Effect of Elevated $p\text{CO}_2$ on Metabolic Responses of Porcelain Crab (*Petrolisthes cinctipes*) Larvae Exposed to Subsequent Salinity Stress

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Abstract

Future climate change is predicted to alter the physical characteristics of oceans and estuaries, including pH, temperature, oxygen, and salinity. Investigating how species react to the influence of such multiple stressors is crucial for assessing how future environmental change will alter marine ecosystems. The timing of multiple stressors can also be important, since in some cases stressors arise simultaneously, while in others they occur in rapid succession. In this study, we investigated the effects of elevated $p\text{CO}_2$ on oxygen consumption by larvae of the intertidal porcelain crab *Petrolisthes cinctipes* when exposed to subsequent salinity stress. Such an exposure mimics how larvae under future acidified conditions will likely experience sudden runoff events such as those that occur seasonally along portions of the west coast of the U.S. and in other temperate systems, or how larvae encounter hypersaline waters when crossing density gradients via directed swimming. We raised larvae in the laboratory under ambient and predicted future $p\text{CO}_2$ levels (385 and 1000 μatm) for 10 days, and then moved them to seawater at ambient $p\text{CO}_2$ but with decreased, ambient, or elevated salinity, to monitor their respiration. While larvae raised under elevated $p\text{CO}_2$ or exposed to stressful salinity conditions alone did not exhibit higher respiration rates than larvae held in ambient conditions, larvae exposed to elevated $p\text{CO}_2$ followed by stressful salinity conditions consumed more oxygen. These results show that even when multiple stressors act sequentially rather than simultaneously, they can retain their capacity to detrimentally affect organisms.

Introduction

Increasing atmospheric carbon dioxide ($\text{CO}_2$) is altering Earth’s climate, creating multiple new stressors with which organisms must cope. Predicted changes include more intense and more frequent storms, shifts in the hydrologic cycle, higher air and seawater temperatures, and the ecological challenge of ocean acidification [1,2,3,4,5]. As the ocean absorbs $\text{CO}_2$ from the atmosphere, seawater pH decreases, which can make growth and survival more difficult for a wide range of marine and estuarine species [6,7,8,9,10]. Estuarine and coastal environments may be particularly sensitive to the effects of climate change, as these ecosystems not only experience both terrestrial and marine perturbations, but also encounter an extensive suite of anthropogenic stressors tied to nutrient loading, fishing, pollution, and habitat degradation [11].

Model predictions of future climate change for the northeastern Pacific Ocean include changes to precipitation and altered temperatures [12,13], in concert with the influence of ocean acidification. Changes in rainfall will affect not only average salinities, but also the frequency and intensity of the most acute runoff events. These events lead to low-salinity extremes, requiring coastal and estuarine organisms to cope with rapid decreases in salinity. Adding complexity to these predicted changes is that areas that receive limited summer rainfall (e.g., Mediterranean climates) can experience hypersalinity and stratified water columns in estuaries and coastal areas; changes in thermal loading due to climate change could increase the frequency and duration of stratification events [2,14,15].

Many emerging studies explore the effects of decreased pH in combination with simultaneous changes in another stressor [9], typically temperature but also salinity in a few studies [16,17,18]. By contrast, very little research exists to assess potential outcomes when multiple stressors are imposed sequentially instead of simultaneously; that is, when one stressor acts first by itself but is followed in rapid succession by another. Understanding how species respond to sequential stressors is particularly important in coastal and estuarine habitats. In these regions, organisms can experience sudden transitions from oceanic conditions to lower salinities due to episodic runoff events, or transitions to higher salinities due to vertical swimming through a stratified water column during mobile life stages. How future high-$p\text{CO}_2$ conditions could affect an organism’s ability to cope with sudden changes in salinity has not yet been examined.

The porcelain crab *Petrolisthes cinctipes* serves as a useful model species for exploring such issues because it possesses life history
characteristics representative of a variety of nearshore invertebrates that will experience changing hydrographic conditions in future decades. Although it occurs primarily in outer-coast intertidal mussel beds and cobble fields from Point Conception, California to the Queen Charlotte Islands, British Columbia [19], it also lives in estuaries where sufficient rocky habitat exists [20,21]. Its planktotrophic larval stage remains relatively close to shore during its moderately long pelagic larval duration [22], making it similar to the larvae of many other invertebrates, including ecologically and commercially important taxa such as barnacles, mussels, oysters, and many other species of crabs [23], so the results of this study may be representative of how many other larvae will respond to future climate change.

Adults of many species in the genus *Petrolisthes* are relatively resilient to stressors such as desiccation [24,25], high temperature [26,27,28], hypoxia [25,29], and hypersaline conditions [30], which is evolutionarily consistent with the routine exposure of these organisms to the variable and stressful conditions of the intertidal zone and estuarine habitats. Though larval stages of many taxa are often more susceptible to stress than adults [31], two previous studies investigated the effects of elevated CO₂ in isolation on *P. cinctipes*, and both concluded that larvae were relatively resilient to pCO₂ stress [32,33]. These findings are consistent with the prediction that crustaceans will likely be one of the classes of organisms that are able to cope with future climate change [6,34].

In this study, we determined how respiration by newly hatched larvae of the porcelain crab *Petrolisthes cinctipes* cultured under current (385 μatm) and future predicted (1000 μatm) levels of atmospheric CO₂ differed when exposed to low (22), normal (34), and high (40) salinity conditions. Though crustacean larvae are relatively good swimmers and can adjust their position in the water column to avoid unfavorable conditions, the mosaic of nearshore conditions results in larvae experiencing a range of salinities and pH. By culturing larvae under different pCO₂ conditions and then exposing them to subsequent changes in salinity, we mimicked how larvae might encounter a plume of hypersaline runoff during their pelagic development or might experience hypersaline conditions when migrating through a stratified coastal embayment.

Oxygen consumption through respiration is a commonly used metric of stress in marine organisms, with stressful conditions usually resulting in a more rapid depletion of oxygen from the water, though metabolism could be suppressed under some conditions of stress [35]. Our investigation of how oxygen consumption differs across pCO₂ and salinity treatments in this species therefore provides insights into how larval stages of nearshore invertebrates may respond to multiple, sequentially imposed stressors characteristic of future environmental conditions.

**Materials and Methods**

Fifty ovigerous *Petrolisthes cinctipes* were collected from Shell Beach (38° 23.03′ N, 123° 06.35′ W) and Twin Coves (38° 27.49′ N, 123° 08.62′ W), California, using permits obtained from the California Department of Fish and Wildlife and California State Parks (no protected species were collected). Crabs were held in individual mesh containers in flow-through seawater at Bodega Marine Laboratory for 2–14 days. Containers were checked daily for hatched larvae, and newly released individuals from multiple broods (n = 24 broods) were transferred in mixed batches of 75 to replicate culture jars. These jars contained 0.45 um filtered seawater bubbled with prescribed pCO₂ levels to maintain carbonate chemistry and water movement, and were held at a constant temperature and normal salinity (approximately 34) [36,37]. At least five females contributed larvae to each culture jar, and broods were split evenly between pCO₂ treatments. Not all females released larvae on the same night, which allowed culture start dates to be staggered and respirometry trials to be run on sequential days using larvae of the same age. The use of larvae from multiple broods helped to minimize brood effects that have been seen in other studies [32,33], facilitating application of findings to the overall population.

Larvae were cultured at 14°C under two pCO₂ conditions: 385 μatm (ambient) and 1000 μatm (elevated). The elevated pCO₂ treatment, maintained by bubbling a premixed, NIST-traceable, air-CO₂ gas mixture through the culture water, was selected to replicate global-average conditions projected for 2100 by fossil-fuel intensive climate model projections (A1FI scenario) [15], but also mimics transient conditions encountered even today [36]. Larvae were held at a density of 75 larvae per 3 L, and were fed newly hatched *Artemia* sp. nauplii at a density of 1 nauplius/ml [38]. The water in each culture jar was changed every two days by siphoning approximately 95% of the water from the jar and replacing it with new filtered seawater that had been equilibrated to the appropriate temperature and pCO₂ treatment. During water changes, dead larvae and uneaten *Artemia* sp. were removed, and water exiting the jars was sampled for carbonate chemistry adhering to recommended practices (Table 1) [39]. We measured pH (total scale) [40] using a glass electrode (Accumet XL60; Thermo Fisher Scientific, Waltham, Massachusetts, USA) and repeated analysis of certified TRIS reference material (measured in millivolts; batch no. 8; A. Dickson, Scripps Institute of Oceanography). Total alkalinity (TA) was measured by autotitration (Metrohm 809; Metrohm, Herisau, Switzerland), using the same certified reference material. Dissolved inorganic carbon (DIC) samples were processed using coulometric titration at the Monterey Bay Aquarium Research Institute (MBARI).

Respirometry trials were conducted in seawater at ambient pCO₂ using a dissolved oxygen meter (model 781B Strathkelvin Instruments Ltd., Glasgow, UK) with a Clark-type microcathode polarographic electrode with a 22 micron diameter platinum cathode and silver/silver chloride anode connected by a buffered potassium chloride electrolyte solution (model 1302). We used a low permeability polypropylene membrane so the electrode could be used in solutions with minimal stirring, though we also visually checked to ensure the movement of larvae was sufficient to maintain water flow over the surface of the membrane. Data were transmitted via a data interface unit to a computer and the oxygen consumption rates were monitored using the Strathkelvin 949 Oxygen System (Version 2.2, Strathkelvin Instruments Ltd., Glasgow, UK).

The electrode was placed in a PVC holder with double O rings to seal the chamber. The holder and electrode were inserted into a test chamber (26.34 mm×47.09 mm ID) holding 25 ml of test solution. To maintain the temperature at 14°C, this test chamber was jacketed in a dish (37.69 mm×75.30 mm ID) and kept in a constant temperature refrigeration unit. For each new trial, the electrode was calibrated using 2% sodium sulfite and air-saturated seawater to determine zero and saturated oxygen values, respectively. Before each series of experiments, a control chamber with no larvae was monitored to establish background microbial oxygen consumption rates, which were then subtracted from the values obtained during trial runs for our analyses. During each trial, eight first-stage larvae were removed from the culturing
system and placed in the chamber for respirometry analysis, and visual analysis indicated that their activity was sufficient to maintain flow over the surface of the electrode. Seawater oxygen saturation state was measured every two minutes for one hour, though we only analyzed data from the start of the trial until the oxygen in the test chamber reached 5 mgL\(^{-1}\) to eliminate potential effects of hypoxia in the chamber on larval respiration (Figure 1). Trials with larvae from each \(p\)CO\(_2\) treatment were run sequentially in pairs (i.e., one trial with larvae from ambient \(p\)CO\(_2\) conditions was followed by one trial with larvae from elevated \(p\)CO\(_2\) conditions), and 13 pairs of trials were run at salinities of 34 over a period of 14 days.

Distilled water was added to filtered, ambient-\(p\)CO\(_2\) seawater (salinity 34) to make salinity 22 solution, and artificial seawater (Instant Ocean, Spectrum Brands, Inc.) was added to make salinity 40 solution. This procedure created two altered salinity treatments for testing effects of salinity stress on larvae drawn from each of our preceding \(p\)CO\(_2\) treatments. We acknowledge that these manipulated solutions have slightly different alkalinities and aragonite saturation states than natural seawater, and may have minor differences in trace elements [18]. Though we were unable to analyze carbonate system parameters of our test solutions during the experiment due to low volumes, we created solutions using the same methods to determine pH and total alkalinity for post-hoc estimates of alkalinity and aragonite saturation state. We also calculated these carbonate system parameters for hypothetical ocean waters of the same salinities as our treatments, using data collected in local coastal and estuarine waters [41].

Larvae were allowed to acclimate in two solution treatments for ten minutes prior to respirometry trials to attenuate a startle response, and the system was calibrated before each trial. Ten minutes was sufficient for larvae to begin active swimming following their transfer. Paired trials (4 pairs at salinity 22 and 3 pairs at salinity 40) with larvae from each \(p\)CO\(_2\) treatment were run sequentially over a period of seven days.

We used ANOVAs to test for respiratory rate differences between salinity treatments (ambient and stressful) and between \(p\)CO\(_2\) treatments (ambient and elevated), and Levene tests revealed our data met the assumption of equal variances. We used a Tukey HSD test to analyze differences among all four treatments. We also used ANOVAs to verify that our \(p\)CO\(_2\) treatments provided statistically different carbonate system conditions for developing larvae, and we used a Welch’s ANOVA for the single parameter (\(\Omega\) aragonite) that did not meet the assumption of equal variances. All statistical analyses were conducted in JMP 9 (SAS Institute, Cary, NC).

**Results**

Larvae experienced significantly different carbonate system conditions in our ambient and predicted future \(p\)CO\(_2\) treatments in the culturing apparatus (Table 1), including pH (ANOVA, \(F_{1,173} = 4451.7\), \(p<0.0001\)), \(\Omega\) aragonite (Welch’s ANOVA, \(F_{1,177} = 1925.2\), \(p<0.0001\)), \(p\)CO\(_2\) (ANOVA, \(F_{1,177} = 2627.0\), \(p<0.0001\)), and dissolved inorganic carbon (ANOVA, \(F_{1,159} = 137.0\), \(p<0.0001\)). Analysis of altered-salinity solutions revealed that alkalinity and aragonite saturation state ranged from 1479–2880 \(\mu\)mol/kg and 0.63–2.16, respectively (Table 2). These values are extremely close to those estimated for natural coastal and estuarine waters in our area at those salinities (Table 2).

There were no differences in oxygen consumption between larvae at ambient conditions and those exposed to a single stressor (Figure 2). Elevated \(p\)CO\(_2\) alone had no effect on larval oxygen consumption (Figure 3; \(F_{1,40} = 2.027\), \(p = 0.1626\)), and larvae consumed less oxygen at ambient salinities than at stressful salinities (Figure 3; \(F_{1,40} = 9.529\), \(p = 0.0038\)). Crab larvae raised under elevated \(p\)CO\(_2\) conditions that were subsequently exposed to high or low salinities consumed significantly more oxygen than ambient conditions experienced higher pH (\(p<0.0001\)) and lower DIC (\(p<0.0001\)) than those under elevated \(p\)CO\(_2\) conditions.

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**Table 1. Culturing system carbonate conditions.**

| \(p\)CO\(_2\) Treatment | 385 \(\mu\)atm | 1000 \(\mu\)atm |
|------------------------|--------------|-----------------|
| pH (Total)              | 7.997±0.004  | 7.706±0.003     |
| \(\Omega\) Aragonite    | 2.033±0.019  | 1.127±0.007     |
| Total Alkalinity (\(\mu\)mol/kg) | 2243.5±5.1 | 2254.6±3.0     |
| Dissolved Inorganic Carbon (\(\mu\)mol/kg) | 2091.9±6.4 | 2178.3±4.0     |
| \(p\)CO\(_2\) (\(\mu\)atm) | 450.1±6.1    | 949.8±7.5       |

Mean values (± S. E.) of carbonate system conditions in larval culturing jars under ambient (385 \(\mu\)atm) and elevated (1000 \(\mu\)atm) \(p\)CO\(_2\) conditions. Larvae under ambient conditions experienced higher pH (\(p<0.0001\)) and lower DIC (\(p<0.0001\)) than those under elevated \(p\)CO\(_2\) conditions.

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Figure 1. Larval oxygen consumption through time. Dissolved oxygen (mean total oxygen less background bacterial consumption rates, ± S.E.) during respirometry trials under ambient or dual-stressor conditions. Horizontal dashed line shows the lower limit of data analyzed in this study; data for oxygen levels under 5 mgL\(^{-1}\) are presented for completeness.

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Table 2. Salinity treatment carbonate conditions.

| Salinity | Treatment Solution | Predicted Natural Seawater |
|----------|--------------------|----------------------------|
| 22       | pH 7.700           | 7.701                      |
|          | pCO₂ 703.9         | 689.3                      |
|          | Total Alkalinity (μmol/kg) 1479 | 1453                     |
|          | Ω Aragonite 0.63   | 0.62                       |
|          | Dissolved Inorganic Carbon (μmol/kg) 1445 | 1419                     |
| 40       | pH 7.805           | 7.804                      |
|          | pCO₂ 928.1         | 951.5                      |
|          | Total Alkalinity (μmol/kg) 2880 | 2943                     |
|          | Ω Aragonite 2.16   | 2.21                       |
|          | Dissolved Inorganic Carbon (μmol/kg) 2707 | 2768                     |

Calculated carbonate conditions of treatment solutions in which the salinity has been altered by adding distilled water (salinity 22) or Instant Ocean artificial seawater mix (salinity 40), compared to estimated carbonate conditions in natural coastal and estuarine waters of the same salinity [41].

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Figure 2. Multi-stressor effects on larvae. Oxygen consumption (mean ± S.E.) by larvae subjected to salinity stress, CO₂ stress, both stressors, and ambient conditions; bars connected by the same letter are not significantly different. Larvae subjected to ambient conditions consumed significantly less oxygen than larvae subjected to both salinity and CO₂ stress (p = 0.0149). There were no differences in oxygen consumption between larvae raised at ambient conditions and larvae subjected to a single stressor.

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larvae raised under ambient conditions and tested subsequently under normal salinities (Figure 2; $F_{3,40} = 3.976$, $p = 0.0149$).

**Discussion**

Throughout our experiment, differences in carbonate chemistry existed not only between our $pCO_2$ treatment conditions, but also among our salinity treatments used in the respirometry chamber. By moving larvae to solutions of altered salinities, alkalinities, and aragonite saturation states, we simulated the natural movement of larval among water masses of varying physical characteristics in the field.

Crab larvae subjected to only a single stressor (salinity or $CO_2$) showed no difference in oxygen consumption compared to larvae raised under ambient conditions (Figure 2), demonstrating that larvae in our study were resilient to a single stressor. However, larvae subjected to both stressors consumed significantly more oxygen than larvae raised under ambient conditions (Figure 2). This finding indicates that these larvae were negatively affected by sequentially imposed stressors, even though they were largely unaffected by single stressors. This has important implications for understanding how species will respond to the multiple stressors expected with future climate change.

Our finding that the combined future increases in $pCO_2$ and changes to the hydrologic cycle could negatively affect crab larvae is somewhat surprising, most previous studies that have investigated the effect of future climate change on crustacean larvae have suggested that they will likely be largely unaffected [42,43,44,45,46,47], including two previous studies on the same species used in our study [32,33]. These studies, however, have primarily investigated single environmental stressors, or have imposed multiple stressors simultaneously. By applying multiple stressors sequentially in an environmentally relevant scenario that mimics realistic future conditions, we interpret that studies that investigate stressors individually might overestimate the resilience of crustacean larvae to future climate change. This investigation indicates that the timing of application of multiple stressors is an important factor to consider when studying the effects of future climate change. Not all stressors will act on the same timeframe, and some stressors such as changes in salinity may be sudden but temporary events.

Stressful conditions appear to negatively impact these organisms by increasing metabolic rates, which would increase food demands. This effect has implications for performance and survival, particularly during the larval phase, when individuals require energy not only for their daily metabolic needs, but also for the energetically costly process of metamorphosis—a final step before recruiting into benthic populations. Larvae with reduced energetic reserves have been shown to select poorer settlement sites [48] and have lower reproductive output as adults [49] than larvae that settle with larger reserves.

Stressors associated with future climate change also have been shown to impair the sensory abilities of multiple taxa in tropical systems [50,51,52] and in intertidal crustaceans in the Atlantic [53,54]. Larvae of the genus *Petrolisthes* are gregarious settlers that rely on waterborne cues to find adult conspecifics that provide protection to newly settled postlarvae [55,56], so if their sensory abilities are impaired by the metabolic stress documented in our study, it would have a negative impact on their settlement success.

Finally, although adults of the genus *Petrolisthes* are highly resilient to abiotic stressors found in the intertidal zone [25,26,27,30], our work shows that larvae may be less resilient to stressors than previously believed, particularly when multiple stressors are imposed in succession, in a similar fashion to how they might be encountered by larvae swimming among water masses. This point indicates that even species that have evolved to live in abiotically stressful habitats such as the intertidal zone might be strongly affected by future climate change through susceptibilities manifesting during certain life stages.

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Author Contributions
Conceived and designed the experiments: SHM SZ EHS JDH. Analyzed the data: SHM JDH TMH. Wrote the paper: SHM SZ EHS BG JDH TMH.

References
1. Min SK, Zhang X, Zwiefer FW, Hegerl GC (2011) Human contribution to more-intense precipitation extremes. Nature 470: 378–381.
2. Hartery CDG, Hughes AR, Hulgenen KM, Miner BG, Sorensen CJ, et al. (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9: 228–241.
3. Karl TR, Trenberth KE (2005) Modern global climate change. Science 302: 1719–1723.
4. Pall P, Alina T, Stone DA, Scott PA, Naozawa T, et al. (2011) Anthropogenic greenhouse gas contribution to flood risk in England and Wales in autumn 2000. Nature 470: 382–385.
5. IPCC (2014) Chapter 5. Coastal Systems and Low-lying Areas. In: Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, et al., editors. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.
6. Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters 13: 1419–1434.
7. Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO2 problem. Annual Review of Marine Science 1: 169–192.
8. Kleypas JA, Buddemeier RW, Archer D, Gattuso JP, Langdon C, et al. (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science 284: 112–117.
9. Kroeker KJ, Kordas RL, Crim RN, Hendriks IE, Ramajo I, et al. (2013) Impact of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology 19: 1084–1096.
10. Borsuk M, Preckzel R (2013) Multi-factorial impacts of warming and acidification of the ocean on marine invertebrates’ life histories. Integrative and Comparative Biology 53: 582–596.
11. Najjar RG, Pyke CR, Adams MB, Breitburg D, Hershner C, et al. (2010) Impacts of climate-change impacts on the Chesapeake Bay. Estuarine, Coastal and Shelf Science 86: 1–20.
12. Diffenbaugh NS, Snyder MA, Sloan LC (2004) Could CO2-induced land-cover change mask the effects of warming and acidification of the ocean on marine invertebrates’ life histories? Integrative and Comparative Biology 53: 582–596.
13. Karl TR, Trenberth KE (2003) Modern global climate change. Science 302: 1719–1723.
14. Diffenbaugh NS, Snyder MA, Sloan LC (2004) Could CO2-induced land-cover change mask the effects of warming and acidification of the ocean on marine invertebrates’ life histories? Integrative and Comparative Biology 53: 112–137.
15. Kepfer LM, Snyder MA, Sloan LC, Zavaleta ES, Fulfort B (2005) Modeled regional climate change and California endemic oar fish ranges. Proceedings of the National Academy of Sciences 102: 16201–16206.
16. Di Lorenzo E, Miller AJ, Schneider N, McWilliams JC (2005) The warming of the Pacific Ocean. Physical Oceanography 35: 336–362.
17. IPCC (2007) Climate Change 2007. The Physical Science Basis. The Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.
18. Kabli JE, Shaffer DJ (2013) Effects of salinity on survival of the exotic seagrass Zostera japonica subjected to extreme high temperature stress. Botanica Marina 56: 73–82.
19. Dickson GH, Matoo OB, Tourekt RT, Sokolova BM, Benaïs E (2013) Environmental salinity modulates the effects of elevated CO2 levels on juvenile hard-shell clams, Mercenaria mercenaria. Journal of Experimental Biology 216: 2607–2618.
20. Dickson GH, Ivanova AV, Matoo OB, Porter HO, Lunag G, et al. (2012) Interactive effects of salinity and elevated CO2 levels on juvenile eastern oysters, Crassostrea virginica. Journal of Experimental Biology 215: 29–43.
21. Morris RH, Abbott DP, Haderle EG (1980) Interzonal Invertebrates of California. Palo Alto, CA: Stanford University Press.
22. Rolleke AL, Hay ME (2007) Population dynamics of the non-native crab Petrolisthes cinctipes (Anomura: Porcellanidae) on the southern coast of Chile. Scientia Marina 71: 273–278.
23. Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integrative and Comparative Biology 53: 597–608.
24. Hemminger A, Ford TD, Hill TM, Russell DM, Sato KN, et al. (2012) Persistent carryover effects of plankonic exposure to ocean acidification in the Olympia oyster. Oyster Ecology 9: 273–278.
25. Gaylord B, Hill TM, Sanford E, Lezna E, Jacobs LA, et al. (2011) Functional impacts of ocean acidification in an ecologically critical foundation species. The Journal of Experimental Biology 214: 2358–2394.
26. Strathmann M (1987) Reproduction and development of marine invertebrates of the northern Pacific coast. Seattle: University of Washington Press.
27. Dickson AG, Sabine CL, Sturman JR (2007) Guide to best practices for ocean CO2 measurements. PICES Special Publication 3: 1–191.
28. Zeebe RE, Wolf-Gladrow D (2001) CO2 in seawater: equilibrium, kinetics, isotopes. Amsterdam: Elsevier.
29. Smith SV, Holbaug J (1997) Annual cycle and interannual variability of ecosystem metabolism in a temperate climate embayment. Ecological Monographs 67: 509–533.
30. Donohue RJ, Calosi P, Bates AH, Laverock B, Rastrick S, et al. (2012) Impact of exposure to elevated pCO2 on the physiology and behaviour of an important ecosystem engineer, the burrowing shrimp Upogebia danae. Aquatic Ecology 46: 73–86.
31. Long WC, Swiney KM, Koy RJ (2013) Effects of ocean acidification on the embryos and larvae of red king crab, Paralithodes camtschaticus. Marine Pollution Bulletin 69: 38–47.
32. Nogueira EF, Sorkin D, Ruel D, Ruel H, Reish C, et al. (2012) Early life history of the Pacific oyster Crassostrea gigas under conditions of elevated CO2. Biogeosciences 9: 2607–2618.
33. McDonald MR, McClintock JB, Amsler CD, Rittschof D, Angus RA, et al. (2007) Reproduction and development of marine invertebrates of the northern Pacific coast. Seattle: University of Washington Press.
34. Smith SV, Holbaug J (1997) Annual cycle and interannual variability of ecosystem metabolism in a temperate climate embayment. Ecological Monographs 67: 509–533.
35. Donohue RJ, Calosi P, Bates AH, Laverock B, Rastrick S, et al. (2012) Impact of exposure to elevated pCO2 on the physiology and behaviour of an important ecosystem engineer, the burrowing shrimp Upogebia danae. Aquatic Ecology 46: 73–86.
36. Long WC, Swiney KM, Koy RJ (2013) Effects of ocean acidification on the embryos and larvae of red king crab, Paralithodes camtschaticus. Marine Pollution Bulletin 69: 38–47.
37. Storlazzi CD, Emery LL, Reish C, Reish H, Reish C, et al. (2012) Early life history of the Pacific oyster Crassostrea gigas under conditions of elevated CO2. Biogeosciences 9: 2607–2618.
50. Munday PL, Dixson DL, McCormick MI, Meekan M, Ferrari MCO, et al. (2010) Replenishment of fish populations is threatened by ocean acidification. Proceedings of the National Academy of Sciences 107: 12930–12934.
51. Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecology Letters 15: 338–346.
52. Ferrari MCO, McCormick MI, Munday PL, Meekan M, Dixson DL, et al. (2012) Effects of ocean acidification on visual risk assessment in coral reef fishes. Functional Ecology 26: 553–558.
53. de la Haye KL, Spicer JI, Widdicombe S, Briffa M (2012) Reduced pH sea water disrupts chemo-responsive behaviour in an intertidal crustacean. Journal of Experimental Marine Biology and Ecology 412: 134–140.
54. de la Haye KL, Spicer JI, Widdicombe S, Briffa M (2011) Reduced sea water pH disrupts resource assessment and decision making in the hermit crab Pagurus bernhardus. Animal Behaviour 82: 495–501.
55. Jensen GC (1994) Competency, settling behavior, and postsettlement aggregation by porcelain crab megalopae (Anomura: Porcellanidae). Journal of Experimental Marine Biology and Ecology 153: 49–61.
56. Jensen GC (1989) Gregarious settlement by megalopae of the porcelain crabs Petrolisthes cinctipes (Randall) and P. eriomerus Stimpson. Journal of Experimental Marine Biology and Ecology 131: 223–231.