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# Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen

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Coastal upwelling regimes are some of the most productive ecosystems in the ocean but are also among the most vulnerable to ocean acidification (OA) due to naturally high background concentrations of $CO_2$. Yet our ability to predict how these ecosystems will respond to additional $CO_2$ resulting from anthropogenic emissions is poor. To help address this uncertainty, researchers perform manipulative experiments where biological responses are evaluated across different $CO_2$ partial pressure ($pCO_2$) levels. In upwelling systems, however, contemporary carbonate chemistry variability remains only partly characterized and patterns of co-variation with other biologically important variables such as temperature and oxygen are just beginning to be explored in the context of OA experimental design. If co-variation among variables is prevalent, researchers risk performing OA experiments with control conditions that are not experienced by the focal species, potentially diminishing the ecological relevance of the experiment. Here, we synthesized a large carbonate chemistry dataset that consists of carbonate chemistry, temperature, and oxygen measurements from multiple moorings and ship-based sampling campaigns from the California Current Ecosystem (CCE), and includes fjord and tidal estuaries and open coastal waters. We evaluated patterns of $pCO_2$ variability and highlight important co-variation between $pCO_2$, temperature, and oxygen. We subsequently compared environmental $pCO_2$ – temperature measurements with conditions maintained in OA experiments that used organisms from the CCE. By drawing such comparisons, researchers can gain insight into the ecological relevance of previously published OA experiments, but also identify species or life history stages that may already be influenced by contemporary carbonate chemistry conditions. We illustrate the implications co-variation among environmental variables can have for the interpretation of OA experimental results and suggest an approach for designing experiments with $pCO_2$ levels that better reflect OA hypotheses while simultaneously recognizing natural co-variation with other biologically relevant variables.

**Keywords:** California Current, climate change, hypoxia, multistressor experiment, pH.

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## Introduction

Coastal upwelling systems located along the eastern boundary of ocean basins are some of the most productive ecosystems in the ocean but are also among the most vulnerable to OA (Feely *et al.*, 2008; Gruber *et al.*, 2012). The net transport of deep, nutrient-rich waters to the sunlit surface by upwelling-favourable winds promotes high rates of primary production which in turn supports productive foodwebs and major fisheries (Fréon *et al.*, 2009). However, subsurface and newly upwelled waters naturally exhibit low $O_2$ and high $CO_2$ concentrations due to the remineralization of organic material exported from surface layers. Consequently, they have a reduced capacity to buffer against changes in carbonate chemistry resulting from ocean uptake of anthropogenic $CO_2$ relative to open-ocean surface waters (Feely *et al.*, 2008; Fassbender *et al.*, 2011; Harris *et al.*, 2013). In eastern Pacific systems such as the California Current, the $CO_2$ burden and $O_2$ drawdown due to respiration are high because the source waters transported to upwelling centres along the coast have been isolated from the surface for a few decades (Feely *et al.*, 2008; Hauri *et al.*, 2009). In the California Current, anthropogenic $CO_2$ has already lowered pH by ~0.1, causing the depth of undersaturation with respect to aragonite to shoal and expanding the spatial extent of undersaturated surface waters (Feely *et al.*, 2008; Gruber *et al.*, 2012; Harris *et al.*, 2013). In only a few more decades, models suggest that the depth of undersaturation may shoal into the upper 75 m of the water column in some regions year-round (Gruber *et al.*, 2012; Hauri *et al.*, 2013). Given the economic, ecological, and biogeochemical importance of eastern boundary upwelling regions, understanding how species that compose these ecosystems will respond to OA has emerged as a high research priority (Fabry *et al.*, 2008; Gruber, 2011; Doney *et al.*, 2012).

To evaluate the sensitivity of species to OA, researchers commonly rely on manipulative experiments where organisms are exposed to different carbonate chemistry conditions. Typically, experiments include "control" conditions that attempt to simulate contemporary or preindustrial $CO_2$ concentrations and "acidified" treatments that correspond to potential future $CO_2$ uptake by the oceans. For studies focused on organisms from low productivity, open-ocean surface waters researchers can rely on IPCC scenarios of atmospheric $pCO_2$ concentrations to identify potential carbonate chemistry treatments because assumptions of air–sea $pCO_2$ equilibrium are often nearly met (Barry *et al.*, 2010; Orr, 2011). However, in upwelling systems, $CO_2$ levels are more variable relative to open ocean waters due to the outcropping of high-$CO_2$ subsurface waters and high rates of primary production and respiration that strongly modulate seawater carbonate chemistry (Hales *et al.*, 2005; Feely *et al.*, 2008; Borges and Abril, 2011; Fassbender *et al.*, 2011). Consequently, OA experiments that use organisms from these habitats and that rely on IPCC future atmospheric $CO_2$ scenarios to devise control and acidified seawater treatments may inadequately replicate contemporary carbonate chemistry or include treatments that fail to reflect realistic future OA hypotheses (Barry *et al.*, 2010; Andersson and Mackenzie, 2012; McElhany and Busch, 2012).

Recognition of the importance of including environmentally relevant $pCO_2$ levels in OA experiments has grown considerably, and has led to the use of seawater chemistry monitoring programmes to inform treatment design in several recent OA studies (Hofmann *et al.*, 2014). 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 $O_2$ over multiple spatial and temporal scales (Reum *et al.*, 2014). This may have important implications for the design and interpretation of ocean acidification (OA) experiments because of the potential for non-additive interactions between carbonate chemistry, temperature, and $O_2$ on organismal performance and ecological interactions (Wernberg *et al.*, 2012, Harvey *et al.*, 2013; Koch *et al.*, 2013; Kroeker *et al.*, 2013). For many organisms, aerobic capacity and metabolic scope (the amount of energy that can be allocated to activities beyond those required for basic existence) may be influenced strongly by temperature, and adversely impacted by reductions in ambient oxygen availability or increases in $CO_2$ concentrations (Pörtner, 2010, 2012).

Given the combined effects that temperature and environmental $O_2$ and $pCO_2$ have on organismal physiology and experimental evidence indicating non-additive interactions on response variables related to fitness and ecosystem function (Pörtner and Farrell, 2008; Pörtner, 2010; Harvey *et al.*, 2013; Kroeker *et al.*, 2013), knowledge of their co-variability is essential for designing OA experiments that adequately characterize biological performance under contemporary relative to future acidified conditions. Yet for workers focused on laboratory OA experiments, these relationships are rarely incorporated into experimental designs. This poses important potential drawbacks. Foremost, if carbonate chemistry strongly covaries with temperature or $O_2$, researchers risk running experiments with control-water characteristics that are atypical of the habitat to which a focal organism/life stage may have acclimated or adapted to. Recently, $pCO_2$ was shown to range widely (~200–2500 μatm) and co-vary with temperature and $O_2$ in a fjord located in the northeast Pacific (Reum *et al.*, 2014). In that system, the strength and direction of the relationships changed with season, but also between subregions that differed in terms of vertical mixing. Consequently, within a given season and region, organisms occurring in low $pCO_2$ waters experience temperatures and $O_2$ levels that differed from those experienced by organisms that occupy high $pCO_2$ waters, and this has direct implications for how ecologically relevant OA experiments should be designed (Reum *et al.*, 2014).

Here, we expand on the topic and evaluate the potential importance of this issue for upwelling systems. To do so, we have assembled a large dataset of carbonate chemistry measurements from a variety of habitats throughout the California Current Ecosystem (CCE), a major eastern boundary upwelling system that supports highly productive foodwebs. Along the coast, equatorward winds during spring and summer drive surface flow offshore in an Ekman layer, leading to the upwelling of cold, salty, $O_2$-poor, and nutrient- and $CO_2$-rich subsurface water. In fall and winter, the wind direction reverses resulting in downwelling and shoreward advection of oceanic waters which are relatively warm, fresh, $O_2$-saturated, nutrient-deplete, and near air–sea $CO_2$ equilibrium (Evans *et al.*, 2011; Harris *et al.*, 2013). The dataset presented here consists of $pCO_2$ measurements from multiple moorings and ship-based sampling campaigns that collectively span 14° of latitude, and includes data from estuary and open coastal water habitats. Although the extent of data is constrained in either time or space for any single sampling campaign, the synthesis of many datasets offers an overview of the potential range of $pCO_2$, temperature, and $O_2$ conditions experienced by organisms from this region. Subsequently, we compared environmental $pCO_2$ and temperature measurements to conditions in OA experiments performed on organisms obtained from populations that reside within the CCE. In doing so, we place these

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experiments in a larger environmental context and draw on observations that may help guide OA researchers in considering experimental designs that are more appropriate for organisms that occur in the CCE and similar coastal systems.

## Material and methods

### Carbonate chemistry data

Our analysis focused on coastal waters within the domain of the northern and central CCE (Figure 1). The region extends from northern Vancouver Island, British Columbia (50°N) to Point Conception, California (34°N), and is typified by distinct wind-driven upwelling (May–October) and downwelling (November–April) oceanographic seasons (Checkley and Barth, 2009). The region includes large expanses of open coastal habitat and many inlets and estuaries that range considerably in terms of surface area, depth, substrate, and connectivity with the open ocean (Hickey and Banas, 2003). To obtain an overview of the potential range of temperatures and carbonate chemistry conditions, we retrieved all publicly available records of measurements sampled within our region of interest from the Carbon Dioxide Information Analysis Center (CDIAC) and Surface Ocean $CO_2$ Atlas (SOCAT, Bakker *et al.*, 2013). The dataset includes carbonate chemistry measurements from discrete water samples collected at depth, shipboard measurements of surface water $pCO_2$, and mooring $pCO_2$ time-series of surface waters off the coast of California and Washington (Supplementary Table S1). In addition to CDIAC and SOCAT data, we also included unpublished carbonate chemistry data obtained from the 2011 and 2012 NOAA Ocean Acidification Programme West Coast Cruise survey, which sampled waters along the continental shelf of western North America; mooring $pCO_2$ time-series of surface waters from an open coast location (Newport, Oregon), and a shallow tidal estuary (Netarts Bay, Oregon); and published carbonate chemistry data from late summer and fall surveys in a large fjord estuary complex (Strait of Juan de Fuca and Puget Sound, Washington; Supplementary Table S1). Further details of the survey, sampling dates, sampling method, and habitats from which data were obtained are provided in Supplementary Table S1. Although the dataset does not include all habitat types that occur within the CCE, we believe these data are sufficiently representative enough to offer an overview of key patterns of carbonate chemistry, temperature, and $O_2$ covariation. For the purposes of the present study, we included all measurements extending up to 200 km from the coast.

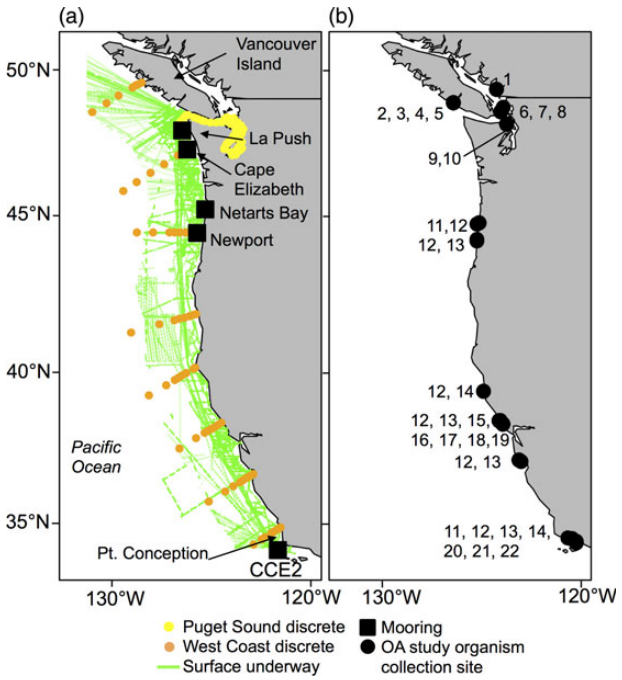


**Figure 1.** Map of coordinates where (a) environmental carbonate chemistry data were obtained from the northern and central CCE from moorings and ship-based underway and discrete samples, (b) the locations where organisms (or their broodstock) included in published OA experiments were collected. In (a), samples coded as Puget Sound also include measurements from the adjoining Strait of Juan de Fuca. For additional details on environmental carbonate chemistry datasets, see Supplementary Table S1.

For our analysis, we focused on $pCO_2$ rather than other parameters of the carbonate system because $pCO_2$ is directly changed by anthropogenic $CO_2$ emissions and is the most commonly used treatment variable in species-exposure OA experiments. Further, direct measurements of $pCO_2$ were more widely available than other parameters in the CDIAC and SOCAT databases, and when not available were readily estimated from other measured parameters of the carbonate system (see Supplementary Table S1 for details on estimation methods). We recognize that organisms may potentially be more sensitive to other variables of the carbonate system such as pH or to changes in the calcium carbonate saturation state of seawater (Barton *et al.*, 2012; Waldbusser and Salisbury, 2013) and that waters with similar $pCO_2$ values may differ with respect to aragonite or calcite saturation states if total alkalinities differ. However, $pCO_2$ is routinely reported in OA studies and is the parameter that allowed us to best standardize comparisons among experiments and between experiments and environmental carbonate chemistry measurements. Further, the focus on $pCO_2$ prevented the use of some carbonate chemistry datasets such as those including information only on pH (e.g. Hofmann *et al.*, 2011). We limited our analysis to $pCO_2$ measurements taken from the top 50 m of the water column because the species and life history stages for which OA experiments have been performed from the northern and central CCE typically occur in waters within this depth range, although individuals of some populations may occur for periods below this depth.

We evaluated separate relationships between $pCO_2$ and temperature and $pCO_2$ and $O_2$ within summer upwelling and winter downwelling periods. Co-variation patterns for $pCO_2$ and temperature were examined for data pooled from shipboard underway and discrete sample measurements collected along the West Coast continental shelf. In addition, we also examined co-variation patterns for Puget Sound and the Strait of Juan de Fuca discrete sample measurements (hereafter referred to as "Puget Sound"). A subset of these data were examined in an earlier study (Reum *et al.*, 2014), but we include them here for completeness and to facilitate comparisons among habitats represented within the CCE. We chose to present co-variation patterns for Puget Sound separately from the West Coast because Puget Sound exhibits slow exchange with open coastal waters and high rates of primary productivity and respiration (Feely *et al.*, 2010). The resulting $pCO_2$–temperature relationships therefore likely differ considerably from patterns observed in shelf waters. For the time-series data, we estimated $pCO_2$–temperature relationships for upwelling and downwelling seasons, but examined co-variation patterns separately for each mooring to evaluate site-level differences.

To evaluate $pCO_2$ and $O_2$ relationships and facilitate comparisons across survey types and seasons, we converted all $O_2$ concentration

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measurements to micromole per kilogramme. As with the $pCO_2$ and temperature data, we examined co-variation within data pooled from shipboard West Coast measurements, Puget Sound, and each mooring and by season. To improve assumptions of normality in the residual error structure for the $pCO_2$–temperature and $pCO_2$–$O_2$ relationships, we $\log_{10}$-transformed $pCO_2$ values before estimating linear relationships using least-squares regression. For presentation purposes, the liner relationships were back-transformed to the original $pCO_2$ scale. Our goal was to estimate overall mean relationships within seasons and survey types to facilitate visual inspection and to evaluate co-variation patterns in an exploratory manner. We therefore did not test for significant differences between seasons or survey types. All linear relationships were fitted using the R version 2.11 statistical software package (R Development Core Team, 2011).

### OA experimental studies

We searched the published literature for OA experiments that included organisms obtained directly from habitats within the northern and central CCE or that originated from broodstock collected from the region. To do so, we searched Google Scholar, ISI databases (Web of Science, Current Contents), and references included in recently published reviews and meta-analyses that address OA (Dupont *et al.*, 2010; Wernberg *et al.*, 2012; Harvey *et al.*, 2013; Kroeker *et al.*, 2013; Wittmann and Pörtner, 2013). We used the search terms "ocean acidification", "carbon dioxide", "experiment", and "manipulation". We included all studies published through 15 October 2013 that were found under these search criteria.

From each study, we retrieved information on the collection site of the organism (or their broodstock) and the temperatures and $pCO_2$ levels at which the experiment was performed. For nearly all studies, experimental $O_2$ concentrations were not reported nor could saturation conditions be safely assumed. We therefore focused our comparison of environmental and experimental conditions to $pCO_2$ and temperature. Following the authors' interpretation of the results, we recorded whether the dependent biological variables differed significantly in a positive or negative direction or showed no significant difference relative to the control $pCO_2$ level specified by the author. If authors did not explicitly designate a control $pCO_2$ level in their study, we considered treatments with $pCO_2$ levels closest to present-day air $pCO_2$ levels (~400 μatm) as the control to facilitate comparisons across studies. When more than one response variable was tested in an experiment, we coded the net outcome of the experiment at a given treatment level based on the result of the variable most sensitive to $pCO_2$.

From the outset, we recognized that the species and life history stages for which published OA experimental data were available held distributions that spanned large sections of the northern and central CCE or were planktonic with high dispersal potential. Further, the exact dispersal patterns of many of these species are not well understood. Although larvae of some species may be functionally limited to a subset of the CCE system (e.g. some species may occur primarily in bays or estuaries), "spillage" into adjoining habitats and waters through advective processes is also probable. We therefore used the full dataset of field $pCO_2$ and temperature measurements to demarcate the potential $pCO_2$–temperature space organisms may encounter. We acknowledge that the actual $pCO_2$ levels and temperatures experienced by organisms will differ for populations across locations, seasons, and due to possible interannual variation in upwelling and climate forcing. Our main intention, however, was to draw comparisons between the conditions maintained in OA experiments and the full range of environmental $pCO_2$ and temperature values based on empirical observations. In the absence of detailed information on the fine-scale distribution and movement patterns of most species and life history stages, and environmental $pCO_2$–temperature measurements of matching resolution, comparisons at finer spatio-temporal scales were not possible.

## Results

### $pCO_2$, temperature, and $O_2$ co-variation

Temperature and $pCO_2$ values in the upper 50 m of the CCE spanned 6–19°C and 100–1500 μatm, respectively, based on the pooled shipboard underway and discrete water sample measurements along the open coast. $pCO_2$ values tended to increase with decreasing water temperature during summer upwelling months (Figure 2a). In Puget Sound, a similar but steeper relationship was apparent relative to the open coastal waters, reflecting $CO_2$-enriched waters (Figure 2a). 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 2a).

Although fewer measurements were available during winter in general (Supplementary Table S1), the data showed weak co-variation between $pCO_2$ and temperature in open coastal waters and the overall range of $pCO_2$ values and temperatures narrowed relative to summer (Figure 2b). This is due to the relative absence of the cold, high- $pCO_2$ upwelled source waters. In contrast, winter $pCO_2$ and temperature values in Puget Sound positively covaried, and the range of $pCO_2$ values and temperatures also narrowed relative to summer (Figure 2b).

Time-series data collected from moored platforms in open coastal waters from four locations also indicated co-variation between $pCO_2$ and temperature (Figure 3a–c). During summer upwelling months, $pCO_2$ again generally declined with increasing temperature (Figure 3a–c). The range of $pCO_2$ levels among stations, however, differed substantially. Time-series data collected at moorings in shelf waters off Washington and near Point Conception, CA. (Figure 3a and b) ranged in $pCO_2$ from 200 to 600 and 300 to 600 μatm, respectively, while off the coast of Oregon the range spanned 200–1100 μatm (Figure 3c). The differences among stations reflect considerable spatial variation in the supply of newly upwelled waters.

Time-series data collected from Netarts Bay, a shallow tidal estuary in northern Oregon, indicated summer range in $pCO_2$ values was also considerable, spanning 300–800 μatm and reflected high rates of primary production and respiration (Figure 3d). The overall relationship between $pCO_2$ and temperature, however, was also negative. In winter, co-variation between $pCO_2$ and temperature at all mooring stations was weaker relative to summer but variable across stations (Figure 3d). In addition, the range of temperatures and $pCO_2$ values were generally lower in winter relative to summer in two of the five stations (Figure 3c and d).

Measurements of $O_2$ were available for a subset of $pCO_2$ records included in the complete environmental $pCO_2$ and temperature dataset (Supplementary Table S1). For all survey types, mooring time-series, and seasons, $pCO_2$ levels decreased with increasing $O_2$ concentration (Figure 4). For most moorings on the shelf, $O_2$ ranged from 230 to 350 μmol $kg^{-1}$ (both seasons combined). Summer $O_2$ levels at Newport Oregon, however, ranged from 120 to 350 μmol $kg^{-1}$ (Figure 4). Summer $O_2$ measurements from discrete samples from

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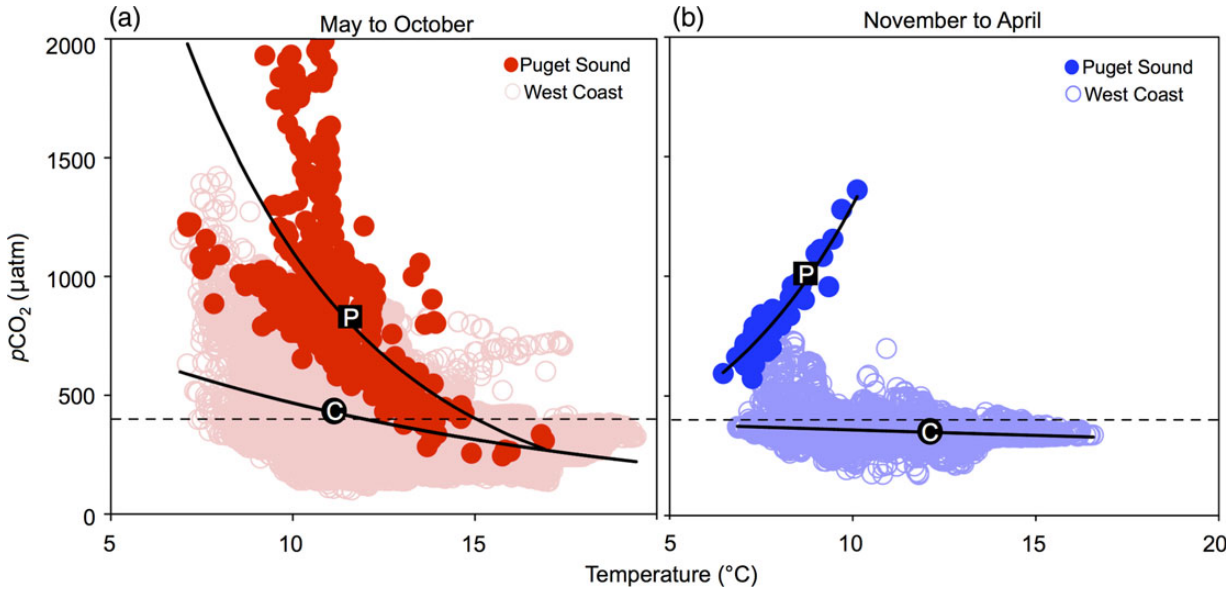


**Figure 2.** (a) Relationship between $pCO_2$ and temperature in the top 50 m of the water column in the northern and central CCE during summer upwelling season which approximately spans May through October and (b) winter (November through April) when downwelling-favourable winds predominate. All non-time-series data are displayed. Measurements of $pCO_2$ from cool waters in Puget Sound are elevated relative to other regions sampled in the CCE. Regression lines are overlaid to aid evaluation of patterns. Lines labelled P and C denote relationships for Puget Sound and open coastal locations, respectively. For reference, approximate present-day $pCO_2$ levels (~390 µatm) are indicated by the dashed horizontal line.

Puget Sound and the West Coast ranged more widely, from 40 to 400 µmol $kg^{-1}$, where concentrations below ~60 µmol $kg^{-1}$ reflect hypoxic conditions (Figure 4). The general 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.

## OA studies

In total, 26 experiments from 22 published OA studies included organisms (or broodstock) from the northern and central CCE (Figure 1b) and provided sufficient information on treatment water conditions to compare with the CCE $pCO_2$–temperature space defined by combining all environmental datasets (Table 1). In terms of taxonomic diversity, 50, 35, and 15% of experiments evaluated responses in echinoderms, molluscs, and teleosts, respectively. However, 35% of all experiments focused on responses in a single species, the echinoderm *Strongylocentrotus purpuratus* (Table 1). Of the experiments, 81% examined gamete, egg, or larval performance (survival, growth, calcification rates), while the remaining studies evaluated performance metrics (growth, development, or calcification rates) in juvenile or adult life history stages (Table 1). One study measured genetic diversity in echinoderm larvae after exposure to different $pCO_2$ treatments to evaluate evolutionary potential.

To facilitate visual inspection and comparison of experimental $pCO_2$ and temperature treatments with the $pCO_2$–temperature space defined by the complete set of environmental measurements in our dataset, we examined echinoderm and non-echinoderm studies separately (Figure 5). Overall, OA experiments included $pCO_2$ treatment levels that extended from 200 to 4000 µatm; 42% of experiments included a single elevated $pCO_2$ treatment in addition to a control at a given temperature, 38% considered two different elevated $pCO_2$ treatments, and 19% included three or more elevated treatments (Figure 5). In total, 72% of experiments included at least one control and one elevated $pCO_2$ treatment that occurred within the observed range of $pCO_2$ and temperature values in the CCE. Of those, 63% (10 out of 16) observed negative biological effects, 18% observed positive effects, and 18% observed no effect relative to control $pCO_2$ levels. Only two studies included $pCO_2$ treatments below approximate present-day atmospheric levels (~400 µatm; Figure 5).

Compared with the $pCO_2$–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; Figure 5). 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 conditions in Alaskan waters, the source stock was collected near Puget Sound (Table 1).

A review of each study indicated that Intergovernmental Panel on Climate Change (IPCC) estimates of future global surface ocean mean $pCO_2$ levels were used as the sole rationale for selecting OA treatments in 45% of studies, while 31% cited a combination of regional modelling studies, local field measurements, and IPCC estimates to support their choice of experimental $pCO_2$ treatment levels. Of the remaining studies, 13% provided no rationale for their choice of $pCO_2$ treatment levels, one based the high $pCO_2$ treatment level on observations of contemporary upwelling conditions, and one noted natural high carbonate chemistry variability in coastal upwelling systems which necessitated the need to test biological responses to a wide range of $pCO_2$ levels. In terms of temperature, 80% of studies did not provide a rationale for their choice of

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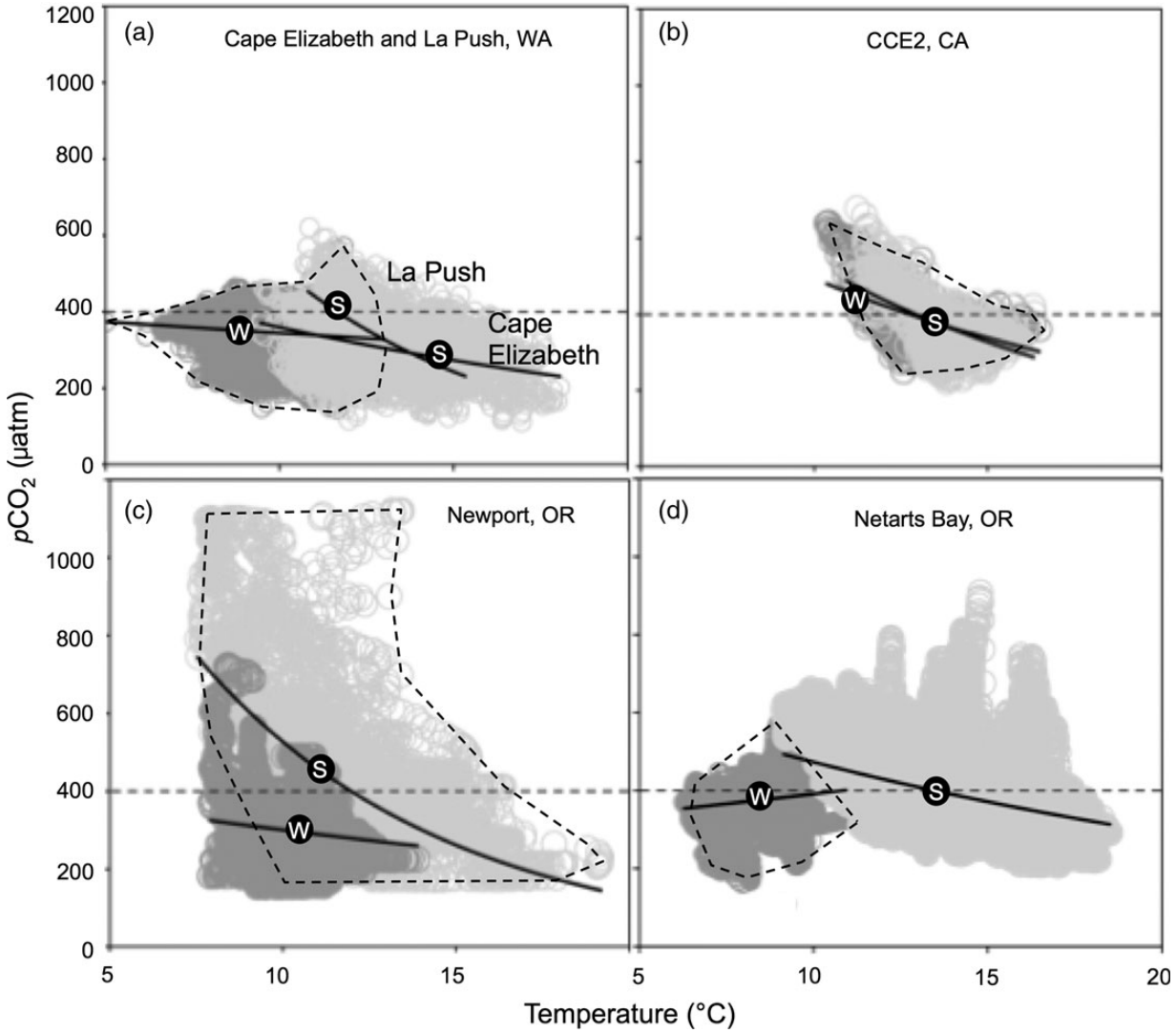


**Figure 3.** Relationship between $pCO_2$ and temperature of surface waters measured during summer (light grey open circle symbols) and winter (dark grey) at open coastal moorings in (a) Washington, (b) California, and (c) Oregon, and (d) a tidal estuary in Oregon. Regression lines are overlaid and labelled S and W to indicate summer or winter, respectively. Dashed line convex hulls demarcate data ranges where seasons overlap. For reference, approximate present-day $pCO_2$ levels (~390 µatm) are indicated by the dashed horizontal line.

experimental levels. The remaining studies cited similarity to local field conditions as a rationale for selecting temperatures. Of the two studies that performed experiments that included crossed temperature and $pCO_2$ treatments meant to correspond to conditions in the CCE, the warmer treatments were chosen in reference to IPCC global average temperature increase projections.

## Discussion

Single-species response experiments have offered important initial evidence that some species and life history stages may be adversely impacted by OA (Kroeker *et al.*, 2013; Wittmann and Pörtner, 2013), but there is a pressing need for the development of experiments that include more appropriate reference treatments that reflect $pCO_2$ levels species have acclimated or adapted to and acidified treatments that more closely reflect natural heterogeneity in carbonate chemistry (Barry *et al.*, 2010; Andersson and Mackenzie, 2012; McElhany and Busch, 2012; Waldbusser and Salisbury, 2013). Our analysis shows that an additional source of concern is natural co-variation between carbonate chemistry and other biologically relevant variables including temperature and $O_2$. These findings have direct consequences for designing experiments that aim to include control treatments that are similar to natural water conditions and for selecting elevated $pCO_2$ treatment levels that more closely correspond to OA hypotheses. Further, by placing the findings of published OA experiments into a larger environmental context, we gain information on the ecological relevance of experimental water conditions and insight into the potential sensitivity of some species and life stages to carbonate chemistry conditions that already occur in the CCE.

Although researchers increasingly recognize the importance of basing experimental $pCO_2$ levels on *in situ* carbonate chemistry observations (Yu *et al.*, 2011; Evans *et al.*, 2013; Hofmann *et al.*, 2014), the implications of natural co-variation with temperature or $O_2$ to experimental design and inference are only beginning to be explored. Given experimental evidence and theoretical expectations of interactive or synergistic effects between $pCO_2$, temperature, and $O_2$ on organisms

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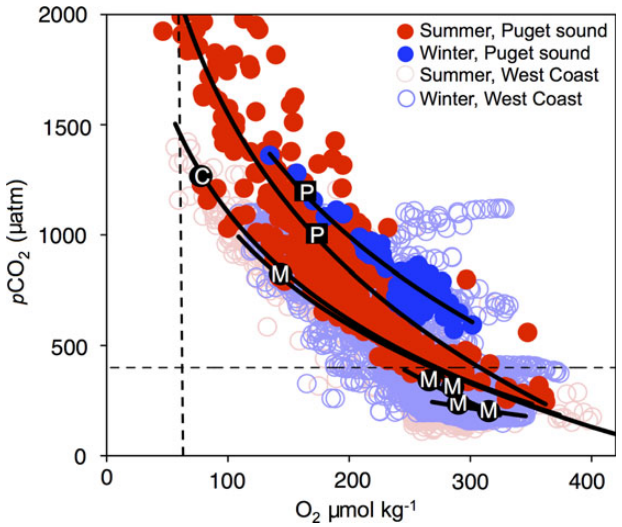


**Figure 4.** Relationships between $pCO_2$ and $O_2$ in the top 50 m of the water column during summer upwelling (May through October) and winter downwelling seasons (November through April). Regression lines are overlaid for summer and winter; lines labelled M, P, and C correspond to mooring, Puget Sound, and open coast datasets, respectively. For reference, approximate present-day $pCO_2$ levels (~390 µatm) are indicated by the dashed horizontal line, and the hypoxia threshold (60 µmol kg$^{-1}$) is indicated with a dashed vertical line.

(Pörtner, 2010, 2012; Harvey *et al.*, 2013), we suggest that failure to account for natural co-variation among these variables in habitats from the CCE may lead to results with diminished relevance for making predictions. To illustrate this point, we present an example multistressor experimental scheme typical of published OA experiments in which temperature (three levels at 8, 12, and 16°C) is crossed with two $pCO_2$ levels that correspond to approximate global surface ocean present-day (400 µatm) and future (800 µatm) conditions (Figure 6a). Under the conventional method, treatments are fully orthogonal which permits estimates 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, is widely applied in multistressor experiments, and facilitates the development of mechanistic models. However, if a goal of a study is to evaluate the potential sensitivity of organisms to future OA as is often the case, the design may be inadequate, given natural $pCO_2$–temperature co-variation within different habitats and water masses. For example, assuming an organism of interest 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, Oregon, mooring $pCO_2$ levels of 800 µatm already occur at 8°C under present-day conditions and "control" 400 µatm waters do not (Figure 6a). At temperatures above 13°C, the mean $pCO_2$ values approach air–sea equilibrium conditions. We do not doubt that simple crossed experimental designs will provide information on the interactive effects of $pCO_2$ and temperature, but we do question the efficacy of the design for testing OA hypotheses on future ecological response to OA, given naturally occurring $pCO_2$–temperature relationships and the wide range of both variables in the CCE.

In light of potential co-variation between carbonate chemistry and other important environmental variables, how should researchers select $pCO_2$ treatments that correspond to OA hypotheses? As a starting point, we recommend that OA experimental designs include multiple controls that reflect the span of $pCO_2$ levels and temperatures likely to be experienced by the organism under study (Figure 5b). To design $pCO_2$ treatments that represent future OA scenarios in productive coastal systems, we suggest that researchers focus on changes in the anthropogenic contribution to *in situ* dissolved inorganic carbon, DIC (e.g. Feely *et al.*, 2008, 2010; Barry *et al.*, 2010; Melzner *et al.*, 2012; Shaw *et al.*, 2013). At the Newport, Oregon, mooring, newly upwelled waters exhibit $pCO_2$ values that are elevated relative to air–sea equilibrium conditions due to the remineralization of organic material before surfacing (Evans *et al.*, 2011). However, after surfacing $CO_2$ concentrations can be drawn down rapidly by photosynthesis (Hales *et al.*, 2005; Hales *et al.*, 2006; Evans *et al.*, 2011), often at rates that typically far exceed $CO_2$ equilibration times across the air–sea interface (e.g. van Geen *et al.*, 2000; Fassbender *et al.*, 2011). 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, $pCO_2$ 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 (ΔDIC + *in situ* DIC), along with a second parameter from the carbonate system, could then be used to recalculate the carbonate system to estimate treatment $pCO_2$ levels.

Because the Newport, Oregon, time-series consists only of $pCO_2$, we first needed to estimate *in situ* DIC. To do so, we estimated total alkalinity (TA) from salinity measurements using a linear model parameterized with data from the CCE (Gray *et al.*, 2011). The relationship has relatively low residual error (approximately ± 20 µmol kg$^{-1}$) and was shown previously to adequately predict TA for the purposes of estimating the carbonate system when using $pCO_2$ as the second parameter (Harris *et al.*, 2013). We used the estimated TA and *in situ* $pCO_2$, salinity, and temperature measurements to solve the carbonate system and calculate *in situ* DIC. To estimate ΔDIC, we solved the carbonate system based on estimates of TA, and *in situ* temperature, and salinity, but assuming seawater equilibrium with average atmospheric $pCO_2$ levels during the period of the moored observations (~390 µatm; Harris *et al.*, 2013) and those predicted under an emissions scenario for the year 2100 (788 µatm; Intergovernmental Panel on Climate Change IS92a "continually increasing" emissions scenario). The present-day air–sea equilibrium DIC estimate was subtracted from the future equilibrium estimate to obtain ΔDIC. Although the same present day and future $pCO_2$ levels were used to calculate ΔDIC for all samples, ΔDIC values differ among samples because estimates of DIC at air–sea $pCO_2$ equilibrium vary based on the TA, temperature, and salinity of the sample. Across all water samples, ΔDIC spanned ~75–105 µmol kg$^{-1}$ depending on the TA and temperature of samples. We then obtained future $pCO_2$ estimates by solving the carbonate system using the estimated TA and *in situ* DIC + ΔDIC values (Figure 6b). Versions of the method have been described previously (e.g. Barry *et al.*, 2010) and can be used to estimate preindustrial carbonate chemistry conditions (e.g. Feely *et al.*, 2008; Harris *et al.*, 2013).

Under this approach, and assuming the same number of treatments is used as depicted in Figure 6a, the effects of temperature and $pCO_2$ can no longer be separated because orthogonality in the design is lost (Figure 6b). However, a more realistic set of control treatments are included that offer a firmer basis for drawing

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**Table 1.** Brief summary of experimental outcomes of reviewed OA studies that focus on organisms (or their broodstock) collected from the CCE.

| Study number | Reference | Collection site | Species (common name) | Life stage | Duration (days, hours) | $T$ (°C) | Control $pCO_2$ (µatm) | Treatment $pCO_2$ (µatm) | Outcome relative to control |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Gooding *et al.* (2009) | Jaricho Beach, BC | *Pisaster ochraceus* (purple sea star) | Juvenile | 70 d | 12, 15 | 380 | 780 | Increased growth rate, reduced calcified mass; feeding and growth increased with temperature |
| 2 | Reuter *et al.* (2011) | Barkley Sound, BC | *Strongylocentrotus franciscanus* (red sea urchin) | Sperm/eggs | 1 h | 10.2 | 400 | 800, 1800 | Decreased range of sperm concentrations over which high fertilization success was likely |
| 3 | Nienhuis *et al.* (2010) | Barkley Sound, BC | *Nucella lamellose* (Frilled dogwinkle) | Adults | 6 d | 9.0 | 380 | 780, 1585 | Enhanced shell dissolution |
| 4 | Sunday *et al.* (2011) | Barkley Sound, BC | *Strongylocentrotus franciscanus* (red sea urchin) | Larvae | 1 d | 12.0 | 400 | 1000 | Reduced larval size, large variation among families |
| 4 | Sunday *et al.* (2011) | Barkley Sound, BC | *Mytilus trossulus* (bay mussel) | Larvae | 1 d | 12.0 | 400 | 1000 | Reduced larval size, small variation among families |
| 5 | Crim *et al.* (2011) | Barkley Sound, BC | *Haliotis kamtschatkana* (northern abalone) | Larvae | 8 d | 12.0 | 400 | 800, 1800 | Reduced survival, increased abnormalities in shell structure, reduced size in normal-shelled larvae |
| 6 | Chan *et al.* (2011) | Orcas Island, WA | *Dendraster excentricus* (Pacific sand dollar) | Larvae | 10 d | 20.0 | 380 | 1000 | Reduced body size and stomachs; no effect on swimming speed |
| 7 | Timmins-Schiffman *et al.* (2012) | San Juan Island, WA | *Crassostrea gigas* (Pacific oyster) | Larvae | 3 d | 20.0 | 468 | 847, 1065 | Increased calcification at day 1, but smaller average size on day 3 |
| 8 | O'Donnell *et al.* (2013) | San Juan Island, WA | *Mytilus trossulus* (bay mussel) | Adults | 20 d | 10.4 | 432 | 299, 575, 736, 980, 1057, 1180, 1322, 1498 | Weaker, less flexible byssal threads at treatments above 1200 µatm |
| 9 | Hurst *et al.* (2012) | Port Townsend, WA | *Theragra chalcogramma* (walleye pollock) | Yearlings | 42 d | 8.8 | 414 | 478, 815, 1805 | No effect on growth but increase in otolith deposition rate, biological implication is unclear |
| 9 | Hurst *et al.* (2012) | Port Townsend, WA | *Theragra chalcogramma* (walleye pollock) | Sub-yearlings warm | 196 d | 8.3 | 596 | 828, 1285, 2894 | Increase growth at $pCO_2$ higher than 900 µatm; no change in condition factor |
| 9 | Hurst *et al.* (2012) | Port Townsend, WA | *Theragra chalcogramma* (walleye pollock) | Sub-yearlings cool | 196 d | 2.4 | 386 | 225, 643, 1543 | No difference in growth or condition factor across treatments |
| 10 | Hurst *et al.* (2013) | Port Townsend, WA | *Theragra chalcogramma* (walleye pollock) | Eggs/larvae | 35 d | 8.1 | 442 | 296, 871, 1844 | No difference in egg hatch rate, size, or survival, but longer time till hatching; authors think elevated $pCO_2$ treatments have minor effect |
| 11 | Evans *et al.* (2013) | Fogarty Creek, OR | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 96 h | 12.8 | 435 | 813, 1255 | Transcriptome up-regulation of genes related to calcification at intermediate $pCO_2$, no change at high level |
| 12 | Pespeni *et al.* (2013) | OR to CA., various locations | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 17 d | 14.1 | 428 | 897 | Reduction in larval body length, change in allele frequency, no change in timing of settlement or competence to metamorphose |
| 13 | LaVigne *et al.* (2012) | OR to CA, various locations | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 50 d | 14.1 | 490 | 1001 | No change Sr/Mg composition in spines; though a difference was observed for a Santa Barbara population of urchins |

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|---|---|---|---|---|---|---|---|---|---|
| 14 | Kelly *et al.* (2013) | Northern and southern CA, various locations | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 5 d | 13.0 | 424 | 1210 | Reduced size in larvae from dams and sires taken from northern and southern locations; no difference in metabolism |
| 15 | Place and Smith (2012) | Bodega Bay, CA | *Strongylocentrotus purpuratus* (purple sea urchin) | Embryos | 7 d | 15.1 | 378 | 1486, 4110 | No disruption to cell cycle in fertilized eggs |
| 16 | Hettinger *et al.* (2012) | Tomales Bay, CA | *Ostreola lurida* (Olympia oyster) | Larvae/ juveniles | 45 d | 20.0 | 739 | 933, 1355 | Reduced larval and juvenile growth |
| 17 | Hettinger *et al.* (2013a) | Tomales Bay, CA | *Ostreola lurida* (Olympia oyster) | Larvae | 22 d | 19.4 | 520 | 1075 | Reduced number of settlers; no difference in shell size or larval dry weight |
| 18 | Hettinger *et al.* (2013b) | Tomales Bay, CA | *Ostreola lurida* (Olympia oyster) | Larvae/ juveniles | 127 d | 20.0 | 485 | 1060 | Reduced larval survival and growth; reduced growth in juveniles outplanted to an estuary |
| 19 | Gaylord *et al.* (2011) | Tomales Bay, CA | *Mytilus californianus* (California mussel) | Larvae | 8 d | 15.4 | 380 | 540, 970 | Thinner, weaker shells |
| 20 | Padilla-Gamiño *et al.* (2013) | Santa Barbara, CA | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 75 h | 13, 18 | 400 | 1100 | Reduced body size and respiration rate; difference in transcriptome observed |
| 21 | Matson *et al.* (2012) | Santa Barbara, CA | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 6 d | 15.6 | 365 | 1038, 1444 | Reduced arm length; no difference in utilization of energy lipid reserves and protein content remained unchanged |
| 22 | Yu *et al.* (2011) | Santa Barbara, CA | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 6 d | 15.6 | 372 | 1057, 1469 | Slight reduction in size |
| 22 | Yu *et al.* (2011) | Santa Barbara, CA | *Strongylocentrotus purpuratus* (purple sea urchin) | Larvae | 6 d | 15.6 | 432 | 1441 | Slight reduction in size |



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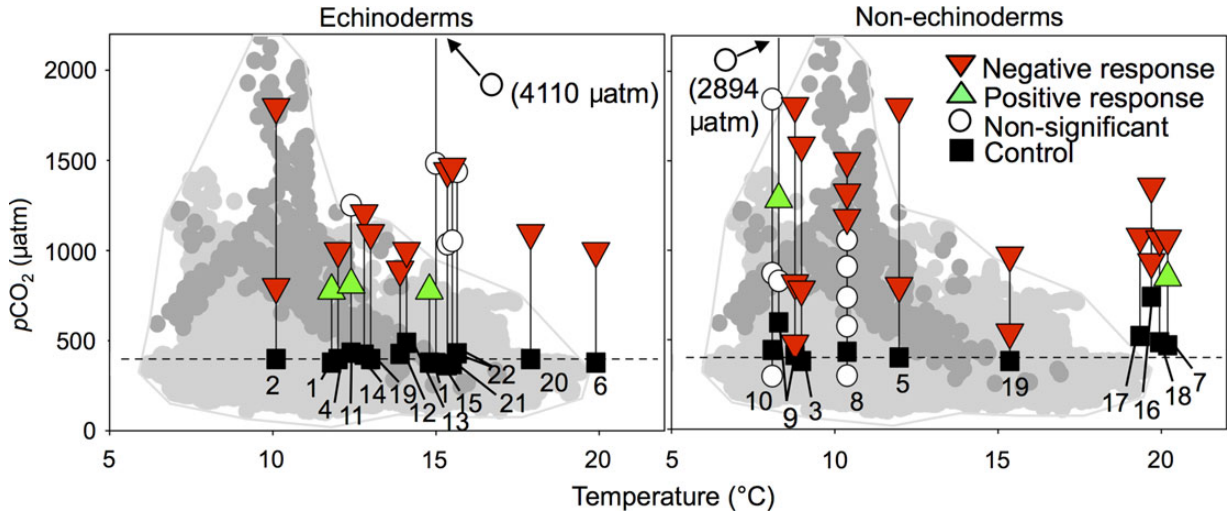


**Figure 5.** Environmental $pCO_2$ and temperature measurements (top 50 m) from the northern and central CCE and conditions maintained in OA experiments performed on organisms from the region. Dark grey circles correspond to environmental measurements from Puget Sound, Washington; light grey circles correspond to environmental measurements from all other regions. $pCO_2$ treatment levels included in an individual experiment at a given temperature are connected by solid black lines. Numbers denote study code (see Table 1). A convex hull (solid grey line) demarcating the extent of all environmental $pCO_2$ and temperature measurements is depicted to aid visual comparisons. For reference, approximate present-day atmospheric $pCO_2$ levels (~390 µatm) are indicated by the dashed horizontal line.

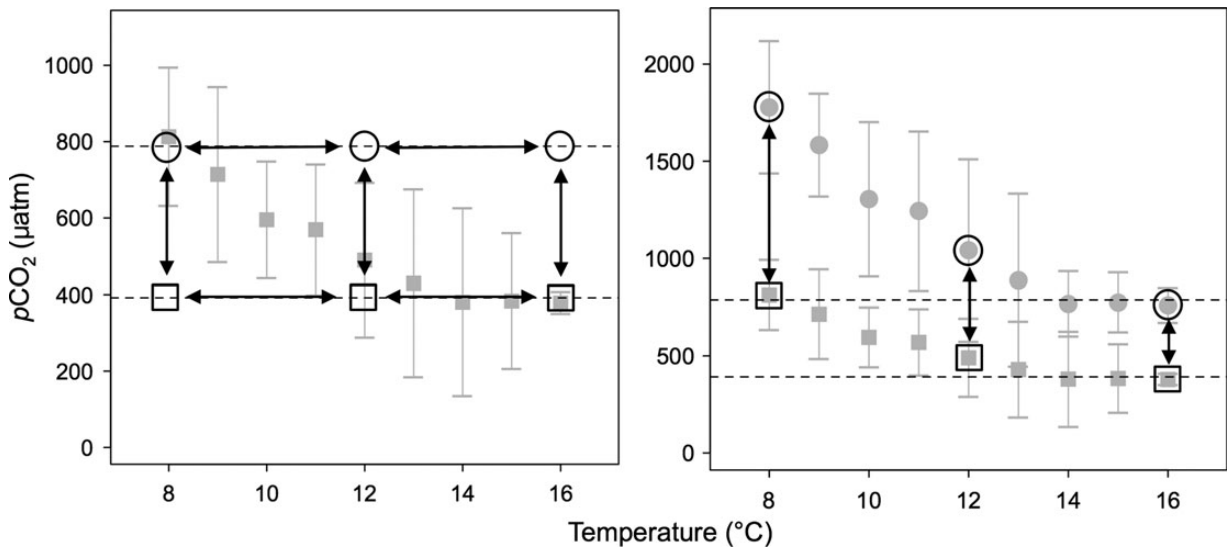


**Figure 6.** Schematic of potential experimental approaches to evaluate OA effects, given co-variation between $pCO_2$ and temperature. To illustrate the benefits and drawback of each approach, *in situ* $pCO_2$ and temperature measurements from the N10 mooring near Newport, Oregon, during summer upwelling season (2008) are depicted (grey, filled squares; bars indicate standard deviation). (a) A conventional temperature (three levels: 8, 12, and 16°C) by $pCO_2$ experimental design in which control $pCO_2$ values are based on approximate present-day global average surface ocean $pCO_2$ levels and the acidified treatments corresponding to IPCC emissions scenario IS92a projections for 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* $pCO_2$ and temperature measurements. Under this design, three controls are included to account for natural co-variation in temperature and $pCO_2$. Treatment levels that more closely correspond to an OA hypothesis were obtained by specifying an increase in DIC attributed to anthropogenic $CO_2$ emissions (see Discussion for details). The future DIC estimate and estimates of TA were used to recalculate the carbonate system to obtain target $pCO_2$ treatment levels. We calculated $pCO_2$ using the R library "seacarb" (Lavigne and Gattuso, 2010) with dissociation constants from Lueker *et al.* (2000).

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 $pCO_2$–temperature controls.

The method we use to estimate future $pCO_2$ requires several important assumptions. First, the approach implicitly assumes that TA, salinity, and temperature will remain unchanged and that future difference in DIC between the observed *in situ* values and

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those calculated assuming present-day air–sea equilibrium will remain the same. Further, to estimate ΔDIC, we assumed that all water properties measured at the time of sampling are the same as when the water mass last approached air–sea equilibrium. If waters were cooler at that time, this would result in a slight overestimate of ΔDIC of $\sim$1.6 μmol $kg^{-1}$ for each degree Celsius. Following earlier studies, we also assumed that waters upwelled at the mooring location possessed an anthropogenic $CO_2$ burden that approximated present-day atmospheric $pCO_2$ conditions (Harris *et al.*, 2013). In other locations within the CCE, such assumptions may not be justified because subsurface waters upwelled on to the shelf may last have had contact with the surface decades prior and therefore would contain less anthropogenic $CO_2$ (Feely *et al.*, 2008; Harris *et al.*, 2013).

The OA $pCO_2$–temperature relationship depicted in Figure 6b corresponds to one simple hypothesis for how present-day carbonate chemistry conditions might change with a simple augmentation of $CO_2$. However, marine organisms will be subjected to multiple stressors in the future, including warmer temperatures, lower ambient $O_2$ concentrations, cultural eutrophication, and pollution (Boyd, 2011; Gruber, 2011; Doney *et al.*, 2012). Treatments corresponding to an OA + warming hypothesis could be created by adding to *in situ* measurements of both temperature and DIC and recalculating the carbonate system to obtain appropriate estimates of $pCO_2$ (Melzner *et al.*, 2012). If a hypoxia + OA treatment is sought, any assumed reduction in $O_2$ owing to aerobic respiration necessarily corresponds to an increase in $CO_2$ (Melzner *et al.*, 2012; Sunda and Cai, 2012). The corresponding increase in DIC beyond that attributed to OA could be estimated based on the molar ratio of $O_2$ consumed to $CO_2$ released in the respiratory consumption of organic matter (Sunda and Cai, 2012). We caution, however, that the appropriateness of these simple methods for estimating treatment levels should be thoughtfully considered in the light of the physical and biological attributes of the system under study. Such simplifications do not take into account the indirect and cascading impacts that changes in individual properties such as temperature will have on ecosystem metabolism and thus the distribution and concentration of $O_2$ and $CO_2$ (e.g. Keeling *et al.*, 2010; Gruber, 2011; Doney *et al.*, 2012), nor would they reflect potential changes in large-scale circulation patterns or productivity regimes which might fundamentally alter relationships between $pCO_2$ and other variables (e.g. Rykaczewski and Dunne, 2010). Despite these uncertainties, developing experimental designs that include controls that reflect present-day $pCO_2$–temperature–$O_2$ relationships in upwelling systems should become the cornerstone of experiments that aim to quantify the potential response of organism to future predicted changes in their environment. The development of experimental systems that permit simultaneous control over $pCO_2$, temperature, and $O_2$ conditions remains a technical challenge, but a growing number of OA research facilities are acquiring the capacity to do so (Bockmon *et al.*, 2013).

Our compilation of environmental carbonate chemistry data is meant to offer an initial overview of the ranges of $pCO_2$, temperature, and $O_2$ in the CCE and the extent to which their relationships vary between seasons and regions. We examined patterns of co-variation between $pCO_2$ and temperature at coarse seasonal time-scales, but note that co-variation patterns may differ depending on temporal scale. On diel time-scales, solar heating and photosynthesis and respiration may drive strong cyclical patterns in temperature, $pCO_2$, and $O_2$ (Barton *et al.*, 2012; Frieder *et al.*, 2012; Waldbusser and Salisbury, 2013), while abrupt events such as storms or the advection of a different water mass into a region can result in rapid change in water characteristics that may persist for several days (Frieder *et al.*, 2012). Over interannual time-scales, large-scale climate phenomena like *El Niño*/Southern Oscillation can influence upwelling patterns, coastal productivity, carbonate chemistry (Chavez *et al.*, 1999; Friederich *et al.*, 2002), and thus potentially relationships among $pCO_2$ and temperature and $O_2$. To date, studies evaluating patterns of co-variation between $pCO_2$ and other biologically relevant variables over a range of temporal scales are sparse, but the topic is an area of research we are currently exploring. Incorporating both temporal dynamics and multiple stressors in experimental systems is technically challenging, but may be necessary for ecologically relevant predictions.

## Conclusions

OA is expected to have far-reaching impacts on the structure and function of marine ecosystems by altering biogeochemical processes and the productivity and distribution of species (Fabry *et al.*, 2008; Doney *et al.*, 2009, 2012; Mora *et al.*, 2013; Waldbusser and Salisbury, 2013). Our ability to predict the response of complex ecological systems to OA, however, remains limited and is highly constrained by major uncertainties in the response of species to both direct (e.g. physiology, neurological impairment) and indirect (e.g. trophic interactions) processes that may be vulnerable to OA (Fabry *et al.*, 2008; Hofmann *et al.*, 2010). While authors have noted the need for OA researchers to use $pCO_2$ levels that correspond to ambient conditions a study species or life history stage is likely to experience (Andersson and Mackenzie, 2012; McElhany and Busch, 2012), patterns of co-variation with temperature and $O_2$ have yet to be incorporated into OA experimental designs (Reum *et al.*, 2014). As demonstrated here, this issue should be of concern to researchers in upwelling systems and other coastal environments where water conditions are highly dynamic over a range of spatial and temporal scales and where co-variation between $pCO_2$, temperature, and $O_2$ are generally expected. Because inferences on the potential response of organisms to future conditions are necessarily premised on the notion that experimental controls reflect present-day conditions, we strongly recommend researchers consider how $pCO_2$ naturally varies with other biologically important variables in their experimental designs. Importantly, the studies we reviewed for the CCE indicate that several species may be sensitive to carbonate chemistry conditions that already occur, and suggest that present-day variability in carbonate chemistry may be more important to contemporary ecological patterns than previously thought. 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 OA experimental design in other marine systems.

## Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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