Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm

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Anthropogenic climate change poses a serious threat to biodiversity. In marine environments, multiple climate variables, including temperature and CO_2 concentration ($[CO_2]$), are changing simultaneously. Although temperature has well-documented ecological effects, and many heavily calcified marine organisms experience reduced growth with increased $[CO_2]$, little is known about the combined effects of temperature and $[CO_2]$, particularly on species that are less dependent on calcified shells or skeletons. We manipulated water temperature and $[CO_2]$ to determine the effects on the sea star *Pisaster ochraceus*, a keystone predator. We found that sea star growth and feeding rates increased with water temperature from 5 °C to 21 °C. A doubling of current $[CO_2]$ also increased growth rates both with and without a concurrent temperature increase from 12 °C to 15 °C. Increased $[CO_2]$ also had a positive but nonsignificant effect on sea star feeding rates, suggesting $[CO_2]$ may be acting directly at the physiological level to increase growth rates. As in past studies of other marine invertebrates, increased $[CO_2]$ reduced the relative calcified mass in sea stars, although this effect was observed only at the lower experimental temperature. The positive relationship between growth and $[CO_2]$ found here contrasts with previous studies, most of which have shown negative effects of $[CO_2]$ on marine species, particularly those that are more heavily calcified than *P. ochraceus*. Our findings demonstrate that increased $[CO_2]$ will not have direct negative effects on all marine invertebrates, suggesting that predictions of biotic responses to climate change should consider how different types of organisms will respond to changing climatic variables.

calcification | climate change | feeding rate | ocean acidification | *Pisaster ochraceus*

Anthropogenic climate change poses one of the most serious threats to biodiversity. The emission of greenhouse gases and other human activities are already driving rapid change in the Earth's climate system, and rates of change are expected to accelerate in the current century (1). In some cases (e.g., ocean pH) environmental conditions are expected to exceed values that have not been reached in many millions of years (2). Although the ecological effects of anthropogenic climate change are already being felt (3, 4), the full repercussions for natural ecosystems and human societies remain poorly understood.

In marine environments, the 2 most important abiotic changes are likely to be increased water temperature and elevated carbon dioxide concentration ($[CO_2]$) (4). The mean global surface temperature has increased ≈0.76 °C in the past 150 years and is predicted to rise an additional 1°–4 °C by the end of this century (1). Both positive and negative biological responses to increased temperature have been well documented and include vertical and latitudinal range shifts, altered feeding and growth rates, and acute responses such as coral bleaching (5–8). Less studied are the biological effects of ocean acidification (OA), which collectively refers to increased oceanic $[CO_2]$ and the corresponding reduction in pH and carbonate availability (2, 9). Seawater pH has dropped ≈0.1 units since the Industrial Revolution and is expected to decrease by another 0.15–0.35 units by the year 2100 (1). Furthermore, the reduction in carbonate availability, a component of calcium carbonate ($CaCO_3$) required by many marine calcifying organisms (2, 9), is believed to be a major driver of decreased growth rates in mollusks, gastropods, coccolithophorids, and other heavily calcified species with experimental increases in $[CO_2]$ (10–12). However, the effects of ocean acidification on species that are less dependent on calcified shells or skeletons are poorly understood.

In addition to the important effects of individual climatic variables, simultaneous changes in multiple climate variables have the potential to yield surprising physical and biological responses that could not be predicted by responses to single climate variables alone. For instance, CO_2 solubility is temperature dependent, and therefore pH may show less extreme changes when increases in $[CO_2]$ and temperature are combined (2). Similarly, multiple stressors may have synergistic, antagonistic, or additive effects on marine species (13–15). For example, elevated temperature and $[CO_2]$ have synergistic negative effects on the calcification rates of tropical corals (14). Ocean acidification may also increase organisms' susceptibility to other stressors; elevated $[CO_2]$ reduces thermal tolerance in crabs (16) and heat-shock protein production in echinoid larvae (17). To fully understand the biological consequences of climate change, multiple climate variables must be experimentally manipulated in tandem.

Pisaster ochraceus (Brandt, 1835), a primarily intertidal sea star, is an ideal study organism to address the aforementioned questions. The importance of temperature on *P. ochraceus* biology has already been established; upwelling-associated cooling of seawater has been shown to reduce *P. ochraceus* feeding rates and alter growth rates (7). However, the extent to which these biological rates will increase with incremental increases in water temperature and the limits to this increase remain unknown. *P. ochraceus* also presents an interesting test of the effects of OA on less-calcified members of a phylum that has so far shown primarily negative responses to OA. Rather than a continuous, heavily calcified endo- or exoskeleton, *P. ochraceus* instead has hundreds of tiny calcareous ossicles embedded within and connected by soft tissue (18, 19), and these ossicles make up a relatively small proportion of *P. ochraceus*' total body mass. Finally, *P. ochraceus* plays an important role in determining rocky intertidal community structure along the western coast of North America (6, 7, 20–22) and commonly feeds on heavily calcified species, such as mussels, that are predicted to experience reduced growth with OA (23, 24). If *P. ochraceus*' response to climate change differs substantially from its prey's response, this could have important implications for the strength of the predator–prey interaction. Overall community responses to cli-

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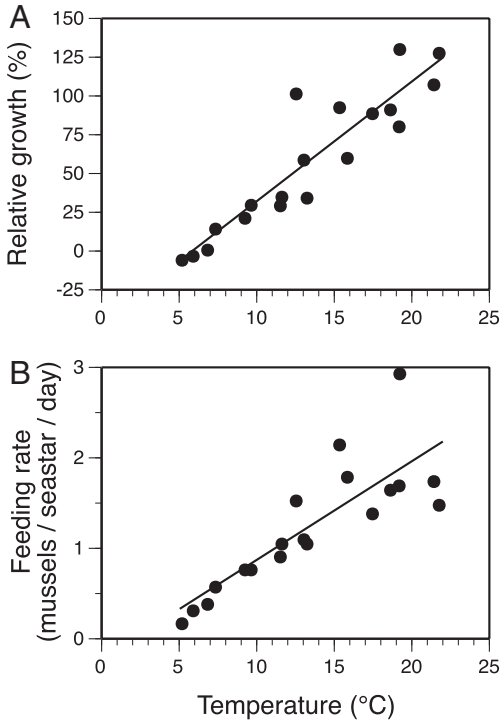

Fig. 1. Sea star growth and feeding rates increased linearly with water temperature. (*A*) Mean relative sea star growth (percentage of change from initial wet mass) with water temperature. (*B*) Mean number of mussels consumed daily per sea star with water temperature. Each data point is a within-tank mean of 2 sea stars.

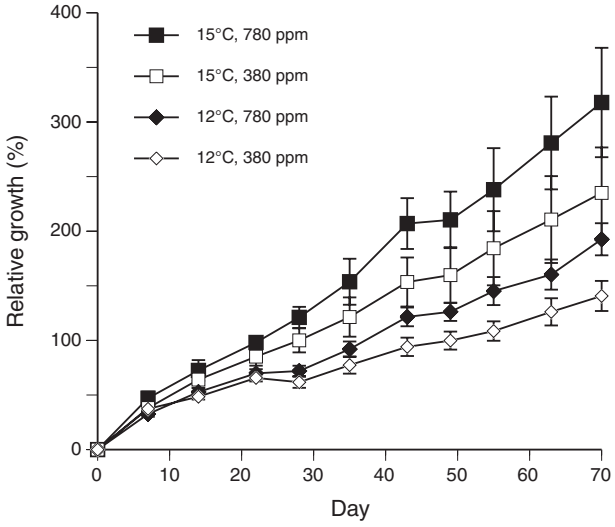

Fig. 2. Mean sea star growth (percentage of change from initial wet mass) over time with increased water temperature and [CO_2]. Error bars represent ±1 standard error (SE) of the mean.

mate change will likely depend heavily on such responses of key ecological species and how species-specific responses alter key interspecific interactions.

In this study, we reared juvenile *P. ochraceus* in the laboratory at water temperatures ranging from 5 °C to 21 °C to determine whether growth and feeding rates will increase across the range of water temperatures this species is likely to experience this century throughout much of its geographic range. We then reared additional juvenile *P. ochraceus* in factorial combinations of 2 temperature and 2 [CO_2] treatments to determine the effects of simultaneous and climatically realistic increases in water temperature and [CO_2] on the growth, feeding, and calcified mass of *P. ochraceus*. We hypothesized the following: (*i*) *P. ochraceus* growth and feeding rates will increase with temperature toward an as-of-yet unmeasured maximum; (*ii*) elevated [CO_2] alone will have no effect on sea star growth or feeding rates, but the proportion of sea stars' total body mass consisting of calcareous material will decline with increased [CO_2]; and (*iii*) increased temperature and [CO_2] will have no interactive effect on *P. ochraceus*' overall growth, but will have an antagonistic effect on the proportion of calcified material.

Results and Discussion

Under manipulated water temperature alone, the relative growth (change in wet mass/initial wet mass) of juvenile *P. ochraceus* increased linearly with temperature from 5 °C to 21 °C (Fig. 1*A*; linear regression, $P < 0.001$, df = 18, $R^2 = 0.84$), with no indication of a peak in growth over the measured temperature range. As no apparent growth maximum was reached in our experiment, our results suggest that juvenile *P. ochraceus* growth rates will likely increase with future oceanic warming throughout much of this species' range (1, 25). Temperature also had a positive effect on feeding rates (Fig. 1*B*; linear regression, $P < 0.0001$, df = 18, $R^2 = 0.69$). It is somewhat less clear if an optimum feeding rate exists within our manipulated range. Although a nonlinear model was not significantly better than a linear model, *P. ochraceus* feeding did appear to level out around 16 °C, and an optimum may lie somewhere between 15 °C and 20 °C. This contention is supported by unpublished data on adult sea stars (S. Pincebourde, personal communication). Further studies of sea star feeding and growth responses to temperature at multiple life stages and with greater temperature resolution above 15 °C would help elucidate the nature of the temperature–growth–feeding relationship.

In a factorial experiment, both temperature and [CO_2] had significant effects on seawater pH, with no interaction between them [2-way ANOVA; temperature, $F_{1,19} = 6.45$, $P = 0.021$; CO_2, $F_{1,19} = 4.91$, $P = 0.039$; temperature (temp) × CO_2 interaction, $F_{1,19} = 2.09$, $P = 0.165$]. In the control (380 ppm) CO_2 treatments, the seawater pH was 7.85 ± 0.02 at 12 °C and 7.88 ± 0.02 at 15 °C; in the high (780 ppm) CO_2 treatments, the pH was 7.79 ± 0.01 at 12 °C and 7.82 ± 0.04 at 15 °C.

Increased temperature and [CO_2] had positive and additive effects on sea star growth rates (Fig. 2; 2-way ANOVA; temperature, $F_{1,18} = 9.81$, $P = 0.006$; CO_2, $F_{1,18} = 5.04$, $P = 0.038$; temp × CO_2, $F_{1,18} = 0.002$, $P = 0.967$). Relative to control treatments, high [CO_2] alone increased relative growth by ≈67% over 10 weeks, while a 3 °C increase in temperature alone increased relative growth by 110%.

The effects of temperature and [CO_2] on sea star feeding rates were more complex since sea star feeding rates generally increase with sea star size. When the effect of sea star size was taken into account as a statistical covariate, elevated [CO_2] had only a marginally positive effect on feeding rates, while a 3 °C temperature increase significantly raised sea star feeding rates by ≈47% (Fig. 3; ANCOVA; temperature, $F_{1,17} = 12.3$, $P = 0.003$; CO_2, $F_{1,17} = 3.18$, $P = 0.081$; temp × CO_2, $F_{1,17} = 0.104$, $P = 0.707$; mean wet mass, $F_{1,17} = 96.8$, $P < 0.0001$).

We also observed changes in the relative proportions of the 3 main sea star body components, which were dry soft tissue, calcified tissue, and water. The relative calcified mass (of total wet mass) showed no change with temperature, but declined significantly overall with increased [CO_2], from a mean of 11.5% at control [CO_2] to 10.9% at high [CO_2]. However, there was a significant interaction between temperature and [CO_2], where the effect of increased [CO_2] on relative calcified material was

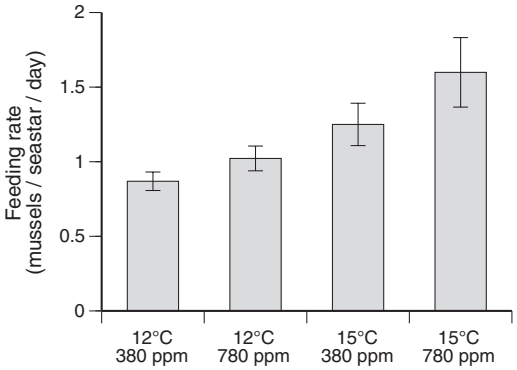

Fig. 3. Mean number of mussels consumed daily per sea star under 4 factorial temperature and [CO_2] combinations. Error bars represent ±1 SE of the mean.

reduced in the high temperature treatments (Fig. 4; ANCOVA; temperature, $F_{1,17} = 2.21$, $P = 0.115$; CO_2, $F_{1,17} = 4.84$, $P = 0.042$; temp × CO_2, $F_{1,17} = 4.84$, $P = 0.042$; sea star wet mass, $F_{1,17} = 8.48$, $P = 0.01$). Although the relative noncalcified wet mass [(dry soft tissue mass + water mass)/total wet mass] increased with [CO_2], the ratio of dry soft tissue mass to water mass remained constant at ≈1:4 regardless of temperature or [CO_2] (2-way ANOVA; temperature, CO_2, and temp × CO_2, all $P > 0.3$).

Our findings show similarities but also key differences from previous studies on the effects of climate change on marine organisms. Increased [CO_2] reduces calcification rates in a variety of marine invertebrates, leading to reduced growth rates (Table 1). Although we found that the relative calcified mass of sea stars declined with increased [CO_2], *P. ochraceus*' overall growth rate did not suffer as a consequence. This seeming disagreement with the responses of other marine invertebrates to elevated [CO_2] could be explained by differences in the amount and location of their calcareous tissue. Unlike urchins, mollusks, and brittle stars, *P. ochraceus* lacks a continuous calcified test, shell, or endoskeleton that encases a large portion of its soft tissue, making it less likely that a reduction in the growth of *P. ochraceus*' calcareous material would physically limit soft tissue growth or function (Table 1; ref. 19). Furthermore, *P. ochraceus*' calcified ossicles make up a relatively small proportion of its total body mass (R.G. and E.T., unpublished data). It may be that elevated [CO_2] decreased the rate at which *P. ochraceus* added calcareous material as it does in other species, but the lack of a continuous calcified shell or test in *P. ochraceus* allowed soft tissue growth to continue despite reduced calcification. Whatever the cause, we found no apparent negative effects of reduced calcification on the growth, feeding, and survival of *P. ochraceus* during our experiment. However, the long-term fitness consequences of reduced calcification in sea stars are unknown.

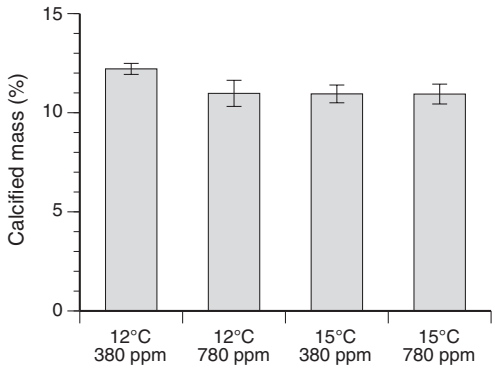

Fig. 4. Mean proportion of sea star wet mass consisting of calcified material under 4 factorial temperature and [CO_2] combinations. Error bars represent ±1 SE of the mean. To account for the confounding effect of sea star size on feeding rate, data were adjusted to the approximate median sea star wet mass (12 g).

Despite the reduction in relative calcified mass with increased [CO_2], the overall effect of [CO_2] on growth was positive. The reasons for the observed increase in growth with elevated [CO_2] are somewhat unclear. The ratio of dry soft tissue mass to water mass remained unchanged by temperature or [CO_2], suggesting that the change in relative calcified mass must have been caused at least in part by an increase in the rate of wet soft tissue growth. Because we could not measure change in calcified mass over the course of the experiment, it is unclear whether the rate of calcified tissue growth simply remained the same as that of sea stars reared at control [CO_2] (thereby failing to keep pace with the increased soft tissue growth) or declined compared to that of control [CO_2] sea stars. Experiments specifically testing sea star calcification rates under control and high [CO_2] conditions will be necessary to answer this question.

Although the unchanged ratio of dry soft tissue mass to water mass demonstrates that the greater growth of sea stars reared at high [CO_2] was primarily because of increased wet soft tissue growth, it does not explain the mechanism behind this increase. The nonsignificant trend of increased feeding with increased [CO_2] suggests that although feeding rate may be partially responsible for the increase in growth rate, there are likely additional factors contributing to this change. It is possible that elevated [CO_2] increases resource use efficiency; for example, the slightly lower pH of high-CO_2 seawater could aid in the digestion of prey tissue, making feeding less energetically costly. Alternatively, low level stressors such as low doses of toxins can elicit positive responses such as increased growth in plants, invertebrates, and vertebrates, a phenomenon referred to as hormesis (26); the stress of reduced pH or carbonate availability may elicit a similar response in sea stars. Identification of the precise mechanism driving the increase in wet soft tissue growth with elevated [CO_2] will require further, more physiologically based experiments.

Several studies have found predominantly negative and nonadditive effects of multiple climate variables on the growth and survival of marine organisms (13–15). The lack of a similar negative or synergistic response in *P. ochraceus* could be explained by the relative thermal tolerances and environments of sea stars vs. many previously studied species. For example, tropical corals often live at or near their thermal tolerance for water temperature (8) and are generally experimentally manipulated at or near these levels (e.g., ref. 14). Therefore, the effect of any additional stress may be magnified. *P. ochraceus*, in contrast, was well within its thermal range in our experiments and, as we have shown here, is unlikely to surpass its optimal water temperature with future climate change in much of its geographic range. Our findings also suggest that the nature of an interaction between climate variables depends on the response variable being measured, even for the same species. In the case of *P. ochraceus*, we found that temperature and [CO_2] had an antagonistic interaction in their effects on relative calcified material, whereas they had a positive and additive effect on overall growth rates. These within-species differences in the interaction between and effects of combined temperature and [CO_2] add an additional level of complexity when attempting to categorize the interactions between multiple climate variables.

Our findings suggest that caution should be exercised when predicting species' responses to climate change on the basis of broad phylogenetic relationships alone. Negative responses to OA in ophiuroids (brittle stars, ref. 27) and echinoids (sea urchins, ref. 11), both in the phylum Echinodermata, have led to overgeneralized predictions that echinoderms will respond neg-

Table 1. Marine invertebrate growth responses to ocean acidification

Phylum, class	Species	$CaCO_3$ skeleton	$[CO_2]$ (ppm)	Calc.*	Growth†	Reference
Echinodermata						
Echinoidea	*Echinometra mathaei*	A	550	ND¶	−	1
Ophiuroidea	*Amphiura filiformis*	A/B	≈1,000‡	+	+/−	7
Asteroidea	*Pisaster ochraceus*	B	780	−	+	Present study
Mollusca						
Bivalvia	*Crassostrea gigas*	A	740	−	ND	6
Bivalvia	*Mytilus edulis*	A	740	−	ND	6
Gastropoda	*Strombus luhuanus*	A	550	ND	−	1
Gastropoda	*Clio pyramidata*	A	≈780‡	−	ND	2
Cephalopoda	*Sepia officinalis*	B	4,000	=	=	37
Arthropoda						
Crustacea	*Palaemon pacificus*	A	1,000	ND	=	38
Crustacea	*Acartia tsuensis*	A	2,380	ND	=	39
Cnidaria						
Anthozoa	*Montipora capitata*	A	≈745	−	−	40
Anthozoa	*Acropora cervicornis*	A	750	ND	−	41

Growth responses of marine invertebrates to ocean acidification with regard to taxon and skeletal type are shown. We distinguish between 2 types of $CaCO_3$ skeletons: (A) encasing skeleton within which the majority of somatic and reproductive tissue is enclosed (e.g., shells and tests) and (B) nonencasing skeletal structures that are embedded within the soft tissues (e.g., spicules and ossicles). Note that this is not intended to be an exhaustive list of studies, but rather a representative sample of studies using climatically realistic increases of $[CO_2]$.

*Effect on calcified material.

†Effect on overall growth.

‡In some studies the seawater pH alone was reported; in such cases, we estimated the $[CO_2]$ on the basis of similar studies with similar pH changes.

¶No data.

atively to OA. However, the lack of a negative effect of OA on sea star growth in our study demonstrates that this prediction cannot be extended to all echinoderms. We also suggest that the differences in responses to OA in *P. ochraceus* vs. previously studied echinoderms could be because of the lack of a continuous calcified endo- or exoskeleton in *P. ochraceus*. Further studies should be conducted on the responses of other less-calcified members of taxa in which other members have shown negative responses. Although obvious examples exist, such as nudibranchs and many cephalopods within the predominantly shelled Mollusca, it could be that even subtle variation in the location or relative amount of calcareous tissue—as is seen among species of bivalves, for example—is an important consideration when predicting biological responses to OA.

The ecological implications of our findings should also be considered, because *P. ochraceus* plays a keystone role in rocky intertidal communities (6, 7, 20–22). An increase in *P. ochraceus* growth, even if only within the juvenile life stage, could lead to higher lifetime feeding rates because faster-growing sea stars would likely reach adult size classes sooner, thereby spending greater time in larger size classes that have higher per capita feeding rates. This increase in predation rates will be even more pronounced if *P. ochraceus*' prey, many of which are heavily calcified, respond negatively to climate change, potentially resulting in a mismatch between predator and prey through changes in their relative sizes. Increased sea star growth rates could also have population-level consequences. Faster-growing sea stars would spend less time in vulnerable small size classes (28), potentially increasing survival rates and lifetime fecundity. We acknowledge, however, that these predicted responses could be moderated by density-dependent effects and negative feedbacks on sea star survival and growth. Needless to say, changes in sea star population growth will be complex and difficult to fully predict.

As we have demonstrated here, responses to anthropogenic climate change, including ocean acidification, will not always be negative. This is an especially important consideration when attempting to make taxon-specific predictions about responses to OA. Furthermore, species-specific responses could have serious ecological consequences when interacting species show different or opposing responses to climate change (23, 29–31). In the rapidly expanding study of the biological consequences of ocean acidification, there is an understandable tendency to focus on calcified organisms that are likely to show easily measured and generally negative responses to experimental acidification. Some ecologically important species, however, may directly benefit from acidification, even within phyla that have traditionally been assumed to respond negatively to OA.

Methods

Study Species and Collection Site. The sea star *P. ochraceus* is a marine intertidal predator found from Alaska to Baja California (32). *P. ochraceus* commonly feeds on mussels, barnacles, and gastropods, with its dominant prey source being mussels of the genus *Mytilus* (24). In protected areas such as the Strait of Georgia where our study was conducted, *Mytilus trossulus* makes up the majority of *P. ochraceus*' diet (24). For this reason, and to facilitate comparisons with earlier work (6, 7), *M. trossulus* was used as prey in this study.

Juvenile sea stars (3–7 g initial wet mass) were used for all experiments in this study. Juveniles were chosen because they exhibit greater scope for growth. Furthermore, because they are not yet reproductively mature (reproductive maturity generally is not achieved until at least 70–95 g wet mass; ref. 33), excess energy is not put toward reproductive growth. All animals used in this study were collected from Jericho Beach in Vancouver, British Columbia, Canada (49.27° N, 123.2° W), in January 2008 (temperature experiment) and April 2008 (temp × CO_2 experiment). The water temperature in this area ranges from a monthly mean of ≈6 °C in February to ≈16 °C in August (34) and is predicted to increase overall by ≈1.5 °C by 2040 (35). Once collected, all sea stars were held in a recirculating seawater system maintained at 13 °C for at least 4 weeks before experimentation.

Sea Star Growth and Feeding Rates with Temperature. Juvenile *P. ochraceus* (wet mass = 4.65 ± 0.19 g; all values reported are mean ± SE) were randomly assigned to one of 24 aquaria, which were set to different temperatures ranging from 5 °C to 21 °C. Each ≈246-L tank was an independent unit with recirculating natural seawater bubbled constantly with ambient air and equipped with a multistage filter system that included a biological filter and UV sterilizer. Water temperatures were maintained to ±0.5 °C of the set temperature using external chillers and were measured at least 3 times a week with a mercury thermometer. The mean temperature for each tank was used

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for statistical analyses. Each sea star was housed in its own 8 × 10 × 10-cm plastic container with mesh sides and top to allow water to flow through. Two containers were then randomly assigned to each tank; there was no trend between the experimental temperature and initial sea star size (simple linear regression: $P > 0.1$, df = 37, $R^2 = 0.02$). At the beginning of the experiment, initial wet mass was measured to the nearest 0.01 g. For all wet mass measurements, each sea star was removed from the water, gently patted dry with a paper towel, immediately weighed on a scale, and then returned to the water. The sea stars acclimated in their assigned tanks at 12.8 °C for 4 days without food, then the tanks were changed to their experimental temperatures over an 8-h period, and finally the sea stars acclimated for an additional 6 days without food.

After the acclimation period, 20 small mussels (15 ± 2 mm shell length) were placed in each container. Empty shells were removed, recorded, and replaced with live mussels every other day. No sea star ran out of mussels during the course of the experiment. Wet mass was measured weekly. After 21 days of feeding, sea stars were wet weighed and all mussels were removed. To control for any effect of water temperature on water retention in the sea stars, tank temperatures were brought back to 12.8 °C over an 8-h period and sea stars reacclimated to this temperature for an additional 48 h without food before their final wet mass was measured.

Sea Star Growth and Feeding Rates with Temperature and [CO_2]. Juvenile sea stars (wet mass = 4.25 ± 0.10 g) were randomly assigned to 1 of 4 treatments: 12 °C and 380 ppm CO_2 ($n = 5$), 12 °C and 780 ppm CO_2 ($n = 6$), 15 °C and 380 ppm CO_2 ($n = 6$), and 15 °C and 780 ppm CO_2 ($n = 5$). These combinations were chosen to approximate current and predicted future levels of change by the year 2100 (Intergovernmental Panel on Climate Change IS92a emissions scenario). The tanks and containers were the same as those described in the previous experiment. Tanks were assigned to treatments using a stratified random design. Temperature was maintained using external tank chillers as above, while CO_2 concentrations were maintained using mass flow controllers to constantly bubble the tanks with either ambient air (containing ≈380 ppm CO_2) run through an air compressor or the appropriate mixture of compressed CO_2 (2% CO_2 with balance air; Praxair) and ambient air from an air compressor. The tanks were covered with lids to help maintain the desired [CO_2] in the tank headspace and seawater.

Two sea stars, each in their own container, were randomly assigned to each tank. The mean initial sea star wet mass did not differ between treatments (1-way ANOVA: $F_{3,40} = 1.02$, $P = 0.395$). Sea stars acclimated in their tanks without food for 9 days while the tanks equilibrated to experimental conditions. The initial wet weights were measured, and then sea stars were fed mussels (shell length = 17 ± 2 mm) ad libitum for the remainder of the experiment. Tank temperatures were measured at least 3 times a week. Tank pH's were also measured frequently to ±0.01 pH units with a portable pH meter (YSI 556-MPS) calibrated at the appropriate experimental temperatures (12 °C and 15 °C). The mean standard deviation of within-tank pH over time was ±0.015, which was far smaller than the SD between tanks as well as between treatments. The mean pH of each tank was used to determine treatment means. Sea stars were wet weighed weekly. After 10 weeks, final wet mass was measured. Sea stars were then dried to constant mass in an oven at 70 °C and placed in ≈125 mL of a 10% bleach solution for 48–72 h to remove their soft tissue. The solution was then vacuum filtered on No. 1 Whatman filter paper to collect the calcified material, which was then dried at 70 °C to constant mass and reweighed.

Statistical Analyses. To avoid pseudoreplication, all biological variables measured were averaged for the 2 sea stars in each tank, and these tank means were used in all statistical analyses. The only exceptions to this were the analyses to determine if the mean initial sea star wet weights were the same across all treatments; individual sea star wet weights were used to calculate these means in both experiments. In the temperature-only experiment, separate simple linear regressions were used to determine the effect of water temperature on relative sea star growth rate [(final grams wet mass – initial grams wet mass)/initial grams wet mass × 100] and per capita feeding rate (number of mussels consumed daily per sea star). Although a second-order polynomial model was initially used to analyze the relationship between temperature and feeding rate, the polynomial term was nonsignificant. Therefore, we present a linear model in the results.

In the 2 × 2 factorial experiment, a 2-way ANOVA was also used to determine the effects of temperature and [CO_2] on relative sea star growth (percentage of gain relative to initial sea star wet mass). Separate ANCOVAs were used to determine the effects of temperature and [CO_2] on sea star feeding rates (number of mussels consumed daily per sea star), percentage of wet mass consisting of calcareous material, and the ratio of dry soft tissue mass to water mass. Because absolute sea star size can affect all of these variables, sea star wet mass was initially included as a covariate in all analyses. When size effects were nonsignificant ($P > 0.1$), they were removed from the analyses. Feeding rate and relative growth data for the factorial experiment were log transformed to equalize variances, and all data were analyzed using JMP 8 (SAS Institute).

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1. Intergovernmental Panel on Climate Change (2007) *Climate Change: The Physical Science Basis* (Cambridge Univ Press, Cambridge, UK).
2. Feely RA, et al. (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* 305:362–366.
3. Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42.
4. Harley CDG, et al. (2006) The impacts of climate change in coastal marine systems. *Ecol Lett* 9:228–241.
5. Southward AJ, Hawkins SJ, Burrows MT (1995) Seventy years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the Western English Channel in relation to rising sea temperature. *J Therm Biol* 20:127–155.
6. Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. *Science* 283:2095–2097.
7. Sanford E (2002) The feeding, growth, and energetics of two rocky intertidal predators (*Pisaster ochraceus* and *Nucella canaliculata*) under water temperatures simulating episodic upwelling. *J Exp Mar Biol Ecol* 273:199–218.
8. Hughes TP, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* 301:929–933.
9. Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J Mar Sci* 65:414–432.
10. Riebesell U, et al. (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* 407:364–367.
11. Shirayama Y, Thornton H (2005) Effect of increased atmospheric CO_2 on shallow water marine benthos. *J Geophys Res* 110: C09S08, doi:10.1029/2004JC002618.
12. Michaelidis B, Ouzounis C, Paleras A, Portner HO (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar Ecol Prog Ser* 293:109–118.
13. Hoffman JR, Hansen LJ, Klinger T (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J Phycol* 39:268–272.
14. Reynaud S, et al. (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biol* 9:1660–1668.
15. Darling ES, Cote IM (2008) Quantifying the evidence for ecological synergies. *Ecol Lett* 11:1278–1286.
16. Metzger R, Sartoris FJ, Langenbuch M, Portner HO (2007) Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. *J Thermal Biol* 32:144–151.
17. O'Donnell MJ, Hammond LM, Hofmann GE (2009) Predicted impact of ocean acidification on a marine invertebrate: Elevated CO_2 alters response to thermal stress in sea urchin larvae. *Mar Biol* 156:439–446.
18. Cavey MJ, Markel K (1994) In *Microscopic Anatomy of Invertebrates: Echinodermata*, eds Harrison FW, Chia F-S (Wiley-Liss, New York), Vol 14, pp 247–344.
19. LeClair EE (1993) Effects of anatomy and environment on the relative preservability of Asteroids: a biomechanical comparison. *Palaios* 8:233–243.
20. Paine RT (1966) Food web complexity and species diversity. *Am Nat* 100:65–75.
21. Paine RT (1969) A note on trophic complexity and community stability. *Ecology* 50:950–961.
22. Navarrette SA, Menge BA, Daley BA (2000) Species interactions in intertidal food webs: Prey of predation regulation of intermediate predators? *Ecology* 81:2264–2277.
23. Hall-Spencer JM, et al. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454:96–99.
24. Harley CDG, et al. (2006) Color polymorphism and genetic structure in the sea star *Pisaster ochraceus*. *Biol Bull* 211:248–262.
25. Blanchette CA, Helmuth B, Gaines SD (2007) Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at Point Conception, California, USA. *J Exp Mar Biol Ecol* 340:126–148.
26. Calabrese EJ, Baldwin LA (2001) Hormesis: A generalizable and unifying hypothesis. *Crit Rev Toxicol* 31:353–424.
27. Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proc R Soc B* 275:1767–1773.
28. Sewell MA, Watson JC (1993) A ''source'' for asteroid larvae?: Recruitment of *Pisaster ochraceus*, *Pycnopodia helianthoides* and *Dermasterias imbricate* in Nootka Sound, British Columbia. *Mar Biol* 117:387–398.
29. Edwards M, Richardson AJ (2004) Impact of climate change on marine phenology and trophic mismatch. *Nature* 430:881–884.
30. Winder M, Schindler DE (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology* 85:2100–2106.

31. Wootton JT, Pfister CA, Forester JD (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *PNAS* 105:18848–18853.
32. Morris RH, Abbott DP, Haderlie EC (1980) *Intertidal Invertebrates of California* (Stanford Univ Press, Stanford, CA).
33. Menge JM, Menge BA (1974) Role of resource allocation, aggression and spatial heterogeneity in coexistence of two competing intertidal starfish. *Ecol Monogr* 44:189–209.
34. National Oceanographic Data Center (2001) *World Ocean Atlas Data Set* (NODC, Silver Spring, MD).
35. Mote PW, Salathé EP, Dulière V, Jump E (2008) *Scenarios of Future Climate Change for the Pacific Northwest* (Univ of Washington, Seattle), report prepared by the Climate Impacts Group, Center for Science in the Earth System, Joint Institute for the Study of the Atmosphere and Oceans.
36. Gazeau F, et al. (2007) Impact of elevated CO_2 on shellfish calcification. *Geophysical Research Lett* 34: L07603, doi: 10,1029/2006GL028554.
37. Gutowska MA, Portner HO, Melzner F (2008) Growth and calcification in the cephalopod *Sepia officinalis* under elevated seawater pCO_2. *Mar Ecol Prog Ser* 373:303–309.
38. Kurihara H, Matsui M, Furukawa H, Hayashi M, Ishimatsu A (2008) Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J Exp Mar Biol Ecol* 367:41–46.
39. Kurihara H, Ishimatsu A (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Mar Poll Bull* 56:1086–1090.
40. Jokiel PL, et al. (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs* 27:473–483.
41. Reneger DA, Riegl BM (2005) Effect of nutrient enrichment and elevated CO_2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. *Mar Ecol Prog Ser* 293:69–76.