Title: EXAMINING THE REPRODUCTIVE SUCCESS OF BULL KELP (*NEREOCYSTIS LUETKEANA*, PHAEOPHYCEAE, LAMINARIALES) IN CLIMATE CHANGE CONDITIONS[1]

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Abstract:

Climate change is affecting marine ecosystems in many ways including rising temperatures and ocean acidification. From 2014-2016, an extensive marine heat wave extended along the west coast of North America and had devastating effects on numerous species during this period, including bull kelp (*Nereocystis luetkeana*). Bull kelp is an important foundation species in coastal ecosystems that can be affected by marine heat waves and ocean acidification; however, these impacts have not been investigated on sensitive early life stages. To determine the effects of changing temperatures and carbonate levels on Northern California's bull kelp populations, we collected sporophylls from mature bull kelp individuals in Point Arena, CA. At the Bodega

Marine Laboratory, we released spores from field-collected bull kelp, and cultured microscopic gametophytes in a common garden experiment with a fully factorial design crossing modern conditions (11.63 ± 0.54°C and pH 7.93 ± 0.26) with observed extreme climate conditions (15.56 ± 0.83°C and 7.64 ± 0.32 pH). Our results found that both increased temperature and decreased pH influenced growth-and egg production of bull kelp microscopic stages. Increased temperature resulted in decreased gametophyte survival and offspring production. In contrast, decreased pH had less of an effect, but resulted in increased gametophyte survival and offspring production. Additionally, increased temperature significantly impacted reproductive timing by causing female gametophytes to produce offspring earlier than under ambient temperature conditions. Our findings inform better predictions of the impacts of climate change on coastal ecosystems and provide key insight into environmental dynamics regulating the bull kelp lifecycle.

Keywords: bull kelp, climate change, kelp forests, marine heat waves, ocean acidification, reproduction

Abbreviations: CCM, carbon concentrating mechanisms; CO_2, carbon dioxide; ENSO, El Niño Southern Oscillations; GLMM, generalized linear mixed models; LMM, linear mixed models ; OA, ocean acidification

Introduction:

Globally, marine systems are under pervasive threats from climate change. Chief among these threats are marine heat waves and ocean acidification (OA) (Cooley et al., 2022). Changing temperature and OA have negative impacts on the critical structure-forming foundational species of the world's oceans, namely kelps and corals, especially in terms of reduced reproduction (Smith et al., 2022; Straub et al., 2019) and juvenile mortality (Harvey et al., 2013; Kroeker et al., 2013; Przeslawksi et al., 2014). In the ocean, early life stages are already subject to high mortality rates due to a number of environmental bottlenecks, and increased temperature and decreased pH can further increase juvenile mortality through reduced recruitment and growth of the microscopic-stages of canopy-forming kelps (Gaitan-Espitia et al., 2014; Hollarsmith et al., 2020; Lind & Konar, 2017; Shukla & Edwards, 2017), reduced calcification and increased disease in juvenile invertebrates (Ban et al., 2013; Kroeker et al., 2013; Miner et al., 2018; Small et al., 2016), and altered larval fish behavior (Ferrari, 2011; Munday, 2010).

Kelp forests are critical to temperate, nearshore subtidal, and intertidal marine systems worldwide, and they sustain numerous economically important recreational and commercial fisheries (Bennett et al., 2016; Blamey & Bolton, 2018; Carr & Reed, 2016). In addition, kelp forests provide numerous ecosystem functions and services such as shelter of structural habitat and food sources to surrounding ecosystems, buffering coastlines from wave energy, ameliorating the effects of ocean acidification, reduction of current speeds and larval delivery to the shore, and modification of seawater chemistry (Carrano et al., 2020; Carrano et al., 2021; Hamilton et al., 2022; Malone et al., 2022).

Globally, the effects of marine heat waves are already having extreme effects on kelp forests (Arafeh-Dalmau et al., 2019; Camus et al., 2021, Filbee-Dexter et al., 2020; Straub et al.,

2019). From 2014-2017, Northern California lost 90% of its bull kelp (*Nereocystis luetkeana*) canopy cover over an area of roughly 350 km (Rogers-Bennett & Catton, 2019). This loss of kelp forest cover has been attributed to a dramatic increase in purple urchin (*Strongylocentrotus purpuratus*) density due to loss of keystone predators, coupled with a pervasive system of marine heat waves (McPhearson et al., 2021). The results of such widespread canopy loss were drastic changes in community structure and composition (Beas-Luna et al., 2020) and the collapse of the several fisheries in the area, such as that of the red sea urchin (*Mesocentrotus franciscanus*) (Rogers-Bennett & Okamoto, 2020) and the closure of the world's largest recreational abalone fishery (*Haliotis rufescens*) (Reid et al., 2016; Rogers-Bennett & Catton, 2019).

Numerous studies in recent years have documented the effects of increased temperature on bull kelp canopies (Berry et al., 2021; Hamilton et al., 2020; Rogers-Bennett & Catton, 2019), and these studies have found that decreases in adult bull kelp canopy abundance have been related to local and large-scale processes associated with warm water (Pfister et al., 2017; Schiel et al., 2004). Bull kelp exposure to warm temperatures also reduces adult blade morphological plasticity to changes in hydrodynamic flow regimes (Suprataya et al., 2020), but the physiological impacts of warm waters on bull kelp need to be further studied. Studies of bull kelp microscopic developmental stages in British Columbia and Alaska have found that increased temperatures have resulted in reductions in settlement and reduced germination and growth (Lind & Konar, 2017; Muth et al., 2019; Schiltroth, 2021), but the impact of rising temperatures on microscopic bull kelp stages in the southern portion of their range in northern California remains unclear. California bull kelp populations represent the range extreme of bull kelp, existing in low-latitude areas that are the most exposed to El Niño-Southern Oscillation (ENSO) warm water events compared to more northern populations. As a result, California bull

kelp populations could either be more warm-water adapted than the higher latitude populations previously studied, or they could be existing much closer to the thermal maxima and therefore be very vulnerable. As the bulk of kelp die-offs during the 2014 to 2016 marine heat wave occurred near the lower-latitude portion of kelp species' ranges (Arafeh-Dalmau et al., 2019; Beas-Luna et al., 2020; Cavanaugh et al., 2019; Finger et al., 2021; Rogers-Bennett & Catton, 2019), it is necessary to further study how future marine heat waves may affect the ability of these foundation species to remain in their lower latitude ranges.

In addition to the increasing threat of marine heat waves, coastal temperate ecosystems are also subject to stress from ocean acidification (OA), which, on average, has already caused a global lowering of surface water pH by 0.11 pH units (Feely et al., 2004; Feely et al., 2009; Gattuso et al., 2015a). Variability of pH levels in nearshore systems is normal to a degree as seasonal oceanographic shifts like upwelling bring deep offshore waters to the surface and expose nearshore ecosystems to reduced pH levels. This exposure varies with local bathymetry and coastal topography, which often changes the intensity of upwelling events along the coast (Feely et al., 2008). While pH variation in the California Current System generally stays between 7.720 and 8.413 pH units (Feely et al., 2018), climate change projections predict an increasing frequency and duration of low-pH extremes (Bakun et al., 2015; García-Reyes et al., 2015), which may result in an average decrease of up to 0.4 pH units (Feely et al., 2008). Low pH may impact physiological functions among a variety of organisms. Studies have shown that OA will more proportionately impact organisms that form calcium carbonate skeletons (Kroeker et al., 2013), but we must also understand how the compounding stress of these combined threats will impact our critical temperate nearshore systems.

Kelps are very efficient at processing multiple carbon species in the water column and require CO_2 for photosynthesis. Kelps are able to uptake CO_2 from the water column either via diffusive entry, or through carbon concentrating mechanisms (CCMs) that allow them to convert the more abundant form of dissolved inorganic carbon, HCO_3^-, into the less abundant CO_2 (Maberly, 1990; Raven, 2003). There is some evidence to suggest that the excess of carbon predicted for future ocean conditions may increase kelp growth in climate change conditions (Brown et al., 2014, reviewed in Veenhof et al., 2021). For example, increased pCO_2 has been shown to have beneficial impacts on mature bull kelp net apparent productivity (Thom, 1996) and growth (Swanson & Fox, 2007). At the microscopic stage, however, the effects of *p*CO2 and pH on kelp can be variable (Edwards, 2022), ranging from having negative effects (Gaitán-Espitia et al., 2014), to no effect (Fernández et al., 2015; Hollarsmith et al., 2020), to positive effects on growth and photosynthesis (Shukla & Edwards, 2017).

Understanding how different life stages respond to environmental stress is critical when trying to predict population resilience to disturbance events. Laminariales, or the large canopy-forming kelps, have a multistage process of development that presents numerous areas for the imposition of bottlenecks from climate stress. However, to our knowledge, no studies have yet investigated the role that pH may play in embryonic sporophyte (sporeling) bull kelp development, nor the combined threats of increased temperature and ocean acidification on any bull kelp life stage.

In this study, we ask how increased temperatures and decreasing pH will affect bull kelp 1) gametophyte development, 2) egg and sporeling production, and 3) sporeling growth. Based on the observed negative effects of the 2014-17 marine heat wave on bull kelp adult sporophytes, we hypothesized that increased temperature will generally result in decreased growth, survival,

and reproduction. In contrast, we hypothesized that decreased pH will have less of an effect than temperature on growth and egg production, but will generally result in increased growth, survival, and reproduction.

Materials and Methods:

Bull Kelp Life Cycle

In California, one of the dominant canopy-forming kelp species is bull kelp (*Nereocystis luetkeana*). The range of bull kelp extends from the eastern Aleutian Islands, Alaska, in the north to Point Conception, California, in the south. Within its California range, it is considered to be the dominant canopy-forming kelp species in Northern California, between San Francisco and the California-Oregon border. Bull kelp experience sea surface temperatures that annually average between 12 and 15 °C at the southernmost edge of its distribution in Point Conception and between 9 and 12 °C near Point Arena in Northern California (National Data Buoy Center [NDBC], 2023a; NDBC, 2023b). Bull kelp is an annual species and is thought to be a more opportunistic, resilient colonizer, especially in areas with too much wave stress for the persistence of giant kelp (*Macrocystis pyrifera*) (Foster & Schiel, 1985; Graham, 1997; Graham et al., 2007).

Bull kelp have a heteromorphic life cycle consisting of a large diploid sporophyte and a microscopic haploid gametophyte. Adult sporophytes develop patches of sori on their blades at the ocean surface, and at maturity, begin to release spores. The released zoospores then settle on hard substrate at the benthos, where they grow into microscopic male and female gametophytes. The female gametophytes begin to produce eggs, and then release the lamoxirene pheromone to trigger sperm release from nearby males (Lüning & Müller, 1978). Once the sperm fertilizes the egg, a new sporophyte begins to develop (Reed, 1990).

Collection

Blades with sori from approximately 10 individuals were collected at the surface by boat from a single kelp bed in Point Arena, California (38.916271°N, 123.725644°W) in October 2017. Sori were cleaned in iodine and fresh water, layered in a cooler with wet paper towels separating individual sori, and transported to the Bodega Marine Laboratory (BML, 38.318164°N, 123.072019°W) for sporulation. Spore densities were determined using a hemocytometer (model number CTL-HEMM-GLDR, LW Scientific, Lawrenceville, U.S.A.), and were introduced into the experimental Petri dishes to facilitate a settlement density of approximately 8 spores/mm^2.

Ex situ culturing experiment

We conducted a fully factorial common garden experiment that consisted of four treatments representing ambient and high temperature and ambient and low pH, with ten replicates per treatment, for a total of forty experimental Petri dishes (Fisher Brand 100 mm × 15 mm). Temperature was maintained at 15.6 ± 0.8°C and 11.6 ± 0.5°C using walk-in incubators at Bodega Marine Laboratory (BML), and pH was maintained at 7.93 ± 0.26 pH and 7.64 ± 0.32 pH using chemical additions of equal parts 1M HCl and 1M NaHCO3 ($NaHCO3 + HCl \rightarrow NaCl + H2CO3$) (Riebesell et al., 2011). Petri dishes were randomly arranged on shelves within the incubators. Temperatures were chosen to represent ambient sea surface temperatures for our ambient temperature treatment, whereas our high temperature treatment represented the 4°C increase in SST observed during the 2014-17 marine heat wave (Gentemann et al., 2017). Ambient and low pH were chosen to represent the pH of incoming seawater at BML and pH during an extreme upwelling event (Feely et al., 2008), respectively. Light was set at 14:10 photoperiod and 30-45 umol m^{-2} s^{-1} to mimic summer conditions when the potential for exposure

177 to higher temperatures and lower pH through upwelling is greatest. The pH of incoming,
178 manipulated, and outgoing seawater was measured to 0.01 pH units immediately after collection
179 using a spectrophotometer. Total alkalinity (T_{alk}, μmol kg^{-1}) was measured using potentiometric
180 acid titration. We changed the water in all experimental dishes every 2 to 3 days for the duration
181 of the 27-day experiment in order to maintain low pH conditions and prevent anoxia or nutrient
182 limitation. We added standard 20 mL L^{-1} Provasoli nutrient mix to all treatment water to prevent
183 nutrient limitation during growth (Provasoli, 1968).

184 *Photo Analysis*

185 Beginning one week after spore inoculation, Petri dishes were photographed weekly with
186 a Micropublisher 5.0 RTV digital camera (QImaging, Surrey, Canada) mounted on an inverted
187 microscope at 40× magnification, resulting in four weeks of photos documenting gametophyte
188 and sporeling growth and reproduction. Within each dish, three points were randomly selected to
189 be photographed, with different points being photographed each week. Each photo encompassed
190 1.08 mm^2 of the Petri dish (7,853 mm^2 bottom surface area).

191 After the growth experiment was completed, each photo was analyzed using ImageJ
192 (Rasband, 2019). Week 1 and 2 photos did not contain any gametophytes large enough to
193 identify by sex, so only Weeks 3 and 4 were used for analysis. Count data was obtained from
194 each photo for female gametophytes, male gametophytes, eggs, and sporeling (Figure 1). Every
195 female counted was also categorized as "productive" (having produced at least one egg or
196 sporeling) or "non-productive" (having no eggs or sporelings). The proportion of productive
197 females in each photo was calculated by dividing the number of productive females by the total
198 number of females counted.

Count data were also used to calculate three additional variables: average number of eggs per female, average number of sporelings per female, and average number of offspring per female ((# eggs + # sporelings)/# females). We used these three ratios to distinguish whether differences in numbers of eggs and juveniles were simply a result of differences in parent gametophyte numbers, or whether they were a result of reduced production by females. These three ratios were also used to approximate which stages of reproduction were taking place at Weeks 3 and 4. Sporeling sizes were also obtained by using the freehand trace tool in ImageJ and measuring the number of pixels encapsulated. Sizes were then converted to μm^2 using a conversion factor of 71330 pixels per 62,500μm^2, which was calculated by measuring the area of a photo of a 0.0625 mm^2 hemocytometer cell at 40× magnification.

Statistical Analysis

All count outcome variables were analyzed using linear mixed models with temperature, pH, and their interaction as fixed effects and Dish ID as a random effect. In order to meet the parametric assumptions of normality of residuals and homogeneity of variances, all count data was subjected to a square-root transformation as needed before being analyzed. We tested the significance of our fixed effects by conducting log-likelihood tests via model comparison using the ANOVA function, where one model included the effect of interest while the other model excluded it.

We analyzed the proportion of productive female gametophytes using a generalized linear mixed model (GLMM) with a beta distribution. Size data were also analyzed with a GLMM using a gamma distribution. GLMMs included temperature, pH, and their interactions as fixed effects, and Petri dish ID as a random effect. Average number of gametophytes per photo in a given dish was also calculated and included in the size model as a covariate to account for

possible density dependence. We also separately analyzed the relationship between average size of sporelings per photo and the covariate (average number of gametophytes per photo) using a linear regression model that included only the covariate as a fixed effect.

All count and size data were only analyzed for Week 4 of our experiment, but calculated ratios of eggs per female (eggs/fem), sporelings per female (sporelings/fem), and offspring per female (offspring/fem) were analyzed for both Weeks 3 and 4 in order to draw conclusions about differences in rates of fertilization or maturation. Specifically, we used the ratio of offspring/fem to ask whether females, regardless of treatment, showed equal fecundity, and the ratios of eggs/fem and sporelings/fem were calculated to inform us about which stage reproduction was within each treatment. We tested the significance of our fixed effects via model comparison using the ANOVA function, where one model included the effect of interest while the other model excluded it. Hypothesis testing was conducted via log-likelihood tests for count and offspring ratio LMMs and Chi-squared tests for size GLMMs. All analyses were performed using R version 4.1.2 (R Core Team, 2021) and the packages *nlme* (Pinheiro et al., 2022), *lme4* (Bates et al., 2015), and *glmmTMB* (Brooks et al., 2017).

Results:

Female and Male Gametophyte Development

High temperature decreased the density of females present after four weeks (Log-Likelihood = 51.1283, DF = 36, $p < 0.0001$) (Figure 2, Table 1). Neither pH nor the interaction between pH and temperature had a significant effect on female gametophyte numbers (Table S1). Female gametophyte numbers in Week 4 did vary among dishes (Log-Likelihood = 7.8795, $p = 0.005$).

Males density also decreased at high temperatures (Log-Likelihood = 45.393, DF = 36 , p < 0.0001), but they varied from females in that their densities increased under lower pH conditions (Log-Likelihood = 8.6378, DF = 36 , p = 0.0033). Neither the pH:temperature interaction term nor the variation among dishes had any significant effect on male gametophyte numbers (Table S2). In summary, these results indicate that temperature caused a significant decrease in female and male gametophyte numbers, whereas low pH only caused a significant increase in male gametophyte numbers.

Egg and Sporeling Counts

After four weeks, high temperatures decreased the numbers of both eggs (Table S3, Log-Likelihood = 33.73, DF = 36, p < 0.0001) and sporelings (Table S4, Log-Likelihood = 36.6391, DF = 36, p < 0.0001) (Figure 3). Low pH increased numbers of eggs (Log-Likelihood = 4.3958, DF = 36, p = 0.036), but there were no significant effects on sporeling counts (Log-Likelihood = 1.0702, DF = 36, p = 0.3009). The interaction term for pH:temperature was insignificant for counts of both eggs and sporelings. Sporeling counts did not vary among dishes (Log-Likelihood = 3.0544, p = 0.0805), but eggs did vary by dish (Log-Likelihood = 5.2080, p = 0.0225). Overall, temperature caused the greatest decreases in both egg and sporeling numbers, whereas low pH caused a significant increase in eggs only.

Proportion of Productive Females

The proportion of productive females (percent of females producing eggs or sporelings) was uniformly high across all treatments, but the high temperature treatments consistently resulted in nearly 100% of females reaching productivity by Week 4 (Figure 4, Table S5). We found that the proportion of productive females was not significantly affected by the interaction between temperature and pH (Chi-Sq = 0. 5117, p = 0.4744) nor the individual effect of pH (Chi-

Sq = 1.1619, p = 0.2811). High temperature was the only variable to result in a significant increase in the proportion of productive females (Chi-Sq = 28.187, p < 0.0001).

Ratios of Offspring per Female

For mean number of eggs per female (egg/fem), we found a marginally significant effect of the interaction between temperature and pH in Week 3 (Log-Likelihood = 3.7737, DF = 36, p = 0.0521) but not Week 4 (Log-Likelihood=0.0976, DF = 36, p = 0.7547) (Table 2, Figure 5). Investigating temperature and pH individually in Week 3, we found that low pH (Log-Likelihood = 3.7345, DF = 36, p = 0.0533) resulted in a marginally significant decrease in the egg/fem ratio under ambient temperature treatments, but an increased egg/fem ratio under high temperature treatments (Table S6). We did not detect an effect of temperature in Week 3 (Log-Likelihood = 0.1406, DF = 36, p = 0.7077). In Week 4, low pH was found to be significantly associated with a higher egg/fem (Log-Likelihood = 9.3663, DF = 36, p = 0.0022), whereas low temperature resulted in lower egg/fem (Log-Likelihood = 13.114, DF = 36, p = 0.0003). The variation among dishes was insignificant in both Week 3 (Log-Likelihood = 0.2318, p = 0.6302) and Week 4 (Log-Likelihood = 0.5643, p = 0.4525).

High temperatures increased the sporelings per female ratio (sporelings/fem) in both Week 3 (Log-Likelihood = 45.2639, DF = 36, p < 0.0001) and Week 4 (Log-Likelihood = 9.1867, DF = 36, p = 0.0024). Low pH decreased the sporeling/fem ratio in Week 3 (Log-Likelihood = 16.7485, DF = 36, p < 0.0001) but not in Week 4 (Log-Likelihood = 0.1262, DF = 36, p = 0.7225). Neither the interaction term pH:temperature nor the variation among Dishes were significant in either week (Table S7).

Ratios of total offspring per female (offspring/fem) increased with high temperatures in Week 3 (Log-Likelihood = 30.4186, DF = 36, p < 0.0001) but not Week 4 (Log-Likelihood =

0.2279, DF = 36, p = 0.6331), whereas low pH increased offspring/fem in Week 4 (Log-Likelihood = 5.2345, DF = 36, p = 0.0221) but not Week 3 (Log-Likelihood = 1.3622, DF = 36, p = 0.2432). Neither the interaction between temperature and pH nor the variation among Dishes were significant in either week (Table S8).

Across all responses, high temperature had the greatest impacts in Week 3, resulting in lower ratios of sporelings/fem and offspring/fem, whereas low pH was most significant in Week 4, resulting in high eggs/fem and offspring/fem.

Growth of Sporeling Bull Kelp

When analyzing the global trend across all treatments, we found that sporeling size was significantly influenced by the average number of gametophytes within each dish in Week 4 (R^2 = 0.639, $p < 0.0001$), indicating possible density dependence where increased number of gametophytes resulted in significantly smaller sporelings (Table 3, Figure 6). When included in the GLMM, the 3-way interaction between pH, temperature, and the average number of gametophytes within each dish was significant (Chi-Sq = 6.3387, p = 0.0118), but all 2-way interactions were insignificant (Table S9).

In order to examine the role of each fixed factor (pH, temperature, and the covariate: average number of gametophytes within each dish) in the 3-way interaction, we subset our model within the two pH and two temperature levels to elucidate the significance of the covariate and the other non-subset factor. When low pH and ambient pH treatments were separately analyzed, the average number of gametophytes within each dish was never independently significant, but high temperatures resulted in a significant increase in size by itself under low pH conditions (Chi-Sq = 10.051, p = 0.0015) and the temperature by covariate interaction was significant under ambient pH conditions (Chi-Sq = 5.3001, p = 0.02132). When the two temperature treatments

were analyzed separately, the covariate was again never significant by itself, but pH resulted in a significant decrease in sporeling sizes under ambient temperature conditions (Chi-Sq = 3.955, p = 0.04673), and the pH by covariate interaction had a significant effect on size under high temperature conditions (Chi-Sq = 6.0391, p = 0.01399) (Figure 6). In summary, sporeling sizes were most significantly increased under high temperatures, but both low pH and the number of gametophytes present reduced this effect.

Discussion:

Our results demonstrated that both temperature and pH do significantly impact bull kelp reproduction and development, but the effects were more varying and nuanced than we predicted. Our most consistent finding was that high temperatures decreased the number of gametophytes that survived and/or developed and the total numbers of eggs and sporelings produced. The number of male gametophytes, female gametophytes, eggs, and sporelings were always lower in high temperature treatments than ambient temperature treatments, regardless of pH. These results align with previous findings that bull kelp exposed to increased temperature conditions had reduced spore germination rates and reduced gametophyte growth (Lind & Konar, 2017; Muth et al., 2019; Schiltroth, 2021). Increased temperatures also result in decreased gametophyte growth and survival and sporophyte recruitment in numerous other kelp species including giant kelp (*Macrocystis pyrifera)* (Camus et al., 2021, Hollarsmith et al., 2020), stalked kelp (*Pterygophora californica*) (Howard, 2014), spiny kelp (*Ecklonia radiata*) (Alsuwaiyan, 2021), paddleweed (*Ecklonia cava*) (Oh et al., 2015), sugar kelp (*Saccharina latissima*), skinny kelp (*Saccharina angutissima*) (Augyte et al., 2019), dragon kelp (*Eualaria fistulosa*) (Lind & Konar, 2017), and other taxa (reviewed in Edwards, 2022). High temperatures can also modulate the ratios of female to male kelp gametophytes, where more equatorward populations may see

lower frequencies of males under high temperatures (Leal et al., 2017a; Oppliger et al., 2011). While we did not analyze sex ratios in this study, we did generally see more females than males across treatments for this relatively low latitude population of bull kelp, which may affect fertilization rates of eggs produced.

Low pH had no significant effect on the number of female gametophytes or sporelings in our study, but there was a significant increase in male gametophytes and eggs. Other studies have found varying impacts of pH on kelp gametophyte growth and survival (reviewed in Veenhof et al. 2021 and Edwards, 2022). Several studies have found overall positive effects of low pH on *M. pyrifera* gametophyte growth, survival, and size (Roleda et al., 2012; Leal et al., 2017a), whereas other studies found that elevated *p*CO2 had little effect on rates of growth and photosynthesis (Fernández et al., 2015) or reproduction (Hollarsmith et al., 2020), or even negative effects (Gaitán-Espitia et al., 2014). The variation in kelp organismal responses across studies, species, and location indicates that while in general ocean acidification does not seem to be a particular factor of concern for kelp, there is much more to be understood about the impacts of ocean acidification on kelp reproduction.

One hypothesized mechanism that may explain our results is that bull kelp female gametophytes become reproductive sooner under high temperature conditions. While the overall number of gametophytes and sporelings declined under high temperature conditions, the female gametophytes that survived were more productive on average, and produced more sporelings earlier than female gametophytes under ambient temperature conditions. We also saw that our results align with this proposed mechanism via slower reproduction and development under ambient temperature conditions. In Week 3, high temperature treatments had higher ratios of both eggs/fem and sporelings/fem than ambient temperature treatments. By Week 4, however,

the eggs/fem ratio in ambient temperature treatments exceeded that of high temperature treatments, and the sporelings/fem ratio was similar regardless of temperature treatment. The later increases in egg/fem and sporelings/fem ratios in ambient temperatures and lack of difference in the offspring/fem ratios across treatments in Week 4 seem to indicate that female gametophytes have equal individual reproductive capacity under both our temperature treatments, but females growing under high temperature treatments were progressing through reproduction earlier.

The accelerated timeline of bull kelp microstage development could be due to rate limitation of metabolic processes under lower temperatures. The Q10 coefficient for seaweed metabolic processes, the factor by which a reaction increases for every 10°C rise in temperature, varies by seaweed species, but generally results in a doubling of the rate of active uptake and general cell metabolism, and thus the uptake of carbon for photosynthesis and nitrate and other nutrients for other processes (Davison, 1991; Hurd et al., 2014; Raven & Geider, 1988). Due to limited amounts of diffusible CO_2 in ocean water, canopy-forming macroalgae generally rely on carbon concentrating mechanisms (CCMs) that utilize and alter enzymatic functions to supply CO_2 to the cell (Hepburn et al., 2011; Raven, 2003). The faster growth rates of kelp microstages at high temperatures may thus be an effect of altered CCMs that change chemical transformations, enzymatic and lipid functions and properties, rates of membrane transport, and thus carbon availability (Davison, 1991; Raven & Geider, 1988). Previous studies have also shown that in other seaweeds, increased temperatures have sped up reproductive timing. In a study examining the effects of temperature on time to egg production in several California kelp species, egg release of bull kelp as well as *M. pyrifera* and *P. californica* occurred much earlier under our high temperature (16°C) than our ambient temperature (12°C) (Howard, 2014).

Additionally, the results of Leal et al. (2017b), while not focused on the size and growth of sporelings after fertilization, did find that high temperatures did result in increased gametophyte growth rates leading up to fertilization in *M. pyrifera* and wakame (*Undaria pinnatifida*). While increased rates of development have been seen among many seaweed species, research on the physiology and metabolic processes of bull kelp microstages is lacking and would benefit from further study.

Recent advances in kelp reproduction studies have given needed attention to delayed development of microscopic stages and the resulting "bank of microscopic forms" (Carney & Edwards, 2006; Hoffman & Santelices, 1991; Schoenrock et al., 2021), but less focus has been placed on the factors that may accelerate microscopic kelp development. In terrestrial plants, increased temperatures have been found to result in an acceleration of pollen tube growth and stigma and ovule development, which correspond to an overall reduction of the length of time females are receptive to pollination (Hedhly et al., 2009). Reviews of other marine organisms, specifically benthic invertebrates, have shown that increased sea surface temperatures may increase the rate and timing of development and spawning (Przeslawski et al., 2008). In order to better understand the ability of populations to recover from extreme climate disturbance events, more research is needed to better understand the effect of climate stressors on survival, time to development, and propagule production.

Our results interestingly reflect natural seasonal fluctuations in northern California's coastal waters (García-Reyes & Largier, 2012). The upwelling season (April to June) is characterized by the upwelling of cold, dense, nutrient rich water that is also more acidic. During relaxation season (July to October), coastal waters become warmer, less acidic, and exhibit less primary productivity and chlorophyll-a (García-Reyes & Largier, 2012). The majority of visible

405 bull kelp juveniles appear in upwelling season and most adults become reproductive by the end
406 of July during the relaxation season, but these two events of visible recruitment and spore release
407 have been observed to occur in all seasons, albeit at much lower rates (Maxell & Miller, 1996;
408 Dobkowski et al., 2019). Consequently, gametophytes and sporelings that develop in the spring
409 will likely be most exposed to low temperatures and low pH, but the vast majority of
410 gametophytes and sporelings that develop in the fall will be exposed to high temperatures. As
411 such, it is conceivable that high temperatures in September and October would affect the first
412 month of sporeling and juvenile development, whereas low pH in the spring would likely be
413 more important for late stage microscopic sporelings and small, visible juveniles.
414 In contrast, low pH conditions seemed to impact reproductive efforts differently based on
415 temperature conditions. The lowest proportion of productive females was observed in low pH
416 treatments under ambient temperatures (Figure 4), and these females seemed to produce more
417 offspring per female, later in the experiment. Other studies, however, have seen an increase in
418 pre-fertilization gametophyte sizes under low pH conditions for *M. pyrifera* and *U. pinnatifida*
419 (Leal et al., 2017a; 2017b). We did see an increase in post-fertilization bull kelp sporeling size
420 under low pH conditions, but only when temperatures were also increased. The late increase in
421 production of eggs and smaller sporeling sizes under ambient temperatures may potentially
422 signal that a delay in reproduction occurs under low pH and ambient temperature conditions.
423 While the specific mechanisms responsible for lower growth under low pH/increased CO_2
424 conditions are not well understood, we suggest further study into this area would be an
425 interesting new direction for further research.
426 Our results potentially contrast with those of Dobkowski et al. (2019) in that we found
427 that low pH (most often seen in the Spring upwelling season) resulted in slower reproduction and

428 growth whereas high temperature (most often seen in late summer and fall) accelerated it. In
429 their study, Dobkowski et al. (2019) witnessed the quickest recruitment of visible bull kelp
430 juveniles (indicating faster microscopic development times) in the spring (upwelling season), and
431 slowest recruitment (implying slower microscopic development times) in the late summer and
432 fall (relaxation season). A potential explanation for the different observed reproductive rates is
433 that Dobkowski et al. (2019) conducted their experiments in the field, where they were exposed
434 to a full array of abiotic conditions, whereas our experiments were conducted in a laboratory
435 setting where only temperature and pH were manipulated, and all other variables were held
436 constant, including nutrients. Previous studies have shown that delayed development of
437 microscopic kelp stages is often closely tied to insufficient nutrient and light regimes (Carney &
438 Edwards, 2010), both of which are present between September to March due to dampened
439 upwelling conditions and reduced daylength. As such, the slow development over winter in
440 natural populations suggests that changing day length and nutrient supply from upwelling could
441 be more important than temperature and pH fluctuations in promoting the development of
442 microscopic kelp stages.

443 Our results suggest that there may be some density-dependent effects on sporeling growth
444 at these microscopic stages. The difference in sporeling sizes between treatments was most
445 significantly correlated with temperature, but also showed at least a marginally significant
446 correlation with the number of gametophytes present in both weeks (Figure 5). However, due to
447 the fact that high temperatures consistently resulted in significant decreases in gametophyte
448 numbers, the relationships of both temperature and number of gametophytes to gametophyte size
449 are confounded, and direct causation cannot be determined. As a result, more research is needed

450 to see whether these increased sizes were really a result of high temperatures or whether they
451 were a result of lowered density of individuals.
452 In natural populations, there are numerous density-dependent effects that impact kelp
453 reproduction and recruitment. At initial spore settlement, high densities of gametophytes are
454 needed for fertilization between male and female gametophytes to occur, so Allee effects may
455 occur if spores settle at a density of less than 1 spore/mm^2 (Reed, 1990). The direction of
456 density-dependence then reverses somewhere between the gametophyte stage and the point
457 where a juvenile becomes easily detectable to the naked eye, and numerous kelps, including bull
458 kelp, exhibit subsequent increases in mortality due to competition for space, grazing, and
459 overgrowth of other species until they reach the adult life stage (Dobkowski et al., 2019; Reed et
460 al., 1991; Schiel & Foster, 2006). Due to the number of mortality agents that occur in a natural
461 environment and need for close proximity between gametophytes to allow for fertilization,
462 reductions in gametophyte numbers and densities from high temperatures could still have
463 detrimental effects on the replenishment of bull kelp forests.
464 The results of this research indicate that climate change will significantly affect bull kelp
465 reproduction via increased temperatures, and, to a lesser extent, ocean acidification. Increasing
466 frequency and intensity of extreme temperature events such as marine heat waves will likely lead
467 to a massive decrease in the survival of gametophytes and decreased, but accelerated, production
468 of embryonic sporophytes. Lowered pH, mimicking ocean acidification, resulted in an increase
469 in numbers of male gametophytes and sporelings, as well as a slower reproduction rate. Warming
470 waters from climate change will interact with seawater chemistry, and the potentially increased
471 access of kelps to easily diffusive CO_2 molecules or increased rates of carbon concentrating
472 mechanisms under warming climate conditions may have significant impacts on metabolic rates

473 affecting growth and reproduction. The ability of bull kelp to recover from extreme climate
474 events depends on the ability of all lifestages to withstand abiotic stress. In order for managers
475 and scientists to intervene successfully through restoration, an understanding of physiological
476 processes and potential bottlenecks and challenges present at each life stage is necessary. This
477 study informed how bull kelp microstages survive under extreme conditions that are becoming
478 increasingly common, which can help to improve projections for this species into the future and
479 help to explain the consequences of extreme events that lead to major die-offs.
480

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488

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Tables and Figures:

Figure 1: The microscopic stages of bull kelp: A) Female gametophyte (image area = 0.065 mm^2); B) Male gametophyte (image area = 0.065 mm^2); C) Female gametophyte producing an egg (image area = 0.077 mm^2); D) Female gametophytes with sporelings (image area = 0.065 mm^2). Scale bars in lower left hand corner represent 0.1mm.

Figure 2: Female and male gametophytes present in each photo after 4 weeks of growth. The box plots summarize the mean (diamond) and median (box midline) for each treatment, the first and third quartiles (upper and lower box limits), outliers within 1.5 times the inter-quartile range (vertical lines), and outliers beyond that range (dots).

Figure 3: Eggs and sporelings present in each photo after 4 weeks of growth. The box plots summarize the mean (diamond) and median (box midline) for each treatment, the first and third quartiles (upper and lower box limits), outliers within 1.5 times the inter-quartile range (vertical lines), and outliers beyond that range (dots).

Figure 4: Proportion of productive female gametophytes after 4 weeks of growth. The box plots summarize the mean (diamond) and median (box midline) for each treatment, the first and third quartiles (upper and lower box limits), outliers within 1.5 times the inter-quartile range (vertical lines), and outliers beyond that range (dots).

Figure 5: Eggs, sporelings, and total offspring (eggs + sporelings) per female after 3 and 4 weeks of growth. The box plots summarize the mean (diamond) and median (box midline) for each

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867 inter-quartile range (vertical lines), and outliers beyond that range (dots).
868
869 Figure 6: Left panel shows the average size of sporelings after 4 weeks of growth. The left panel
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873 the relationship of the covariate (mean number of gametophytes) to the response variable (mean
874 sporeling size). Data and trends are represented by different dash and dot styles and colors for
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881
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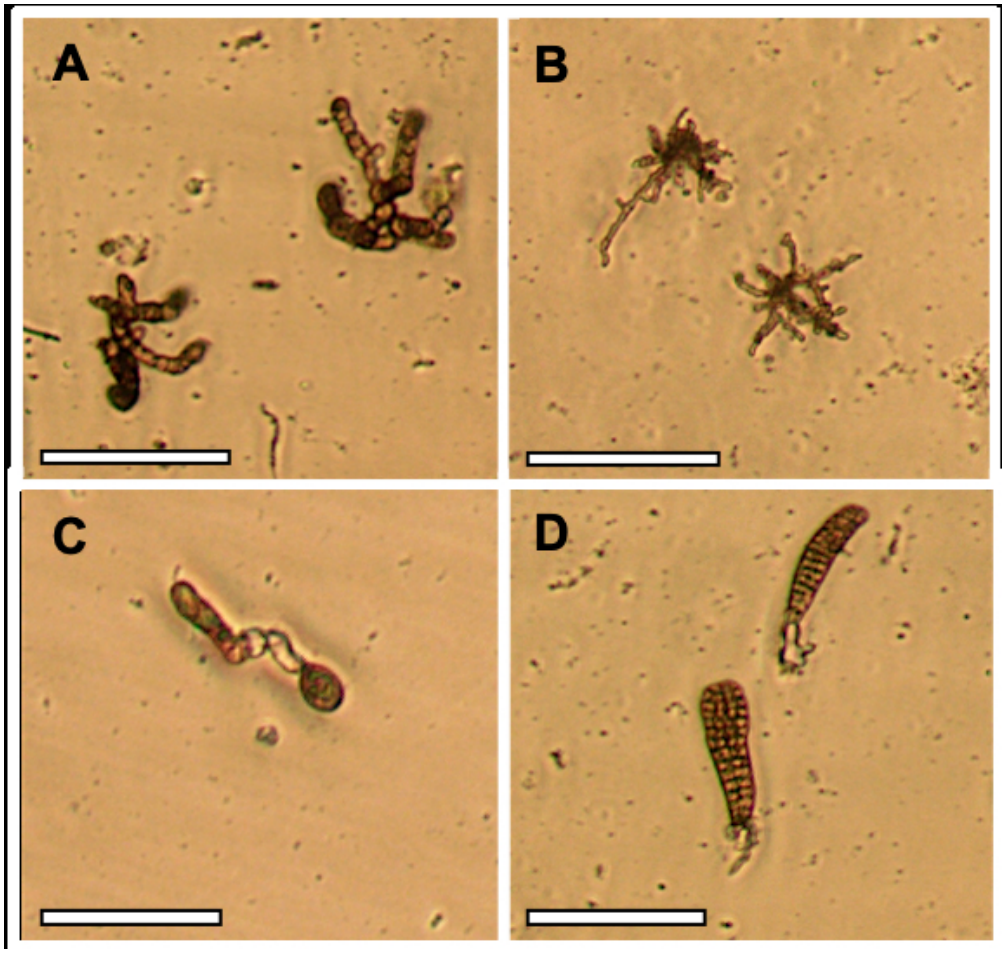

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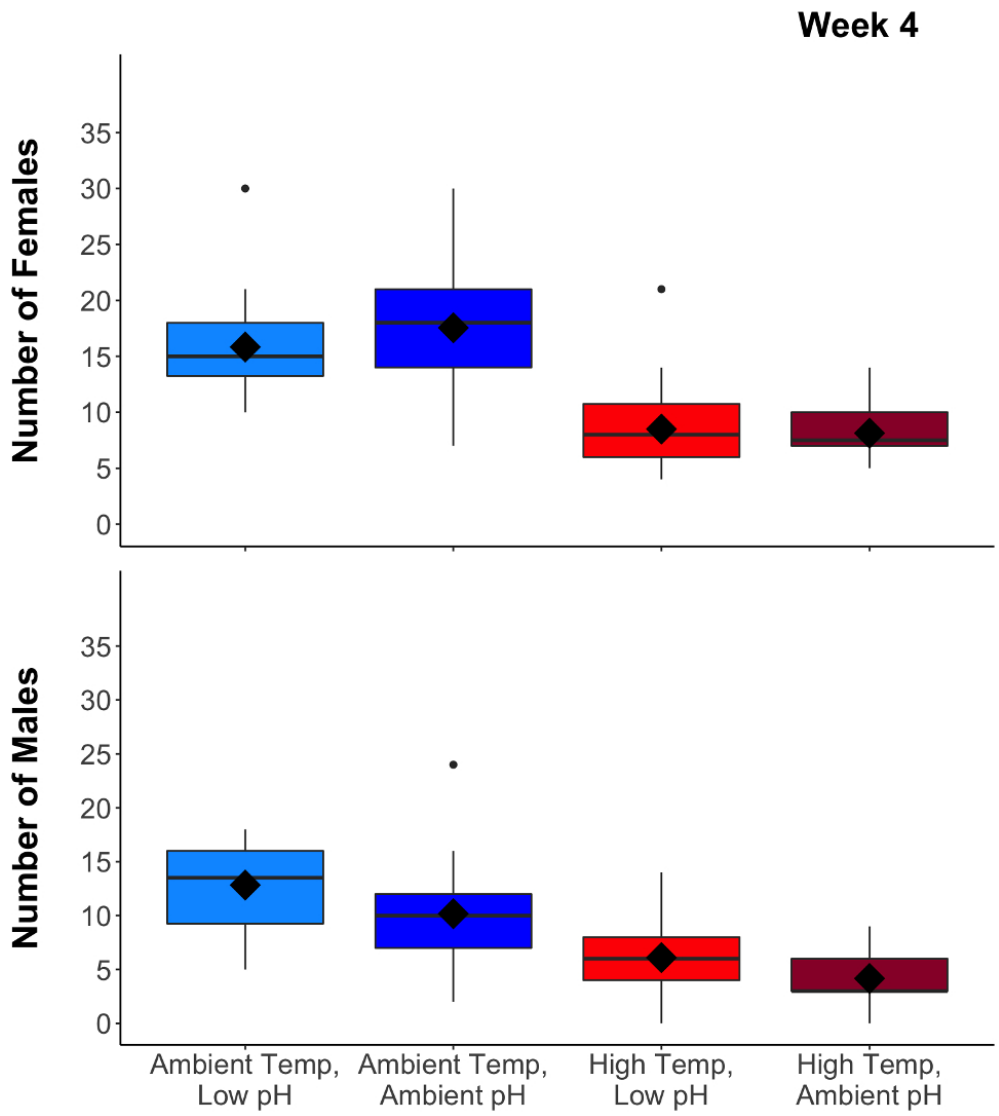
48x45mm (300 x 300 DPI)



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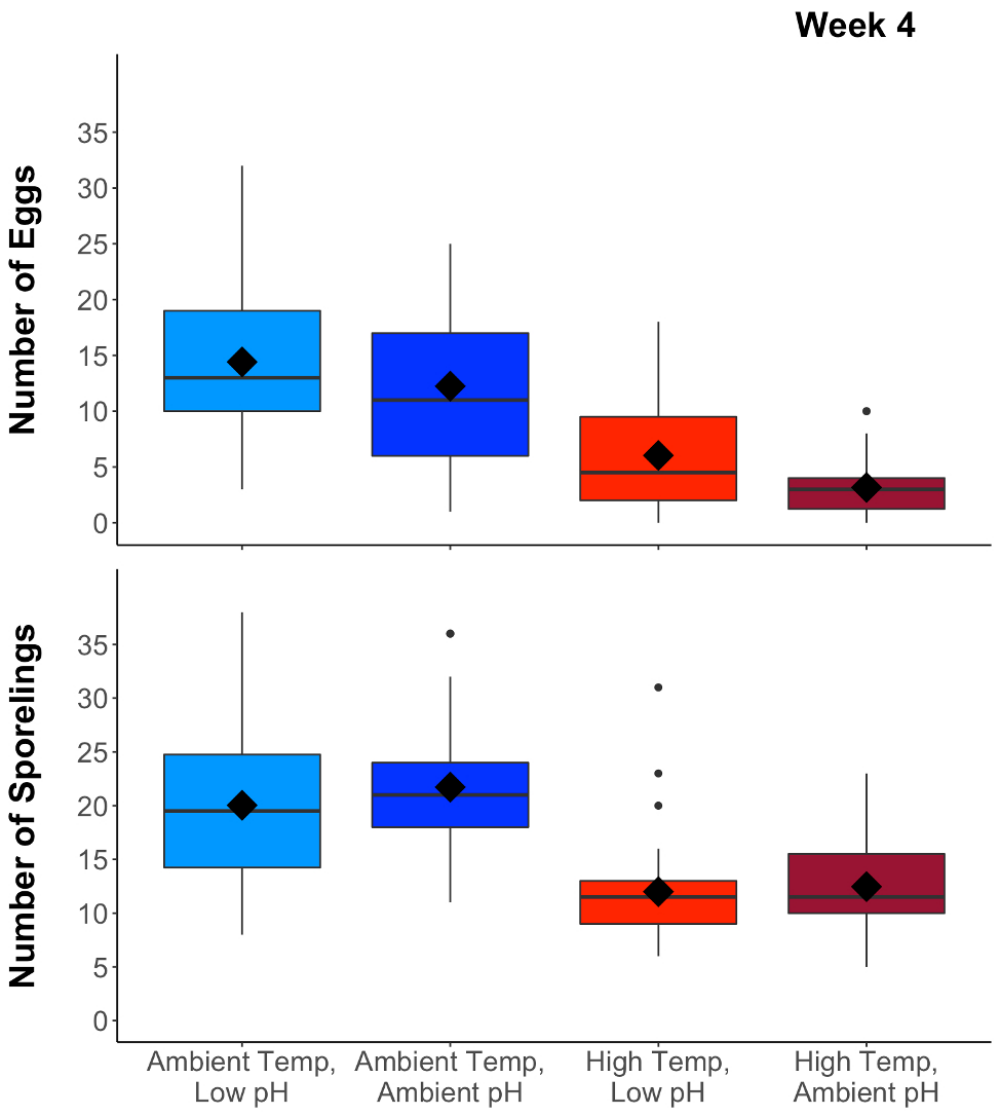
82x92mm (300 x 300 DPI)



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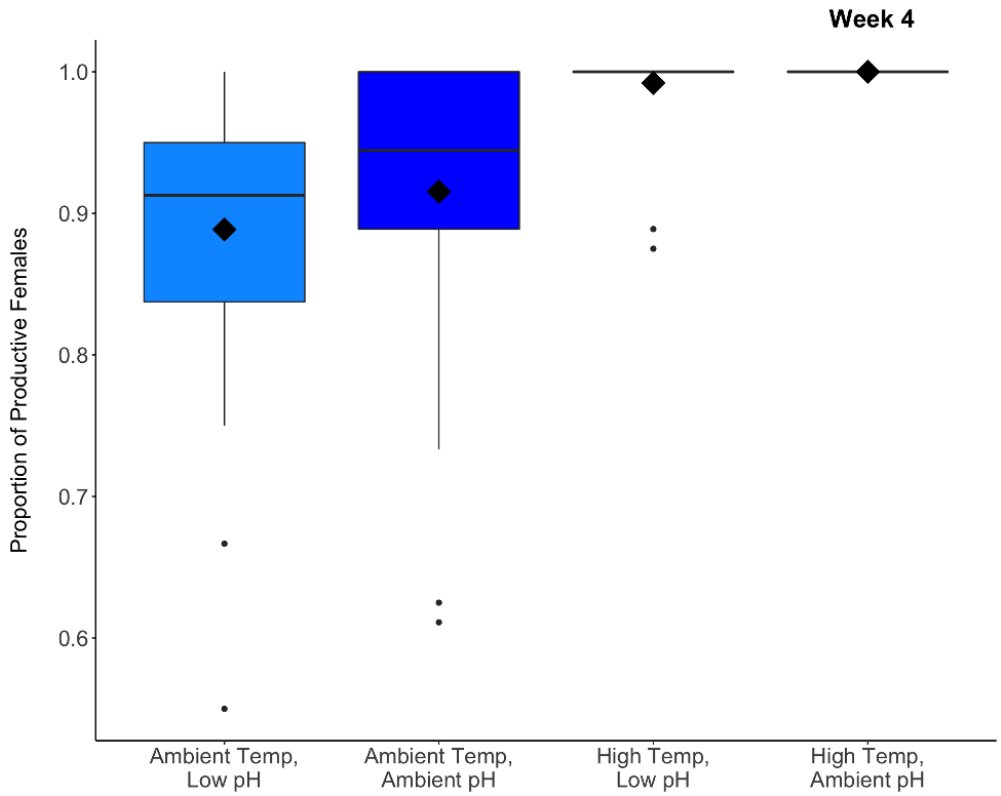
82x91mm (300 x 300 DPI)



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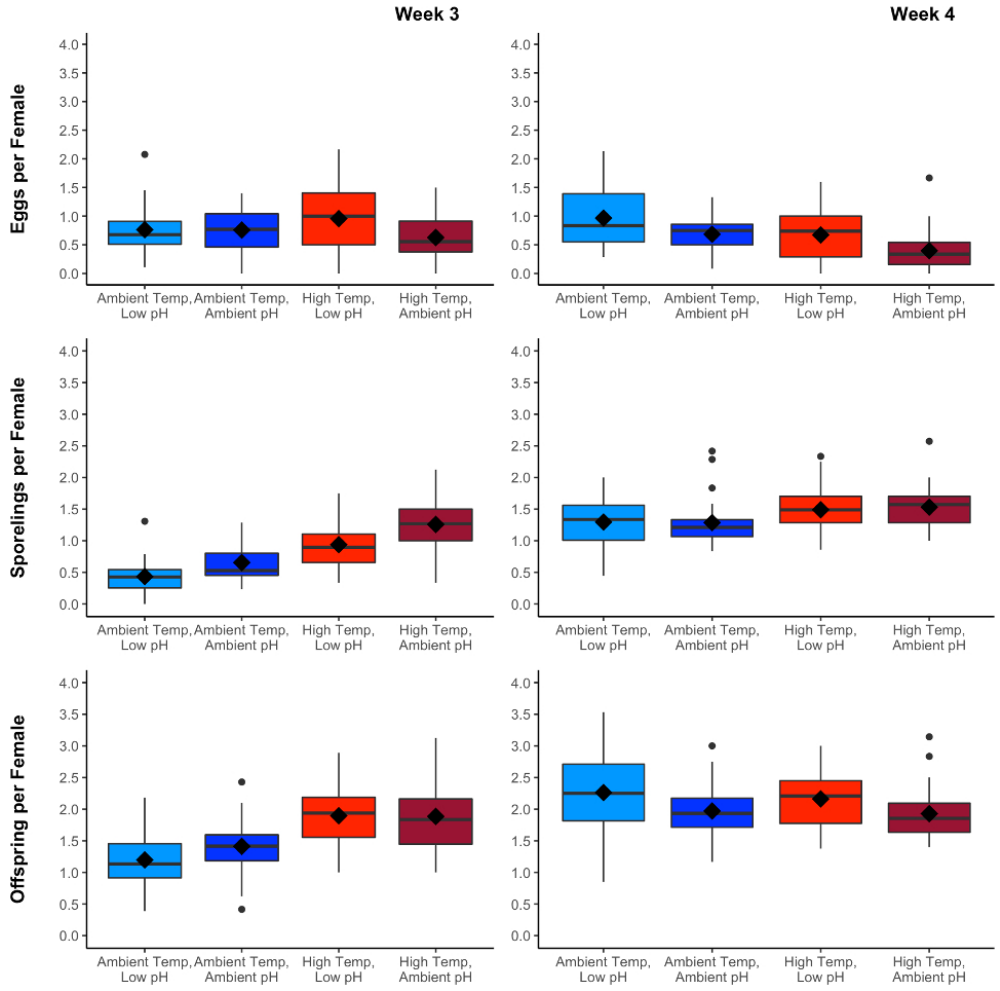
82x65mm (300 x 300 DPI)



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82x81mm (300 x 300 DPI)

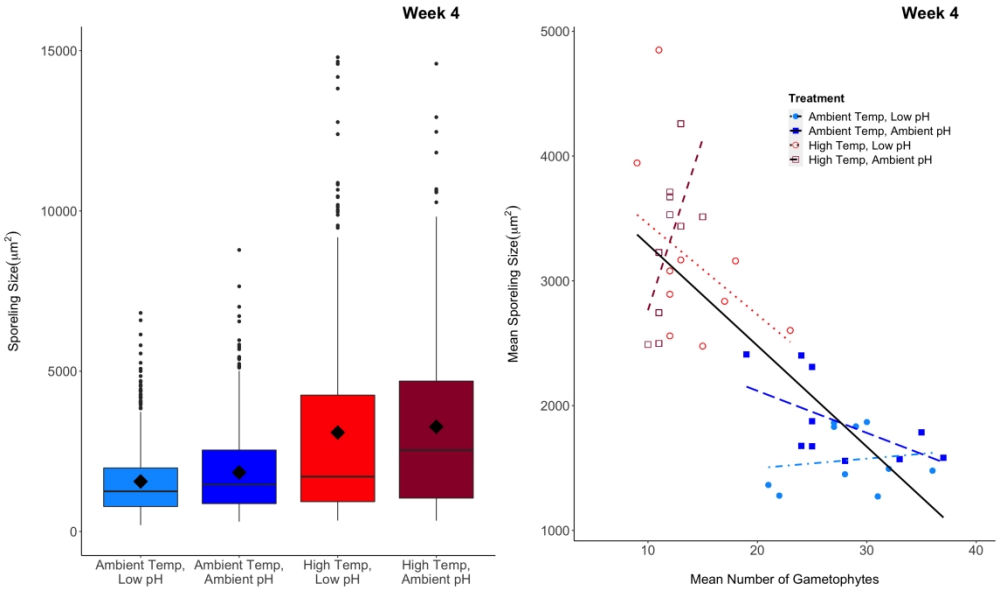

Figure 6: Left panel shows the average size of sporelings after 4 weeks of growth. The left panel shows box plots that summarize the mean (diamond) and median (box midline) for each treatment, the first and third quartiles (upper and lower box limits), outliers within 1.5 times the inter-quartile range (vertical lines), and outliers beyond that range (dots). The right panel shows the relationship of the covariate (mean number of gametophytes) to the response variable (mean sporeling size). Data and trends are represented by different dash and dot styles and colors for each treatment: Ambient Temp and Low pH (light blue, solid circles, dot-dash line), Ambient Temp and Ambient pH (dark blue, solid squares, long dash line), High Temp and Low pH (red, open circle, dotted line), and High Temp and Ambient pH (dark red, open square, short dash line).The trend across all groups is represented by the solid black line. Heterogeneous slopes and different ranges of values for each treatment indicate that the different treatments are confounded with differences in the covariate.

243x143mm (300 x 300 DPI)

Linear Mixed Model Results					
Variable	**Variable Type**	**Effect**	**Log-Likelihood**	**DF**	**P-value**
Females	Count	Temperature	51.128	36	**<0.001**
		pH	0.518	36	0.472
		Temperature:pH	0.813	36	0.367
		Petri Dish ID (random)	7.880		**0.005**
Males	Count	Temperature	45.393	36	**<0.001**
		pH	8.638	36	**0.003**
		Temperature:pH	0.016	36	0.901
		Petri Dish ID (random)	1.082		0.298
Eggs	Count	Temperature	33.730	36	**<0.001**
		pH	4.396	36	**0.036**
		Temperature:pH	0.190	36	0.663
		Petri Dish ID (random)	5.208		**0.023**
Juveniles	Count	Temperature	36.639	36	**<0.001**
		pH	1.070	36	0.301
		Temperature:pH	0.199	36	0.656
		Petri Dish ID (random)	3.054		0.081
Eggs per Female (Week 3)	Ratio	Temperature	0.141	36	0.708
		pH	3.735	36	**0.053**
		Temperature:pH	3.774	36	**0.052**
		Petri Dish ID (random)	0.232		0.630
Eggs per Female (Week 4)	Ratio	Temperature	13.114	36	**<0.001**
		pH	9.366	36	**0.002**
		Temperature:pH	0.098	36	0.755
		Petri Dish ID (random)	0.564	36	0.453
Juveniles per Female (Week 3)	Ratio	Temperature	45.264	36	**<0.001**
		pH	16.749	36	**<0.001**
		Temperature:pH	0.067	36	0.796
		Petri Dish ID (random)	0.118		0.731
Juveniles per Female (Week 4)	Ratio	Temperature	9.187	36	**0.002**
		pH	0.126	36	0.723
		Temperature:pH	0.100	36	0.751
		Petri Dish ID (random)	1.145		0.285
Offspring per Female (Week 3)	Ratio	Temperature	30.419	36	**<0.001**
		pH	1.362	36	0.243
		Temperature:pH	1.709	36	0.191
		Petri Dish ID (random)	0.065		0.799
Offspring per Female (Week 4)	Ratio	Temperature	0.228	36	0.633
		pH	5.234	36	**0.022**
		Temperature:pH	0.006	36	0.937
		Petri Dish ID (random)	1.053		0.305

Linear Mixed Model results for count and offspring to female ratio data.

185x245mm (300 x 300 DPI)

Generalized Linear Mixed Model Results

Variable	Data Subset	Effect	Chi-Sq	DF	P-value
Proportion of Females Productive	All Data	Temperature	28.187	36	<0.001
		pH	1.162	36	0.281
		Temperature:pH	0.512	36	0.474
Size	All Data	# Gametophytes:Temperature:pH	6.339		0.012
	Ambient Temperature	pH	3.955		0.047
		# Gametophytes (covariate)	1.173		0.279
		# Gametophytes:pH	2.267		0.132
	High Temperature	pH	0.081		0.776
		# Gametophytes (covariate)	0.988		0.320
		# Gametophytes:pH	6.039		0.014
	Low pH	Temperature	10.051		0.002
		# Gametophytes (covariate)	0.319		0.572
		# Gametophytes:Temperature	1.611		0.204
	Ambient pH	Temperature	2.082		0.149
		# Gametophytes (covariate)	3.181		0.074
		# Gametophytes:Temperature	5.300		0.021

Generalized Linear Mixed Model results for proportion productive females and size data.

194x116mm (300 x 300 DPI)