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Elevated pCO_2 is less detrimental than increased temperature to early development of the giant kelp, *Macrocystis pyrifera* (Phaeophyceae, Laminariales)

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Abstract: Global climate change is increasing ocean temperature and partial pressure of CO_2 (pCO_2) in coastal and marine ecosystems. Research in this field has largely focused on how limited CO_3^{2-} availability and low pH adversely affect early development of calcifying organisms, but noncalcareous organisms are comparatively understudied despite their prevalence in many coastal communities. We investigated how present-day and future levels of ocean temperature (12°C vs 15°C, respectively) and pCO_2 (400 μatm vs 1500 μatm, respectively) influence successful germling production, gametophyte survival, growth, and sex ratio, and embryonic sporophyte production and growth in the habitat-forming kelp *Macrocystis pyrifera* over a 15-wk period in San Diego, California, USA. Our results indicate that relative to present-day conditions, successful germling production was reduced fourfold under elevated temperature alone, and fivefold under combined elevated temperature and pCO_2 (i.e. "future conditions"). Similarly, survival and growth of male and female gametophytes were lower under elevated temperature alone than under either present-day, elevated pCO_2 alone, or future conditions. Gametophyte sex ratios skewed slightly toward males across all treatments. Sporophyte recruitment and growth were greatest and occurred earliest under elevated pCO_2 alone, but were delayed under elevated temperature alone. Although elevated pCO_2 and temperature adversely affected germling production independently and cumulatively, elevated pCO_2 enhanced gametophyte and sporophyte survival under both present-day and elevated temperatures. Thus, under projected climate change conditions, elevated pCO_2 may be less detrimental than increased temperature for development beyond germling production. Given that *M. pyrifera* is globally distributed and provides numerous ecosystem services including the potential to mitigate ocean acidification, impacts of climate change on its complex life history merit further exploration.

Key words: Carbon dioxide, Climate change, Gametophyte, Germling, Global warming, Kelp forest, Marine habitat, Ocean acidification, Sex ratio, Sporophyte

INTRODUCTION

Climate change is dramatically altering biological processes and community assemblages in coastal ecosystems worldwide (Walther *et al*. 2002; Harley *et al*. 2006; Seifert *et al*. 2015; Poloczanska *et al*. 2016). Fossil fuel combustion and land use change have increased atmospheric [CO_2] from *c*. 280 parts per million (ppm) in the preindustrial era to their current level of *c*. 400 ppm, whereas global air and sea-surface temperatures have concomitantly increased [Intergovernmental Panel on Climate Change (IPCC) 2013]. The ocean has retained 20 times more heat than the atmosphere since the mid-20th century and absorbed ~50% of CO_2 released since 1800, leading to increased seawater temperature and ocean acidification (Sabine *et al*. 2004; Levitus *et al*. 2005). By 2150, sea surface temperatures are projected to increase by an additional 1.4–5.8°C and seawater pH is projected to decrease by an additional 0.3 units, with a tripling of atmospheric CO_2 concentrations (Caldeira & Wickett 2003; RCP 8.5; IPCC 2013), further affecting coastal organisms.

Decreased seawater pH reduces CO_3^{2-} availability and impairs $CaCO_3$ structure formation in calcareous organisms (Jokiel *et al*. 2008; Doney *et al*. 2009; reviewed by Kroeker *et al*. 2013a). Although ocean acidification and warming negatively affect many adult organisms (e.g. Gazeau *et al*. 2010; Tateda *et al*. 2015), it is their early life stages that may be especially vulnerable (Munday *et al*. 2011; Rivest & Hofmann 2014). Given that these life stages often serve as population bottlenecks (Underwood & Fairweather 1989), identifying how they are affected may be crucial to a larger understanding of how climate change will affect coastal ecosystems. Therefore, much of the research in this field has been devoted to understanding how changes in ocean conditions cumulatively affect early development when $CaCO_3$ deposition first occurs (Green *et al*. 2004; Kurihara 2008; Dupont *et al*. 2013), and if these effects persist through subsequent life stages (Beckerman *et al*. 2002; Hettinger *et al*. 2012; Parker *et al*. 2012). However, there remains a general lack of understanding about how noncalcifying organisms will respond to changing ocean conditions (Porzio *et al*. 2011; reviewed by Harley *et al*. 2012; Koch *et al*. 2013). The net effect of ocean acidification on fleshy macroalgae in particular is equivocal since elevated partial pressure of CO_2 (pCO_2) may be beneficial for photosynthesis and therefore mitigate the negative effects of increasingly acidic seawater (Hepburn *et al*. 2011; Cornwall *et al*. 2012; Brown *et al*. 2014; Fernandez *et al*. 2015; Sunday *et al*. 2017).

Kelp forests serve critical roles in temperate to subpolar marine environments. The giant kelp *Macrocystis pyrifera* (Linnaeus) C.Agardh (Phaeophyceae: Laminariales) provides complex, three-dimensional habitat and forms the base

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of food webs for several economically important organisms (Turner *et al*. 1968; Graham *et al*. 2007; Schiel & Foster 2015). Kelps exhibit heteromorphic life histories that alternate between microscopic and macroscopic phases (Neushul 1963). Specifically, haploid zoospores are released from reproductive sori on the sporophylls of diploid sporophytes and disperse across a range of distances (Carney *et al*. 2013). Upon settlement, germlings evacuate the original germ wall of the zoospore and undergo gametogenesis, resulting in male and female gametophytes that produce sperm and eggs, respectively. After motile sperm fertilize the female gametophytes, zygotes are formed that eventually develop into diploid sporophytes.

Investigating the development of sensitive microscopic life stages may ultimately be the most informative for understanding how climate change will affect *M. pyrifera* populations. Successful kelp sporophyte recruitment relies on a multitude of physical and biological factors, including irradiance, water temperature, nutrients, and substrate availability (Lüning & Neushul 1978; Deysher & Dean 1986a, Amsler & Neushul 1990; Reed *et al*. 2004). However, little is known about how climate change will affect sporophyte recruitment or development into macroscopic adults (Harley *et al*. 2012; Brown *et al*. 2014). Although elevated temperature can be either beneficial or detrimental to primary production in their microscopic and macroscopic life stages (e.g. Moreau *et al*. 2015; Schoenrock *et al*. 2015), macroalgae are capable of acclimating to a wide variety of environmental conditions, which may facilitate their survival in warmer, more acidic waters (Duarte & Ferreira 1995; Eggert *et al*. 2007). For example, high pCO_2 conditions have been shown to accelerate oogonium formation, but not affect germination rates or sporogenesis in female gametophytes of the kelp *Laminaria hyperborea* (Olischläger *et al*. 2012). Further, zoospores of the intertidal kelp *Egregia menziesii* decrease swimming speeds under elevated pCO_2 and temperature conditions (Hoos 2015). In *M. pyrifera* sporophytes, elevated pCO_2 and temperature synergistically increase meristem growth and photosynthetic carbon uptake (Brown *et al*. 2014), but also decrease germination rates and zoospore survival (Gaitán-Espitia *et al*. 2014). However, low pH coincident with elevated pCO_2 does not appear to differentially affect the survival of male and female *M. pyrifera* gametophytes or alter their sex ratios (Roleda *et al*. 2012; Leal *et al*. 2017). Although these studies are not comprehensive, they exemplify the immense variation in the sensitivity of different kelp life stages to climate change stressors and underscore our need to understand how each of these stages will respond to global climate change.

In this study, we quantified the independent and interactive effects of elevated pCO_2 and temperature on *M. pyrifera* microscopic life stages by measuring changes in density (hereafter "production") and the longest axis lengths of individuals (hereafter "growth") in each treatment. Specifically, we assessed variation in germling production, gametophyte survival, growth, and sex ratios, and in embryonic sporophyte production and growth between present-day and elevated temperatures (12°C vs 15°C, respectively) and pCO_2 (400 µatm vs 1500 µatm, respectively) levels that may be expected along the southern California coast. We hypothesized that increased pCO_2 and temperature together would antagonistically affect the development of these early life stages.

MATERIAL AND METHODS

To create seawater with different temperature and pCO_2 conditions, 10 litres of seawater were collected near the Point Loma kelp forest, San Diego, California every week for 15 wk (April–August 2014) and held in two airtight 5-litre Nalgene carboys with no headspace. For each collection, the carboys were immediately transferred to San Diego State University's (SDSU's) Coastal and Marine Institute Laboratory and held in the dark at 12°C within a temperature-controlled room until the seawater pCO_2 concentrations could be manipulated (usually within 24 h). The seawater within the carboys was first analyzed for total alkalinity (TA) and total inorganic carbon (TIC) using potentiometric acid titration as described by Millero *et al*. (1993). These values were then used to calculate seawater pCO_2 within the carboys using the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/) as described in Riebesell *et al*. (2010). The precisions of our TA and TIC estimates were checked every other day (three to four times per week) using Certified Reference Materials (provided by A. Dickson, Scripps Institution of Oceanography) and were approximately ±5 µmol kg^{-1} and ±2 µmol kg^{-1}, respectively. After this, a certified CO_2–air mixture of 1500 ppm CO_2 (Praxair, San Diego, California USA) was bubbled into one of the carboys through an air stone for 2 h to elevate seawater pCO_2 conditions to *c*. 1500 µatm pCO_2 (7.3 pH units) as may be seen by the year 2200 (Caldeira & Wickett 2003), whereas ambient air was similarly bubbled into the other carboy to create present-day pCO_2 seawater conditions of *c*. 400 µatm pCO_2 (7.9 pH units). After 2 h of bubbling, potentiometric titrations of the seawater were again done to confirm that the desired pCO_2 levels had been reached, and additional bubbling of the gasses was done if needed (See Results, Fig. 1). After this, 4 litres of seawater was siphoned from each carboy and transferred into separate 1-litre airtight Nalgene bottles while being careful not to introduce air bubbles. This resulted in eight 1-litre Nalgene bottles, four filled with 400 µatm pCO_2 seawater and four filled with 1500 µatm pCO_2 seawater. Two bottles from each pCO_2 treatment were then placed into a Percival 6EL incubator (Percival Scientific Inc., Perry, Iowa USA) that was set at 12°C, whereas the remaining two bottles from each treatment were placed in a second incubator set at 15°C. This resulted in orthogonal combinations of seawater with two temperatures and two pCO_2 levels; hereafter referred to as present-day (12°C, 400 µatm pCO_2), elevated temperature alone (15°C, 400 µatm pCO_2), elevated pCO_2 alone (12°C, 1500 µatm pCO_2), and future (15°C, 1500 µatm pCO_2) conditions.

To investigate the effects of elevated seawater temperature and pCO_2 on *M. pyrifera* (Linnaeus) C. Agardh germling production, fertile sporophylls (sori-bearing reproductive blades) were collected from haphazardly selected *M. pyrifera* sporophytes within the Point Loma kelp forest (32°69.7′N, 117°26.6′W) in April 2014. The sporophylls were transported to the laboratory in dark coolers where they were wiped clean

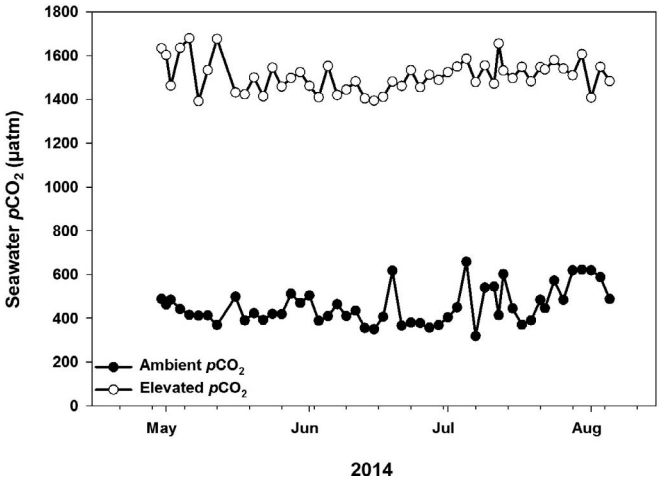


Fig. 1. Temporal variation in seawater pCO_2 within the carboys used to make the present-day and elevated-temperature (i.e., 400 µatm pCO_2) and the elevated-pCO_2 and future (i.e., 1500 µatm pCO_2) treatments over the 15-wk experiment (30 April to 5 August 2014).

with damp paper towels to remove epibiota and desiccated in the dark at 4°C for 3 h as described by Carney (2011). Sporophylls were then immersed in 12°C, 400-µatm pCO_2 filtered seawater to induce zoospore release. The density of swimming zoospores within the stock solution was estimated using a hemocytometer, and the solution was diluted with new filtered seawater until densities of *c.* 7×10^6 swimming zoospores ml^{-1} were obtained. Five-millilitre aliquots of this zoospore solution were then added to separate 100×15 mm Petri dishes ($n = 10$ dishes per temperature–pCO_2 combination) containing 85 ml of seawater from one of the four temperature–pCO_2 combinations described above (i.e. present-day, elevated temperature alone, elevated pCO_2 alone, and future conditions). The dishes were then placed into one of two Percival 6EL incubators and held in the dark overnight at their respective temperatures (12°C and 15°C) to allow zoospore settlement (Carney & Edwards 2010). The following morning, the lights within the incubators were turned on and set to a 12:12 light:dark photoperiod with irradiances of 15–20 µmol photons m^{-2} s^{-1}, which were representative of the levels observed at the benthos near the collection site (M. S. Edwards unpublished data). Treatment seawater was changed every 24 h and successful germling production within the Petri dishes was estimated after 72 h by examining three haphazardly selected fields of view (FOV = 1.19 mm^2) in each dish using an inverted microscope at ×400 magnification. Each FOV was photographed using LAS EZ (Leica Microsystems, Heerbrugg, Switzerland) and Image J software was used to count germlings that had successfully evacuated the zoospore germ wall (i.e. they had undergone gametogenesis).

To examine the effects of elevated seawater temperature and pCO_2 on the survival, growth, and development of *M. pyrifera* gametophytes and embryonic sporophytes, zoospores were again released into 12°C, 400-µatm pCO_2 seawater as described above. The density of swimming zoospores in the solution was determined using a hemocytometer, and the zoospore solution was diluted with new filtered seawater until densities of *c.* 7×10^6 swimming zoospores ml^{-1} were obtained. To facilitate settlement densities of *c.* 100–150 spores mm^{-2}, which we considered ideal for our cultures of *M. pyrifera* (see Reed *et al.* 1991), 5-ml aliquots of this zoospore solution were added to Petri dishes ($n = 5$ dishes per temperature-pCO_2 combination) containing 85 ml of filtered seawater from one of the four temperature–pCO_2 combinations described above (i.e. present-day, elevated temperature alone, elevated pCO_2 alone, and future conditions). Separate Petri dishes were then held in the incubators under their respective temperature–pCO_2 conditions for 15 wk, during which time they were evaluated for gametophyte survival and growth, and for embryonic sporophyte production and growth. Seawater in each dish was replaced with new seawater of the corresponding temperature–pCO_2 conditions every 48 h, with replacement seawater prepared ~24 h before each water change as described above. We did not initially elevate NO_3^- concentrations because zoospores exhibit high settlement in nutrient-enriched environments and the presence of nutrients might have influenced initial settlement densities (Amsler & Neushul 1990). But starting in week 11, 0.5 ml of nitrate (8.82×10^{-4} M $NaNO_3$; Guillard's F/2) was added to each 500-ml Nalgene bottle described above to compensate for the seawater becoming nutrient depleted ($\leq$ 1 µmol NO_3^- l^{-1}). Gametophyte survival and growth as well as embryonic sporophyte production and growth within the dishes were assessed each week using an inverted microscope at ×400 magnification, and LAS EZ and Image J software. Male and female gametophytes were differentiated from one another on the basis of their distinct morphologies. Specifically, male gametophytes were identified by their smaller cells and filamentous branching, whereas female gametophytes were identified by their larger cells and little to no branching. Changes in the densities of each were used to estimate gametophyte survival and embryonic sporophyte production, whereas changes in the longest axis length of 15 male and 15 female gametophytes within each FOV during weeks 3–11 were used to estimate gametophyte growth. Weeks 12–15 were omitted from this analysis because the largest gametophytes had begun producing sporophytes and thus disappeared, thereby confounding reliable estimates of growth. Consequently, the longest axis length of 10 embryonic sporophytes was measured in each FOV during weeks 12–15 to estimate their growth in each of the four temperature–pCO_2 treatments. To estimate gametophyte sex ratios, male and female gametophytes were both counted ($n \geq 85$ gametophytes per replicate Petri dish) and their sex ratios were determined on the basis of the proportion of males in each dish [sex ratio = # males/(# males + # females)] as per Roleda *et al.* (2012) and Leal *et al.* (in press).

All statistical analyses were done using SYSTAT v12. Before analyses, data were checked for normality by graphical examination of their residuals, and for equality of variances using Bartlett's or Levene's tests. Any data not meeting the assumptions of parametric statistics were transformed and retested to ensure the problems were corrected. If problems with normality could not be corrected with transformation, the appropriate nonparametric test was used. Data for seawater NO_3^- concentration were non-

Table 1. Two-way model I ANOVA testing differences in *Macrocystis pyrifera* germling densities among temperatures (12°C vs 15°C) and seawater pCO_2 conditions (400 vs 1500 µatm pCO_2). Boldface type denotes significance ($P < 0.05$). Data were log transformed before analysis to correct for problems with normality. The relative amount (%) of variation explained by each factor is denoted by its magnitude of effect (ω^2) as calculated according to Graham and Edwards (2001).

Source	*v*	Mean square	*F*	*P*	ω^2
Temperature	1	3.995	97.131	< **0.001**	0.69
pCO_2 treatment	1	0.214	5.213	**0.028**	0.03
Temperature × pCO_2 treatment	1	0.037	0.906	0.348	< 0.01
Error	36	0.041			0.28

normal and could not be fixed by transformation. Therefore, a Mann–Whitney *U* test was used to confirm that NO_3^- levels within the seawater indeed differed between the weeks before (weeks 1–10) vs after (weeks 11–15) the Guillard's F/2 NO_3^- addition (see Results). Data for germling production (# individuals mm^{-2}) within the Petri dishes were heteroscedastic and therefore log transformed, which corrected the problem. A two-way model I ANOVA was then used to evaluate if germling production differed between pCO_2 conditions and seawater temperatures as described above. Proportional gametophyte survival data (# gametophytes at week 11/# gametophytes at week 1) were square root and arcsin transformed, and a two-way model I ANOVA was used to evaluate if proportional gametophyte survival at week 11 (i.e. before embryonic sporophyte production began) differed among the pCO_2 and temperature conditions described above. Differences in the longest axis length (i.e. growth measured in micrometres) of male and female gametophytes among the pCO_2 and temperature treatments were evaluated after 11 wk using separate two-factor model I ANOVAs. Independent binomial tests were used to assess if the ratio of male to female gametophytes differed from 50:50 in any of the pCO_2 and temperature treatments. Data for embryonic sporophyte production (# individuals mm^{-2}) at week 15 (i.e. the end of the experiment) were heteroscedastic and square root transformed, which corrected the problem. After this, differences in embryonic sporophyte production among pCO_2 and temperature treatments were evaluated with a two-way model I ANOVA. Last, a two-way model I ANOVA was used to evaluate if the sporophytes' longest axis length (i.e. growth in micrometres) varied between pCO_2 and temperature conditions at the end of the experiment.

In evaluating our statistical results, we recognize the contrasting chances of making type I (i.e. accepting the existence of patterns that are not real) and type II (i.e. failing to identify patterns that are real) errors. Given the consequences for understanding patterns of kelp reproduction under future ocean conditions, we use a significance level of 0.05 for all analyses but discuss trends and present means (± standard error) for all results with significance levels below 0.1. In addition, we provide full statistical results in table form along with estimates of their magnitude of effects [i.e. percent variance explained (ω^2); see Graham & Edwards (2001)] for all analyses.

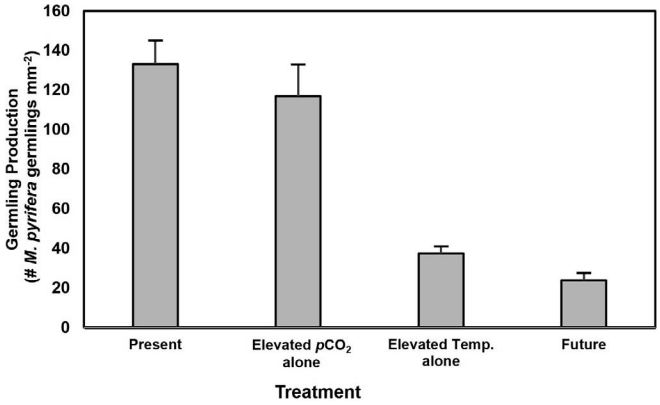


Fig. 2. *Macrocystis pyrifera* germling production (# germlings mm^{-2}, mean + 1 standard error) after 72 h in Petri dishes with two pCO_2 treatments (400 µatm vs 1500 µatm) and two temperature treatments (12°C vs 15°C) ($n = 10$ dishes per temperature–pCO_2 combination).

RESULTS

Seawater temperature and pCO_2 manipulations

Seawater pCO_2 within the carboys used to make the present-day and elevated temperature alone treatments (455 ± 12 µatm pCO_2, mean ± 1 standard error) was consistently *c*. 1000 µatm lower than in the seawater used to make the future and elevated pCO_2 alone treatments (1509 ± 11 µatm pCO_2) over the 15-wk experiment (Fig. 1). Further, the temporal patterns of variability within each pCO_2 level are well within the natural ranges observed within kelp forests during a single month (Koweek *et al.* 2017) or between different locations within a kelp forest on a given day (M.S. Edwards, unpublished data), and thus we do not expect them to significantly affect our results. In addition, temperatures within the temperature-controlled room and the two Percival incubators remained constant at their set temperatures of 12°C and 15°C, resulting in consistent differences among the four temperature-pCO_2 treatments over the 15-wk experiment. In contrast, NO_3^- concentrations in the seawater were significantly lower during weeks 1–10 (i.e. before the Guillard's F/2 NO_3^- addition) than during weeks 11–15 (i.e. after the NO_3^- addition) (1.05 ± 0.17 vs 5.23 ± 0.22 µmol $NO_3^- l^{-1}$, means ± 1 standard error, respectively) (Mann–Whitney *U*, $P = 0.004$), but generally followed natural fluctuations observed near the benthos in the Point Loma kelp forest as described by Carney & Edwards (2010).

Germling production

Overall, *M. pyrifera* germling production was approximately four times greater under 12°C (125 ± 10 germlings mm^{-2}, mean ± 1 standard error) than under 15°C (31 ± 3 germlings mm^{-2}) (ANOVA; $P < 0.001$), and 25% greater in 400 µatm pCO_2 seawater (85 ± 12 germlings mm^{-2}) than in 1500 µatm pCO_2 seawater (70 ± 13 germlings mm^{-2}) ($P = 0.028$) (Table 1, Fig. 2). Further, these two factors did not interact with each other ($P = 0.348$). Thus, although the absolute germling densities varied among the four treatment combinations, the relative differences in germling production between the two

Table 2. Two-way model I ANOVA testing differences in proportional *Macrocystis pyrifera* gametophyte survival at week 11 among temperatures (12°C vs 15°C) and seawater pCO_2 conditions (400 µatm vs 1500 µatm pCO_2). Data were square root and arcsin transformed before analysis to correct for problems with normality. The relative amount (%) of variation explained by each factor is denoted by its magnitude of effect (ω^2) as calculated according to Graham and Edwards (2001).

Source	*v*	Mean square	*F*	*P*	ω^2
Temperature	1	0.063	1.038	0.323	< 0.01
pCO_2 treatment	1	0.162	2.669	0.122	0.07
Temperature × pCO_2 treatment	1	0.205	3.366	0.085	0.10
Error	16	0.061			0.83

temperatures were consistent under both pCO_2 concentrations, and likewise the relative differences in germling production between the two pCO_2 concentrations were consistent under both temperatures. Indeed, the greatest overall germling production was observed in dishes with present-day conditions of 12°C and 400 µatm pCO_2 seawater (133 ± 12 germlings mm^{-2}), and was more than five times greater than the lowest overall production, which was observed in dishes with future conditions of 15°C and 1500 µatm pCO_2 seawater (24 ± 4 germlings mm^{-2}). Together, this indicates that although elevated temperature and elevated pCO_2 negatively affect germling production on their own, antagonistic effects on production resulted from their co-occurrence, as may be expected with future climate change.

Gametophyte survival and growth

Overall, gametophyte survival during the first 11 wk of the experiment (i.e. before embryonic sporophyte production began) did not vary significantly between the two temperatures (ANOVA, $P = 0.323$) or the two pCO_2 levels ($P = 0.122$) (Table 2, Fig. 3). These factors also did not interact with each other ($P = 0.085$), although a general pattern was observed in which gametophyte survival in the elevated temperature-alone treatment (where 28% of the gametophytes survived) was approximately one-half of that in the other three treatments (where 55% to 60% of the gametophytes survived) (Fig. 3).This suggests that elevated temperature alone may be more detrimental to gametophyte survival than elevated pCO_2 alone or when temperature and pCO_2 are both elevated such as expected under future conditions.

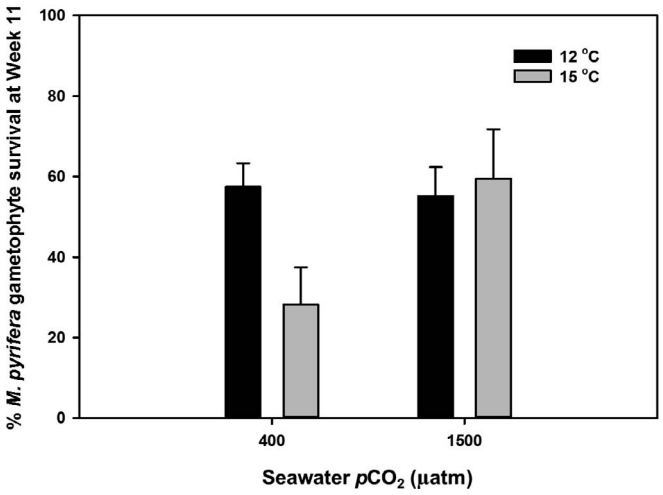


Fig. 3. Proportional survival of *Macrocystis pyrifera* gametophytes (mean + 1 standard error) after 11 wk in Petri dishes before sporophyte recruitment with two pCO_2 treatments (400 µatm vs 1500 µatm) and two temperature treatments (12°C vs 15°C) ($n = 5$ dishes per temperature–pCO_2 combination).

Table 3. Separate two-way model I ANOVAs testing differences in the longest axis lengths (i.e., growth) of male (upper table) and female (lower table) *Macrocystis pyrifera* gametophytes at week 11 among temperatures (12°C vs 15°C) and seawater pCO_2 conditions (400 µatm vs 1500 µatm pCO_2). Boldface type denotes significance ($P < 0.05$). The relative amount (%) of variation explained by each factor is denoted by its magnitude of effect (ω^2) as calculated according to Graham and Edwards (2001).

Source	*v*	Mean square	*F*	*P*	ω^2
Male gametophytes					
Temperature	1	0.0001	0.001	0.975	< 0.01
pCO_2	1	0.109	1.675	0.214	0.02
Temperature × pCO_2	1	0.708	10.88	**0.005**	0.32
Error	16	0.065			0.65
Female gametophytes					
Temperature	1	0.008	0.801	0.384	< 0.01
pCO_2	1	0.037	3.631	0.075	0.11
Temperature × pCO_2	1	0.037	3.687	0.073	0.11
Error	16	0.01			0.79

Male and female gametophytes grew in all temperature–pCO_2 treatment combinations during the first 11 wk of the experiment, with similar responses to the different treatments. Specifically, neither temperature nor pCO_2 alone affected growth in either male (ANOVA, $P = 0.975$ and 0.214, respectively) or female ($P = 0.384$ and 0.075, respectively) gametophytes (Table 3, Fig. 4). However, for male gametophytes, temperature and pCO_2 interacted with each other ($P = 0.005$) such that male gametophyte lengths were greatest under present-day and future conditions. These factors did not interact with each other for female gametophyte growth ($P = 0.073$). Still, general patterns emerged by week 11 of the

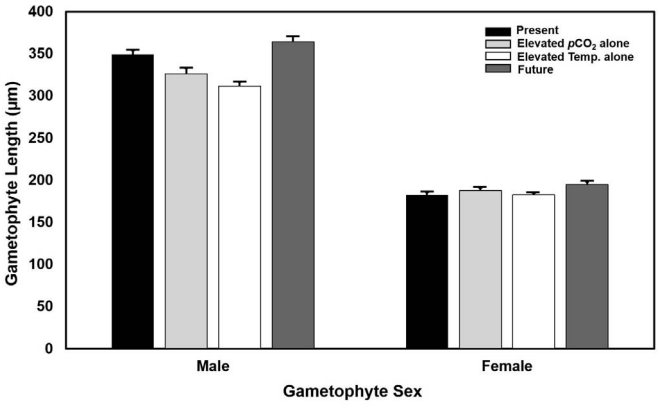


Fig. 4. Length of male and female *Macrocystis pyrifera* gametophytes (micrometres, mean + 1 standard error) after 11 wk under two pCO_2 treatments (400 µatm vs 1500 µatm) and two temperature treatments (12°C vs 15°C) ($n = 15$ dishes per temperature–pCO_2 combination).

Table 4. Results of independent binomial tests of the proportional differences in the numbers of male and female *Macrocystis pyrifera* gametophytes observed at week 11 under present-day (12°C × 400 μatm pCO_2), elevated pCO_2 alone (12°C × 1500 μatm pCO_2), elevated temperature alone (15°C × 400 μatm pCO_2), future conditions (15°C × 1500 μatm pCO_2), and total combined across all treatments. Included are the total number of gametophytes observed in each treatment, the number of males and females observed, and the expected number of each assuming equal sex ratios. Boldface type denotes significance ($P < 0.05$). Note that although there were significantly more male gametophytes observed at week 11, no differences were observed for any of the independent treatment combinations.

Treatment	Total (both sexes)	Males	Females	Expected no. of each	*P*
Present-day conditions	1223	634	589	611.5	0.207
Elevated pCO_2 alone	1608	831	777	804	0.186
Elevated temperature alone	535	282	253	267.5	0.226
Future conditions	926	477	449	463	0.373
Total (all treatments combined)	4292	2224	2068	2146	**0.018**

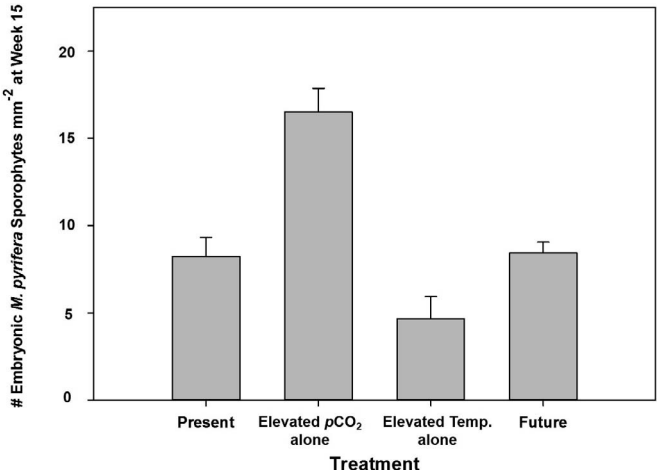


Fig. 5. *Macrocystis pyrifera* sporophyte production at week 15 (# individuals mm^{-2}, mean + 1 standard error) under present conditions (12°C × 400 ppm), elevated pCO_2 alone (12°C × 1500 ppm), elevated temperature alone (15°C × 400 ppm), and future-conditions treatments (15°C × 1500 ppm).

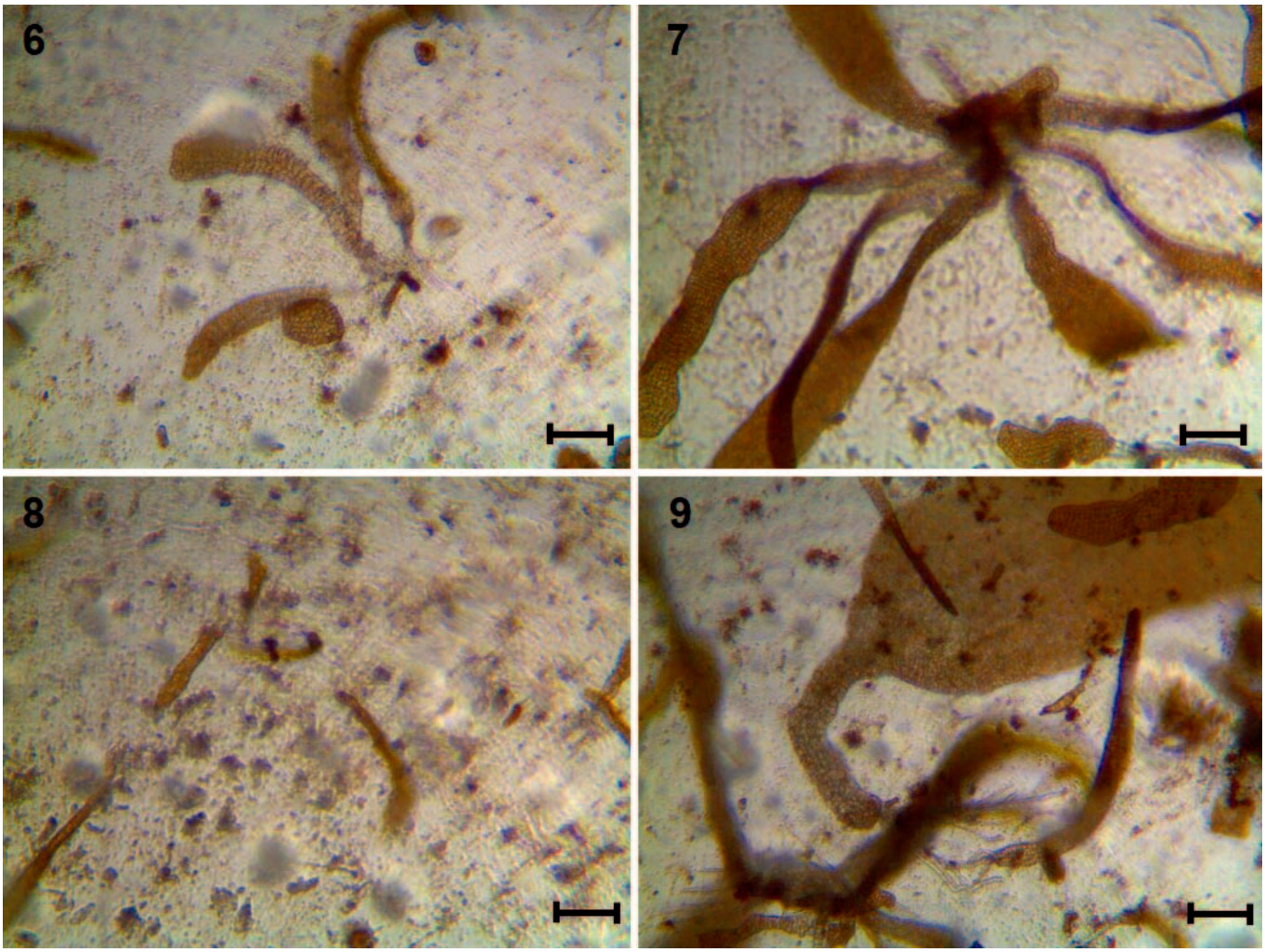
experiment. First, male gametophytes were nearly twice as large as their female counterparts across all treatment combinations (Fig. 4). Second, both male and female gametophytes were generally smallest (i.e. they exhibited the least growth) in the elevated temperature-alone treatment (311 ± 5 μm and 182 ± 2 μm, respectively) and largest (i.e. they exhibited the greatest growth) in the future-conditions treatment (364 ± 6 μm and 195 ± 3 μm, respectively) (Fig. 4). They were also intermediate in the present-day (349 ± 5 μm and 182 ± 3 μm, respectively) and elevated pCO_2 alone (326 ± 7 μm and 188 ± 3 μm, respectively) treatments. Together, this indicates that elevated temperature alone generally resulted in the lowest growth, whereas elevated temperature and pCO_2 together (i.e. future conditions) generally resulted in the greatest growth in both gametophyte sexes. In contrast, the impacts of elevated pCO_2 alone were intermediate and variable between the sexes. These conditions, however, did not affect the sex ratios of the surviving gametophytes. Specifically, from the over 4292 gametophytes observed at week 11 of the experiment, 2224 (51.8%) were males and 2068 (48.2%) were females (Table 4). Although this was a significant departure from a 50:50 sex ratio (binomial test, $P = 0.018$), no significant departures from a 50:50 sex ratio were observed in any of the treatment combinations (Table 4).

Embryonic sporophyte production and growth

Embryonic sporophyte production was observed in all temperature–pCO_2 treatment combinations by week 15 of the experiment, but the timing of their first observation varied across the treatments. Specifically, embryonic sporophytes were first observed during week 12 in both the future-conditions and elevated pCO_2-alone treatments, during week 13 in the present-day conditions treatment, and last during week 14 in the elevated temperature-alone treatment. Further, overall embryonic sporophyte production measured during week 15 of the experiment varied significantly among both seawater pCO_2 (ANOVA, $P < 0.001$) and temperature ($P < 0.001$) conditions (Figs 5, 6; Table 5). These factors did not interact with each other ($P = 0.06$), but general patterns emerged, with the greatest embryonic sporophyte production observed under elevated pCO_2 alone (2.8 ± 0.4 sporophytes mm^{-2}, mean ± standard error), the least production observed under elevated temperature alone (0.3 ± 0.1 sporophytes mm^{-2}), and intermediate production observed under present-day (0.7 ± 0.2 sporophytes mm^{-2}) and future (0.7 ± 0.1 sporophytes mm^{-2}) conditions (Fig. 5). Together, this indicates that elevated pCO_2 generally enhanced embryonic sporophyte production and partially ameliorated the negative impacts of elevated temperature (Figs 5–9). Last, although sporophyte growth was not significantly affected by increased seawater temperature (ANOVA, $P = 0.129$), the sporophytes produced under 1500 μatm pCO_2 were significantly larger than those produced under 400 μatm pCO_2 by week 15 of the experiment ($P = 0.007$) regardless of temperature (Fig. 10, Table 6). Therefore, taken together, our data indicate that elevated seawater pCO_2 may be more beneficial to embryonic sporophytes than elevated temperatures while also facilitating earlier recruitment and faster growth under both present-day and elevated temperatures.

Table 5. Two-way model I ANOVA testing differences in *Macrocystis pyrifera* embryonic sporophyte production at week 15 among temperatures (12°C vs 15°C) and seawater pCO_2 conditions (400 μatm vs 1500 μatm pCO_2). Boldface type denotes significance ($P < 0.05$). Data were square root transformed before analysis to correct problems with heteroscedascity. The relative amount (%) of variation explained by each factor is denoted by its magnitude of effect (ω^2) as calculated according to Graham and Edwards (2001).

Source	*v*	Mean square	*F*	*P*	ω^2
Temperature	1	9.784	27.44	< **0.001**	0.43
pCO_2	1	10.524	29.25	< **0.001**	0.46
Temperature × pCO_2	1	1.468	4.08	0.06	0.05
Error	16	0.359			0.06



Figs 6–9. *Macrocystis pyrifera* sporophytes at week 15 (FOV = 1.19 mm^{-2}, magnification: ×100) under different treatments.
Fig. 6. Present conditions (12°C × 400 ppm).
Fig. 7. Elevated pCO_2 alone (12°C × 1500 ppm).
Fig. 8. Elevated temperature alone (15°C × 400 ppm).
Fig. 9. Future conditions (15°C × 1500 ppm).

DISCUSSION

As anthropogenic activities increase atmospheric greenhouse gas concentrations, oceanic absorption of heat and CO_2 will continue to increase seawater temperature and acidity (Caldeira & Wickett 2003; Levitus *et al*. 2005). Although not all ecosystems will exhibit the same magnitude of change (Hofmann *et al*. 2011; Sunday *et al*. 2017), warming and acidification are generally expected to reduce community biodiversity and homogenize ecosystems (Harley 2011; Kroeker *et al*. 2013b). Organisms may prioritize the regulation of physiological processes under stressful conditions, which can lead to offsets in predatory/competitive ability and alter biotic interactions (Kroeker *et al*. 2014; Jellison *et al*. 2016). Elevated pCO_2 and temperature independently and interactively elicit variable physiological responses across a multitude of taxa and at different life-history stages (Doney *et al*. 2009; Haigh *et al*. 2015), thereby limiting our capacity to forecast how climate change will affect biological processes across multiple scales.

Species with complex life histories may respond differently to environmental change as they transition through each developmental phase (Harley *et al*. 2012). Marine invertebrate larvae, for example, demonstrate sensitivity to warming that is exacerbated by elevated pCO_2 (reviewed by Byrne 2011; Kroeker *et al*. 2013b). Indeed, elevated temperatures accelerate development of larval urchins and barnacles, but simultaneous increases in acidity impede this progression (Baragi & Anil 2015; Hardy & Byrne 2015). Likewise, microscopic kelp life stages are generally more vulnerable to climate-change stressors than their macroscopic counterparts because of physiological limitations that prevent resilience and repair (Henry & Cole 1982; Hoos 2015). Therefore, quantifying the survival and growth of early kelp life stages under elevated temperature and pCO_2 conditions gives insight into the sensitivity of these life-

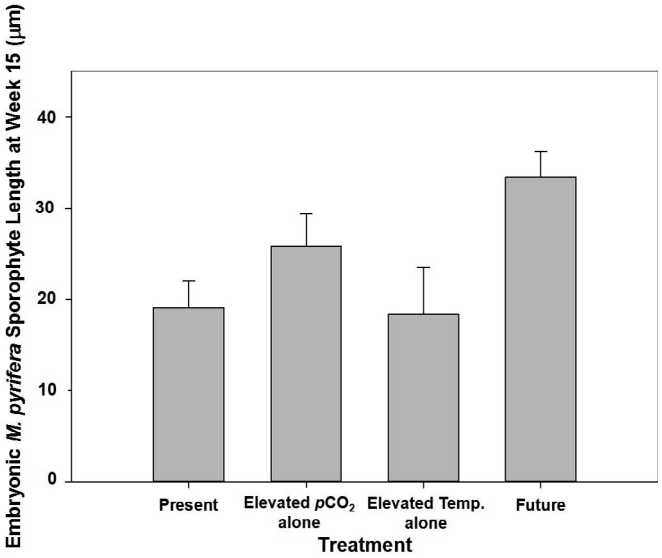


Fig. 10. Length of *Macrocystis pyrifera* sporophytes at week 15 (micrometres, mean + 1 standard error) under present conditions (12°C × 400 ppm), elevated pCO_2 alone (12°C × 1500 ppm), elevated temperature alone (15°C × 400 ppm), and future conditions treatments (15°C × 1500 ppm).

history stages and can help us predict effects on kelp assemblages and the ecosystem services they provide.

In this study, we found that increased temperature and pCO_2 (i.e. 15°C and 1500 μatm pCO_2) antagonistically affected germling production in the giant kelp, *M. pyrifera*, whereas elevated temperature alone reduced successful propagule settlement under present-day pCO_2 levels (i.e. 400 μatm). Likewise, gametophyte survival and growth over 11 wk were both lowest under elevated temperatures, but they did not vary among the other treatment combinations. Indeed, growth and survival of male and female gametophytes were both greatest under future conditions, a reversal from what was observed during germling production and a trend that continued into the sporophyte life stage (see also Brown *et al*. 2014). Further, although we observed a slightly greater proportion (< 2%) of male gametophytes when integrated across all treatment combinations, gametophyte sex ratios were not significantly affected by changes in temperature and pCO_2. Similarly, Roleda *et al*. (2012) and Leal *et al*. (in press) both found nonsignificant differences in kelp gametophyte sex ratios across multiple pH treatments, which Leal *et al*. (in press) attributed to seasonality and not elevated pCO_2. Our data not only corroborate these conclusions, but they also suggest that elevated temperature does not significantly affect sex ratios either. However, our findings do suggest that elevated temperature inhibits development of all microscopic kelp life stages, whereas elevated pCO_2 reduces germling production and enhances gametophyte and sporophyte development.

Kelp sensitivity to elevated temperatures is well documented in microscopic (e.g. Deysher & Dean 1986b) and macroscopic (e.g. Steneck *et al*. 2002) stages alike, but less is known about their responses to interactive effects of temperature and pCO_2 (Harley *et al*. 2012). Gaitán-Espitia *et al*. (2014) detected high *M. pyrifera* zoospore mortality

Table 6. Two-way model I ANOVA testing differences in *Macrocystis pyrifera* embryonic sporophyte longest axis lengths (i.e., growth) at week 15 among temperatures (12°C vs 15°C) and seawater pCO_2 conditions (400 μatm vs 1500 μatm pCO_2). Boldface type denotes significance ($P < 0.05$). The relative amount (%) of variation explained by each factor is denoted by its magnitude of effect (ω^2) as calculated according to Graham and Edwards (2001).

Source	*v*	Mean square	*F*	*P*	ω^2
Temperature	1	111.13	2.58	0.129	0.05
pCO_2	1	414.07	9.61	**0.007**	0.29
Temperature × pCO_2	1	4.82	0.110	0.743	< 0.01
Error	15	43.07			0.66

under elevated temperature and pCO_2 levels both when examined alone and interactively. Our results similarly indicated reduced germling production under elevated temperatures, but elevated pCO_2 alone did not significantly affect germling production. As germling production is contingent upon successful zoospore settlement, it is important to note the discrepancy between these two studies. Differences in zoospore production and germling settlement may be attributed to the different pCO_2 levels used, as the seawater used by Gaitán-Espitia *et al*. (2014) was more acidic (1800 μatm pCO_2) than the levels used in our study (1500 μatm pCO_2). However, this may also demonstrate that zoospores are more susceptible to acidic seawater than germlings. Consequently, future studies should investigate smaller incremental changes in pCO_2 on zoospore settlement and germling production as well as the relationship between zoospore settlement and germling production in warmer, more acidic seawater. However, responses of kelp forests to temperature and pCO_2 may vary given that kelp forest population dynamics are largely driven by regional trends (Krumhansl *et al*. 2016).

Overall, our results indicate that relative to elevated pCO_2, increased temperatures stunt early *M. pyrifera* development, thereby reducing germling densities, gametophyte viability, and embryonic sporophyte production. Although Fernández *et al*. (2015) demonstrate that decreased pH does not alter photosynthetic activity in *M. pyrifera* gametophytes, enhancing CO_2 availability concurrently with higher temperatures (i.e. future-conditions treatment) may increase their metabolic activity and enable greater carbon uptake, thereby facilitating high gametophyte and sporophyte growth rates, as has been shown for meristematic *M. pyrifera* sporophyte tissues (Brown *et al*. 2014). Despite the potential benefits for kelp development that increased temperature and pCO_2 together may provide, NO_3^- addition was necessary for facilitating growth and development in both studies, thus suggesting that elevated pCO_2 does not compensate for nutrient depletion under elevated temperatures.

Embryonic sporophyte production and growth were dependent upon adding NO_3^- to the seawater after week 10, which simulated upwelling events and internal waves that periodically provide NO_3^- to kelp forests (McPhee-Shaw *et al*. 2007). During summer 2014, an anomalous intrusion of warm water (i.e. "the blob") raised Southern California sea-surface temperatures by up to 4°C (Leising *et al*. 2015), and natural seawater was *c*. 1 μmol l^{-1} NO_3^- throughout the

experiment. Since sporophyte recruitment requires 4 μmol l^{-1} NO_3^- (Zimmerman & Kremer 1986; Carney & Edwards 2010) and gametophytes began transitioning into sporophytes within 2 wk of adding NO_3^-, limited nutrient availability likely delayed gametophyte development and sporophyte recruitment across all treatments (Carney 2011). After nutrient supplementation, sporophyte recruitment first occurred in the elevated pCO_2 alone and future treatments. Thus, higher pCO_2 levels may provide a competitive advantage to embryonic sporophytes, as initial recruitment to a kelp forest patch generally allows these earlier recruits to dominate because of faster, unencumbered growth rates in the absence of other recruits (Dayton *et al*. 1984; Carney *et al*. 2013). This is substantiated by similar sporophyte lengths in the elevated pCO_2-alone and future-conditions treatments at the end of the experiment, with threefold greater embryonic sporophyte production under elevated pCO_2. Therefore, given that nutrient requirements of gametophytes are met, elevated pCO_2 may increase the probability of survival to adulthood under present-day temperatures.

The benefits of elevated pCO_2 may not compensate for the negative impacts of elevated temperature under low nutrient conditions, which may elicit dormancy in microscopic kelps (Carney 2011). Specifically, kelp gametophytes can persist in a delayed state where they grow without reproducing (reviewed by Carney & Edwards 2010), and this may be especially evident during El Niño events, which are characterized by low nitrate availability and warm temperatures (Ladah *et al*. 1999; Hernández-Carmona *et al*. 2001). This may explain why female gametophytes tend to exhibit rapid growth under warm conditions, but oftentimes do not form reproductive structures (Lee & Brinkhuis 1988; Nelson 2005). Since temperature is a chief component of reproductive phenology in autotrophs (Dieck 1993; Sherry *et al*. 2007), this adaptation may be beneficial for kelp forest populations as nutrient concentrations are expected to decline in a warming ocean with increased coastal stratification (Capotondi *et al*. 2012).

In this study, we identified the independent and interactive effects of elevated temperature and pCO_2 on early development in *M. pyrifera*. Germling production, gametophyte survival, growth, sex ratios, and embryonic sporophyte production were all greatly reduced when exposed to elevated temperatures alone; only sporophyte growth was contingent upon pCO_2 more than temperature. Our results further indicate that although elevated temperature is largely detrimental to early *M. pyrifera* development, these impacts may be mitigated by the presence of elevated pCO_2. This may be especially relevant given that these two factors are both expected to increase in the future. Despite the vast array of research concerning the physiology and ecology of kelps and their role in regulating regional biodiversity, the effects of climate change on this species remain understudied. The susceptibility of early life stages to these environmental stressors could dramatically alter the recruitment and ontogeny of this habitat-forming species, and alter the biogenic structure of rocky reefs (e.g. Connell *et al*. 2011). Kelp forests are dynamic ecosystems and important contributors to global primary productivity. Thus, as key players in the oceanic carbon system and potential mitigants of ocean acidification (Hurd 2015), understanding how climate-change stressors affect kelp forests at multiple scales could elucidate carbon fluxes in the coastal zone and facilitate effective management, policy decisions, and mitigation of anthropogenic climate change.

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