

Carbonate chemistry co-variation with temperature and oxygen in coastal environments and the design of ecologically relevant ocean acidification experiments

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Introduction

Ocean acidification (OA) is expected to have major impacts on marine ecosystems by directly influencing organismal performance (e.g., growth, development, survival) and indirectly through shifts in food web structure or competitive interactions. Our ability to predict the effects of OA on most species is currently limited but growing, and CO₂ exposure experiments are central to efforts to increase understanding.

Typically, experiments include control conditions that attempt to simulate contemporary or preindustrial seawater CO₂ concentrations and acidified treatments that correspond to potential future CO₂ uptake by the oceans. For studies focused on organisms from low productivity, open-ocean surface waters, researchers can rely on IPCC simulations of future atmospheric CO₂ partial pressure (pCO₂) to identify potential carbonate chemistry treatments because assumptions of air-sea pCO₂ equilibrium are often nearly met (1). In contrast, pCO₂ can be far

from air-sea equilibrium in many coastal systems, and considerable spatial and temporal variation can exist due to multiple processes, including high rates of primary production and respiration, freshwater inputs, and upwelling (2, 3). To estimate the potential impact of OA on organisms from these regions, control pCO₂ levels that reflect contemporary ambient conditions are needed. Recognition of this issue has led researchers to use data from coastal seawater chemistry monitoring programs to inform treatment levels in several recent OA experiments.

Less appreciated from an experimental perspective, however, is the possibility that carbonate chemistry conditions may also naturally co-vary with other biologically relevant variables, including temperature and O₂ (4). This may have important implications for the design of appropriate controls and treatments. Organismal physiology and interspecific interactions are strongly influenced by temperature and O₂ and may have non-additive interactions with carbonate chemistry (5). Consequently, the

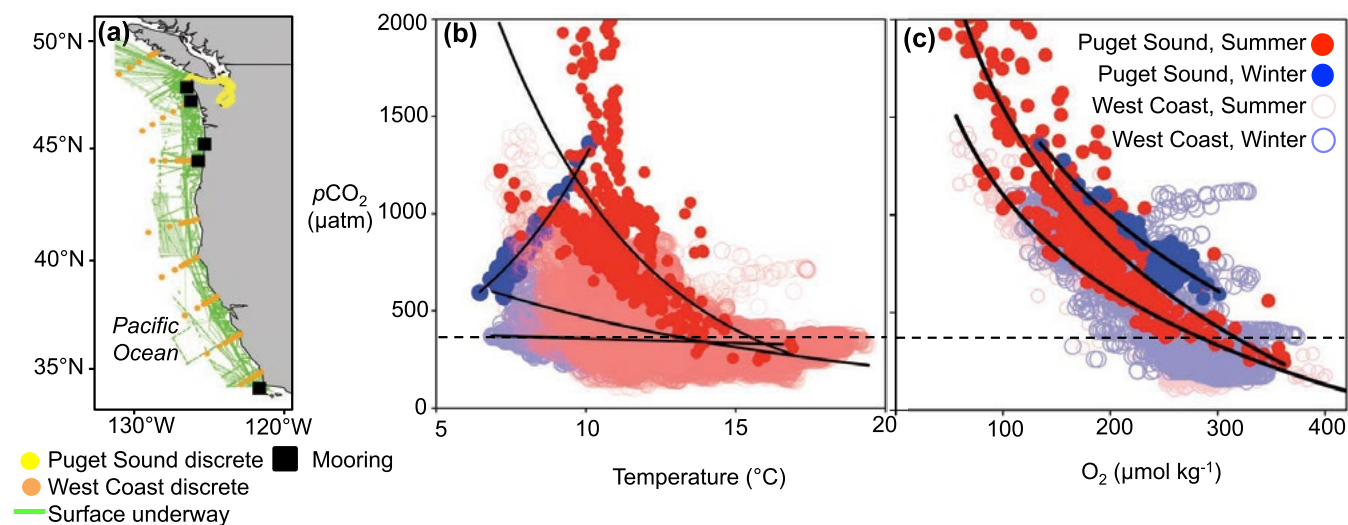


Figure 1. (a) Map of coordinates from which environmental carbonate chemistry data were obtained from moorings and ship-based underway and discrete water samples. Relationship between (b) pCO₂ and temperature and (c) pCO₂ and oxygen (O₂) in the CCE during upwelling (summer) and downwelling (winter) oceanographic seasons, respectively. Regression lines are overlaid to aid evaluation of patterns. For reference, approximate present-day pCO₂ levels (~390 µatm) are indicated by the dashed horizontal line. In (a), samples coded as Puget Sound also include measurements from the adjoining Strait of Juan de Fuca. Figures modified from 4.

temperature and O_2 level of seawater used in experiments may influence the estimated effect of OA. A danger is that temperature and O_2 levels that do not normally co-occur might be selected for the control pCO_2 treatment. If the desire is to use experimental results to help draw inferences on the likely future impacts of OA, such controls might provide inaccurate baselines, reducing the relevance of the experiment (4). The need for identifying appropriate control conditions also extends to multi-stressor climate change experiments in which temperature, O_2 or other variables might be crossed.

The remainder of this article aims to explore some of the challenges researchers face in designing OA experiments when study organisms come from waters in which carbonate chemistry covaries with other biologically important variables. To illustrate the issues, environmental data were assembled from a variety of habitats in the California Current Ecosystem (CCE), a major eastern boundary upwelling system that supports highly productive food webs. First, seasonal and regional covariation patterns between pCO_2 and temperature and O_2 were identified. Next, experimental conditions from published OA studies from the CCE were placed into an environmental context by comparing them to *in situ* pCO_2 and temperature measurements. Last, the implications of covariation between pCO_2 and temperature for OA experimental design were examined for a specific location on the Oregon coast.

Covariation between pCO_2 and temperature and O_2

Covariation patterns between carbonate chemistry and temperature and O_2 were examined for the region extending from northern Vancouver Island, British Columbia (50°N) to Point Conception, California (34°N; Fig. 1a). The data set included measurements from estuary and open coastal water habitats that extended up to 200 km from the coast and down to 50 m depth. Covariation patterns were examined during the upwelling (May – October) and downwelling (November–April) oceanographic seasons. To assess overall patterns of covariation, data were pooled across habitats, except for data from Puget Sound, which were examined separately. Puget Sound is a large, complex fjord that exhibits slow exchange with open coastal waters, high rates of primary productivity and respiration, and therefore pCO_2 -temperature relationships that likely differ substantially from elsewhere in the CCE (6). Full details of the data set are available in 4.

Overall, temperature and pCO_2 values spanned 6 to 19°C and 100 to 1500 μatm , respectively, and covariation patterns between pCO_2 and temperature varied depending in part on season and region (Fig. 1b). In general, pCO_2 values tended to increase with decreasing water temperature during summer upwelling months (Fig. 1b). For Puget Sound, a similar but steeper relationship was apparent relative to the open coastal waters, reflecting CO_2 -enriched waters (Fig. 1b). Along the open coast, the range of pCO_2 values was also wider at cool relative to warm temperatures. For instance, pCO_2 values at 9°C ranged from 320 to 1400 μatm , while at 16°C, the range extended from 130 to 420 μatm . In Puget Sound, the pCO_2 range was also wider at cooler temperatures (Figure 1b).

In contrast, during the winter, co-variation between pCO_2 and temperature was weaker in open coastal waters, and the range in pCO_2 values and temperatures narrowed relative to summer (Fig. 1b). This was due to the relative absence of cold, high- pCO_2 upwelled waters. In Puget Sound, winter pCO_2 positively co-varied with temperature, and the range of pCO_2 values and temperatures also narrowed relative to summer (Fig. 1b).

Last, covariation patterns between pCO_2 and O_2 were far more consistent between regions and seasons relative to pCO_2 and temperature (Fig. 1c). Overall, summer O_2 measurements from all locations ranged from 40 to 400 $\mu\text{mol kg}^{-1}$, where concentrations below $\sim 60 \mu\text{mol kg}^{-1}$ reflect hypoxic conditions (Fig. 1c). The negative relationship between pCO_2 and O_2 corresponds to the well-understood effects of aerobic respiration and photosynthesis in marine ecosystems. When aerobic respiration dominates, CO_2 is remineralized and O_2 levels are drawn down, while the reverse occurs when photosynthesis dominates.

Environmental pCO_2 -temperature vs. experimental conditions

Given covariation patterns in the CCE, how well have experimental conditions from published OA studies matched environmental measurements? To answer this, the literature was reviewed for experiments that included organisms (or broodstock) obtained from habitats within CCE. In total, 26 OA experiments (22 published studies) were found (Fig. 2a; see 4 for details). For nearly all studies, experimental O_2 concentrations were not reported nor could saturation conditions be safely assumed. Comparisons of environmental and experimental conditions therefore focused exclusively on pCO_2 and temperature.

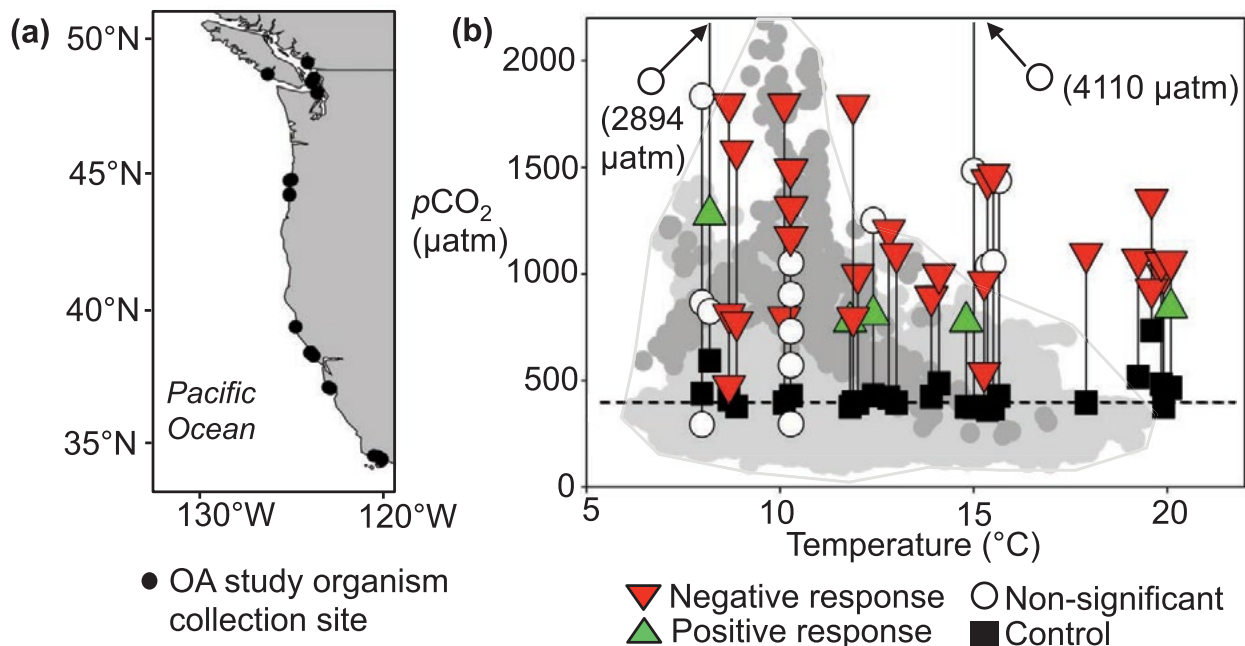


Figure 2. (a) Map of locations where organisms (or their broodstock) included in published OA experiments were collected. (b) Environmental pCO₂ and temperature measurements for the CCE and conditions maintained in OA experiments performed on organisms from the same region. Dark grey circles correspond to environmental measurements from Puget Sound; light grey circles correspond to measurements from all other regions. pCO₂ treatment levels included in an individual experiment are connected by solid black vertical lines. A convex hull (solid grey line) demarcating the extent of all environmental pCO₂ and temperature measurements is depicted to aid visual comparisons. For reference, approximate present-day atmospheric pCO₂ levels (~390 μatm) are indicated by the dashed horizontal line. Figures modified from 4.

Following the authors' interpretation, any significant biological response to the treatment was noted along with the direction of response (Fig. 2b). If authors did not explicitly designate a control pCO₂ level in their study, treatments levels with pCO₂ levels closest to present-day air pCO₂ levels (~400 μatm) were considered as the control to facilitate comparisons across studies. When more than one response variable was tested in an experiment, the net outcome of the experiment was coded at a given treatment level based on the result of the variable most sensitive to pCO₂.

Compared with the pCO₂-temperature space defined by the complete set of environmental measurements in our dataset, five experiments were performed at temperatures that matched or exceeded the warmest observed values (~19°C; Fig. 2b). These included three experiments on the early life history stages of the native *Olympia* oyster, an experiment on sand dollar larvae, and an experiment on the non-native Pacific oyster (which is routinely reared at ~20°C to optimize survival under commercial hatchery conditions). One experiment included a 2.1°C treatment; though this temperature was meant to simulate cool con-

ditions in Alaskan waters, the source stock was collected near Puget Sound. Interestingly, several studies observed negative and positive responses in organisms at pCO₂ and temperature values that occur today in the CCE (Fig. 2b).

How did researchers select pCO₂ levels and temperatures for their experiments? In terms of pCO₂ levels, IPCC estimates of future global surface ocean average pCO₂ levels were cited as justification in 45% of studies, while 31% cited a combination of regional modeling studies, local field measurements, and IPCC estimates to support their choice of experimental pCO₂ treatment levels. Of the remaining studies, 13% provided no rationale for their choice of pCO₂ treatment levels, one based the high pCO₂ treatment level on observations of contemporary upwelling conditions, and one noted naturally high carbonate chemistry variability in coastal upwelling systems that necessitated testing of biological responses to a wide range of pCO₂. For temperature, 80% of studies did not provide a rationale at all. The remaining studies cited similarity to local field conditions. Only two studies performed multistressor experiments, both crossing temperature and pCO₂, and no experiments considered temporal variation in carbonate chemistry conditions.

OA experimental designs

To help illustrate the effect covariation has on experimental design, an example multistressor experimental scheme is presented in Fig. 3a that is typical of published OA studies. Three temperature treatments are included (8, 12, and 16°C) and crossed with two pCO₂ levels that correspond to approximate global surface ocean present-day (400 μatm) and future (800 μatm) conditions (Fig. 3a). All treatments are fully orthogonal, which permits estimation of the effect sizes of the individual predictor variables and of their interaction on the response variable. The method holds merit as a tool for comparing the relative influence that each predictor has on the response variable. However, if a goal of a study is to evaluate the potential sensitivity of organisms to future OA, the design may be inadequate, given natural pCO₂-temperature co-variation.

For example, if the study organism occurs in shelf waters off Oregon during summer upwelling months (e.g., a pelagic larval invertebrate), the assumption that 800 μatm corresponds to a future OA prediction across all temperatures is not accurate. At the Newport, Ore-

gon mooring, pCO₂ levels of 800 μatm already occur at 8°C under present-day conditions and control 400 μatm waters do not (Fig. 3a). At temperatures above 13°C, the mean pCO₂ values approach air-sea equilibrium conditions. The experimental design will certainly provide information on the interactive effects of pCO₂ and temperature, but the utility of the design for drawing inferences on the potential response of wild populations to OA in the region is questionable.

Given potential co-variation between carbonate chemistry and other important environmental variables, how might researchers select pCO₂ treatments that better correspond to OA hypotheses? First, researchers should consider inclusion of multiple controls that reflect the span of pCO₂ levels and temperatures likely to be experienced by the organism under study (Fig. 3b). To design pCO₂ treatments that represent future OA scenarios in highly productive regions, researchers might focus on estimating likely changes in the anthropogenic contribution to *in situ* dissolved inorganic carbon (DIC) (4). At the Newport, Oregon mooring, newly upwelled waters

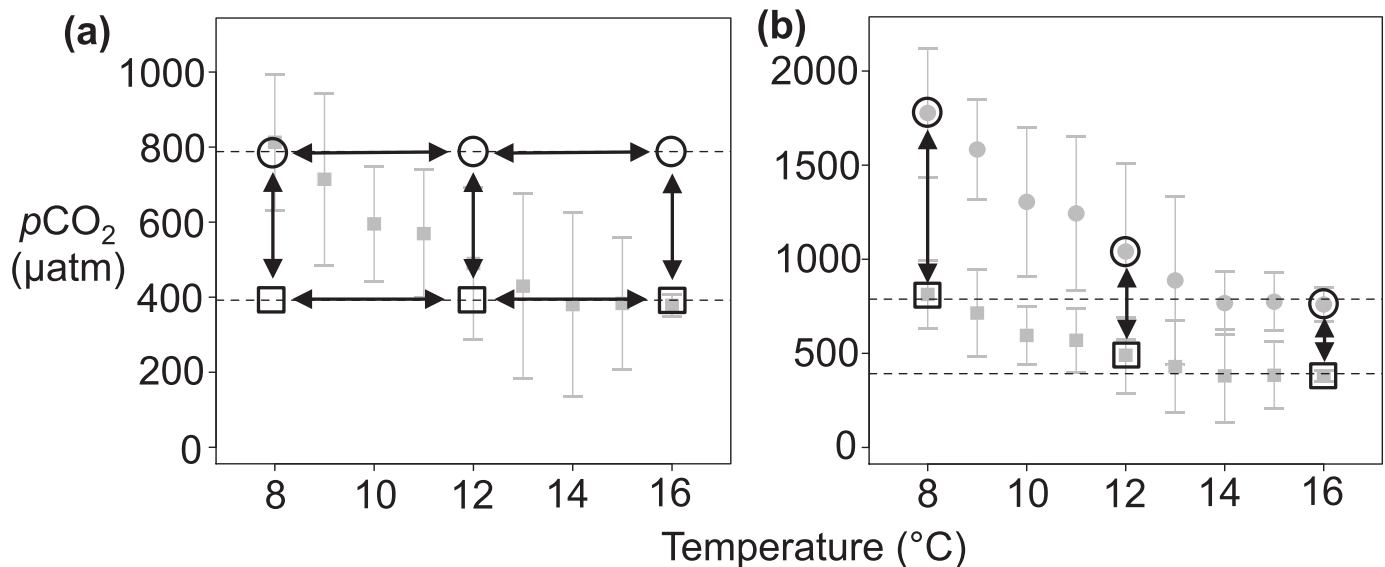


Figure 3. Schematic of potential experimental approaches to evaluate OA effects, given co-variation between pCO₂ and temperature. To illustrate the benefits and drawback of each approach, in situ pCO₂ and temperature measurements from the NH10 mooring near Newport, Oregon during summer upwelling season (2008) are depicted (grey, filled squares; bars indicate standard deviation). **(a)** A conventional temperature (three levels: 8, 12, and 16°C) by pCO₂ experimental design, in which control pCO₂ values are based on approximate present-day global average surface ocean pCO₂ levels and the acidified treatments correspond to IPCC emissions scenario IS92a projections for the year 2100 (390 and 788 μatm; open square and circle symbols, respectively). Arrows indicate statistical comparisons permitted by the design. **(b)** Experimental design informed by in situ pCO₂ and temperature measurements. Under this design, three controls are included to account for natural co-variation in temperature and pCO₂. Treatment levels that more closely correspond to an OA hypothesis were obtained by specifying an increase in DIC attributed to anthropogenic CO₂ emissions (see text and 4 for additional details). The future DIC estimate and estimates of alkalinity were used to recalculate the carbonate system to obtain target pCO₂ treatment levels. Figures modified from 4.

exhibit $p\text{CO}_2$ values that are elevated relative to air-sea equilibrium conditions due to the remineralization of organic material before surfacing (7). However, after surfacing, CO_2 concentrations can be drawn down rapidly by photosynthesis (2, 7), often at rates that far exceed CO_2 equilibration times across the air-sea interface (e.g., 8, 9). Consequently, the anthropogenic CO_2 burden of upwelled waters is primarily acquired when they were last in contact with the atmosphere and before DIC changes due to biological processes post-surfacing. In our example, $p\text{CO}_2$ treatments reflecting future OA hypotheses could be obtained by increasing *in situ* DIC concentrations by an increment (ΔDIC) expected under a given CO_2 emissions scenario. The future DIC estimate ($\Delta\text{DIC} + \textit{in situ}$ DIC), along with a second parameter from the carbonate system, could then be used to recalculate the carbonate system to estimate treatment $p\text{CO}_2$ levels.

Under this approach, and assuming the same number of treatments is used as depicted in Fig. 3a, the effects of temperature and $p\text{CO}_2$ can no longer be separated because orthogonality in the design is lost (Fig. 3b). However, a more realistic set of control treatments are included that offer a firmer basis for drawing inferences about future OA impacts at a given temperature. The experimental design could be improved further by using O_2 concentrations that currently occur at the three different $p\text{CO}_2$ -temperature controls.

Summary

The need for OA researchers to use $p\text{CO}_2$ levels that correspond to ambient conditions a study species or life history stage is likely to experience is now widely recognized in the literature, but patterns of co-variation with temperature and O_2 have yet to be incorporated into OA experimental designs. This issue should be of concern to researchers in coastal systems where water conditions are highly dynamic over a range of spatial and temporal scales and where co-variation between $p\text{CO}_2$, temperature, and O_2 are generally expected. Because inferences on the potential response of organisms to future conditions are premised on the notion that experimental

controls reflect present-day conditions, we strongly recommend that researchers consider how $p\text{CO}_2$ naturally varies with other biologically important variables in their experimental designs.

Although we focused on $p\text{CO}_2$, the challenges associated with covariation in water conditions also extend to experimental efforts to understand the main and interactive effects of other climate change phenomena, including global warming and ocean deoxygenation. We caution that while simple crossed multistressor experiments can provide information on the interactive effects of variables on organisms in a statistical sense, treatments should be considered and interpreted in light of covariation patterns experienced by organisms over their distribution.

With the continued collection of high-quality carbonate chemistry measurements and their archival on freely accessible databases, analyses like the one we present here for the CCE may yield further insight into the relevance of carbonate chemistry variability to contemporary ecological processes, as well as guide climate change experimental designs in other marine systems.

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