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The influence of food supply on the response of Olympia oyster larvae to ocean acidification

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Abstract

Increases in atmospheric carbon dioxide drive accompanying changes in the marine carbonate system as carbon dioxide (CO_2) enters seawater and alters its pH (termed "ocean acidification"). However, such changes do not occur in isolation, and other environmental factors have the potential to modulate the consequences of altered ocean chemistry. Given that physiological mechanisms used by organisms to confront acidification can be energetically costly, we explored the potential for food supply to influence the response of Olympia oyster (*Ostrea lurida*) larvae to ocean acidification. In laboratory experiments, we reared oyster larvae under a factorial combination of pCO_2 and food level. High food availability offset the negative consequences of elevated pCO_2 on larval shell growth and total dry weight. Low food availability, in contrast, exacerbated these impacts. In both cases, effects of food and pCO_2 interacted additively rather than synergistically, indicating that they operated independently. Despite the potential for abundant resources to counteract the consequences of ocean acidification, impacts were never completely negated, suggesting that even under conditions of enhanced primary production and elevated food availability, impacts of ocean acidification may still accrue in some consumers.

1 Introduction

In marine systems, the planktonic food environment influences larval condition at the time of settlement, and is one of a suite of interacting variables that affects larval and juvenile quality in the natural environment (Phillips, 2002; Pace and Manahan, 2007; Giménez, 2010). A second factor receiving growing attention is ocean acidification, which refers to the process of decreasing carbonate ion (CO_3^{2-}) concentrations and pH as atmospheric carbon dioxide (CO_2) increases in concentration and enters seawater (Sabine et al., 2004). Many studies have shown definitively the negative effects of

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high seawater pCO_2 conditions on a multitude of marine larval and juvenile organisms (Doney et al., 2009; Kroeker et al., 2010).

There have been fewer studies examining how effects of ocean acidification might be influenced by shifts in other environmental factors, and the majority of these studies have investigated combined effects of ocean acidification and temperature (reviewed by Byrne, 2011). However, changes in ocean carbonate chemistry are sure to be accompanied by shifts in more than temperature alone. Other environmental factors, such as salinity, dissolved oxygen, and patterns of primary production are also predicted to diverge from present values. Such changes may be especially dramatic in nearshore environments (Feely et al., 2008, 2010; Gruber, 2011). For example, some models project an enhancement in the intensity of upwelling within the California Current System of the eastern Pacific (Bakun, 1990; Snyder et al., 2003). This process, which brings waters that are naturally pCO_2-rich to the surface, already exposes coastal benthic marine invertebrate species to low pH seawater (Snyder and Sloan, 2005; Feely et al., 2008), and may also influence phytoplankton productivity, a major food source for pelagic planktotrophic larval stages. There are several mechanisms by which ocean productivity could moderate ocean acidification effects on planktotrophic larvae. First, upwelling-induced phytoplankton blooms can directly alter seawater chemistry by drawing down total CO_2 through enhanced photosynthesis rates, shifting carbonate and pH levels back towards conditions favorable to shell and skeleton-building (Hauri et al., 2009). Secondly, ocean acidification could induce shifts in phytoplankton C : N ratios and reduce the nutritive value of food for planktotrophic larvae (Riebesell et al., 2007). Finally, ocean acidification could induce shifts in phytoplankton species assemblages (Riebesell et al., 2007; Berge et al., 2010). Food quality and quantity influence larval characteristics that determine metamorphic and post-settlement success (Vargas et al., 2006), and the ability of planktotrophic larvae to ingest a range of food particle sizes and types (e.g. Baldwin and Newell, 1995) could influence larval responses to ocean acidification-induced shifts in plankton composition.

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Because of the potential for ocean productivity to shift in concert with altered seawater chemistry, and because surmounting lower pH conditions can incur increased energetic costs, there has been speculation about how food availability might modulate the effects of ocean acidification on marine taxa (e.g. Parker et al., 2012; Dupont et al., 2013). However, few tests have been performed to determine whether improved or impaired nutrition can ameliorate or exacerbate the negative effects of ocean acidification (but see Holcomb et al., 2010, 2012; Melzner et al., 2011; Thomsen et al., 2013; Comeau et al., 2013). Studies explicitly examining ocean acidification effects on the larval-to-juvenile life stage transition have discovered that rates of metamorphosis, settlement, and subsequent juvenile growth are affected negatively by high pCO_2/low pH conditions (Albright et al., 2008; Talmage and Gobler, 2009; Parker et al., 2010). In Olympia oysters (*Ostrea lurida*), an important estuarine foundation species along the west coast of North America, high pCO_2/low pH conditions induced larval carry-over effects that led to persistent, reduced juvenile growth rates (Hettinger et al., 2012). One hypothesis for reduced juvenile growth is that juveniles are energy depleted from a stressful larval experience and the energetically expensive process of metamorphosis, and thus do not perform as well as individuals reared as larvae in ambient pCO_2 conditions. During metamorphosis, the morphological and physiological changes that occur during the transition from the pelagic larval to the benthic juvenile stage require nutritional reserves. For example, Videla et al. (1998) found that 65 % of the energy reserves in the pediveliger stage of oysters (*Ostrea chilensis*) were used in the metamorphosis process. If larvae are compromised from stress, and have a decreased capacity to accumulate energy (i.e. lipids and proteins), individuals will start the metamorphosis process without the required nutritional reserves (Rodriguez et al., 1990; Videla et al., 1998; Pechenik et al., 1998; Pechenik 2006); the result will be juveniles with reduced performance and condition.

To determine whether food supply can play a significant role in the response of marine organisms to ocean acidification, we exposed larval Olympia oysters (*O. lurida*) to a factorial combination of food and pCO_2 regimes, and tracked growth and survival

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over the full pelagic duration. In these laboratory experiments, we paid particular attention to the possibility that negative effects of high pCO_2 might be ameliorated under high food availability.

2 Methods

2.1 Study system

Olympia oysters (*Ostrea lurida*) are native to bays and estuaries along the east Pacific coast from Alaska to Baja California, Mexico (Baker, 1995). Olympia oysters are a brooding species, and females collect sperm from the water column to fertilize their eggs. Developing trochophore larvae are brooded for approximately 10 days in the mantle cavity before being released by the female as veliger larvae. Veliger larvae develop for 2–3 weeks before they settle and metamorphose into juveniles cemented to hard substrate with one valve of the shell (Baker, 1995).

2.2 Larval culture maintenance

Adult Olympia oysters ($n = 140$) were collected from Tomales Bay, California (38°06′58″ N, 122°51′16″ W) in June 2011, transported to Bodega Marine Laboratory (BML), Bodega Bay, California, cleaned of all epiphytes, and distributed evenly among four 100 L culturing cylinders. Adult oysters were held in the cylinders at 18–22 °C in filtered seawater (0.45 µm), and fed microalgal food (*Isochrysis galbana*) daily. Every other day, water in the cylinders was changed to maintain clean culturing conditions. After 3 days, released larvae were present in two of the four culturing cylinders. Within 12 h from larval release, larvae were counted and distributed by pipette equally among 4.5 L glass jars on day 1 of the experiment ($n = 1000$ larvae per jar), for subsequent rearing through the full pelagic larval period. Each jar held 2 L of filtered seawater at the appropriate pCO_2 level.

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All seawater used during larval rearing was pre-adjusted, prior to addition of larvae, to appropriate pCO_2 levels in 20 L carboys by bubbling filtered seawater for 2–3 days with NIST-traceable CO_2 air mixtures (carboy water). The control seawater pCO_2 target was 500 µatm, approximating an average seawater pCO_2 level that occurs presently during the summer months in Tomales Bay (A. D. Russell, unpublished data). The accompanying seawater high pCO_2 target was 1000 µatm, a realistic and appropriate level designed to represent a doubling of the approximate current pCO_2 level during summer months in our study region (Moss et al., 2010; McElhany and Busch, 2013). To minimize off-gassing and maintain target seawater pCO_2 levels within the jars, the same NIST-traceable CO_2 gas mixtures used to maintain carboy water were pumped continuously into headspaces over the jars. The details of the larval culturing apparatus can be found in Hettinger et al. (2012). Carboys and jars were held in seawater tables maintained at 20 °C (±0.2 °C), within the natural temperature range in our study region during summer months when Olympia oyster larvae are in the water column (Smith and Hollibaugh, 1997).

Every other day, 90% of the seawater in each jar (jar water) was removed by reverse-filtration through 125 µm mesh and replaced with carboy water at the appropriate pCO_2 level. Immediately following each water change, microalgal food (*I. galbana*) was added to each jar at densities of: 100 000, 50 000, or 10 000 cells mL^{-1}. The highest level is known to encourage rapid growth and high survival of larval and juvenile oysters (Strathmann, 1987) ($n = 2$ pCO_2 levels × 3 food levels × 5 replicate jars = 30 jars). In our study region during the time when Olympia oyster larvae are in the water column, chlorophyll *a* concentrations can reach 24.6 mg m^{-3} (±14.2 mg m^{-3}); the lower limit of which approximates our high food level (100 000 cells mL^{-1}; ~ 10 mg m^{-3} Chl *a*) in this study (Kimbro et al., 2009; B. S. Cheng, unpublished data).

A set of modified glass jars (substrate jars) was constructed for ease of quantifying larval settlement by removing the bases from a new set of jars and replacing the bases with grey PVC plates (5 mm thick) attached with aquarium-safe silicone. Each PVC plate was roughened with sandpaper to encourage settlement, and substrate jars were

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conditioned in filtered seawater for two days before use. On day 9 of the experiment, larvae were transferred into substrate jars. Following settlement, the PVC plates were removed from the jars and percent settlement was quantified (see Sect. 2.4 for details).

2.3 Water chemistry sampling

Samples of jar water (previously containing larvae and microalgae) and carboy water (no larvae or microalgae) for total alkalinity (TA) and dissolved inorganic carbon (DIC) were collected every other day when a water change was performed. Seawater pH and temperature were measured using a potentiometric pH/temperature meter (Accumet Excel XL60). Raw pH readings (mV) were calibrated using two seawater buffers (2-amino-2-hydroxymethyl-1,3-propanediol ("TRIS") and 2-aminopyridine/HCl ("AMP") in synthetic seawater). These buffers were made in-house and checked against a certified TRIS buffer (A. Dickson, Scripps Institute of Oceanography, La Jolla, California). Salinity was determined using a YSI Professional Plus multiparameter instrument with a conductivity probe calibrated in a NIST-traceable conductivity calibration solution (YSI, Yellow Springs, OH). TA was measured using automated Gran titration with duplicates (Metrohm 809), and standardized using certified reference material from A. Dickson. A subset of samples was analyzed for DIC at the University of Georgia's infrared CO_2 analysis facility (Cai and Wang, 1998). Other carbonate system parameters (calcite and aragonite saturation states: $\Omega_{calcite}$, $\Omega_{aragonite}$ and seawater pCO_2) were calculated using the carbonate system analysis software CO2SYS (Lewis and Wallace, 1998) employing DIC and TA as the primary input variables, with equilibrium constants K1 and K2 taken from Mehrbach et al. (1973) refit by Dickson and Millero (1987), and KSO_4 from Dickson (1990).

2.4 Sampling of larvae

Larval oysters in the culture jars were sampled at key time points to quantify shell growth and total dry weight (body plus shell). On day 1, prior to their placement in the

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culture jars, 50 larvae were collected haphazardly by pipette, placed on a 125 µm Nitex plankton filter, rinsed twice with distilled water, with all excess distilled water wicked immediately through the back of the filter, and left to dry at room temperature for 24 h. Larvae were photographed individually under a dissecting microscope (Leica M125 with DC290 camera) for analysis using ImageJ software (ver. 1.37, National Institutes of Health) to determine the initial projected area of the shell. Larval shell areas on day 5, 9, and 11 post-larval release were calculated similarly (n = 10 larvae per jar at each time point), and growth was estimated as the increase in projected shell area per day since larval release.

Total dry weights of larvae sampled on days 1, 5, 9, and 11 post-larval release were determined by transferring larvae individually to aluminum vessels (pre-ashed at 500 °C for 3 h), drying at 50 °C for > 24 h, and weighing on a microbalance (Sartorius Ultramicro, Goettingen, Germany). Larvae were dried in their vessels a second time, reweighed to verify their weights, and combusted at 460 °C for 4 h in a muffle furnace (Thermo Scientific FB1415M) to remove all organic matter. Ash-free dry tissue weights were determined from the weight difference before and after combusting; total dry weights were calculated by subtracting ashed weights of the empty aluminum vessels.

Settlement was assessed daily starting at day 11, two days after larval transfers were made into the substrate jars. When < 5 % of larvae remained swimming (used as our assay point for settlement), the bases of each substrate jar were removed, and the number of oysters that had settled and metamorphosed into benthic juveniles was determined by counting settlers under a dissecting microscope (Leica M125).

2.5 Statistical analyses

Larval shell growth rate, total dry weight, and settlement were analyzed using separate, partly nested ANOVAs with jar means as replicates. In this split-plot design, pCO_2 level was the whole-plot factor, food level was the sub-plot factor, and headspaces were nested within pCO_2 level. The water chemistry data were analyzed using this same

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ANOVA structure, and separate ANOVAs were conducted for pH and TA. For each jar, all measurements of jar water and carboy water were averaged across the experiment, and an ANOVA was conducted on these estimates of the average conditions in each jar. In all analyses, data fulfilled assumptions of normality and homogeneity of variance, tested using Shapiro-Wilks' and Bartlett's test, respectively, and thus untransformed data were used. The statistical software JMP (ver. 8.0.1, Statistical Analysis Software) was used for all analyses.

3 Results

3.1 Larval culturing water chemistry

pH values in the pCO_2treatments differed from one another (ANOVA, pCO_2, $F_{1,12}$ = 22850.5, $p < 0.0001$; Table 1). pH of jars within headspaces assigned to the same treatment also varied slightly (ANOVA, headspace[pCO_2], $F_{4,12} = 6.66$, $p < 0.0046$). Specifically, mean pH in the jars from headspace 1 in the 500 µatm pCO_2 treatment differed from headspaces 2 and 3 by 0.009 pH unit. pH levels across the three headspaces in the 1000 µatm pCO_2 level did not differ. pH values also differed as a function of food level (ANOVA, food, $F_{2,12} = 33.82$, $p < 0.0001$; Table 1). pH units decreased approximately 0.01 unit between each of the subsequent food levels in both pCO_2 levels (i.e. the high food level had a pH unit ~ 0.01 lower than the medium food level and the medium food level had a pH unit ~ 0.01 lower than the low food level). This pattern of decreasing pH with increasing food levels (i.e. algal concentrations) is likely from increased respiration of the algae in the low-light conditions that characterized our cultures. Total alkalinity did not differ between the pCO_2 treatments (ANOVA, pCO_2, $F_{1,12} = 0.38$, $p = 0.5471$), among food levels (ANOVA, food, $F_{2,12} = 0.41$, $p = 0.6745$) (Table 1), or among the headspaces assigned to the same treatments (ANOVA, headspace[pCO_2], $F_{4,12} = 0.30$, $p = 0.8711$). DIC samples were used simply to overconstrain the carbonate system and were not analyzed statistically.

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3.2 Larval shell growth

Larval shell growth on day 5 post-larval release was reduced in low compared to medium food levels (ANOVA, food, $F_{2,12} = 4.30$, $p = 0.0391$), but did not differ between the two pCO_2 levels (ANOVA, pCO_2, $F_{1,12} = 3.36$, $p = 0.0917$) (Fig. 1a). Similar patterns were exhibited on day 9; however, at this time point, growth was reduced in low compared to medium and high food levels (ANOVA, food, $F_{2,12} = 10.99$, $p = 0.0019$; pCO_2, $F_{1,12} = 2.08$, $p = 0.1747$) (Fig. 1b). At day 11, shell growth was still reduced in low compared to medium and high food levels (ANOVA, food, $F_{2,12} = 3.95$, $p = 0.0481$) and also in high compared to control pCO_2 conditions (ANOVA, pCO_2, $F_{1,12} = 4.89$, $p = 0.0471$) (Fig. 1c). Indeed, larval shell growth was 9 %, 5 %, and 14 % higher in ambient compared to high pCO_2 conditions, for the high, medium, and low food treatments, respectively. The effects of food on larval shell growth were consistent regardless of pCO_2 level (Day 5, ANOVA, pCO_2^* food, $F_{2,12} = 0.26$, $p = 0.7776$; Day 9, ANOVA, pCO_2^* food, $F_{2,12} = 0.04$, $p = 0.9622$; Day 11, ANOVA, pCO_2^* food, $F_{2,12} = 0.34$, $p = 0.7203$). Larval shell growth also did not vary between headspaces assigned to the same treatment (Day 5, ANOVA, Headspace [pCO_2], $F_{4,12} = 0.57$, $p = 0.6899$; Day 9, ANOVA, Headspace [pCO_2], $F_{4,12} = 1.14$, $p = 0.3847$; Day 11, ANOVA, Headspace [pCO_2], $F_{4,12} = 0.25$, $p = 0.9041$).

3.3 Larval weight

On day 5 post-larval release, total dry weight was reduced in low compared to medium food levels (ANOVA, food, $F_{2,11} = 12.58$, $p = 0.0014$) and was also reduced in elevated compared to control pCO_2 conditions (ANOVA, pCO_2, $F_{1,11} = 16.41$, $p = 0.0019$) (Fig. 2a). On day 9, low food decreased total dry weight compared to medium and high food levels (ANOVA, food, $F_{2,11} = 10.44$, $p = 0.0029$), but pCO_2 level did not have an effect (ANOVA, pCO_2, $F_{1,11} = 1.80$, $p = 0.2064$) (Fig. 2b). The same patterns were exhibited on day 11 for low food (ANOVA, food, $F_{2,11} = 37.54$, $p < 0.0001$) and pCO_2 level (ANOVA, pCO_2, $F_{1,11} = 3.48$, $p = 0.089$) (Fig. 2c). The effect of pCO_2

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on total dry weight was dependent on food level on day 5 (ANOVA, pCO_2^* food, $F_{2,11} = 12.75$, $p = 0.0014$), but not day 9 (ANOVA, pCO_2^* food, $F_{2,11} = 0.87$, $p = 0.4476$) or day 11 (ANOVA, pCO_2^* food, $F_{2,11} = 0.25$, $p = 0.7816$) post-larval release. This response varied between headspaces assigned to the same treatment on day 5 (ANOVA, Headspace [pCO_2], $F_{4,11} = 4.69$, $p = 0.0188$), but not on day 9 (ANOVA, Headspace [pCO_2], $F_{4,11} = 3.16$, $p = 0.0587$) or day 11 (ANOVA, Headspace [pCO_2], $F_{4,11} = 1.01$, $p = 0.4452$) post-larval release.

3.4 Settlement

Settlement occurred on day 16 in the high and medium food treatments in both control and elevated pCO_2 levels, on day 19 in the low food treatment in the control pCO_2 level, and on day 22 in the low food treatment and elevated pCO_2 level. On average, across all food level treatments, survival through settlement was 70 % higher in control compared to high pCO_2 treatments (ANOVA, pCO_2, $F_{1,12} = 61.76$, $p < 0.0001$; Fig. 3). There was also a significant effect of food level on settlement success and settlement was reduced in the low compared to the medium and high food levels (ANOVA, food, $F_{2,12} = 6.79$, $p < 0.0106$; Fig. 3). Settlement was 40 % higher in the ambient pCO_2/high food compared to the ambient pCO_2/low food treatments, and 54 % higher for the high pCO_2/high food compared to the high pCO_2/low food treatments. The effect of pCO_2 level on settlement was consistent across the three food levels (ANOVA, pCO_2^* food, $F_{2,12} = 1.77$, $p = 0.2115$), and the response did not vary between headspaces assigned to the same treatment (ANOVA, Headspace [pCO_2], $F_{4,12} = 1.10$, $p = 0.4021$).

4 Discussion

Ocean acidification-induced reductions in growth and calcification have been reported across all life stages of bivalve species (e.g. Ries et al., 2009; Miller et al., 2009;

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Gaylord et al., 2011; Hettinger et al., 2012). Several mechanisms are thought to drive such responses including altered metabolic rates and modified energy budget allocation (Pörtner et al., 2004; Michaelidis et al., 2005; Wood et al., 2008; Melzner et al., 2011). There has been speculation in the literature regarding how food limitation or proliferation can exacerbate or ameliorate the negative effects of ocean acidification (e.g. Parker et al., 2012; Dupont et al., 2013). However, studies explicitly testing for potential counteractive effects of high food environments on organisms in elevated pCO_2 conditions are few and limited primarily to adult or juvenile life stages (e.g. Holcomb et al., 2010, 2012; Melzner et al., 2011; Thomsen et al., 2013). This gap in knowledge of the possible interactive effects of food supply and ocean acidification on larval life stages diminishes our ability to predict the consequences of global change for natural populations, especially in areas where oceanographic processes and features create seasonally dynamic conditions of waters that can be depleted in phytoplankton and low in pH (e.g. the northern California coast). These instances arise from strong upwelling bringing nutrient and pCO_2-rich water to the nearshore in the days before a phytoplankton bloom occurs (Feely et al., 2008; Kudela et al., 2008). pCO_2 can be rapidly drawn down through photosynthesis during a bloom, and waters may thus have abundant food available as plankton in the absence of high seawater pCO_2(Kudela et al., 2008). During the strong upwelling season along the northern California coast, oyster larvae can also experience upwelled waters when those waters enter and exchange with water in embayments, such as Tomales Bay, California. In this instance, limited food might often accompany high pCO_2/low pH conditions (Kimbro et al., 2009).

This study revealed that reduced food levels and elevated pCO_2conditions generally cause decreased oyster larval shell growth, total dry weights, and settlement. However, the effect of pCO_2 level on each response variable did not depend upon food level, demonstrated by the non-significant interaction terms. The one exception to this pattern was for total dry weight at day 5 post-larval release, which was driven by the high total dry weights measured in the ambient pCO_2/medium food level treatment. This response showed a significant interactive effect. In one of the few studies on interactive

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pCO_2 and food effects, Melzner et al. (2011) found similar results of high pCO_2 and reduced food level on adult mussels. In their study, shell growth was lower in the high pCO_2 and low food treatments, but the effect of pCO_2 on shell growth did not depend on the food level. In general, our results indicate a benign food environment (i.e. one in which food is not limiting) partially ameliorates the negative effects of larval exposure to high pCO_2 conditions.

Previous work demonstrated that larval carry-over effects resulted in a magnified, negative juvenile response in Olympia oysters, and effects persisted well into juvenile life (Hettinger et al., 2012). The detected reduction in juvenile growth might have resulted from larvae starting the energetically expensive metamorphosis process with depleted energy (i.e. protein and lipid) reserves. The effect of diminished larval energy reserves on juvenile growth could be exacerbated in low larval food environments, and these effects could also persist well into juvenile life. Early life stages often act as population bottlenecks (Gosselin and Qian, 1997), and larval carry-over effects persisting after settlement, into the juvenile and later stages, could ultimately influence adult demographics.

Our results suggest that Olympia oyster larvae do not demonstrate the ability to counteract exposure to elevated pCO_2 conditions in high food environments. Furthermore, low food environments and elevated pCO_2 conditions both lead to reduced larval shell growth and whole weights, but these stressors operate additively rather than synergistically. This study shows that even when food supply is abundant, larvae exposed to elevated pCO_2 are smaller and weigh less than larvae in ambient conditions. This difference in larval size and weight will likely influence juvenile growth through larval carry-over effects, and could have significant implications for adult populations.

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Table 1. A table of seawater properties of the two seawater pCO_2 regimes during the culturing experiment (± s.d. computed from averages of the jar replicates).

Food level	TA ($\mu mol\ kg_{sw}^{-1}$)	DIC ($\mu mol\ kg_{sw}^{-1}$)	pH_T	$pCO_{2\,calc}$	Ω_{calc}	Ω_{arag}
			500 µatm			
High	2242 ± 12 (18)	2046 ± 13 (18)	8.05 ± 0.008	539 ± 73	3.3 ± 0.4	2.2 ± 0.2
Med	2240 ± 13 (18)	2040 ± 17 (18)	8.06 ± 0.005	528 ± 91	3.4 ± 0.4	2.2 ± 0.2
Low	2243 ± 13 (18)	2036 ± 11 (18)	8.06 ± 0.004	494 ± 69	3.6 ± 0.3	2.3 ± 0.3
			1000 µatm			
High	2244 ± 11 (18)	2165 ± 13 (18)	7.75 ± 0.005	1094 ± 118	1.9 ± 0.2	1.2 ± 0.1
Med	2245 ± 11 (18)	2163 ± 11 (18)	7.76 ± 0.002	1067 ± 94	2.0 ± 0.2	1.3 ± 0.1
Low	2246 ± 12 (18)	2165 ± 8 (18)	7.76 ± 0.004	1065 ± 102	2.0 ± 0.2	1.3 ± 0.1

The number of samples for TA and DIC are indicated in parentheses.
Salinity and temperature (mean ± s.d.) were 19.4 ± 0.05 °C and 33.4 ± 0.46 psu, respectively.
TA, total alkalinity; DIC, dissolved inorganic carbon.

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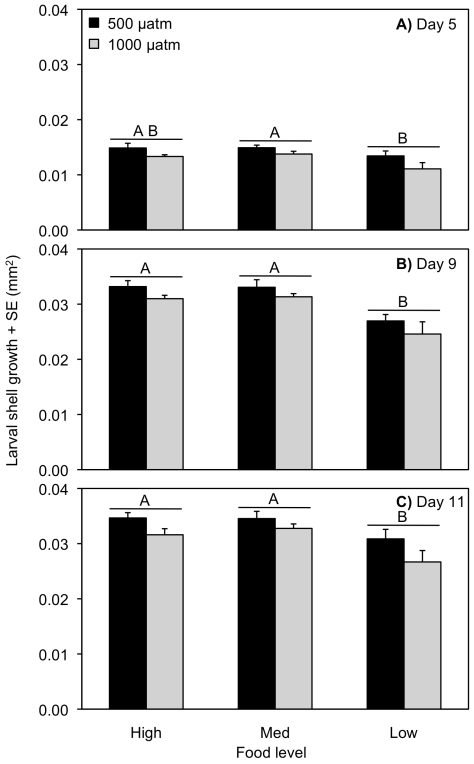

Fig. 1. Effect of pCO_2 and food supply on larval shell growth in Olympia oysters (*Ostrea lurida*). Data are shell growth on **(A)** day 5, **(B)** day 9, and **(C)** day 11 post-larval release in control (black bars) and elevated (grey bars) pCO_2 levels. Food levels in each treatment relate to the following concentrations: 100 000 cells mL^{-1} (high), 50 000 cells mL^{-1} (med), 10 000 cells mL^{-1} (low), and refer to the density of *Isochrysis galbana* in each jar per treatment. Values are means across all jar replicates of a given treatment + 1 SE. Shared letters above bars indicate food levels that did not differ significantly (Tukey HSD, $p > 0.05$).

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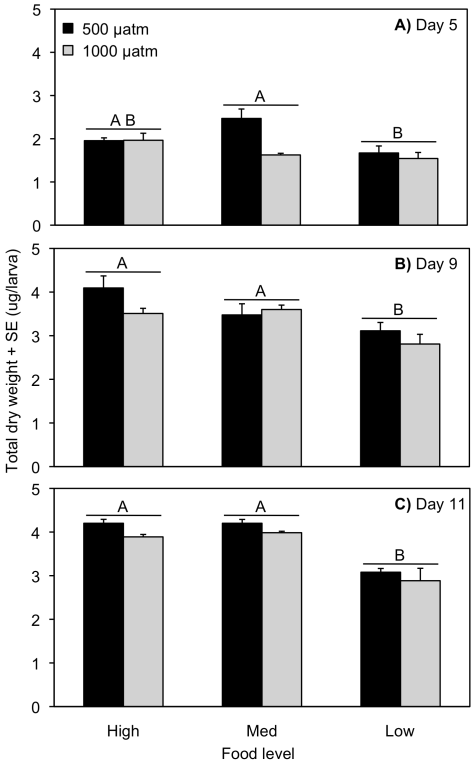

Fig. 2. Effect of pCO_2 and food supply on larval total dry weight in Olympia oysters (*Ostrea lurida*). Data are weight on **(A)** day 5, **(B)** day 9, and **(C)** day 11 post-larval release in control (black bars) and elevated (grey bars) pCO_2 levels. Food levels in each treatment relate to the following concentrations: 100 000 cells mL^{-1} (high), 50 000 cells mL^{-1} (med), 10 000 cells mL^{-1} (low), and refer to the density of *Isochrysis galbana* in each jar per treatment. Values are means across all jar replicates of a given treatment + 1 SE. Shared letters above bars indicate food levels that did not differ significantly (Tukey HSD, $p > 0.05$).

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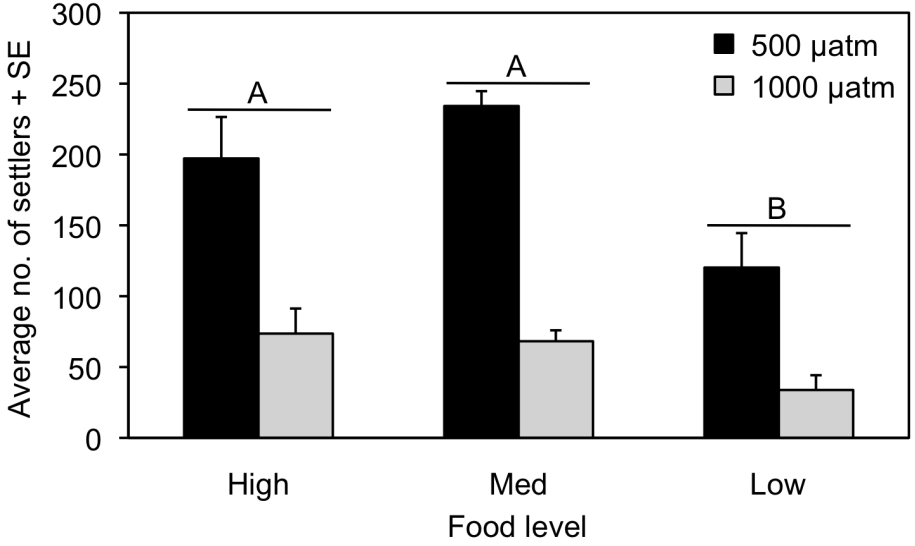

Fig. 3. Effect of pCO_2 and food supply on larval settlement in Olympia oysters (*Ostrea lurida*). Data are the average number of settlers in control (black bars) and elevated (grey bars) pCO_2 levels. Food levels in each treatment relate to the following concentrations: 100 000 cells mL^{-1} (high), 50 000 cells mL^{-1} (med), 10 000 cells mL^{-1} (low), and refer to the density of *Isochrysis galbana* in each jar per treatment. Values are averaged across all jar replicates of each larval pCO_2treatment + 1 SE. Shared letters above bars indicate food levels that did not differ significantly (Tukey HSD, $p > 0.05$).

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