Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry covariation with temperature and oxygen

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Abstract

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 covariation with other biologically important variables such as temperature and oxygen are just beginning to be explored in the context of OA experimental design. If covariation 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 data set 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 covariation 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 of covariation 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 covariation with other biologically relevant variables.

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-favorable winds promotes high rates of primary production which in turn supports productive food webs 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 is high because the source waters transported to upwelling centers 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 programs to inform treatment design in several recent OA studies (Hofmann *et al.* 2014). Less

93 appreciated from an experimental perspective, however, is the possibility that carbonate
94 chemistry conditions may also naturally co-vary with other biologically relevant variables
95 including temperature and O_2 over multiple spatial and temporal scales (Reum *et al.*, 2014). This
96 may have important implications for the design and interpretation of ocean acidification
97 experiments because of the potential for non-additive interactions between carbonate chemistry,
98 temperature, and O_2 on organismal performance and ecological interactions (Wernberg *et al.*,
99 2012, Harvey *et al*., 2013; Koch *et al*., 2013; Kroeker *et al*., 2013). For many organisms, aerobic
100 capacity and metabolic scope (the amount of energy that can be allocated to activities beyond
101 those required for basic existence) may be influenced strongly by temperature, and adversely
102 impacted by reductions in ambient oxygen availability or increases in CO_2 concentrations
103 (Pörtner, 2010, 2012).
104 Given the combined effects that temperature and environmental O_2 and pCO_2 have on
105 organismal physiology and experimental evidence indicating non-additive interactions on
106 response variables related to fitness and ecosystem function (Pörtner and Farrell, 2008; Pörtner,
107 2010; Harvey *et al.*, 2013; Kroeker *et al.*, 2013), knowledge of their co-variability is essential for
108 designing OA experiments that adequately characterize biological performance under
109 contemporary relative to future acidified conditions. Yet for workers focused on laboratory OA
110 experiments, these relationships are rarely incorporated into experimental designs. This poses
111 important potential drawbacks. Foremost, if carbonate chemistry strongly covaries with
112 temperature or O_2, researchers risk running experiments with control-water characteristics that
113 are atypical of the habitat to which a focal organism / life stage may have acclimated or adapted
114 to. Recently, pCO_2 was shown to range widely (~200 to 2500 μatm) and covary with temperature
115 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 data set 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 food webs. Along the coast, equatorward winds during the 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 data set presented here consists of pCO_2 measurements from multiple moorings and ship-based sampling campaigns that collectively span 14 degrees 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 data sets 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 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.

Materials and methods

Carbonate chemistry data

Our analysis focused on coastal waters within the domain of the northern and central California Current Ecosystem (CCE; Fig. 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 numerous 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). The data set 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 (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 Program 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; Table S1). Further details of the survey, sampling dates, sampling method, and habitats from which data were obtained are provided in Table S1. Although the data set 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.

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 ocean acidification 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 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 (Pörtner, 2008; 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 data sets 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

183 performed from the northern and central CCE typically occur in waters within this depth range,
184 although individuals of some populations may occur for periods below this depth.
185 We evaluated separate relationships between pCO_2 and temperature and pCO_2 and O_2
186 within the summer upwelling and winter downwelling time periods. Covariation patterns for
187 pCO_2 and temperature were examined for data pooled from shipboard underway and discrete
188 sample measurements collected along the West Coast continental shelf. In addition, we also
189 examined covariation patterns for Puget Sound and the Strait of Juan de Fuca discrete sample
190 measurements (hereafter referred to as 'Puget Sound'). A subset of these data were examined in
191 an earlier study (Reum *et al.*, 2014), but we include them here for completeness and to facilitate
192 comparisons among habitats represented within the CCE. We chose to present covariation
193 patterns for Puget Sound separately from the West Coast because Puget Sound exhibits slow
194 exchange with open coastal waters and high rates of primary productivity and respiration (Feely
195 *et al.*, 2010). The resulting pCO_2-temperature relationships therefore likely differ considerably
196 from patterns observed in shelf waters. For the time-series data, we estimated pCO_2-temperature
197 relationships for upwelling and downwelling seasons, but examined covariation patterns
198 separately for each mooring to evaluate site-level differences.
199 To evaluate pCO_2 and O_2 relationships and facilitate comparisons across survey types and
200 seasons, we converted all O_2 concentration measurements to μmol kg^{-1}. As with the pCO_2 and
201 temperature data, we examined covariation within data pooled from shipboard West Coast
202 measurements, Puget Sound, and each mooring and by season. To improve assumptions of
203 normality in the residual error structure for the pCO_2-temperature and pCO_2-O_2 relationships, we
204 $\log_{10}$-transformed pCO_2 values prior to estimating linear relationships using least squares
205 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 covariation 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 ocean acidification experiments that included organisms obtained directly from habitats within the northern and central CCE or that originated from brood stock 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 ocean acidification (Dupont *et al.*, 2010; Wernberg *et al.*, 2012; Harvey *et al*., 2013; Kroeker *et al*., 2013; Wittmann and Portner, 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 brood stock) 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 spatiotemporal 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 to 19°C and 100 to 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 the summer upwelling months (Fig. 2a). In Puget Sound, a similar but steeper relationship was apparent relative to the open coastal waters, reflecting CO_2-enriched waters (Fig. 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 (Fig. 2a).

Although fewer measurements were available during the winter in general (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 (Fig. 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 (Fig. 2b).

Time-series data collected from moored platforms in open coastal waters from four locations also indicated covariation between pCO_2 and temperature (Fig. 3a-c). During the summer upwelling months, pCO_2 again generally declined with increasing temperature (Fig. 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 (Fig. 3a,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 to 1100 μatm (Fig. 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 the summer range in pCO_2 values was also considerable, spanning 300 to 800 μatm and reflected high rates of primary production and respiration (Fig. 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 the summer but variable across stations (Fig. 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 (Fig. 3c,d).

Measurements of O_2 were available for a subset of pCO_2 records included in the complete environmental pCO_2 and temperature dataset (Table S1). For all survey types, mooring time series, and seasons, pCO_2 levels decreased with increasing O_2 concentration (Fig. 4). For most open coastal surface 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} (Fig. 4). Summer O_2 measurements from discrete samples from 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 (Fig. 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 brood stock) from the northern and central CCE (Fig. 1b) and provided sufficient information on treatment water conditions to compare with the CCE pCO_2-temperature space defined by combining all environmental data sets (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 data set, we examined echinoderm and non-echinoderm studies separately (Fig. 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 (Fig. 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; Fig. 5).

In comparison to the pCO_2-temperature space defined by the complete set of environmental measurements in our data set, five experiments were performed at temperatures that matched or exceeded the warmest observed values (~19°C; Fig. 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 modeling 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 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 Portner, 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 covariation 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 covariation 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 (Pörtner, 2010, 2012; Harvey *et al.*, 2013), we suggest that failure to account for natural covariation among these variables in habitats from the CCE may lead to results with diminished relevance for making future 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 (Fig. 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 covariation within different habitats and water masses. For example, assuming an organism of interest occurs in shelf waters off Oregon during the 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 (Fig. 6a). At temperatures above 13°C, 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

387 OA given naturally occurring pCO_2-temperature relationships and the wide range of both
388 variables in the CCE.

389 In light of potential covariation between carbonate chemistry and other important
390 environmental variables, how should researchers select pCO_2 treatments that correspond to OA
391 hypotheses? As a starting point we recommend that OA experimental designs include multiple
392 controls that reflect the span of pCO_2 levels and temperatures likely to be experienced by the
393 organism under study (Fig. 5b). To design pCO_2 treatments that represent future OA scenarios in
394 productive coastal systems, we suggest that researchers focus on changes in the anthropogenic
395 contribution to *in situ* dissolved inorganic carbon, DIC (e.g., Barry *et al*., 2010; Melzner *et al.*,
396 2012; Feely et al. 2008; Feely et al. 2010; Shaw *et al.*, 2013). At the Newport, Oregon mooring,
397 newly upwelled waters exhibit pCO_2 values that are elevated relative to air-sea equilibrium
398 conditions due to the remineralization of organic material prior to surfacing (Evans et al. 2011).
399 However, after surfacing CO_2 concentrations in can be drawn down rapidly by photosynthesis
400 (Hales et al. 2005, Evans et al. 2011), often at rates that typically far exceed CO_2 equilibration
401 times across the air-sea interface (e.g., van Green et al., 2002, Fassbender et al., 2011).
402 Consequently, the anthropogenic CO_2 burden of upwelled waters is primarily acquired when
403 they were last in contact with the atmosphere and prior to DIC changes due to biological
404 processes post-surfacing. In our example, pCO_2 treatments reflecting future OA hypotheses
405 could be obtained by increasing *in situ* DIC concentrations by an increment (ΔDIC) expected
406 under a given CO_2 emissions scenario. The future DIC estimate (ΔDIC + *in situ* DIC), along with
407 a second parameter from the carbonate system, could then be used to recalculate the carbonate
408 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 to 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 (Fig. 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 Fig. 6a, the effects of temperature and pCO_2 can no longer be separated because orthogonality in

the design is lost (Fig. 6b). 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 presently occur at the three different pCO_2-temperature controls.

The method we use to estimate future pCO_2 requires several important assumptions (Harris et al. 2013, Melzner et al. 2012). 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 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 over estimate of ΔDIC of ~1.6 μmol kg^{-1} for each degree C. 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 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 Fig. 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

455 temperature and DIC and recalculating the carbonate system to obtain appropriate estimates of
456 pCO_2 (Melzner *et al.*, 2012). If a hypoxia + OA treatment is sought, any assumed reduction in O_2
457 owing to aerobic respiration necessarily corresponds to an increase in CO_2 (Melzner *et al.*, 2012;
458 Sunda and Cai, 2012). The corresponding increase in DIC beyond that attributed to OA could be
459 estimated based on the molar ratio of O_2 consumed to CO_2 released in the respiratory
460 consumption of organic matter (Sunda and Cai, 2012). We caution, however, that the
461 appropriateness of these simple methods for estimating treatment levels should be thoughtfully
462 considered in light of the physical and biological attributes of the system under study. Such
463 simplifications do not take into account the indirect and cascading impacts that changes in
464 individual properties such as temperature will have on ecosystem metabolism and thus the
465 distribution and concentration of O_2 and CO_2 (e.g., Keeling *et al.*, 2010; Gruber, 2011; Doney *et*
466 *al.*, 2012), nor would they reflect potential changes in large-scale circulation patterns or
467 productivity regimes which might fundamentally alter relationships between pCO_2 and other
468 variables (e.g., Rykaczewski and Dunne, 2010). Despite these uncertainties, developing
469 experimental designs that include controls that reflect present-day pCO_2-temperature-O_2
470 relationships in upwelling systems should become the cornerstone of experiments that aim to
471 quantify the potential response of organism to future predicted changes in their environment. The
472 development of experimental systems that permit simultaneous control over pCO_2, temperature,
473 and O_2 conditions remains a technical challenge, but a growing number of OA research facilities
474 are acquiring the capacity to do so (Bockmon *et al.*, 2013).

475 Our compilation of environmental carbonate chemistry data is meant to offer an initial
476 overview of the ranges of pCO_2, temperature, and O_2 in the CCE and the extent to which their
477 relationships vary between seasons and regions. We examined patterns of covariation between

478 pCO_2 and temperature at coarse seasonal time scales, but note that covariation patterns may
479 differ depending on temporal scale. On diel time scales, solar heating and photosynthesis and
480 respiration may drive strong cyclical patterns in temperature, pCO_2 and O_2 (Barton *et al.*, 2012;
481 Frieder *et al.*, 2012; Waldbusser and Salisbury, 2013), while abrupt events such as storms or the
482 advection of a different water mass into a region can result in rapid chang in water characteristics
483 that may persist for several days (Frieder *et al*., 2012). Over interannual time scales, large-scale
484 climate phenomena like El Niño/Southern Oscillation can influence upwelling patterns, coastal
485 productivity, carbonate chemistry (Chavez *et al.*, 1999; Friederich *et al.*, 2002), and thus
486 potentially relationships among pCO_2 and temperature and O_2. To date, studies evaluating
487 patterns of covariation between pCO_2 and other biologically relevant variables over a range of
488 temporal scales are sparse, but the topic is an area of research we are currently exploring.
489 Incorporating both temporal dynamics and multiple stressors in experimental systems is
490 technically challenging, but may be necessary for ecologically relevant predictions.
491

492 ## Conclusions

493 Ocean acidification is expected to have far-reaching impacts on the structure and function of
494 marine ecosystems by altering biogeochemical processes and the productivity and distribution of
495 species (Fabry *et al.*, 2008; Doney *et al.*, 2009; Doney *et al.*, 2012; Mora *et al.*, 2013;
496 Waldbusser and Salisbury, 2013). Our ability to predict the response of complex ecological
497 systems to OA, however, remains limited and is highly constrained by major uncertainties in the
498 response of species to both direct (e.g., physiology, neurological impairment) and indirect (e.g.,
499 trophic interactions) processes that may be vulnerable to OA (Fabry *et al.*, 2008; Hofmann *et al.*,
500 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 (McElhany and Busch, 2012; Andersson and Mackenzie, 2012), patterns of covariation 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 covariation 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 and 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.

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References

546 Alin, S. R., Feely, R. A., Dickson, A. G., Hernández-Ayón, J. M., Juranek, L. W., Ohman, M.
547 D., and Goericke, R. 2012. Robust empirical relationships for estimating the carbonate
548 system in the southern California Current System and application to CalCOFI
549 hydrographic cruise data (2005–2011). Journal of Geophysical Research, 117: C05033.
550 Andersson, A. J., and Mackenzie, F. T. 2012. Revisiting four scientific debates in ocean
551 acidification research. Biogeosciences, 9: 893-905.
552 Barry, J. P., Tyrrell, t., Hansson, L., Plattner, G., and Gattuso, J. 2010. Atmospheric CO_2 targets
553 for ocean acidification perturbation experiments. *In* Guide to best practices for ocean
554 acidification research and data reporting, pp. 53-66. Ed. by U. Riebesell, V. J. Fabry, L.
555 Hansson, and J.-P. Gattuso. Publications Office of the European Union, Luxembourg.
556 Bakker, D. C. E., *et al*., 2013. Earth System Science Data, 6, 1–22, 6, doi:10.5194/essd-6-69-
557 2014, 2014.
558 Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., and Feely, R. A. 2012. The Pacific
559 oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon
560 dioxide levels: Implications for near-term ocean acidification effects. Limnology and
561 Oceanography, 57: 698-710.
562 Bockmon, E., Frieder, C., Navarro, M., White-Kershek, L., and Dickson, A. 2013. Controlled
563 experimental aquarium system for multi-stressor investigation: carbonate chemistry,
564 oxygen saturation, and temperature. Biogeosciences, 10: 5967-5975.
565 Borges, A. V., and Abril, G. 2011. Carbon dioxide and methane dynamics in estuaries. *In*
566 Treatise on Estuarine and Coastal Science. Ed. by D. McClusky, and E. Wolanski. Vol 5,
567 pp. 119–161. Waltham: Academic Press.
568 Boyd, P. W. 2011. Beyond ocean acidification. Nature Geoscience, 4: 273-274.

569 Chan, K.Y.K., Grünbaum, D., O'Donnell, M.J. 2011. Effects of ocean-acidification-induced
570 morphological changes on larval swimming and feeding. The Journal of Experimental
571 Biology, 214: 3857-3867.
572 Chavez, F. P., Strutton, P. G., Friederich, C. E., Feely, R. A., Feldman, G. C., Foley, D. C., and
573 McPhaden, M. J. 1999. Biological and chemical response of the equatorial Pacific Ocean
574 to the 1997-98 El Niño. Science, 286: 2126-2131.
575 Checkley, D. M., and Barth, J. A. 2009. Patterns and processes in the California Current System.
576 Progress in Oceanography, 83: 49-64.
577 Crim, R.N., Sunday, J.M., and Harley, C.D. 2011 Elevated seawater CO_2 concentrations impair
578 larval development and reduce larval survival in endangered northern abalone (*Haliotis*
579 *kamtschatkana*). Journal of Experimental Marine Biology and Ecology, 400: 272-277.
580
581 Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean acidification: The other
582 CO_2 problem. Annual Review of Marine Science, 1: 169-192.
583 Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., Galindo,
584 H. M., *et al.* 2012. Climate change impacts on marine ecosystems. Annual Review of
585 Marine Science, 4: 11-37.
586 Dupont, S., Ortega-Martínez, O., and Thorndyke, M. 2010. Impact of near-future ocean
587 acidification on echinoderms. Ecotoxicology, 19: 449-462.
588 Evans, T. G., Chan, F., Menge, B. A., and Hofmann, G. E. 2013. Transcriptomic responses to
589 ocean acidification in larval sea urchins from a naturally variable pH environment.
590 Molecular ecology, 22: 1609-1625.

591 Evans, W., Hales, B., and Strutton, P. G. 2011. Seasonal cycle of surface ocean pCO_2 on the
592 Oregon shelf. Journal of Geophysical Research, 116: C05012.
593 Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on
594 marine fauna and ecosystem processes. ICES Journal of Marine Science, 65: 414-432.
595 Fassbender, A. J., Sabine, C. L., Feely, R. A., Langdon, C., and Mordy, C. W. 2011. Inorganic
596 carbon dynamics during northern California coastal upwelling. Continental Shelf
597 Research, 31: 1180-1192.
598 Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., *et al.*
599 2010. The combined effects of ocean acidification, mixing, and respiration on pH and
600 carbonate saturation in an urbanized estuary. Estuarine Coastal and Shelf Science, 88:
601 442-449.
602 Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B. 2008. Evidence for
603 upwelling of corrosive "acidified" water onto the continental shelf. Science, 320: 1490-
604 1492.
605 Fréon, P., Barange, M., and Arístegui, J. 2009. Eastern boundary upwelling ecosystems:
606 integrative and comparative approaches. Progress in Oceanography, 83: 1-14.
607 Frieder, C. A., Nam, S. H., Martz, T. R., and Levin, L. A. 2012. High temporal and spatial
608 variability of dissolved oxygen and pH in a nearshore California kelp forest.
609 Biogeosciences, 9: 3917-3930.
610 Friederich, G., Walz, P., Burczynski, M., and Chavez, F. 2002. Inorganic carbon in the central
611 California upwelling system during the 1997-1999 El Niño-La Niña event. Progress in
612 Oceanography, 54: 185-203.

613 Gaylord, B., Hill, T.M., Sanford, E. *et al.*, 2011. Functional impacts of ocean acidification in an
614 ecologically critical foundation species. The Journal of Experimental Biology, 214: 2586-
615 2594.
616 Gooding, R.A., Harley, C.D., and Tang, E. 2009. Elevated water temperature and carbon dioxide
617 concentration increase the growth of a keystone echinoderm. Proceedings of the National
618 Academy of Sciences, 106: 9316-9321.
619 Gray, S. E., DeGrandpre, M. D., Moore, T. S., Martz, T. R., Friederich, G. E., and Johnson, K. S.
620 2011. Applications of in situ pH measurements for inorganic carbon calculations. Marine
621 chemistry, 125: 82-90.
622 Gruber, N. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global
623 change. Philosophical Transactions of the Royal Society A: Mathematical, Physical and
624 Engineering Sciences, 369: 1980-1996.
625 Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., and Plattner, G.-K. 2012. Rapid
626 progression of ocean acidification in the California Current System. Science, 337: 220-
627 223.
628 Hales, B., Karp‐Boss, L., Perlin, A., and Wheeler, P. A. 2006. Oxygen production and carbon
629 sequestration in an upwelling coastal margin. Global Biogeochemical Cycles, 20:
630 GB002517.
631 Hales, B., Takahashi, T., and Bandstra, L. 2005. Atmospheric CO_2 uptake by a coastal upwelling
632 system. Global Biogeochemical Cycles, 19: GB1009.
633 Harris, K. E., DeGrandpre, M. D., and Hales, B. 2013. Aragonite saturation state dynamics in a
634 coastal upwelling zone. Geophysical Research Letters, 40: 2720-2725.

635 Harvey, B. P., Gwynn-Jones, D., and Moore, P. J. 2013. Meta-analysis reveals complex marine
636 biological responses to the interactive effects of ocean acidification and warming.
637 Ecology and evolution, 3: 1016-1030.
638 Hauri, C., Gruber, N., Plattner, G.-K., Alin, S., Feely, R. A., Hales, B., and Wheeler, P. A. 2009.
639 Ocean acidification in the California current system. Oceanography, 22: 60-77.
640 Hauri, C., Gruber, N., Vogt, M., Doney, S. C., Feely, R. A., Lachkar, Z., Leinweber, A., *et al.*
641 2013. Spatiotemporal variability and long-term trends of ocean acidification in the
642 California Current System. Biogeosciences, 10: 193-216.
643 Hettinger, A., Sanford, E., Hill, T.M., Hosfelt, J.D., Russell, A.D., and Gaylord, B. 2013a. The
644 influence of food supply on the response of Olympia oyster larvae to ocean acidification.
645 Biogeosciences, 10: 6629-6638.
646 Hettinger, A., Sanford, E., Hill, T.M., Lenz, E.A., Russell, A.D., and Gaylord, B. 2013b. Larval
647 carry-over effects from ocean acidification persist in the natural environment. Global
648 Change Biology, 19: 3317-3326.
649 Hettinger, A., Sanford, E., and Hill, T.M., *et al.,* (2012) Persistent carry-over effects of
650 planktonic exposure to ocean acidification in the Olympia oyster. Ecology, 93: 2758-
651 2768.
652 Hickey, B. M., and Banas, N. S. 2003. Oceanography of the US Pacific Northwest Coastal
653 Ocean and estuaries with application to coastal ecology. Estuaries, 26: 1010-1031.
654 Himmelman, J. H. 1975. Phytoplankton as a stimulus for spawning in three marine invertebrates.
655 Journal of Experimental Marine Biology and Ecology, 20: 199-214.
656 Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., and
657 Sewell, M. A. 2010. The effect of ocean acidification on calcifying organisms in marine

ecosystems: an organism-to-ecosystem perspective. Annual Review of Ecology, Evolution, and Systematics, 41: 127-147.

Hofmann, G. E., Evans, T. G., Kelly, M. W., Padilla-Gamiño, J. L., Blanchette, C. A., Washburn, L., Chan, F., et al. 2014. Exploring local adaptation and the ocean acidification seascape—studies in the California Current Large Marine Ecosystem. Biogeosciences, 11: 1053-1064.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., *et al.* 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS ONE, 6: e28983.

Hurst, T.P., Fernandez, E.R., and Mathis, J.T. 2013. Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). ICES Journal of Marine Science: 70, 812-822.

Hurst, T.P., Fernandez E.R., Mathis, J.T., Miller, J.A., Stinson, C.M., and Ahgeak, E.F. 2012. Resiliency of juvenile walleye pollock to projected levels of ocean acidification. Aquatic Biology, 17: 247-259.

Juranek, L. W., Feely, R. A., Peterson, W. T., Alin, S. R., Hales, B., Lee, K., Sabine, C. L., *et al.* 2009. A novel method for determination of aragonite saturation state on the continental shelf of central Oregon using multi-parameter relationships with hydrographic data. Geophysical Research Letters, 36: GL040778.

Keeling, R. F., Körtzinger, A., and Gruber, N. 2010. Ocean deoxygenation in a warming world. Annual Review of Marine Science, 2: 199-229.

679 Kelly, M. W., Padilla-Gamiño, J. L., and Hofmann, G. E. 2013. Natural variation and the
680 capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus*
681 *purpuratus*. Global Change Biology, 19: 2536-2546.
682 Koch, M., Bowes, G., Ross, C., and Zhang, X. H. 2013. Climate change and ocean acidification
683 effects on seagrasses and marine macroalgae. Global Change Biology, 19: 103-132.
684 Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M.,
685 *et al.* 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities
686 and interaction with warming. Global Change Biology, 19: 1884-1896.
687 Lavigne, H., and Gattuso, J. 2010. Seacarb: seawater carbonate chemistry with R. R package
688 version 2.4.3.
689 Lueker, T. J., Dickson, A. G., and Keeling, C. D. 2000. Ocean pCO2 calculated from dissolved
690 inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory
691 measurements of CO2 in gas and seawater at equilibrium. Marine chemistry, 70: 105-
692 119.
693 Matson, P.G., Pauline, C.Y., Sewell, M.A., and Hofmann, G.E. 2012 Development under
694 elevated pCO_2 conditions does not affect lipid utilization and protein content in early life-
695 history stages of the purple sea urchin, *Strongylocentrotus purpuratus*. The Biological
696 Bulletin, 223: 312-327.
697 McElhany, P., and Busch, D. S. 2012. Appropriate pCO_2 treatments in ocean acidification
698 experiments. Marine Biology, 160: 1807-1812.
699 Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H.
700 P., *et al.* 2012. Future ocean acidification will be amplified by hypoxia in coastal habitats.
701 Marine Biology, 160: 1875-1888.

Miller, A. W., Reynolds, A. C., Sobrino, C., and Riedel, G. F. 2009. Shellfish face uncertain future in high CO2 world: influence of acidification on oyster larvae calcification and growth in estuaries. PLoS ONE, 4: e5661.

Mora, C., Wei, C.-L., Rollo, A., Amaro, T., Baco, A. R., Billett, D., Bopp, L., *et al.* 2013. Biotic and Human vulnerability to projected changes in ocean biogeochemistry over the 21st century. PLoS Biol, 11: e1001682.

Nienhuis, S., Palmer, A.R., and Harley, C.D.G. 2010. Elevated CO_2 affects shell dissolution rate but not calcification rate in a marine snail. Proceedings of the Royal Society B: Biological Sciences, 277: 2553-2558.

Orr, J. 2011. Recent and future changes in ocean carbonate chemistry. *In* Ocean Acidification. Ed. by J. Gattuso, and L. Hansson. Oxford University Press, New York, NY.

O'Donnell, M. J., George, M. N., and Carrington, E. 2013. Mussel byssus attachment weakened by ocean acidification. Nature Climate Change, 3: 587–590.

Padilla-Gamiño, J. L., Kelly, M. W., Evans, T. G., and Hofmann, G. E. 2013. Temperature and CO_2 additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. Proceedings of the Royal Society B: Biological Sciences, 280: 20130155.

Pespeni, M. H., Sanford, E., Gaylord, B., Tessa, M. H., Hosfelt, J. D., Jaris, H. K., LaVigne, M., Lenz, E. A., Russell, A. D., Young, M. K., and Palumbi, S. R. 2013. Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences, 110: 6937–6942.

Place, S. P., and Smith, B. W. 2012 Effects of seawater acidification on cell cycle control mechanisms in *Strongylocentrotus purpuratus* embryos. PLoS ONE, 7: e34068.

725 Pörtner, H. O. 2010. Oxygen-and capacity-limitation of thermal tolerance: a matrix for
726 integrating climate-related stressor effects in marine ecosystems. The Journal of
727 Experimental Biology, 213: 881-893.
728 Pörtner, H. O. 2012. Integrating climate-related stressor effects on marine organisms: unifying
729 principles linking molecule to ecosystem-level changes. Marine Ecology Progress Series,
730 470: 273-290.
731 Pörtner, H. O., and Farrell, A. P. 2008. Physiology and climate change. Science, 322: 690-692.
732 R Development Core Team. 2011. R: A language and environment for statistical computing. R
733 Foundation for Statistical Computing, Vienna, Austria.
734 Reum, J. C., Alin, S. R., Feely, R. A., Newton, J., Warner, M., and McElhany, P. 2014. Seasonal
735 carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary:
736 implications for the design of ocean acidification experiments. PLoS ONE, 9: e89619.
737 Reuter, K. E., Lotterhos, K. E., Crim, R. N., Thompson, C.A., and Harley, C.D. 2011. Elevated
738 pCO_2 increases sperm limitation and risk of polyspermy in the red sea urchin
739 *Strongylocentrotus franciscanus*. Global Change Biology, 17: 163-171.
740 Rykaczewski, R. R., and Dunne, J. P. 2010. Enhanced nutrient supply to the California Current
741 Ecosystem with global warming and increased stratification in an earth system model.
742 Geophysical Research Letters, 37: GL045019.
743 Shaw, E. C., McNeil, B. I., Tilbrook, B., Matear, R., and Bates, M. L. 2013. Anthropogenic
744 changes to seawater buffer capacity combined with natural reef metabolism induce
745 extreme future coral reef CO_2 conditions. Global Change Biology, 19: 1632-1641.

746 Sunda, W. G., and Cai, W.J. 2012. Eutrophication induced CO_2-acidification of subsurface
747 coastal waters: interactive effects of temperature, salinity, and atmospheric pCO_2.
748 Environmental Science & Technology, 46: 10651-10659.
749 Timmins-Schiffman E, O'donnell, M. J., Friedman, C.S., and Roberts, S. B. 2012. Elevated
750 pCO_2 causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*.
751 Marine Biology, 160: 1973-1982.
752 van Geen, A., Takesue, R. K., Goddard, J., Takahashi, T., Barth, J. A., and Smith, R.L. 2000.
753 Carbon and nutrient dynamics during coastal upwelling off Cape Blanco, Oregon. Deep-
754 Sea Research, 47: 975-1002.
755 Waldbusser, G. G., and Salisbury, J. E. 2013. Ocean acidification in the coastal zone from an
756 organism's perspective: multiple system parameters, frequency domains, and habitats.
757 Annual Review of Marine Science, 6: 221-247.
758 Watson, G., Bentley, M., Gaudron, S., and Hardege, J. 2003. The role of chemical signals in the
759 spawning induction of polychaete worms and other marine invertebrates. Journal of
760 Experimental Marine Biology and Ecology, 294: 169-187.
761 Wernberg, T., Smale, D. A., and Thomsen, M. S. 2012. A decade of climate change experiments
762 on marine organisms: procedures, patterns and problems. Global Change Biology, 18:
763 1491-1498.
764 Wittmann, A. C., and Portner, H.-O. 2013. Sensitivities of extant animal taxa to ocean
765 acidification. Nature Climate Change, 3: 995-1001.
766 Yu, P. C., Matson, P. G., Martz, T. R., and Hofmann, G. E. 2011. The ocean acidification
767 seascape and its relationship to the performance of calcifying marine invertebrates:

Laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO_2/pH. Journal of Experimental Marine Biology and Ecology, 400: 288-295.

Supplemental Material

Table S1. Details of environmental carbonate chemistry data sets obtained from the northern and central CCE.

Figure Legends

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 brood stock) 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 data sets please see Table S1.

Figure 2. (a) Relationship between pCO_2 and temperature in the top 50 m of the water column in the northern and central California Current Ecosystem during the summer upwelling season which approximately spans May through October and (**b**) winter (November through April) when downwelling-favorable 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

791 sampled in the CCE. Regression lines are overlaid to aid evaluation of patterns. Lines labeled P
792 and C denote relationships for Puget Sound and open coastal locations, respectively. For
793 reference, approximate present-day pCO_2 levels (~390 μatm) are indicated by the dashed
794 horizontal line.
795
796 **Figure 3.** Relationship between pCO_2 and temperature of surface waters measured during
797 summer (light grey open circle symbols) and winter (dark grey) at open coastal moorings in (**a**)
798 Washington, (**b**) California, and (**c**) Oregon and (**d**) a tidal estuary in Oregon. Regression lines
799 are overlaid and labeled S and W to indicate summer or winter, respectively. Dashed line convex
800 hulls demarcate data ranges where seasons overlap. For reference, approximate present-day
801 pCO_2 levels (~390 μatm) are indicated by the dashed horizontal line.
802
803 **Figure 4.** Relationships between pCO_2 and O_2 in the top 50 m of the water column during the
804 summer upwelling (May through October) and winter downwelling seasons (November through
805 April). Regression lines are overlaid for summer and winter; lines labeled M, P, and C
806 correspond to mooring, Puget Sound, and open coast data sets, respectively. For reference,
807 approximate present-day pCO_2 levels (~390 μatm) are indicated by the dashed horizontal line,
808 and the hypoxia threshold (60 umol kg^{-1}) is indicated with a dashed vertical line.
809
810 **Figure 5.** Environmental pCO_2 and temperature measurements (top 50 m) from the northern and
811 central California Current Ecosystem and conditions maintained in ocean acidification
812 experiments performed on organisms from the region. Dark grey circles correspond to
813 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 ocean acidification effects given covariation 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 the 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 covariation 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).

837

838

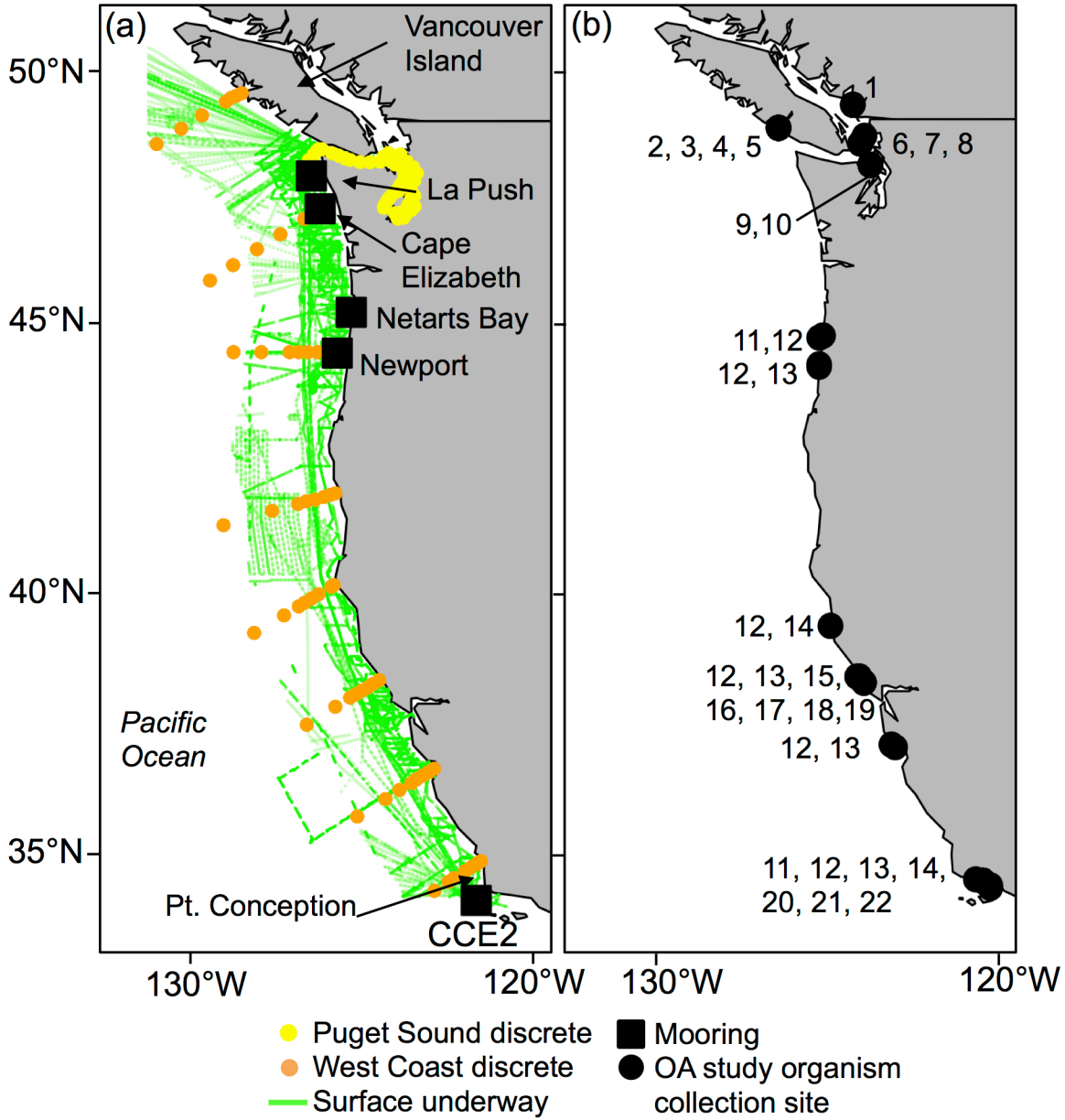
Table 1. Brief summary of experimental outcomes of reviewed OA studies that focus on organisms (or their brood stock) collected from the CCE.

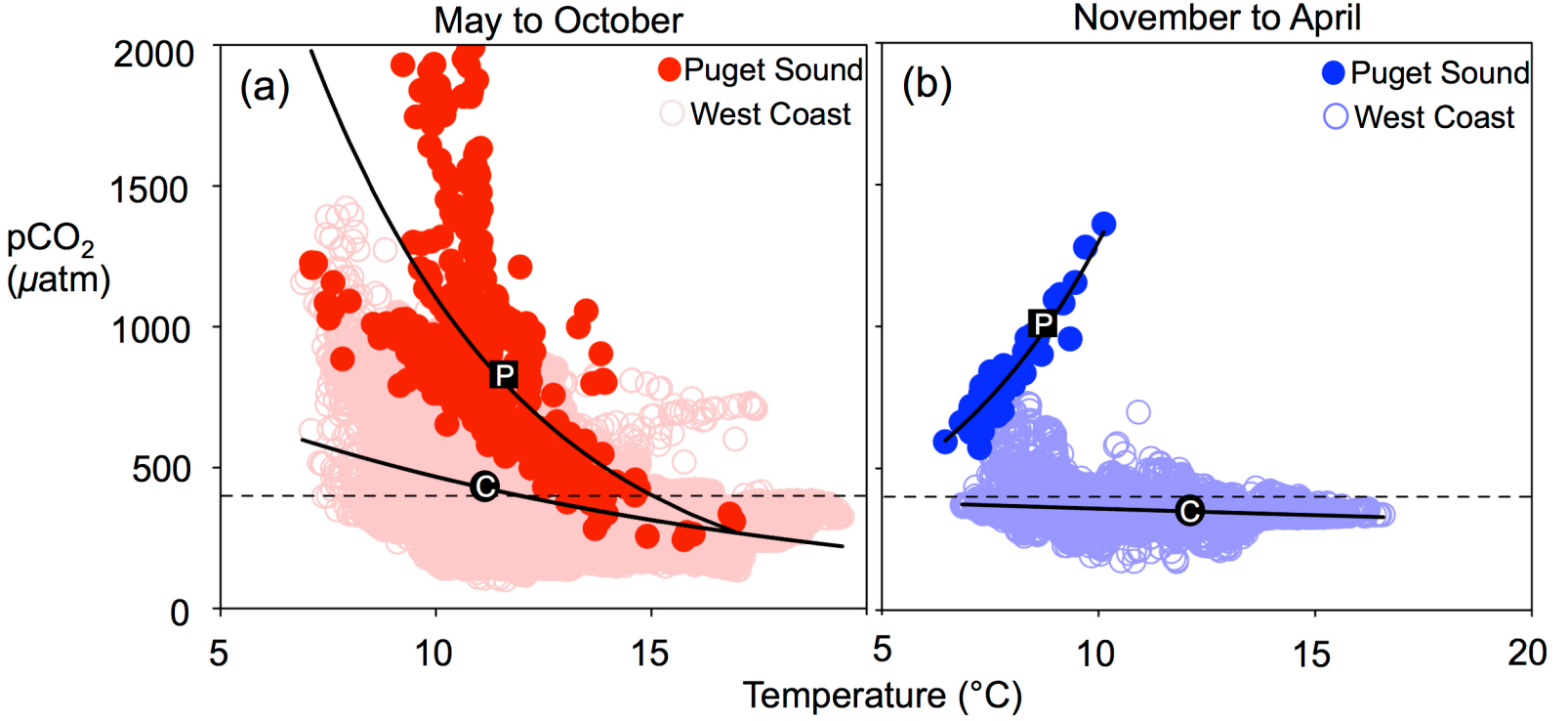
Study Number	Reference	Collection site	Species (Common name)	Life stage	Duration (days, hours)	*T* (°C)	Control pCO_2 (µatm)	Treatment pCO_2 (µatm)	Outcome relative to control
1	Gooding et al. (2009)	Jaricho Beach, BC	*Pisaster ochraceus* (purple sea star)	juvenile	70 d	12, 15	380	780	Increased growth 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)	adult	6 d	9.0	380	780, 1585	Enhanced shell dissolution
4	Sunday et al. (2011)	Barkley Sound, BC	*Strongylocentrotus franciscanus* (red sea urchin)	larval	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)	larval	1 d	12.0	400	1000	Recued larval size, small variation among families
5	Crim et al. (2011)	Barkley Sound, BC	*Haliotis kamtschatkana* (northern abalone)	larval	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)	larval	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)	larval	3 d	20.0	468	847, 1065	Increased calcification at day one, but smaller average size on day 3

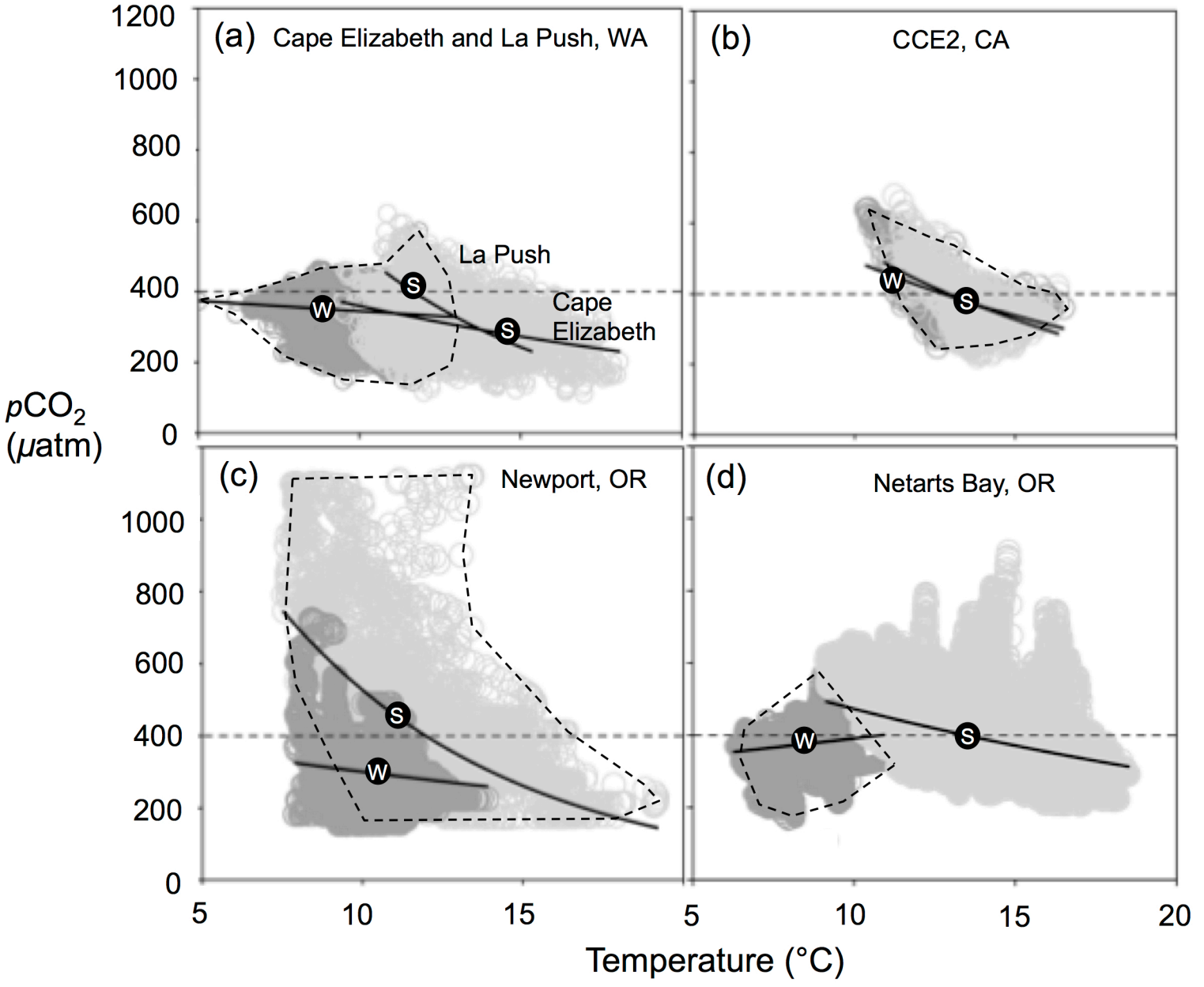
8	Odonnel et al. (2013)	San Juan Island, WA	*Mytilus trossulus* (bay mussle)	adults	20 d	10.4	432	299, 575, 736, 980 1057, 1180, 1322, 1498	Weaker, less flexible byssal threads at treatments above 1200 μatm
9	Hurst et al. (2012)	Port Townsend, WA	*Theragra chalcogramma* (walleye pollock)	yearlings	42 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/larval	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)	larval	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)	larval	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)	larval	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

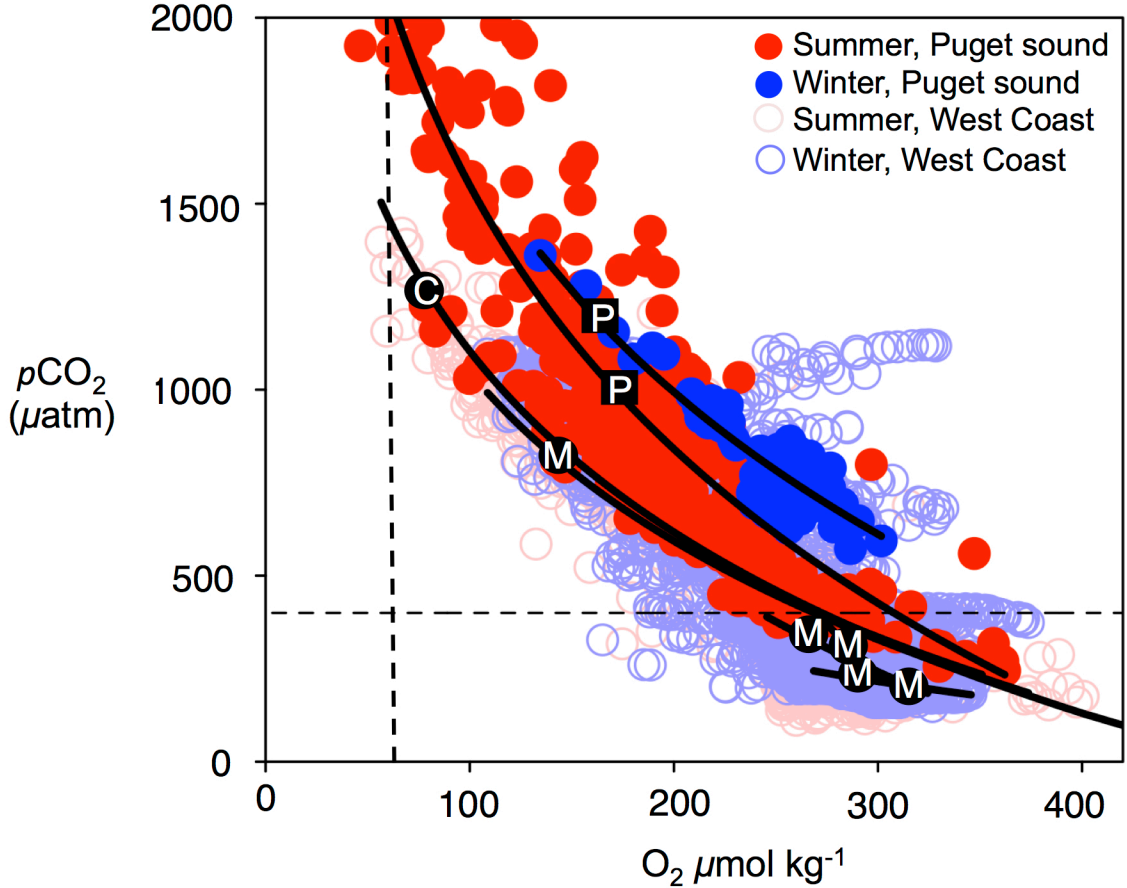
14	Kelly et al. (2013)	Northern and southern CA, various locations	*Strongylocentrotus purpuratus* (purple sea urchin)	larval	5 d	13.0	424	1210	Reduced size in larvae from dams and sires taken from northern and southern locations; no difference in metabolism
15	Place et al. (2012)	Bodega Bay, CA	*Strongylocentrotus 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)	larval/ 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)	larval	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)	larval/ 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)	larval	8 d	15.4	380	540, 970	Thinner, weaker shells
20	Padilla-Gamino et al. (2013)	Santa Barbara, CA	*Strongylocentrotus purpuratus* (purple sea urchin)	larval	75 h	13, 18	400	1100	Reduced body size and respiration rate; difference in transcriptome observed

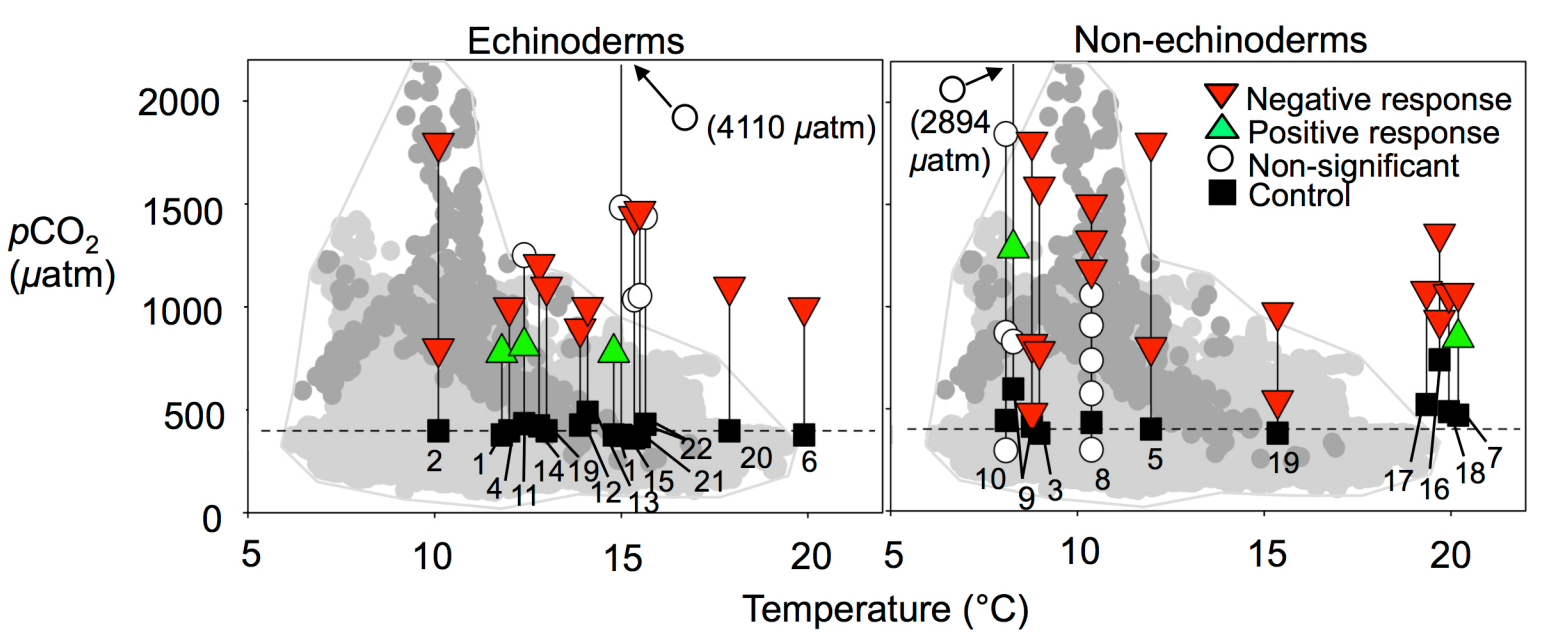
21	Matson et al. 2013	Santa Barbara, CA	*Strongylocentrotus purpuratus* (purple sea urchin)	larval	6 d	15.6	365	1038, 1444	Reduced arm length; no difference in utilization of energy lipid reserves and protein content remained unchanged
22	Yu et al. (2011)	Santa Barbara, CA	*Strongylocentrotus purpuratus* (purple sea urchin)	larval	6 d	15.6	372	1057, 1469	Slight reduction in size
22	Yu et al. (2011)	Santa Barbara, CA	*Strongylocentrotus purpuratus* (purple sea urchin)	larval	6 d	15.6	432	1441	Slight reduction in size

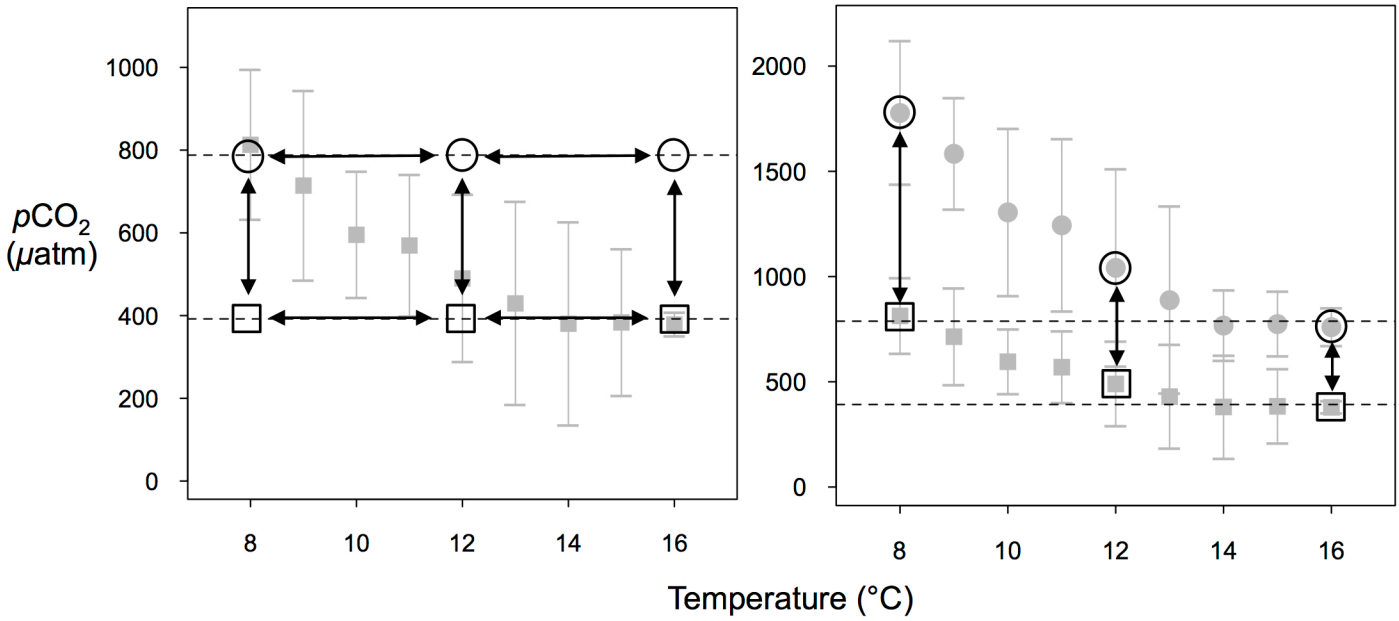

(a)
Vancouver Island
La Push
Cape Elizabeth
Netarts Bay
Newport
Pacific Ocean
Pt. Conception
CCE2
50°N
45°N
40°N
35°N
130°W
120°W
(b)
1
2, 3, 4, 5
6, 7, 8
9,10
11,12
12, 13
12, 14
12, 13, 15,
16, 17, 18,19
12, 13
11, 12, 13, 14,
20, 21, 22
130°W
120°W
Puget Sound discrete
West Coast discrete
Surface underway
Mooring
OA study organism
collection site


May to October
November to April
(a)
(b)
Puget Sound
West Coast
Puget Sound
West Coast
P
C
P
C
2000
1500
1000
500
0
pCO2
(μatm)
5
10
15
5
10
15
20
Temperature (°C)


(a) Cape Elizabeth and La Push, WA
(b) CCE2, CA
(c) Newport, OR
(d) Netarts Bay, OR
La Push
Cape Elizabeth
W
S
1200
1000
800
600
400
200
0
pCO2 (μatm)
5
10
15
20
Temperature (°C)


Summer, Puget sound
Winter, Puget sound
Summer, West Coast
Winter, West Coast
C
P
P
M
M
M
M
M
2000
1500
1000
500
0
0
100
200
300
400
pCO2 (μatm)
O2 μmol kg-1


Echinoderms
Non-echinoderms
pCO2 (μatm)
2000
1500
1000
500
0
5
10
15
20
Temperature (°C)
(4110 μatm)
(2894 μatm)
Negative response
Positive response
Non-significant
Control
2
1
4
11
14
19
12
1
13
15
22
21
20
6
10
9
3
8
5
19
17
16
18
7


pCO_2 (μatm)
1000
800
600
400
200
0
8
10
12
14
16
2000
1500
1000
500
0
Temperature (°C)

Supplement Material

Table S1. Details of environmental pCO_2-temperature and pCO_2-O_2 data sets from the northern and central California Current Ecosystem. We calculated pCO_2 values for two published and two unpublished carbonate chemistry data sets that consisted of total alkalinity (TA) and dissolved inorganic carbon (DIC) measurements. All TA and DIC measurements from those surveys were obtained following Feely et al. (2008, 2010) and had precisions of ~1.5 μmol kg^{-1} and ~2.0 μmol kg^{-1}, respectively. We calculated pCO_2 using the R library 'seacarb' (Lavigne and Gattuso, 2010) with dissociation constants from Lueker *et al.* (2000). Additional information on sampling methods are provided in the cited references.

Sample type	Name and/or approximate location	Sample depth (m)	Dates (pCO_2-temp)	Dates (pCO_2-O_2)	Habitat	Sampling method	Reference
Mooring time series	Chá bă, La Push, Washington (N 47.97, W 124.95)	0.3	Jul-2010 to Oct-2010	Jul-2010 to Oct-2010	Surface shelf waters (mooring at 60 m isobath, 5 km from coast)	Direct (Licor 820 series sensor)	Mathis et al. (2011)
Mooring time series	CCE2, Point Conception, California (N 34.32, W 120.81)	0.3	Jan-2010 to Feb-2011	Jan-2010 to Feb-2011	Surface shelf waters (mooring at 60 m isobath, 5 km from coast)	Direct (Licor 820 series sensor)	Sabine et al. (2011)
Mooring time series	Cape Elizabeth, Washington (N 47.68, W 122.25)	0.3	Jun-2006 to Oct-2010	Jun-2006 to Oct-2010	Surface shelf waters (mooring at 60 m isobath, 5 km from coast)	Direct (Licor 820 sensor)	Mathis et al. (2013)
Mooring time series	NH10, Newport, Oregon (N 44.63, W124.30)	1	Aug-2007 to May-2008	Aug-2007 to May-2008	Surface shelf waters (mooring at 60 m isobath, 20 km from coast)	Direct (Sunburst SAMI-CO_2 sensor)	Evans et al. (2011)
Hatchery seawater	Whiskey Creek Hatchery, Netarts Bay, Oregon	1 to 3 (tidal)	Feb-2010, Jul-		Tidal bay, no significant	Direct (Licor 840 sensor)	Barton et al. (2012), Hales et al. (unpub. data)

intake time series	(N 45.40, W 123.95)		2010		freshwater input		
Shipboard discrete	Puget Sound,and Strait of Juan de Fuca, Washington	0–50	Feb-2008, Aug-2008	Feb-2008, Aug-2008	Fjord estuary	Calculated (from DIC and TA)	Feely et al. (2010)
Shipboard discrete	Puget Sound, Washington	0–50	Sep-2009, Oct-2011, Oct-2011	Sep-2009, Oct-2011, Oct-2011	Fjord estuary	Calculated (from DIC and TA)	Reum et al. (2014)
Shipboard discrete	Southern British Columbia to California	0–50	Aug-2007	Aug-2007	Open coast waters	Calculated (from DIC and TA)	Feely et al. (2011)
Shipboard discrete	Southern British Columbia to California	0–50	Aug-2011, Aug-2012	Aug-2011, Aug-2012	Open coast waters	Calculated (from DIC and TA)	R.A. Feely, S.R. Alin, and others (*unpublished data)*
Shipboard underway	Southern British Columbia through central California, including Puget Sound and Strait of Juan de Fuca (34–51°N, 0–200 km offshore)	2–5	2007–2012		Open coast and fjord estuary surface waters	Direct (Licor 6262 or 7000 sensor)	Bakker et al. (2013), Pfeil et al. (2013), Feely and Sabine (2008a), Feely and Cosca (2012)

References

Bakker, D.C.E., Pfeil, B., Smith, K., *et al.* 2013 An update to the surface ocean CO_2 atlas (SOCAT version 2). Earth System Science Data Discussions, 6, doi: 10.5194/essdd-6-465-2013, 465–512.

Barton, A., Burke, H., Waldbusser, G.G., Langdon, C., and Feely, R.A. 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. Limonology and Oceanography, 57: 698–710.

Evans, T.G., Chan, F., Menge, B.A., and Hofmann, G.E. 2013. Transcriptomic responses to ocean acidification in larval sea urchins from a naturally variable pH environment. Molecular ecology, 22: 1609-1625.

Feely, R.A., Alin, S.R., Newton, J., Sabine, C.L., Warner, M., *et al.* 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuarine Coastal and Shelf Science, 88: 442-449.

Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., and Hales, B. 2008. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science, 320: 1490-1492.

Feely, R., and Sabine, C. 2008a. Sea surface and atmospheric fCO_2 data measured in the coastal Pacific ocean onboard the R/V McArthurII 2007 Cruises. http://cdiac.ornl.gov/ftp/oceans/McArthurII/2007_Data/. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.GCP_McArthurII_2007

Lavigne, H., and Gattuso, J. 2010. Seacarb: seawater carbonate chemistry with R. R package version 2.4.3.

Lueker, T.J., Dickson, A.G., and Keeling, C.D. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Marine Chemistry, 70: 105-119.

Mathis, J., Sutton, A., Sabine, C., Musielewicz, S., Maenner, S., and Bott, R. 2011. High-resolution ocean and atmosphere pCO_2 time-series measurements from mooring LaPush_125W_48N. http://cdiac.esd.ornl.gov/ftp/oceans/Moorings/LaPush_125W_48N/. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.TSM_LaPush_125W_48N

Mathis, J., Sutton, A., Sabine, C., Musielewicz, S., and Maenner, S. 2013. High-resolution ocean and atmosphere pCO_2 time-series measurements from mooring WA_125W_47N. http://cdiac.esd.ornl.gov/ftp/oceans/Moorings/WA_125W_47N/. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/OTG.TSM_WA_125W_47N

Pfeil, B., Olsen, A., Bakker, D.C.E. *et al.* (2013) A uniform, quality controlled surface ocean CO_2 atlas (SOCAT). Earth System Sciences Data, 5, doi: 10.5194/essd-5-125-2013.

Reum, J. C., Alin, S. R., Feely, R. A., Newton, J., Warner, M., and McElhany, P. 2014. Seasonal carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary: implications for the design of ocean acidification experiments. PLoS ONE, 9: e89619.

Sabine, C., Maenner, S., and Sutton, A. 2011. High-resolution ocean and atmosphere pCO_2 time-

series measurements from mooring CCE2_121W_34N. http://cdiac.esd.ornl.gov/ftp/oceans/Moorings/CCE2_121W_34N/. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee. doi:10.3334/CDIAC/otg.TSM_CCE2_121W_34N.