected baseline conditions near modern spring and summer values and future temperature and $p\text{CO}_2$ conditions from the SSP5-8.5 projection (Supp. Figure 11; Figure 4). We adjusted the carbonate system using headspace $p\text{CO}_2$ rather than bicarbonate additions, which allowed alkalinity to remain consistent between treatments. The achieved carbonate system conditions fell within two standard deviations of the North Atlantic STSS annual means for the 2000s and the 2090s under SSP5-8.5 (Supp. Figure 11). Although the absolute temperatures fell between the North Atlantic STSS and STPS biome means, the temperature increase between treatments matched the projected warming (Figure 4 C-D). This example shows how biome

conditions can guide the translation of regional projections into laboratory treatments while accounting for practical constraints on condition setting.

This dataset is particularly useful for phytoplankton experiments because target $p\text{CO}_2$ alone does not fully describe the inorganic carbon environment associated with a given temperature. Phytoplankton differ in how they acquire inorganic carbon and can respond to multiple components of the carbonate system (Rost et al., 2003). Passive diffusion of CO_2 into the cell depends not only on aqueous CO_2 concentrations but also on the replenishment of CO_2 through HCO_3^- dehydration (Reinfelder, 2011). In some phytoplankton, extracellular carbonic anhydrase accelerates this dehydration, replenishing CO_2 near the cell surface and supporting inorganic carbon uptake (Hopkinson et al., 2013). Active HCO_3^- transport is also widespread among phytoplankton, providing direct access to the dominant inorganic carbon pool in seawater and reducing reliance on diffusive CO_2 uptake (Matsuda et al., 2017). Treatments with the same target $p\text{CO}_2$ but different carbonate speciation therefore do not necessarily represent the same inorganic carbon environment for phytoplankton. Constraining the complete carbonate system allows experiments to reproduce the inorganic carbon environment of a region and treatments to be compared on the same basis. In experimental evolution studies, it also specifies the chemical environment maintained as selection acts across generations.

The monthly estimates can also support experiments that vary conditions through time rather than holding them constant. When biological response curves are nonlinear, responses to fluctuating conditions may differ from responses measured at the corresponding mean condition (Denny, 2017). In experimental evolution studies, phytoplankton exposed to fluctuating $p\text{CO}_2$ conditions evolved greater plasticity than populations maintained under constant conditions (Schaum and Collins, 2014). Variable temperature regimes similarly accelerated adaptation to severe conditions

in diatoms and produced faster-evolving populations than stable treatments (Schaum et al., 2018). However, variable experimental regimes introduce additional decisions because both the amplitude and frequency of variation must be considered (Gill et al., 2022). The monthly estimates provided here can inform seasonal means and amplitudes for temperature and carbonate chemistry within each biome, although they do not resolve variability at shorter timescales.

The biome conditions presented here describe broad, open-ocean surface environments and do not capture variation with depth, coastal conditions, or changes occurring at timescales shorter than one month. Coastal environments in particular can experience greater temporal variability in multiple drivers than is represented by the biome means (McElhany and Shallin Busch, 2013; Kroeker et al., 2020). We also excluded seasonally ice-covered regions and held biome boundaries fixed through time, although the locations and areas of these biomes are expected to shift as the ocean changes. The projections were calculated from five CMIP6 models and therefore do not represent the full range of uncertainty across CMIP6. Monthly and annual biome means average across large areas and multiple models, so they should not be interpreted as predictions for a specific location. These conditions are best used to place experimental treatments in a regional and emissions-scenario context, while experiments focused on a particular site may require local observations or higher-resolution projections.

4.5 Future directions

Understanding how drivers scale together in present and future ocean is important for both experimental design and the interpretation of results. With this unified dataset that incorporates seasonality and geospatial variability, we provide a resource that can begin to aid both efforts. While temperature and inorganic carbon are critical ocean drivers under future climate change, they are not the only ones (Boyd et al., 2015). Critically, the geospatial range and intensity of

dissolved oxygen, inorganic nitrogen, and light may all see changes. Work investigating physiology across the full multi-driver parameter space will be critical. Likewise, a unified understanding of how these parameters scale with each other in past and future oceans will inform future work. The complexity of scaling relationships between many drivers increases with each additional driver added. Thus, work synthesizing the most common combination of drivers will be of particular importance to experimental design.

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6 | Data Availability Statement

All untransformed ScenarioMIP CMIP6 model output used in this study can be found at the respective DOIs cited for each model (see Supp. Table 1 and references). Additionally, our calculations of the full carbonate system for output is archived on Zenodo ([10.5281/zenodo.22017672](https://doi.org/10.5281/zenodo.22017672)). Additionally, all biome means, compiled literature data, laboratory measurements, and code generating all figures in this work from raw data is archived on Zenodo ([10.5281/zenodo.23049658](https://doi.org/10.5281/zenodo.23049658)).

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781

782 8 | Figures:

783 **Figure 1) Ocean biomes used to calculate driver means.** Map shows 14 ocean biomes colored
784 by their climate type: equatorial (EQU), subtropical permanently-stratified (STPS), subtropical
785 seasonally-stratified (STSS), and subpolar seasonally-stratified (SPSS). Individual biomes are
786 identified with numbers and separated by both biome type and basin: 1) N. Pacific SPSS, 2) N.
787 Pacific STSS, 3) N. Pacific STPS, 4) Pacific EQU E, 5) Pacific EQU W, 6) S. Pacific STPS, 7)
788 Southern Ocean STSS, 8) Southern Ocean SPSS, 9) N. Atlantic SPSS, 10) N. Atlantic STSS, 11)
789 N. Atlantic STPS, 12) Atlantic EQU, 13) S. Atlantic STPS, and 14) Indian Ocean STPS. Note that
790 seasonally ice-covered regions are not included in this study (grey area).

791 **Figure 2) Annual surface ocean temperature and inorganic carbon drivers for ocean biomes**
792 **from CMIP6 ensemble.** For every panel each point represents the mean annual surface value for
793 a biome averaged across the total biome area and five models for three 10-year time spans: 1850
794 through 1859, 2000 through 2009, and 2090 through 2099. Error bars show standard deviation of
795 the grand mean. Mean conditions for 2090s are shown under a moderate and high-emission
796 scenario (SSP2-4.5 and SSP5-8.5). Surface ocean $p\text{CO}_2$ is shown on the y-axis against **A)** surface
797 ocean temperature and dissolved inorganic carbon parameters **B)** CO_2 , **C)** HCO_3^- , **D)** CO_3^{2-} , **E)**
798 total scale pH, and **F)** total DIC. Panels **G)** to **L)** show the change in these values relative to the
799 modern (2000s) baseline. Color indicates scenario and decade. Point shape indicates biome type.

800 **Figure 3) Comparison of literature treatments with modeled carbonate system conditions.**
801 Colored points are as described in top row of Figure 2. Grey points show individual literature
802 treatments obtained from 196 studies in OA-ICC database for phytoplankton cultures. The x-axis
803 of both panels shows CO_2 concentrations with the y-axis of **A)** showing HCO_3^- ion concentration
804 and **B)** showing CO_3^{2-} ion. Both plots are cropped to the densest region of data. Plot inset in both
805 panels shows the full data range.

806 **Figure 4) Application of biome-based temperature and carbonate system conditions in two**
807 **eukaryotic phytoplankton taxa A)** Phytoplankton cell density's effect on media pH change for
808 *G. huxleyi* and *T. pseudonana*. Cell density threshold that corresponds to a change of 0.1 pH units
809 is shown. **B)** Acclimation of *G. huxleyi* cultures to carbonate conditions over four successive
810 transfers. Carbonate conditions are kept relatively stable by keeping cell density below the
811 threshold shown ($\sim 10^5$ cells mL^{-1}). Panels **C)** and **D)** show alignment of measured *G. huxleyi* and
812 *T. pseudonana* carbonate condition change to projected north Atlantic biome condition change as
813 well as change at BATS station. Error bars on lab conditions are the standard deviation of replicate
814 cultures measured at the final timepoint of the experiment with propagated error from change
815 calculation. Change in lab conditions is referenced to the ambient condition treatment. Error on
816 biome means is the same as Figure 1. Average change in surface conditions for 1990s, 2000s, and
817 2010s is shown for BATS bottle data referenced to the 2000s.

FIGURE 1

Biome Type

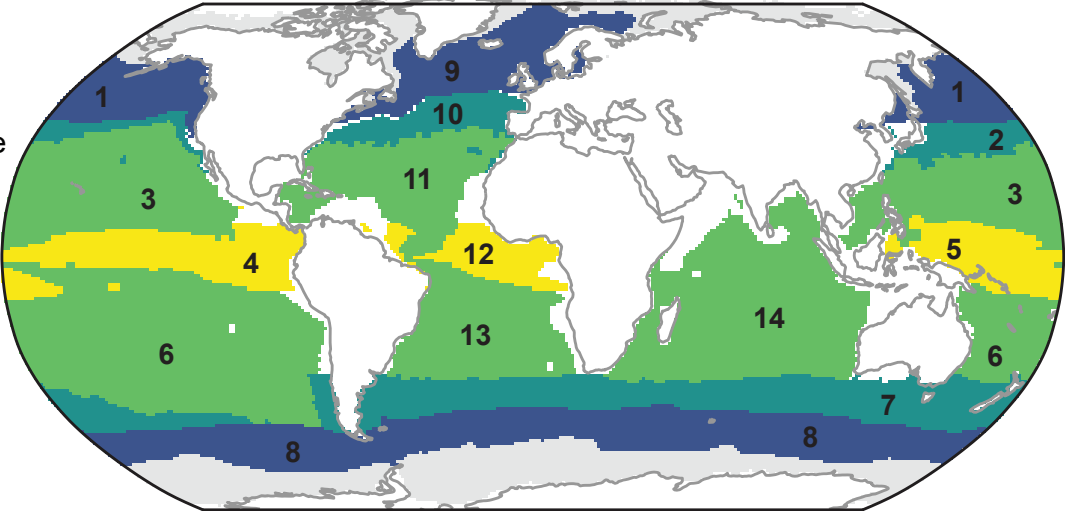
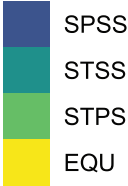


FIGURE 2

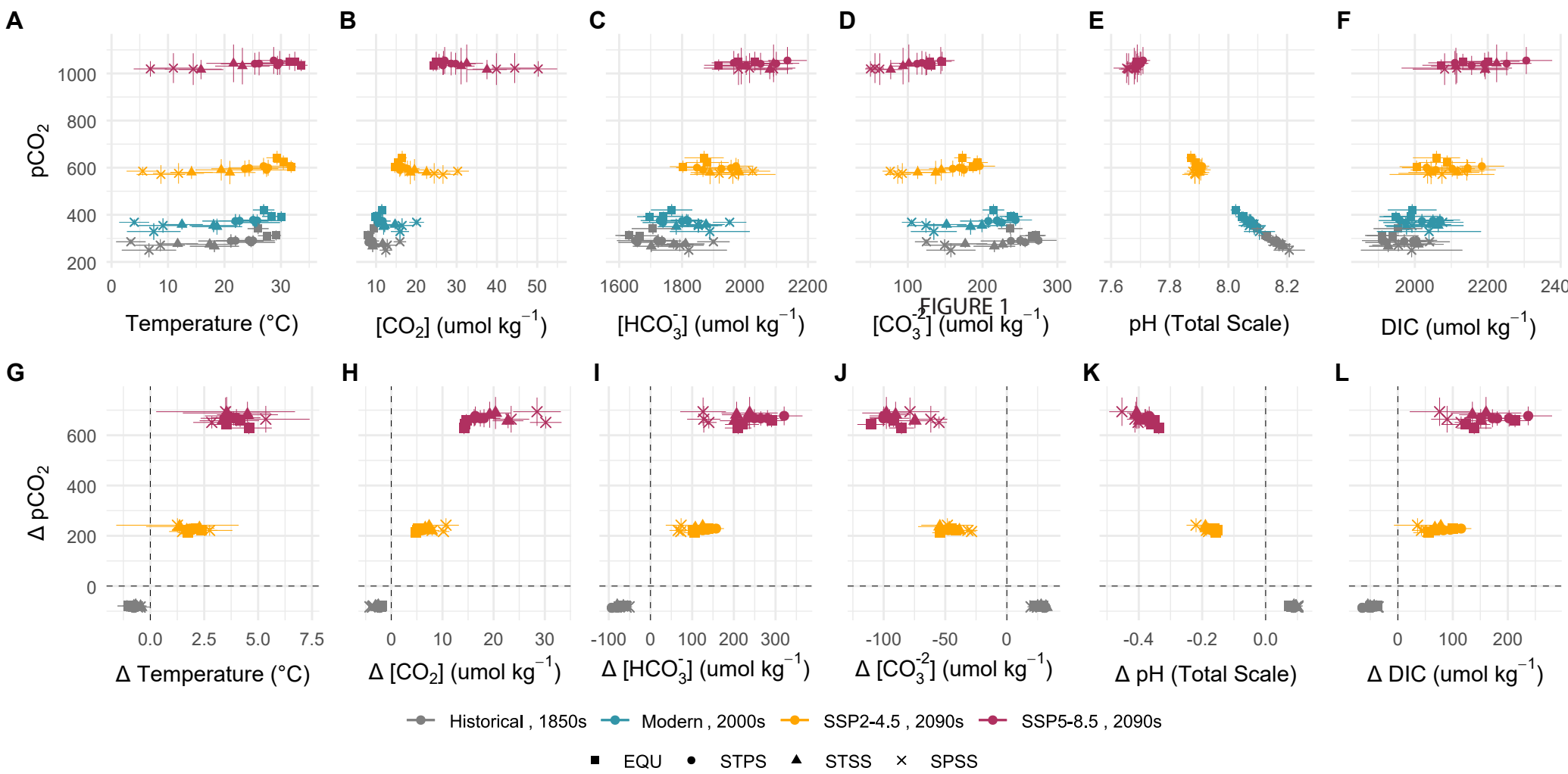
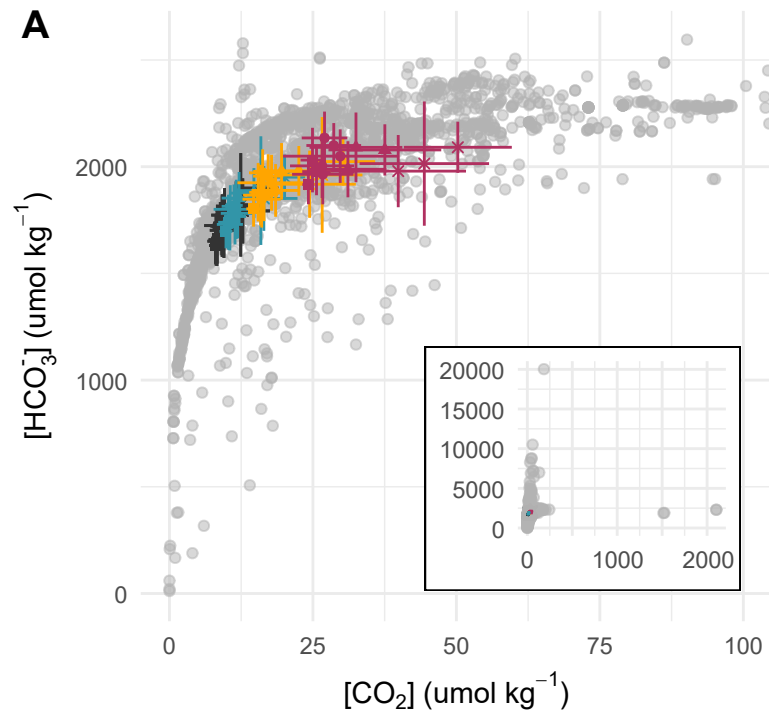
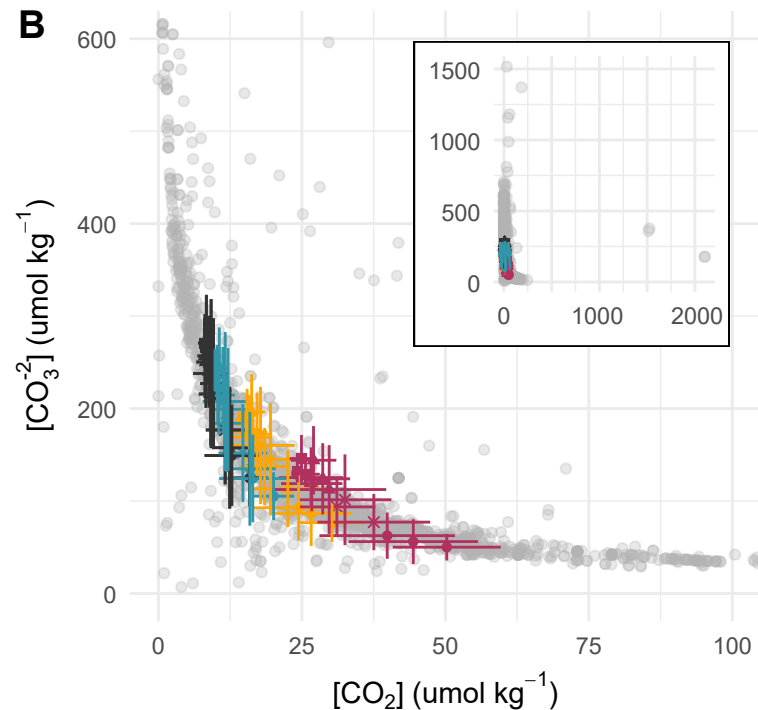


FIGURE 3

A

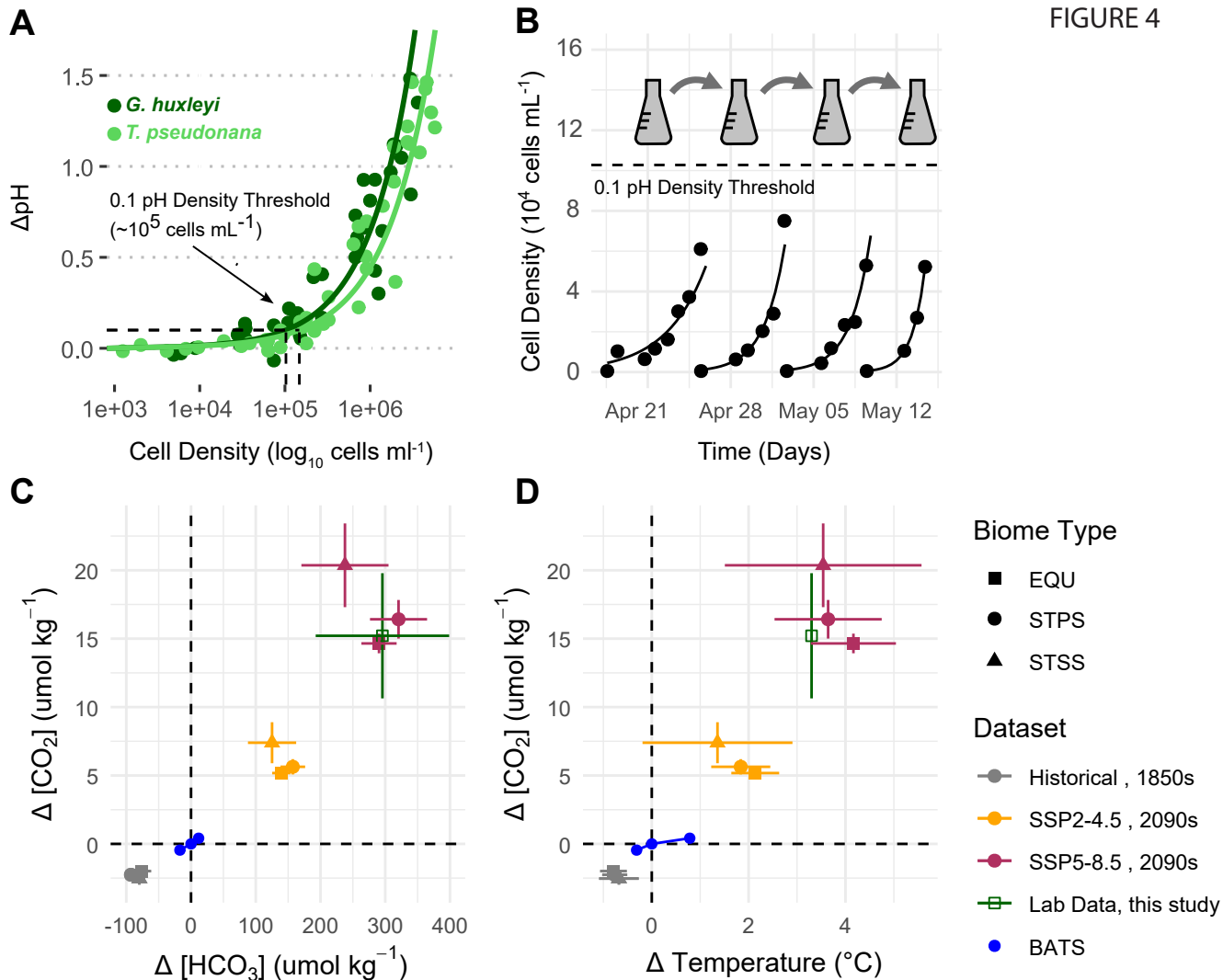


B



— Historical , 1850s — Modern , 2000s — SSP2-4.5 , 2090s — SSP5-8.5 , 2090s
 ■ EQU • STPS ▲ STSS × SPSS
 • OA-ICC Treatment

FIGURE 4



Supplementary Material for

**Using Global Climate Models to Inform Multi-Driver Biological
Ocean Experiments**

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S1.0 Supplemental Methods

Here we provide more detailed information on the OA-ICC data screening process. Additionally, the following sections also give details on media preparation, culturing, harvesting, and carbonate system measurements that were used to match North Atlantic present and future conditions.

S1.1 - OA-ICC Database Screening Details

We accessed PANGAE DOI numbers for all studies on the OA-ICC online portal that 1) targeted phytoplankton, 2) were pelagic in focus, and 3) were laboratory based (Yang et al., 2024). We extracted carbonate and temperature conditions used in studies that met this criteria with the Python package *pangaepy* (Huber et al., 2020). Full screening process can be seen in Supp. Figure 1. Five studies did not return a dataset from the associated DOI and were likely collections of studies on PANGAEA (Felden et al., 2023). To avoid duplication, these studies were dropped at this stage. From each dataset, we extracted carbonate system information from columns that were identified as re-calculated by OA-ICC curators using the package *seacarb* to ensure a consistent set of chemical calculations (Yang et al., 2016, 2024). We did not recalculate $[\text{CO}_2]$, $[\text{HCO}_3^-]$, and $[\text{CO}_3^{2-}]$ values from the database and they follow calculations found in Nisumaa et al., 2010. We used the same constants for our CMIP6 biome calculations with the exception of the hydrogen fluoride dissociation constant where in Nisumaa et al., 2010 used the dissociation constant from Perez and Fraga, 1987. Based on sensitivity analysis in Nisumaa et al., 2010 and the concentration of hydrogen fluoride in seawater, this is unlikely to measurably affect carbonate system ion calculations between the OA-ICC and the CMIP6 dataset. While we did not recalculate carbon species from OA-ICC, many studies did not contain total scale pH. Therefore, we recalculated total pH with $[\text{HCO}_3^-]$ and $[\text{CO}_3^{2-}]$ using temperature and the same carbonic acid dissociation constant as Nisumaa et al. 2010 and this study (Supp. Table 2). Three studies did not appear to contain any re-calculated data following Nisumaa et al. and were not included in the final study.

We programmatically selected any column that contained treatment information (e.g. “treat”, “treatment”, “Exp treat”, etc.) and temperature of the samples. If no treatment information was detected (n=41 studies), it was assumed that the $[\text{CO}_2]$ represented the treatment group. This approach was taken as many studies appeared to alter $[\text{CO}_2]$ but did not list which samples belonged to a treatment group. This approach may inflate the number of treatments in these 41 studies but does not affect our downstream presence/absence analysis at a study level.

Many datasets examined the same samples at multiple time intervals (e.g. before or after culture growth). In the compiled data, we reported each timepoint separately as a condition within the study rather than summing carbonate system conditions across time. This strategy was chosen to represent the full range of carbonate conditions that phytoplankton may be exposed to in a study. Three studies reported multiple carbonate system conditions (e.g. two CO_2 ion concentrations) for the same sample where it could not be determined whether this was separate treatment information or time intervals. These three studies were excluded for clarity. Lastly, ten studies reported multiple temperature treatments for a single carbonate system measurement likely because of acclimation and testing at different temperatures. For these ten studies we used the same approach as with time intervals; carbonate data was duplicated and paired with both temperature treatments to represent the range of conditions used.

S1.2 - Artificial Seawater Media

We cultured phytoplankton in a modified artificial seawater medium with some modifications on existing recipes. The media consisted of an artificial seawater base that matched the concentrations given in Berges et al., 2001 for anhydrous and hydrated salts. This was prepared in ultra purified Milli-Q water (MilliporeSigma, Burlington MA). However, we modified the sodium bicarbonate concentrations from 0.174 g/L to 0.184 g/L as we found this better matched our goal DIC in testing. We amended this seawater base with a 'L1' nutrient addition to match replete nutrient conditions (Guillard and Hargraves, 1993). This matches suggested 'L1' nutrient concentrations from National Center for Marine Algae and Microbiota (NCMA) with the final concentrations presented in Supplementary Table 3 for clarity.

After all components were added, the media was not pH adjusted as suggested by other protocols. We found pH adjustment with a strong acid/base increased variability in carbonate conditions and that media pH stabilized given adequate equilibration time after mixing. Media was stirred with a magnetic stir bar in ambient conditions for ~24h before sterilization with autoclave at 121°C and 23 PSI for 30 minutes. Media was removed hot from the autoclave and shaken vigorously under desired $p\text{CO}_2$ headspace for 48-72 hours to acclimate carbonate conditions. Media was acclimated in the $p\text{CO}_2$ controlled growth chambers that phytoplankton were also grown in (Percival Scientific, Perry IA). Pressure sterilization caused some precipitation of calcium salts that lowered alkalinity and DIC. However, we found the long acclimation time post-sterilization, immediate flask removal from autoclave chamber after sterilization, and aforementioned 5.7% increase in NaHCO_3 concentrations all resulted in consistent media conditions.

S1.2 - pH and DIC Determination

We measured pH and DIC in order to calculate the full carbonate system of our media. As we amended the media with inorganic nutrients (silica, phosphorus, ammonia, etc.) we avoided using alkalinity measurements as these additions could be confounding to calculations of other parameters from alkalinity (Dickson, 2010).

We determined total pH using a reference electrode method (SOP6a, Dickson et al., 2007). Saltwater 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS) solution was prepared as described in Dickson et al. 2007. We choose not to modify the recipe as the salinity of the reference solution (35 PSU) is close to that of our media (~36 PSU) and the protocol notes that differences in this range likely results minor pH errors (<0.005 pH). Measurements were made using a Sartorius pHBasic meter (Göttingen, Germany) with sensitivity down to ± 0.1 mV. Final calculations of total scale pH using sample and standard voltages as well as temperature was performed using R package *seacarb* 'pH' function which follows calculations as in SOP6a (Dickson et al., 2007b; Gattuso et al., 2024)

As the stable carbon isotope composition of the media was separately being tested, DIC concentration was determined using a GasBench II and Delta V Plus IRMS (Thermo Scientific, Bremen, Germany) by the Stable Isotope Facility at University of California Davis. Samples were

carefully sterile filtered with a syringe using 0.2 micron filters into 15 mL gas-sealed vials for analysis. For analysis, 1 to 4 mL are acidified with 85% phosphoric acid to force speciation to aqueous CO₂ and purged via a double needle sampler into a helium carrier gas. Full carbonate system speciation was calculated for samples using pH and DIC via a python implementation of CO2SYS (PyCO2SyS, Humphreys et al., 2022). Constants used were identical to those used for determining carbonate speciation in CMIP-6 models (Supplementary Table 1).

S1.3 Cell Density Threshold Determination

We determined the cell density threshold at which phytoplankton cultures began to substantially alter carbonate conditions. Even in shaken open containers with a constant replenishment of CO₂ from flask headspace, DIC uptake rates outpaced CO₂ diffusion into media once a certain cell density was reached. Previous literature has encouraged the determination of this threshold from cell-carbon relationships (LaRoche et al., 2010). However, given the range of variables in individual culturing set-ups, we directly determined this threshold for our incubation based on changes in pH measurements.

Cultures were inoculated from maintenance at a range of low densities with foam stoppers to allow gas transfer. We tested a range of culture containers, shaking speeds, and headspace *p*CO₂ levels in this determination (see results section 3.5). Culture pH was monitored daily along with cell density via flow cytometry. Aliquots of concentrated cultures were fixed and later enumerated for absolute cell count via flow cytometry (Beckman Coulter Cytotflex) to obtain cell densities. To eliminate clumping issues introduced by fixation, all samples were treated with a surfactant (Sigma-Aldrich TWEEN 80, 10%) upon thawing. Cultures were allowed to grow past the density threshold at which pH changed. We fitted an exponential curve to the relationship between log₁₀ cell density and change in pH (i.e. ΔpH). Given variability in both ΔpH and cell density measurements, we used a change of 0.1 ΔpH on our fitted curve to act as a threshold.

S1.4 Harvest Protocols and Growth Conditions

Cultures were maintained at 22°C and 100 uE before use in experiments; light levels were maintained as such throughout experiments. For acclimation to *p*CO₂ and temperature treatments, cultures were transferred to Erlenmeyer flasks and shaken at 150 rpm with foam stoppers to allow gas transfer. Cultures were transferred at or just below the determined species-specific cell density threshold to ensure limited change to the carbonate system. Cultures were transferred at least four times before harvesting. Carbonate parameters (pH and DIC) were measured at the beginning (pre-inoculation) and end (harvest) of the final acclimation. Additionally, pH was measured throughout the growth of the last flask to monitor any carbonate system shifts in media. Analytical replicates (*n* = 2) were taken at final timepoint for DIC.

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Model	Surface Ocean Parameters	Ensemble members	Top Grid Cell	Citations
CanESM5	T, S, $p\text{CO}_2$, pH	r1ilp2fl, r2ilp2fl, r3ilp2fl, r4ilp2fl, r5ilp2fl	0-6.19 m	Swart et al., 2019a Swart et al., 2019c
CanESM5-CanOE	T, S, $p\text{CO}_2$, pH	r1ilp2fl, r2ilp2fl, r3ilp2fl	0-6.19 m	Swart et al., 2019b Christian et al., 2022
CESM2	T, S, $p\text{CO}_2$, pH	r10ilp1fl, r11ilp1fl, r4ilp1fl	0-10 m	Danabasoglu, 2019 Danabasoglu et al., 2020
CESM2-WACCM	T, S, $p\text{CO}_2$, pH	r1ilp1fl, r2ilp1fl, r3ilp1fl	0-10 m	Danabasoglu, 2019b Danabasoglu et al., 2020
GFDL-ESM4	T, S, $p\text{CO}_2$, pH	r1ilp1fl	0-2 m	John et al., 2018 Dunne et al., 2020

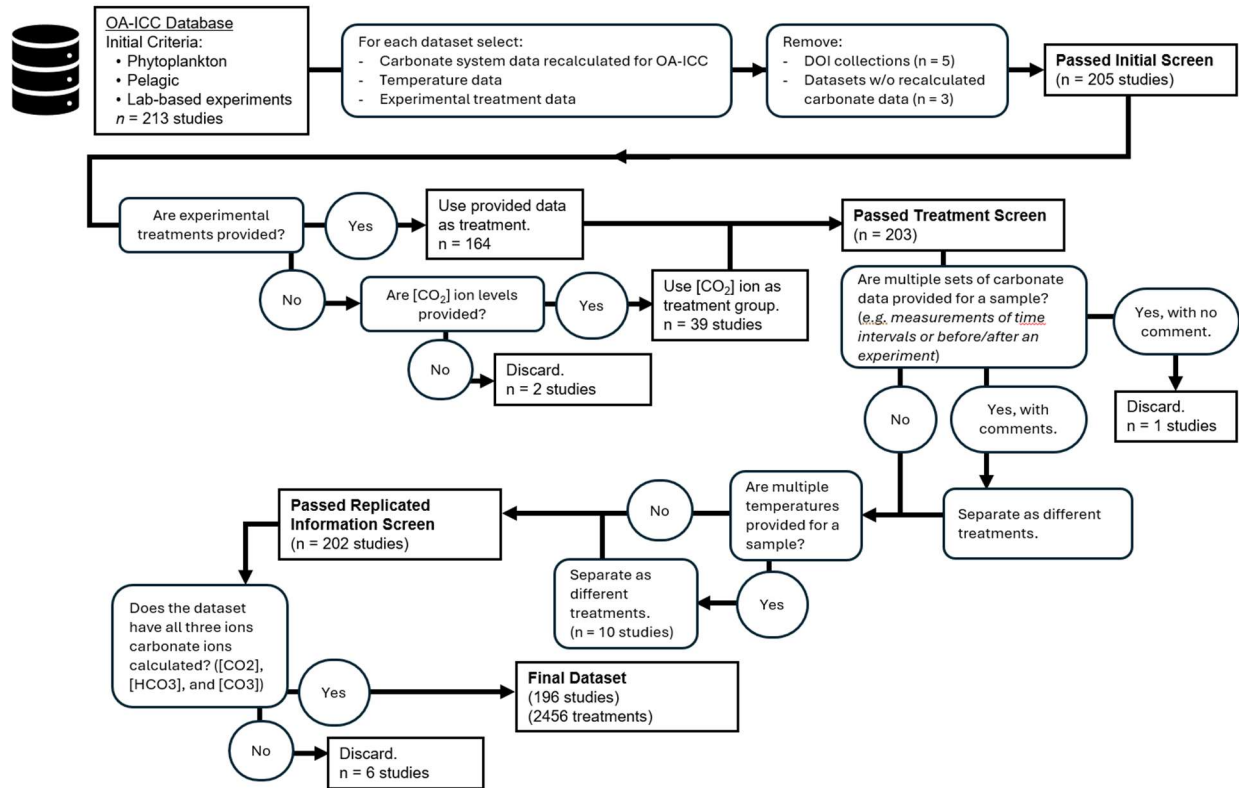
Supplementary Table 1) ESM data from CMIP6 used in ensemble for biome averages.

Name	Abbreviation	Parameter	Relevance	Citation
pH Scale	pH	‘Total Scale’	Seawater	N/A
Carbonic acid dissociation	K_1 and K_2	‘Lueker’	$2 < T < 35$ °C, $19 < S < 43$, Total scale, real seawater	Lueker et al. (2000) <i>Mar. Chem.</i>
Bisulfate ion dissociation	K_s	‘Dickson’	$0 < T < 45$ °C Synthetic seawater	Dickson (1990) <i>J. Chem. Thermodyn.</i>
Boron:salinity relationship	<i>boron:salinity</i>	‘Uppström’	Real seawater	Uppström (1974) <i>Deep-Sea Res.</i>
Hydrogen fluoride dissociation	K_f	‘Dickson and Riley’	Seawater media	Dickson & Riley (1979) <i>Mar. Chem.</i>

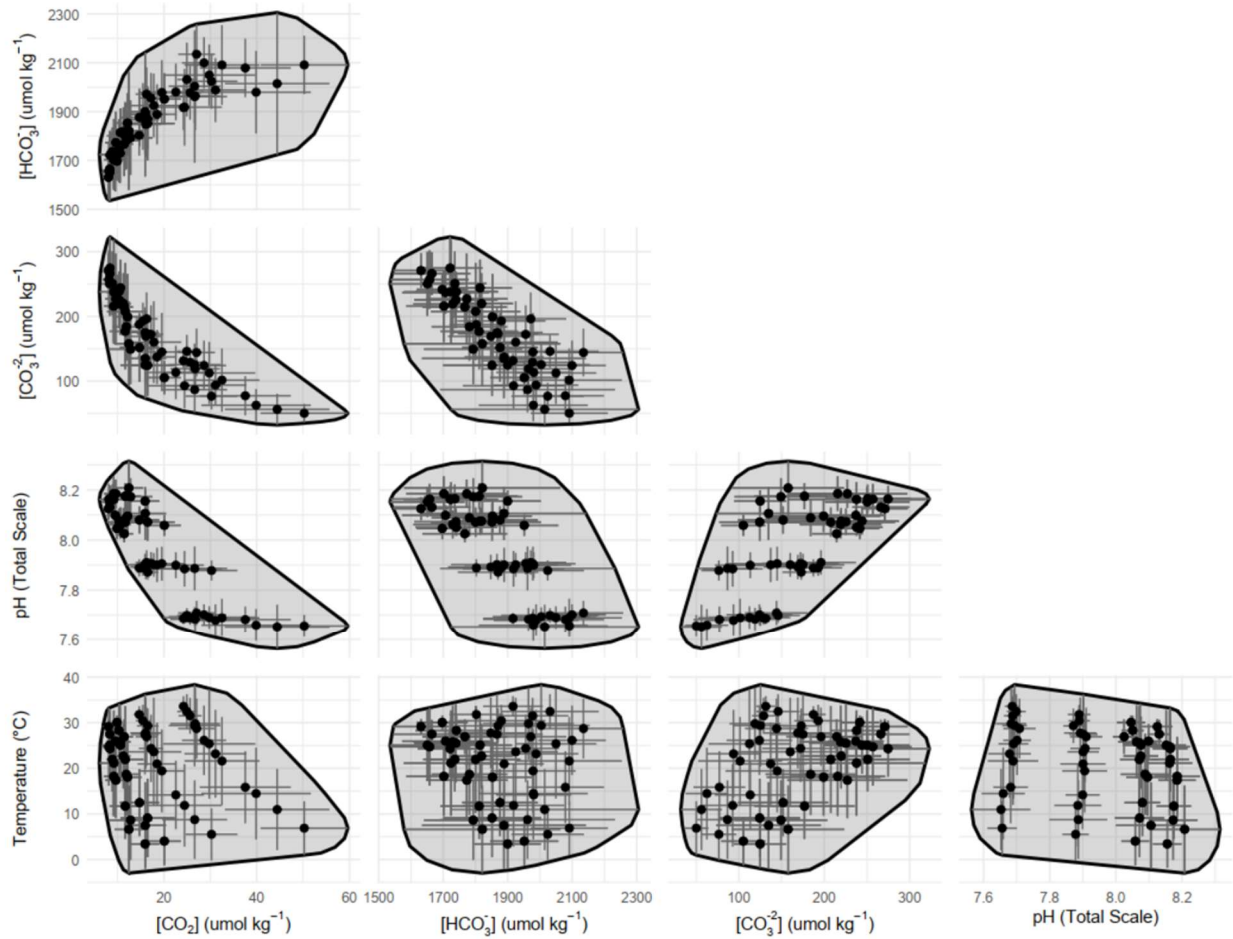
Supplementary Table 2) Chemical constants used for calculation of the carbonate system in this study. The constants were used for calculation of the carbonate system with ‘CO2SYS’ in the ESM model output, as well as with laboratory measurements taken in this study (Figure 3). The python implementation of CO2SYS was used (PyCO2SYS, Humphreys et al. 2022) and follows this implementation’s defaults. Note that calculation of carbonate system parameters for OA-ICC data followed Nisumaa et al. (2010) *Earth Syst. Sci. Data.* and matches these constants except for K_f (See supplement methods section S1-1). See main text for full citations.

	Component	Formula	Molar Concentration in Final Medium
ESAW (Following Berges et al., 2001 w/ modifications)	Sodium chloride	NaCl	3.63×10^{-1} M
	Sodium sulfate	Na ₂ SO ₄	2.50×10^{-2} M
	Potassium chloride	KCl	8.03×10^{-3} M
	Sodium bicarbonate *	NaHCO ₃	2.19×10^{-3} M
	Potassium bromide	KBr	7.25×10^{-4} M
	Boric Acid	H ₃ BO ₃	3.71×10^{-4} M
	Sodium fluoride	NaF	6.67×10^{-5} M
	Magnesium chloride hexahydrate	MgCl ₂ · 6H ₂ O	4.71×10^{-2} M
	Calcium chloride dihydrate	CaCl ₂ · 2H ₂ O	9.14×10^{-3} M
	Strontium chloride hexahydrate	SrCl ₂ · 6H ₂ O	8.18×10^{-5} M
L1 Nutrients (Following Guillard and Hargraves, 1993)	Sodium nitrate	NaNO ₃	8.82×10^{-4} M
	Monosodium phosphate	NaH ₂ PO ₄	3.62×10^{-5} M
	Sodium metasilicate	Na ₂ SiO ₃	1.06×10^{-4} M
L1 Trace Solution (Following Guillard, 1975)	Trace Metal Solution	<i>Various</i>	<i>Various</i>
	f/2 Vitamins Solution	<i>Various</i>	<i>Various</i>
	* Increased from 2.07×10^{-3} Molar		

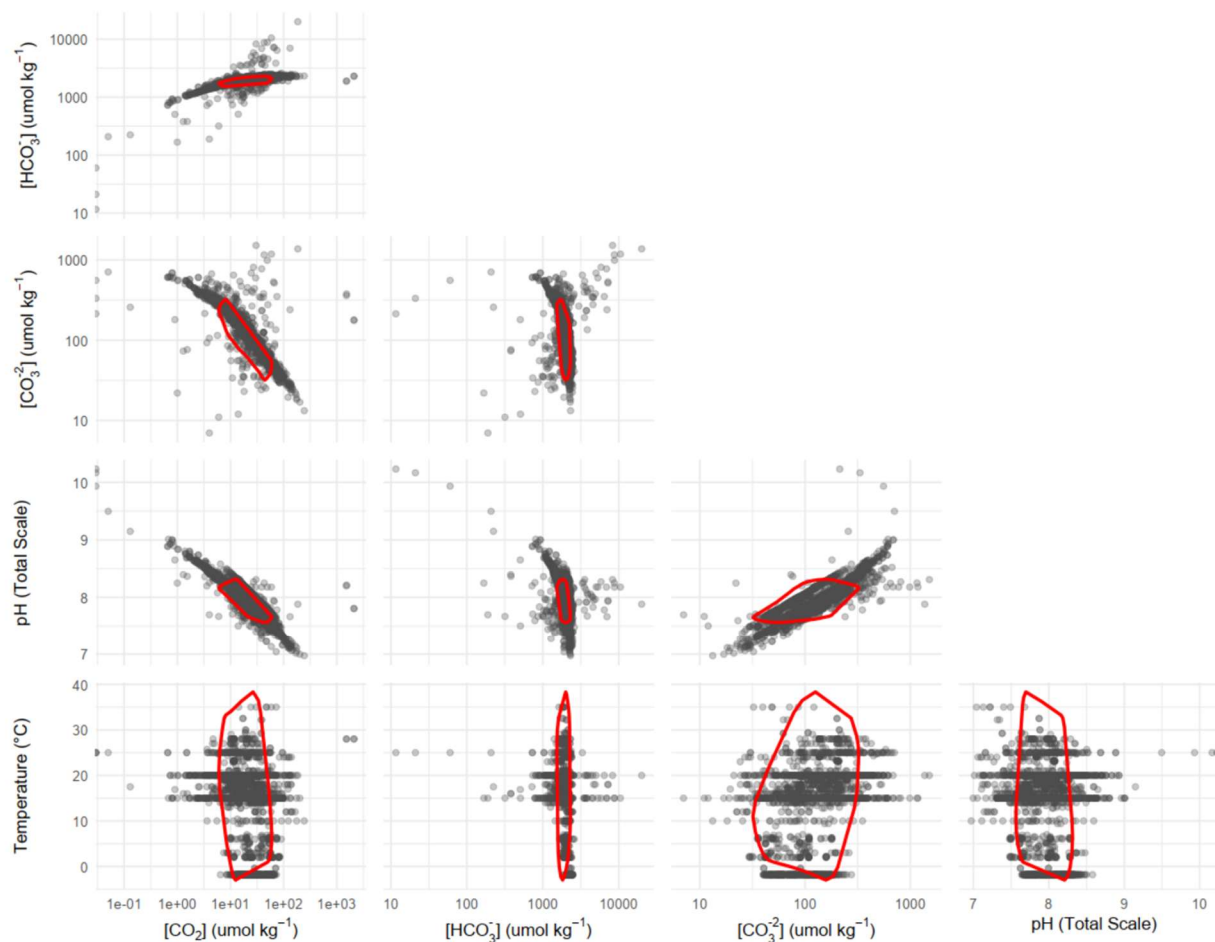
Supplementary Table 3) Media recipe for culturing *G. huxleyi* and *T. pseudonana*. Follows media recipes from National Center for Marine Algae and Microbiota (NCMA) with adjusted sodium bicarbonate concentrations.



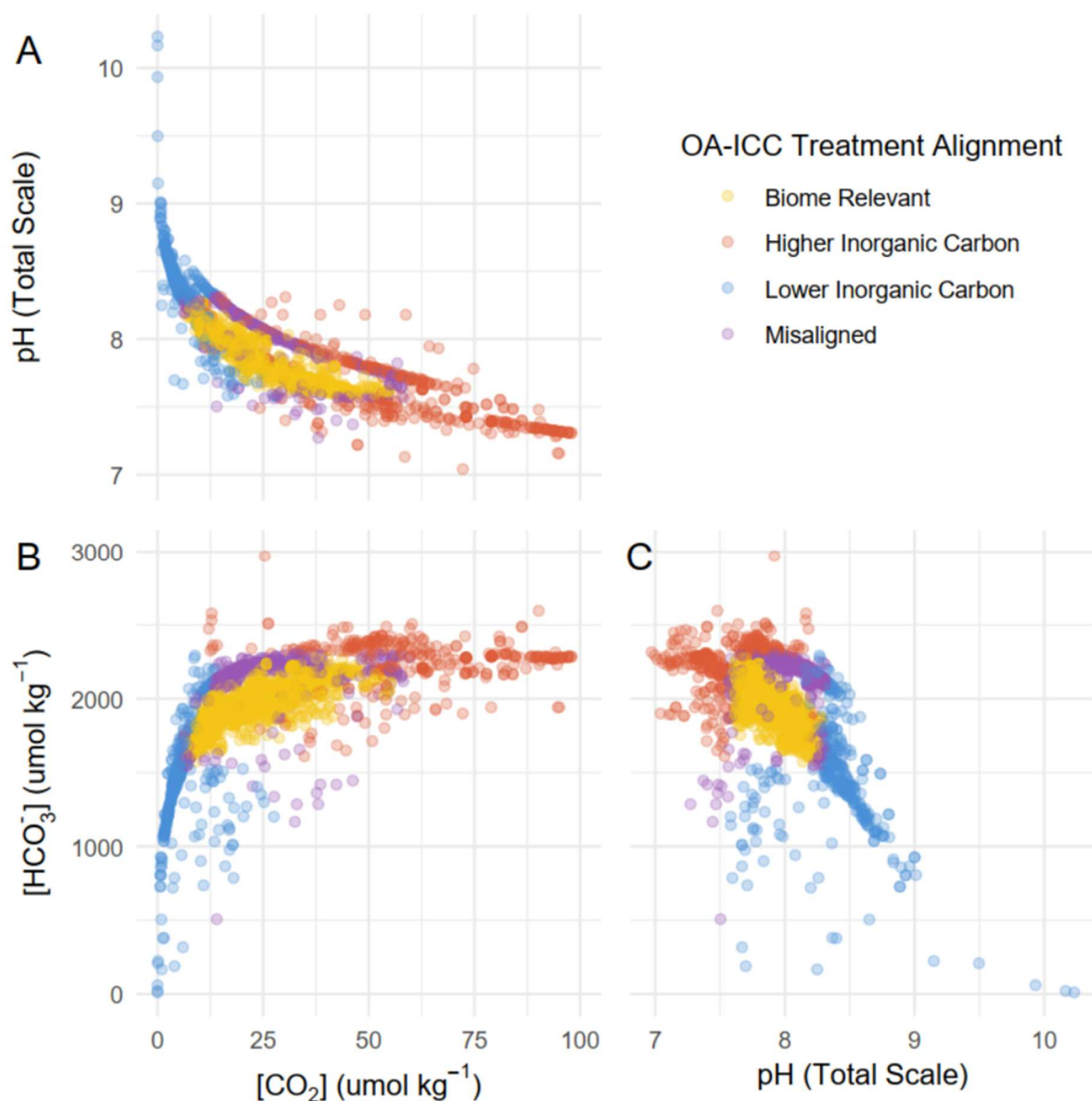
Supplementary Figure 1) OA-ICC Database Screening Process. Flowchart depicts the methodological steps used to both screen studies for relevance as well as separate data from studies into distinct treatments.



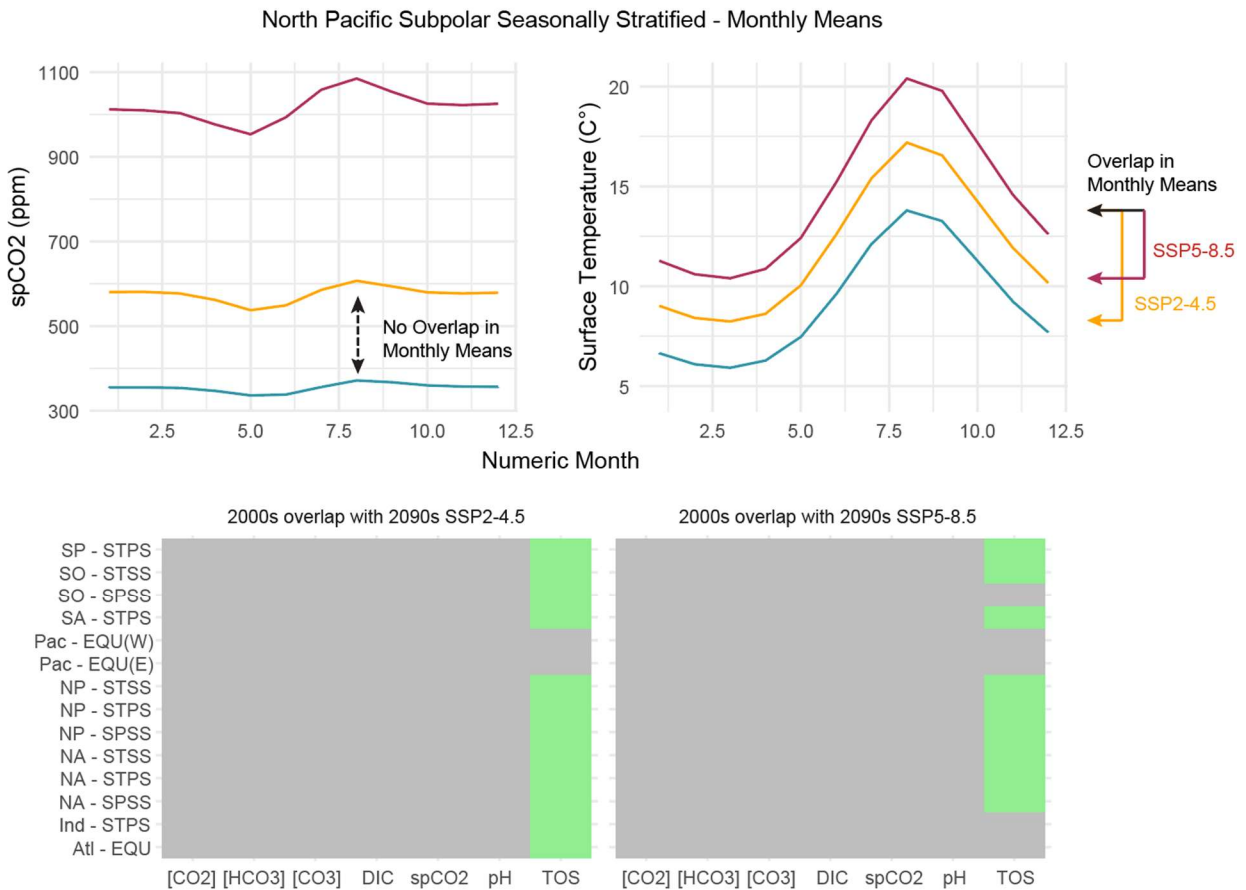
Supplementary Figure 2) Two standard deviation boundaries around mean annual ocean biomes. All pairs between temperature and four carbonate system ions are shown on both axes. Black dots show all 14 ocean biomes across two scenarios and three decades. Error bars show two standard deviations of the mean. Grey areas are computed convex hulls binding an approximately two standard deviation regions. Treatments were considered ‘biome relevant’ if their values fell within all 10 regions.



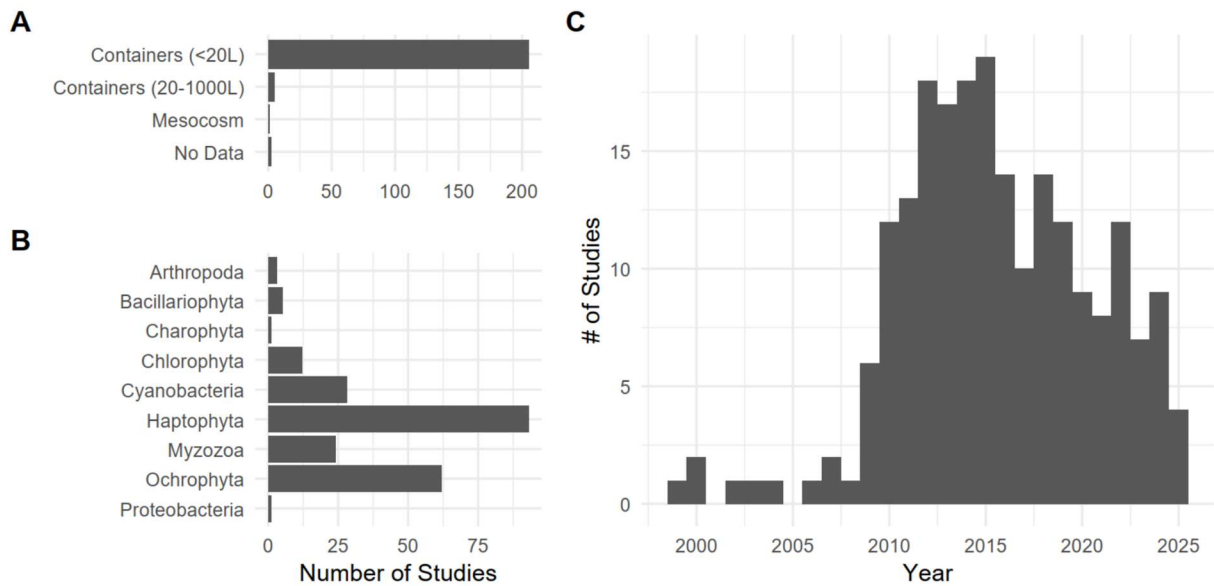
Supplementary Figure 3) Alignment of literature conditions with ‘biome relevant’ two standard deviation zones. All pairs between temperature and four carbonate system ions are shown on both axes. Red areas are bounded regions shown and described in Supp Figure 2 (there shown in black). Points show all 2456 unique treatments detected from OA-ICC literature database. Note the log10 scale for $[\text{CO}_2]$, $[\text{HCO}_3^-]$, and $[\text{CO}_3^{2-}]$; pH and temperature are shown untransformed.



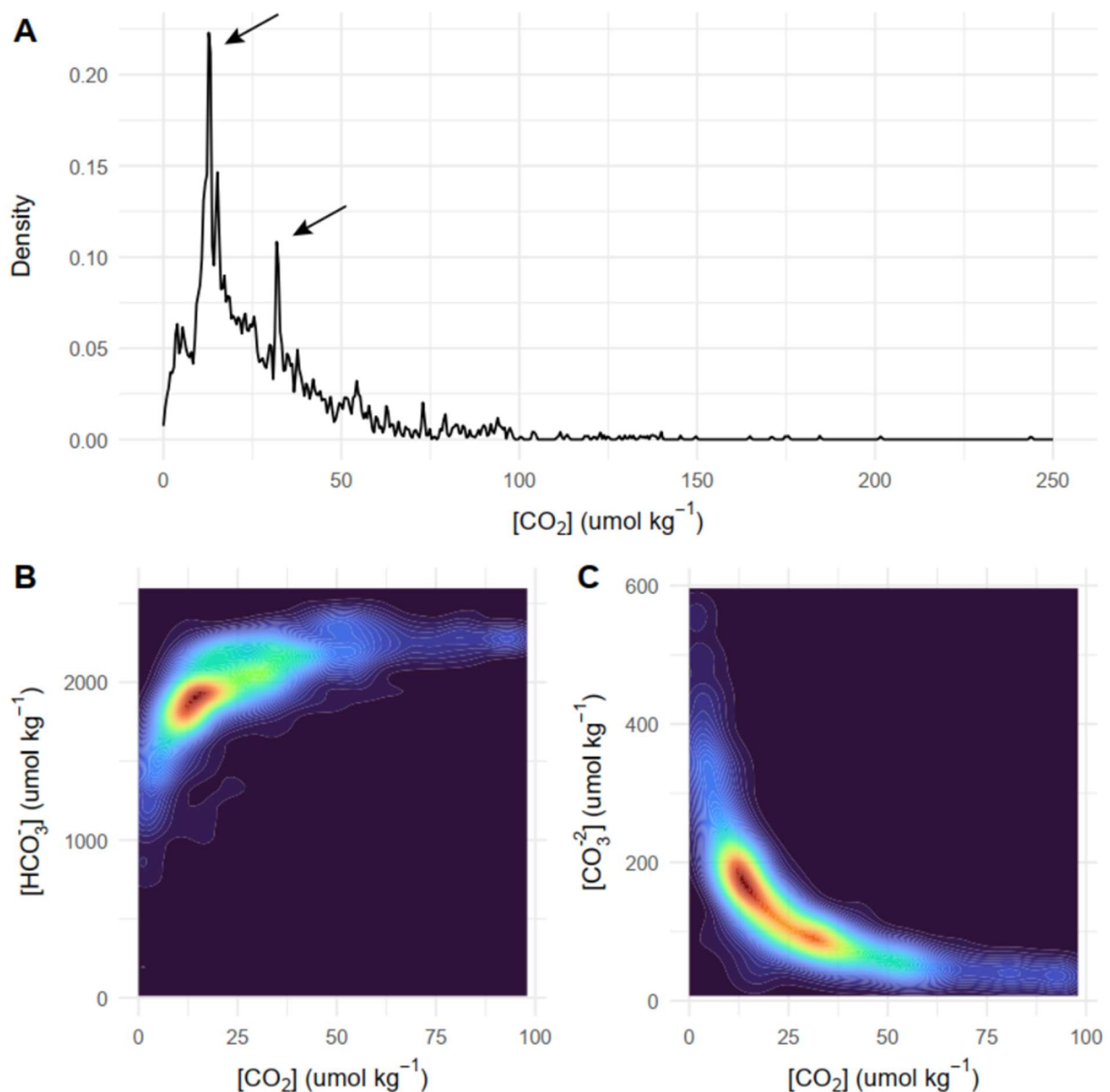
Supplementary Figure 4) Treatment classification for literature data outside of ocean biome regions. Points show all 2456 unique treatments detected from OA-ICC literature database. Axes show pairs of the three variables used in determining directionality of treatments outside of ocean biome region. Treatments shown in yellow were within two standard deviation regions, blue points were classified as 'low' relative to that region, red points were classified as 'high'. Purple points were neither consistently higher nor lower but instead misaligned with the biome region. See main text methods section 2.4 for precise classification details.



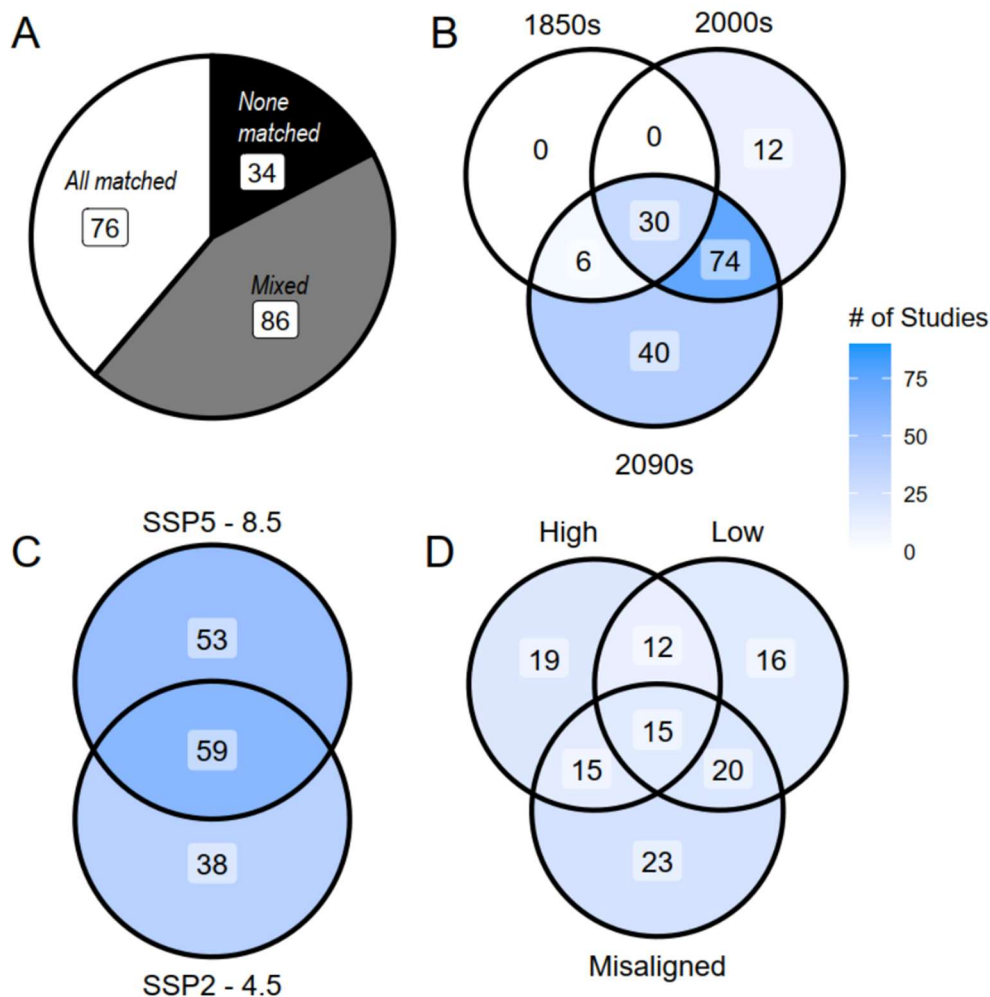
Supplementary Figure 5) Testing CMIP6 biomes for seasonal overlap in drivers with future end-of-century conditions. Top panels show the seasonal cycle of **A)** pCO₂ and **B)** temperature via monthly means (standard deviation not shown) for a single biome (NP-SPSS). Line colors indicate scenario-periods in CMIP6 data; 2000s (blue), SSP2-45 2090s (yellow), and SSP5-8.5 2090s (red). Bottom panel **C)** show heatmaps of biomes versus drivers for each scenario; SSP2-45 (left) and SSP5-8.5 (right). Grey indicated there was no seasonal overlap between 2000s and 2090s for monthly mean values (i.e. 2000s monthly mean max was less than 2090s monthly mean minimum). Green indicates that there was overlap.



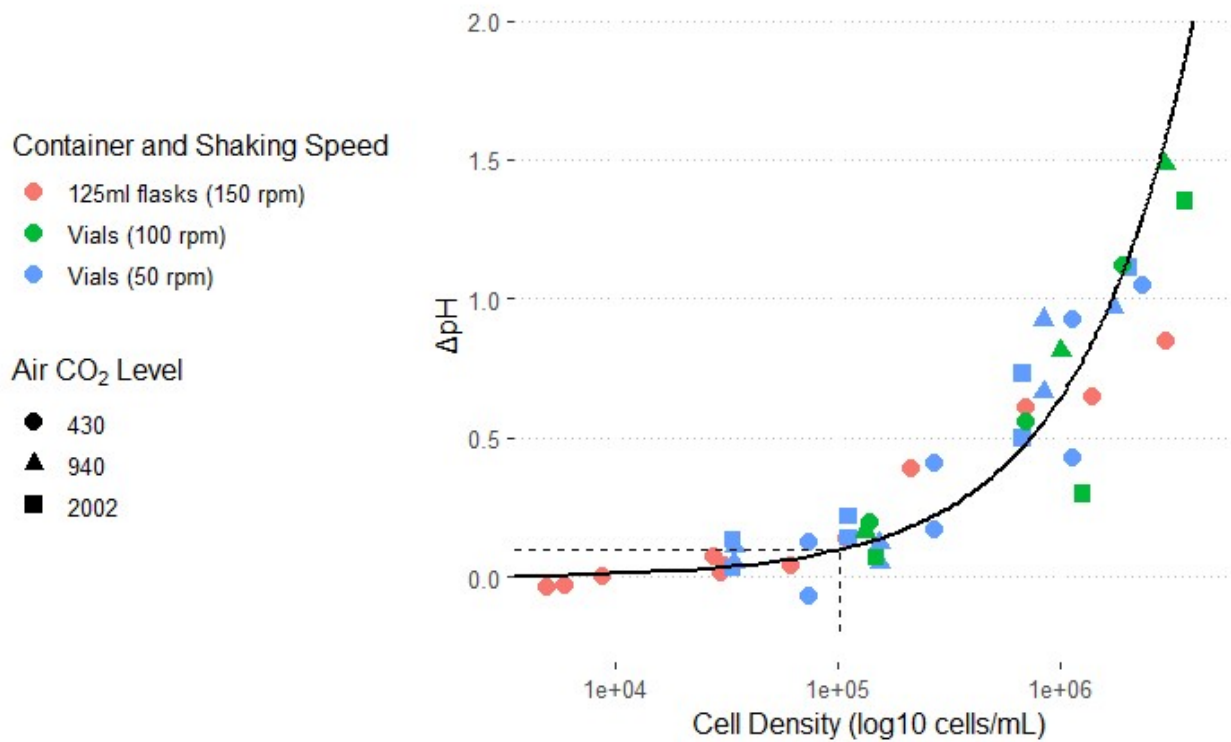
Supplementary Figure 6) OA-ICC Database Metadata. Figure presents metadata for the subset of studies evaluated from OA-ICC. **A)** Size of containers used in studies. **B)** Taxonomic information on organisms in subset. **C)** Histogram showing year of publication for studies.



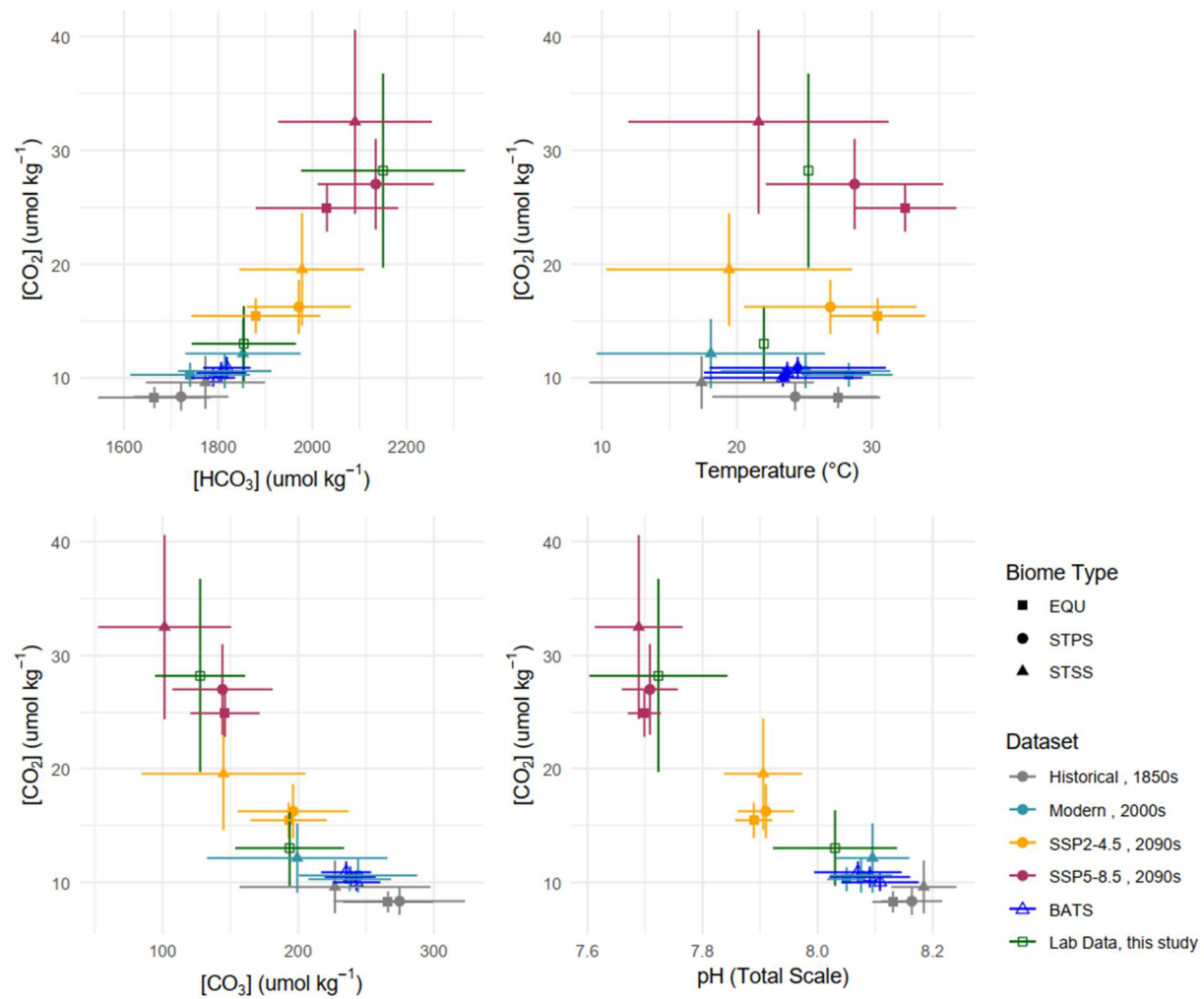
Supplementary Figure 7) Density of inorganic carbon species treatments in literature phytoplankton studies. **A)** Density distribution of $[\text{CO}_2]$ from OA-ICC treatments ($n = 2456$, bandwidth = 0.06). Two areas of density described in the main text are noted with arrows. Bottom panels show two-dimensional density distributions of treatments for **B)** $[\text{CO}_2]$ versus $[\text{HCO}_3^-]$ and **C)** $[\text{CO}_2]$ versus $[\text{CO}_3^{2-}]$.



Supplementary Figure 8) Alignment of OA-ICC treatments with biome conditions at the study level. **A)** Pie chart showing the number of studies out of 196 total where all treatments matched a biome condition, some matched a biome condition, and none matched. **B)** Venn diagram showing the number of studies that had a treatment whose ion composition best matched a biome from the three different decades. **C)** Venn diagram showing the number of studies that had a treatment whose ion composition best matched a biome in 2090s from the two future scenarios. **D)** Venn diagram showing the number of studies that had a treatment whose ion composition was higher than future values, lower than pre-industrial values, or were in range but misaligned with respect to biome averages (see Methods Section 2.4 for definitions).



Supplementary Figure 9) Change in pH with cell density for *G. huxleyi* with various headspace $p\text{CO}_2$ containers used. Change in pH for a culture is plotted against cell density. Shape shows headspace $p\text{CO}_2$ for a culture, color shows container and shaking speed.



Supplementary Figure 10) Alignment of measured *G. huxleyi* and *T. pseudonana* carbonate condition changes to projected north Atlantic biomes (EQU, STSS, STPS) conditions as well as change at BATS station. Error bars on lab conditions are the standard deviation of replicate cultures measured at the final timepoint of the experiment. Error for biome means is the same as Figure 2. Surface conditions for 1990s, 2000s, and 2010s are shown for BATS bottle data. Error bars are the standard deviation of the mean.