Title: Examining the Reproductive Success of Bull Kelp (*Nereocystis luetkeana*) In Climate Change Conditions

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Abstract:

Climate change is affecting marine ecosystems through warming temperatures and ocean acidification. From 2014-2016, an extensive marine heatwave 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, but the impacts of marine heat waves and ocean acidification on specific parts of the bull kelp life cycle have not been fully investigated. 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 and proceeded to culture microscopic bull kelp gametophytes in a common garden experiment with a fully factorial design crossing modern conditions (11.63±0.54°C and 7.93±0.26pH) with projected climate change conditions (15.56±0.83°C and

7.64±0.32pH). Our results found that both increasing temperatures and decreasing pH influenced growth-and egg production of bull kelp microscopic stages. We found that increased temperature and decreased pH had interacting effects on both sporophyte growth and egg production. Increased temperature generally resulted in decreased gametophyte survival and offspring production. In contrast, decreasing pH had less of an effect, but generally resulted in increased gametophyte survival and offspring production. Additionally, we found that increased temperature significantly impacted the rate of production by causing female gametophytes to produce offspring earlier than under low temperature conditions. Our findings inform better predictions of the impacts of climate change on coastal ecosystems as well as provide key insight into environmental dynamics regulating the bull kelp lifecycle.

Introduction:

Globally, marine systems are under pervasive threats from climate change. Chief among these threats are marine heat waves and ocean acidification (OA). Exactly how temperature and OA will impact the critical structure forming foundational species of the world's oceans, namely kelps and corals, is the topic of much study. Kelp forests are critical to temperate, nearshore subtidal and intertidal marine systems worldwide, and sustain numerous economically important recreational and commercial fisheries (Steneck 2002). In addition, kelp forests provide numerous ecosystem 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, modification of seawater chemistry (Hamilton et al. 2022, Malone et al. 2022).

From 2014-2017 Northern California lost 90% of its *Nereocystis luetkeana* kelp canopy cover over an area of roughly 350 km (Rogers-Bennett and Catton 2019). This loss of kelp forest cover has been attributed to a dramatic increase in purple urchin density due to loss of keystone predators, coupled with a pervasive system of marine heatwaves (McPhearson et al. 2021). This widespread loss of kelp canopy resulted in 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 (Rogers-Bennett and Okamoto 2020) and the closure of the world's largest recreational abalone fishery (Rogers-Bennett and Catton 2019, Reid et al. 2016).

Numerous studies in recent years have documented the effects of increasing temperature on bull kelp canopies (Rogers-Bennett and Catton 2019, Hamilton et al. 2020), and have found that decreases in adult bull kelp canopy abundance have been related to local and large scale processes associated with warm water (Schiel et al. 2004, Pfister et al. 2017). Bull kelp exposure

to warm temperatures also reduces tension regulated plasticity in adult blades (Suprataya et al. 2020), but the physiological impacts of warm waters on bull kelp need to be further studied. Studies of bull kelp microscopic stages in British Columbia and Alaska have found that increased temperatures have resulted in reductions in settlement and reduced germination and growth (Schiltroth 2021, Lind and Konar 2017, Muth et al. 2019), but the impact of rising temperatures on microscopic bull kelp stages in the southern portion of their range in northern California remains unclear.

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, exhibit a heteromorphic life cycle, in which they possess a large, multicellular sporophyte, and a microscopic, unicellular gametophyte. The heteromorphic life cycle of macroalgae begins with a large sporophyte. As the sporophyte develops, a sporophyll forms, and at maturity, begins to release spores. The released zoospores then settle on substrate, where they grow into microscopic male and female gametophytes. The female gametophytes release a pheromone to trigger sperm release from nearby males. Once the sperm fertilizes the egg, a new sporophyte begins to develop (Reed 1990). This multistage process of microscopic bull kelp development presents numerous areas for the imposition of bottlenecks from climate stress.

In addition to the increasing threat of marine heat waves, coastal temperate ecosystems are also subject to stress from ocean acidification (OA). 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 natural shifts in pH. This exposure varies with local bathymetry, which often changes the intensity of upwelling events along the coast (Feely et al. 2008). While pH varies naturally, climate change projections predict

an increasing frequency and duration of low-pH extremes (Bakun et al. 2015, García-Reyes et al. 2015) that 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 it is critical that we also understand how the compounding stress of these combined threats will impact our critical temperate nearshore systems.

Kelps are photosynthetic organisms and therefore utilize CO_2. Kelps have the unique ability to concentrate calcium carbonate into their tissues and are therefore very efficient at processing multiple carbon species in the water column. There is some evidence to suggest that the excess of Carbon predicted for future ocean conditions may increase kelp growth in climate change conditions. Bull kelp has been found to be both a receptor and benefactor of the carbon cycle in coastal marine ecosystems. Increased CO_2 levels have beneficial impacts on bull kelp net apparent productivity (Thom 1996) and growth (Swanson and Fox 2007), and in turn, bull kelp has been found to have significant impacts on seawater carbonate chemistry in their surrounding ecosystems (Pfister et al. 2019). However, to our knowledge, no studies have yet investigated the role that pH play in bull kelp juvenile kelp development, nor the combined threats of increased temperature and ocean acidification on any bull kelp lifestage.

After witnessing the large scale die-offs of bull kelp adults as a result of the 2014-2016 marine heatwave, we asked the question: how will increasing temperatures and decreasing pH impact bull kelp 1) gametophyte development, 2) egg and sporeling production, and 3) juvenile growth? We hypothesized that both increasing temperatures and decreasing pH influence growth and egg production of bull kelp microscopic stages. Based on the observed negative effects of the warm blob on bull kelp adults, we hypothesized that increased temperature will generally result in decreased metrics of reproduction (e.g. fewer offspring, less growth, lower survival). In

contrast, we hypothesized that decreasing pH has less of an effect than temperature on growth and egg production, but generally resulted in increased metrics of reproduction (e.g. more offspring, more growth, less survival) due to the fact that more carbonate in the water will increase photosynthesis.

Materials and Methods:

Collection

Sporophylls from approximately 10 individuals were collected at the surface by boat from Point Arena, California (38.916271°N, 123.725644°W) in October 2017. Sporophylls were cleaned in iodine and freshwater 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 at densities of approximately 8 spores mm-2.

Ex-situ culturing experiment

The fully factorial common garden experiment consisted of four treatments representing high and low temperature and high and low pH, with ten replicates per treatment, for a total of forty experimental petri dishes (Fisher Brand 100mx15mm). Temperature was maintained at 15.56±0.83°C and 11.63±0.54°C using incubators at 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). 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 SSTs observed during the Warm Blob (Gentemann et al. 2017). High 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. The pH of incoming, manipulated, and outgoing seawater was measured immediately after collection using a spectrophotometer. Total alkalinity (T_{alk}, μmol kg−1) was measured using potentiometric acid titration. We calculated carbonate chemistry parameters using CO2SYS v2.1 http://cdiac.ess-dive.lbl.gov/ftp/co2sys/) and the R package seacarb (Gattuso et al., 2018), with K1 and K2 dissociation constants based on Mehrbach et al. (1973) refit by Dickson and Millero (1987), and KHSO4 based on Dickson et al. (2007). Water was changed in all experimental dishes every 2-3 days for the duration of the experiment - 27 days. We added standard 20 mL L^{-1} Provasoli nutrient mix to all treatment water (Provasoli 1968).

Photo Analysis

Beginning one week after spore inoculation, petri dishes were photographed weekly with a Micropublisher 5.0 RTV digital camera (QImaging, Surrey, Canada) mounted on an inverted microscope at 4x magnification, resulting in 4 weeks of photos documenting population growth and reproduction. Within each dish, 3 points were randomly selected to be photographed, with different points being photographed each week. Each photo encompassed 1.08mm^2 of the petri dish (15,708mm^2 bottom surface area).

After the growth experiment was completed, each photo was analyzed using ImageJ. Week 1 and 2 photos did not contain any gametophytes large enough to identify, so only Weeks 3 and 4 were used for analysis. Count data was obtained from each photo for female gametophytes, male gametophytes, eggs, and juvenile sporelings (Figure 1). Females were also categorized as “productive” (having produced at least one egg or juvenile) or “non-productive” (have no eggs or juveniles), and the proportion of productive females in each photo was

calculated by dividing the number of productive females by the total number of females counted. Count data was also used to calculate three additional variables: average number of eggs per female (No. eggs/No. females), average number of juveniles per female (No. juveniles/No. females), and average number of offspring per female ((No. eggs + No. juveniles)/No. Females). Juvenile sizes were also obtained by using the freehand trace tool and measuring the number of pixels encapsulated. Sizes were then converted to um^2 using a conversion factor of 71330 pixels per 62,500um^2, which was calculated by measuring the area of a photo of a 0.0625mm^2 hemocytometer cell.

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 before being analyzed.

Average number of eggs, juveniles, and total offspring (juveniles+eggs) per female were subject to different transformations based on each week. Both eggs per female (EPF) and offspring per female (OPF) were analyzed in Week 3 using the raw data, but in Week 4, both variables were subjected to a log transformation to achieve normality and homogeneity of variance. Data for juveniles per female (JPF) were square root transformed in both Weeks 3 and 4.

Size data for weeks 3 and 4 were subjected to a log transformation and analyzed using linear mixed models with with temperature, pH, and their interaction as fixed effects, and Dish ID as a random effect. Average number of gametophytes per photo in a given dish was also calculated and included in the model as a covariate to account for possible density dependence.

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. All analyses were performed using R version 4.1.2 (R Core Team 2021) and the package *nlme* (Pinheiro et al. 2022).

Results:

Female and Male Gametophyte Development

To assess how lowered pH and increased temp affect the development of gametophytes, we examined the average number of female and male gametophytes per photo for both weeks 3 and 4 (Figure 2, Table 1). High temperatures resulted in a significant decline in the number of females present in both Week 3 (Log-Likelihood=20.9715, DF=36 , $p<0.0001$) and Week 4 (Log-Likelihood=51.1283, DF=36 , $p<0.0001$). Neither pH nor the interaction between pH and temperature had a significant effect on female gametophyte numbers, regardless of week. The random effect of Dish had a significant effect on female gametophyte numbers in both Week 3 (Log-Likelihood=5.0547, $p=0.0246$) and Week 4 (Log-Likelihood=7.8795, $p=0.005$), indicating that there was some variance between petri dishes for this test.

Males were similarly negatively affected by high temperatures in both Week 3 (Log-Likelihood=18.9288, DF=36 , $p<0.0001$) and Week 4 (Log-Likelihood=45.393, DF=36 , $p<0.0001$). However, they varied from females in that their numbers were significantly higher under lower pH conditions in Week 4 (Log-Likelihood=8.6378, DF=36 , $p=0.0033$), but not in Week 3 (Log-Likelihood=1.4436, DF=36 , $p=0.2296$). Neither the pH:temperature interaction term nor the random effect Dish had any significant effect on male gametophyte numbers. In summary, these results indicate that temperature caused a significant decrease in female and

male gametophyte numbers regardless of age, whereas low pH only caused a significant increase in male gametophyte numbers in Week 4.

Egg and Sporeling Counts

High temperatures were correlated with significantly lower number of eggs in both Week 3 (Log-Likelihood=6.6296, DF=36 , p=0.01) and Week 4 (Log-Likelihood=33.73, DF=36 , p<0.0001) and a significantly lower number of juveniles in Week 4 only (Log-Likelihood=36.6391, DF=36 , p<0.0001). In Week 3, however, high temperatures were associated with a significantly higher number of juveniles (Log-Likelihood=5.6487, DF=36 , p=0.0175) (Figure 3).

Low pH was associated with a significantly larger number of eggs in Week 4 (Log-Likelihood=4.3958, DF=36 , p=0.036) but not Week 3 (Log-Likelihood=1.1809, DF=36 , p=0.2772), whereas juveniles were significantly fewer under low pH conditions in Week 3 (Log-Likelihood=10.0321, DF=36 , p=0.0015) but not Week 4 (Log-Likelihood=1.0702, DF=36 , p=0.3009). The interaction term for pH:temperature was consistently insignificant for counts of both eggs and juveniles. The random effect of Dish was insignificant for eggs in Week 3 (Log-Likelihood=0.7630, p=0.3824) and Juveniles in Week 4 (Log-Likelihood=3.0544, p=0.0805), but was significant for eggs in Week 4 (Log-Likelihood=5.2080, p=0.0225) and juveniles in Week 3 (Log-Likelihood=4.9355, p=0.0263).

Proportion of Productive Females

Due to the high proportion of productive females in all treatments, we were unable to normalize our data and therefore unable to run linear mixed models (Table 2). However, we were able to visually determine that there were higher proportions of productive females under high

temperatures in both Week 3 (low pH=0.89±0.13, ambient pH=0.95±0.08) and Week 4 (low pH=0.99±0.03, ambient pH=1±0) compared to low temperatures in Week 3 (low pH=0.68±0.20, ambient pH=0.80±y0.15) and Week 4 (low pH=0.88±0.11, ambient pH=0.92±0.10). The variance in proportion of productive females was also much higher in low temperature treatments than high temperature treatments (Figure 4).

Ratios of Offspring per Female

To look at how production was affected, we assessed 3 different calculated variables: mean number of eggs per female, mean number of juveniles per female, and mean number of offspring (eggs + juveniles) per female (Table 2, Figure 5). For mean number of eggs per female (EPF), 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). Investigating temperature and pH individually in Week 3, we found that pH (Log-Likelihood=3.7345, DF=36 , p=0.0533) was marginally significantly correlated with a decreased EPF ratio under low temperature treatments, but an increased EPF ratio under high temperature treatments. Temperature was found to be individually insignificant in Week 3 (Log-Likelihood=0.1406, DF=36 , p=0.7077). In Week 4, pH was found to be significantly correlated with a higher EPF (Log-Likelihood=9.3663, DF=36 , p=0.0022) whereas temperature was significantly correlated with a lower EPF (Log-Likelihood=13.114, DF=36 , p=0.0003). The random effect Dish was insignificant in both Week 3 (Log-Likelihood=0.2318, p=0.6302) and Week 4(Log-Likelihood=0.5643, p=0.4525).

For mean number of juveniles per female (JPF), we found that high temperatures were significantly correlated with a larger JPF 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 was significantly

correlated with a lower JPF 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 random effect Dish were significant in either week.

Increased ratios of total offspring per female (OPF) were significantly correlated 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 more OPF were significantly correlated with low pH 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 random effect Dish were significant in either week.

Growth of Juvenile Bull Kelp

For our last question, we looked at the overall size of juveniles. We first assessed the relationship of the covariate (average number of gametophytes per photo in a given dish) to the average size of gametophytes in each dish (Table 3). The covariate was found to have a significant relationship with gametophyte size in Week 3 (Log-Likelihood=10.9074, DF=36 , $p=0.001$), but was just barely insignificant in Week 4 (Log-Likelihood=3.0196, DF=36 , $p=0.0823$) (Figure 6). When plotting the average gametophyte size versus covariate, we found a negative correlation between the two variables in both Week 3 ($R^2=0.363$) and Week 4 ($R^2=0.639$), indicating a possible situation of density dependence where increased number of gametophytes were correlated with smaller juveniles.

We found temperature to be correlated with a significant increase in juvenile size in both Week 3 (Log-Likelihood=35.7158, DF=36 , $p<0.0001$) and Week 4 (Log-Likelihood=8.2143, DF=36 , $p=0.0042$) (Figure 7). pH had no significant relationship with juvenile sizes in Week 3

(Log-Likelihood=0.2684, DF=36 , p=0.6044), but in Week 4, it was found to be significantly associated with smaller juveniles (Log-Likelihood=8.1251, DF=36 , p=0.0044).

The random effect Dish was found to be significant in both Week 3 (Log-Likelihood=5.0104, p=0.0252) and Week 4 (Log-Likelihood=12.0374 , p=0.0005), indicating there was some variation in size based on petri dish ID. The interaction between pH and temperature was insignificant in both Week 3 (Log-Likelihood=0.0001, DF=36 , p=0.991) and Week 4 (Log-Likelihood=0.1151, DF=36 , p=0.7345).

Discussion:

Climate change represents a threat to kelps through variable effects on community structure and function due to increasing mean sea-surface temperatures (SST), increasing frequency/intensity of marine heatwaves, and ocean acidification. California kelp forests have already experienced significant die-offs over the past several years that have likely been the result of elevated SST and urchin grazing. In this research, we sought to ask whether the 2014-2016 “Warm Blob” marine heatwave had significant impacts on the survival of bull kelp gametophytes and the production and growth of juvenile bull kelp sporophytes. While we did find widely significant effects of both temperature and pH in our experiment, we were not able to overall accept our hypothesis that high temperature would result in overall negative effects on bull kelp reproduction, whereas pH would have overall positive effects on bull kelp reproduction. Both temperature and pH do significantly impact bull kelp reproduction and development, but the effects are more variable and nuanced than we predicted.

The most consistent finding of ours is that high temperatures resulted in significant reduction in the number of gametophytes that survived/settled and the number of offspring produced. The number of males gametophytes, female gametophytes, and eggs was always lower

in high temperature treatments than low temperature treatments, regardless of pH. The only deviation from this trend occurred in Week 3, when the number of juveniles was larger in high temperature treatments than low temperature treatments (Figure 2). By week 4, however, the number of juveniles in high temperature treatments seemed to stagnate and was consequently lower than in low temperature treatments. These results align with previous findings that increased temperature on bull kelp and have found that increased temperatures result in reduced germination rates (Schiltroth 2021), decreased spore settlement, and reduced gametophyte growth (Lind and Konar 2017; Muth et al 2019). Increased temperatures also result in decreased gametophyte survival and sporophyte recruitment in numerous other kelp species including giant kelp (*Macrocystis pyrifera)* (Hollarsmith et al 2020*)*, stalked kelp (*Pterygophora californica*) (Howard 2014), spiny kelp (*Ecklonia radiat*a) (Alsuwaiyan 2021), sugar kelp (*Saccharina latissima*), and dragon kelp (*Eualaria fistulosa*) (Lind and Konar 2017).

Low pH had a much less significant impact on number of gametophytes or offspring than temperature, but there were a few instances where pH did cause a significant decrease in number of males in Week 3 and a significant increase in male gametophytes and eggs in Week 4. Other studies have found varying impacts of pH on kelp gametophyte growth and survival. Several studies have found overall positive effects of low pH on giant kelp (*Macrocystis pyrifera*) gametophyte growth, survival, and size (Roleda et al. 2012, Leal et al. 2017), where as other studies found that elevated pCO2 had little effect on rates of growth and photosynthesis (Fernández et al. 2015) or reproduction (Hollarsmith et al 2020). The variation in kelp organismal responses across studies, species, and location (Roleda and Hurd 2012; Hollarsmith et al 2020) indicates that there is much more to be understood about the impacts of ocean acidification on kelp reproduction.

Another finding of ours is that, as a result of temperature stress, bull kelp gametophytes are putting more effort into reproduction. While the overall number of gametophytes and juveniles declined under high temperature conditions, the female gametophytes that survived were more productive on average, and produced more juveniles earlier than female gametophytes under low temperature conditions. In week 3, low temperature conditions exhibited fewer offspring per female than high temperature conditions, but this ratio evened out across temperature treatments by week 4 (Figure 5). This result suggests that female gametophytes possess similar capacities for offspring production regardless of treatment, but that females become reproductive sooner under high temperature conditions. This idea is also supported by the separate ratios for eggs per female and juveniles per female in each week. The EPF ratio in high temperature treatments in Week 3 was higher or even to that of lower temperature treatments but by Week 4, the higher temperature treatments had an overall lower EPF ratio than the low temperature treatments. The differences in average JPF ratio between high temperature treatments and low temperature treatments in Week 3 was very large, but by Week 4, even though JPF was still significantly larger in high temperature treatments, the differences between the two temperature treatments was not nearly as distinct. In the kelp reproductive cycle, eggs mature into juveniles, so the higher proportion of eggs and juveniles in high temperature treatments in week 3 and the higher EPF and nearly equal JPF in low temperature treatments in Week 4 seem to tell a story of slower development under low temperature conditions. These results are consistent with those of Howard 2014, who found that lower temperatures lead to slower microstage reproduction of bull kelp as well as giant kelp (*Macrocystis pyrifera*) and stalked kelp (*Pterygophora californica*).

In contrast, low pH conditions seemed to lead to slower reproductive efforts under cold temperatures but increased reproductive efforts under high temperatures. The lowest proportion of productive females was observed in low pH treatments under ambient temperatures, and these females seemed to produce more offspring per female, later in the experiment. There was an insignificant interaction between pH and temperature in Week 3, where EPF and JPF ratios were higher under low pH and high temperature conditions, but this relationship was not particularly significant for overall OPF. The greatest increase in productivity between Week 3 and Week 4 under different pH treatments can be found in the large increase in EPF ratios under low pH conditions. This late increase in production of eggs may potentially signal that a delay in reproduction occurs under low pH conditions.

Recent advances in kelp reproduction studies have given needed attention to delayed development of microscopic stages and the resulting “bank of microscopic forms” (Hoffman and Santelices 1991, Carney and Edwards 2006, Schoenrock et al. 2021), but less focus has been placed on the factors that may accelerate microscopic kelp development. In terrestrial plants, increasing 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 increasing 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 time to development and propagule production.

For all variables other than EPF in Week 3, we found no interacting effects of temperature and pH on bull kelp microscopic stages, which contrasts with studies of giant kelp (Macrocystis pyrifera), that did find interactive effects of pH or pCO2 and temperature on spore, gametophyte, and sporeling production (Gaitan-Espitia et al. 2014, Shukla and Edwards 2017, Hollarsmith et al. 2020). Our results interestingly reflect natural seasonal fluctuations in northern California's coastal waters, where conditions of low pH and high temperature rarely overlap. Oceanographic conditions in California's coastal waters are typically determined by three seasons: upwelling season (April to June), relaxation season (July to September), and storm season (December to March) (García-Reyes and Largier 2012). Upwelling season is characterized by the upwelling of cold, dense, nutrient rich water from the deep ocean to the coastline, causing coastal waters to be colder and more acidic. During non-upwelling season, on the other hand, the intensity of upwelling processes decrease, and coastal waters become relatively warmer, more basic, and exhibit less primary productivity and chlorophyll-a content due to shortened daylengths (García-Reyes and Largier 2012). The bull kelp lifecycle has a relatively consistent, yet still flexible, phenology. The majority of visible juveniles appear in the spring and most adults become reproductive by the end of July, but these two events of visible recruitment and spore release have been observed to occur in all seasons, albeit at much lower rates (Maxell and Miller 1996, Dobkowski et al. 2018).

As a result, the need for gametophytes to endure high temperature or low pH conditions is dependent on the life cycle of the parent sporophyte. Gametophytes and sporelings that develop in the spring will likely be most exposed to low temperatures and low pH as a result of upwelling, but the vast majority of gametophytes and sporelings that develop in the fall will be exposed to non-upwelling season late summer conditions. As such, it is conceivable that high

temperatures in September and October would affect the first month of juvenile development, whereas upwelling season would likely be more important for visible juveniles than the microscopic ones.

However, our results potentially contrast with those of Dobkowski et al. 2018 in that we found that low pHs (most often seen in the Spring) resulted in slower reproduction and growth whereas high temperatures (most often seen in late summer and fall) accelerated it. In their study, Dobkowski et al. (2018) witnessed the quickest recruitment of visible juveniles (indicating faster microscopic development times) in the Spring, and slowest recruitment (implying slower microscopic development times) in the late summer and fall. A potential explanation for the different observed reproductive rates is that Dobkowski et al. conducted their experiments in the field, where they were exposed to a full of array of abiotic conditions, whereas our experiments were conducted in a laboratory setting where only temperature and pH were manipulated, and all other variables were held constant. Previous studies have shown that delayed development of microscopic kelp stages is often closely tied to insufficient nutrient and light regimes (Carney and Edwards 2010), both of which are present between September to March due to dampened upwelling conditions and reduced daylength. As such, the slow development over winter in natural populations suggests that nutrients from upwelling and daylength could be more important than temperature and pH fluctuations in promoting the development of microscopic kelp stages.

Our results suggest that there may be some density dependence effects on juvenile growth at these microscopic stages. The difference in juvenile sizes between treatments was most significantly correlated with temperature, but also showed at least a marginally significant correlation with the number of gametophytes present in both weeks (Figure 5). However, due to

the fact that high temperatures consistently resulted in significant decreases in gametophyte numbers, the relationships of both temperature and number of gametophytes to gametophyte size are confounded, and direct causation cannot be determined. As a result, more research is needed to see whether these increased sizes were really a result of high temperatures or whether they were a result of lowered population sizes. In natural populations, there are numerous density-dependent effects that impact kelp reproduction and recruitment. At initial spore settlement, high densities of gametophytes are needed for fertilization between male and female gametophytes to occur, so Allee effects may occur if spores settle at a density of less than 1 spore•mm^2 (Reed 1990). The direction of density-dependence then reverses somewhere between the gametophyte stage and the point where a juvenile becomes easily detectable to the naked eye, and numerous kelps, including bull kelp, exhibit subsequent increases in mortality until they reach the adult life stage (Schiel and Foster 2006, Reed et al. 1991, Dobkowski et al. 2018). Our petri dishes, each containing thousands of spores and gametophytes, contained a bottom surface area of approximately 157.8cm^2, whereas an adult bull kelp holdfast can grow up to an estimated 40cm in diameter and 1256cm^2 surface area (Abbot and Hollenberg 1976). As a result, only one juvenile sporophyte in each petri dish would be expected to survive to adulthood and reproductive maturity in natural environments.

Beyond the fact that this experiment was conducted in a laboratory setting, there were some significant ways in which our experiment varied from a natural setting. The greatest difference is that we only cultured our gametophytes and juveniles for one month, whereas the Warm Blob lasted for over two years (Gentemann et al. 2017). While we were able to determine that survival of microscopic kelp stages was still possible under high temperatures, it may be possible in intermediate kelp stages, between 1 cm and 1m tall, that high temperatures would still

result in significant mortality rates. As such, more research is needed to better understand the tolerances of intermediate bull kelp stages to environmental stresses. Additionally, we only manipulated temperature and pH in this experiment, but during the Warm Blob, there were numerous abiotic and environmental cascades co-occurring, including reduced nutrient input from upwelling (Gentemann et al. 2017) and increased grazing by sea urchins due to predator loss (Rogers-Bennett and Catton 2019). Regardless of whether juvenile kelps are resilient enough to survive high temperatures, the near total grazing rate of the enormous urchin barrens has been enough to severely hinder or completely prevent the reestablishment of kelp canopies in some areas. Lastly, we did obtain the spores we used for this experiment from a bull kelp population that was able to survive the Warm Blob, so there may be some genetic heritability of tolerance to warmer water conditions, but this remains to be experimentally studied.

The results of this research indicate that climate change will significantly affect bull kelp reproduction via increasing temperatures, and, to a lesser extent, ocean acidification. Increasing frequency and intensity of extreme temperature events such as marine heatwaves will lead to a massive decrease in the survival of gametophytes and decreased, but accelerated, production of juvenile sporophytes. Lowered pH as a result of ocean acidification resulted in an increase in numbers of male gametophytes and juvenile sporelings, as well as a slower reproduction rate. Currently, managers and scientists are focused on understanding the natural processes that guide kelp recovery and whether those may require some degree of management intervention. In order to better understand the ability of species to recover from climate change, more research is needed to understand the ability of intermediate stages between propagules and adults to withstand abiotic stress.

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Tables:

Week 3

Treatment	**Eggs**	**Females**	**Juveniles**	**Males**
Ambient Temp, Low pH	10.9 (7.03)	14.2 (4.21)	5.83 (3.47)	13.3 (5.23)
Ambient Temp, Ambient pH	10.67 (6.92)	13.77 (3.47)	8.73 (3.84)	10.37 (3.64)
High Temp, Low pH	8.53 (5.88)	8.77 (3.4)	7.97 (3.75)	7.27 (3.97)
High Temp, Ambient pH	7.27 (8.58)	9.97 (7.65)	11.1 (6.19)	8.1 (5.83)

Week 4

Treatment	**Eggs**	**Females**	**Juveniles**	**Males**
Ambient Temp, Low pH	15.6 (10.48)	15.83 (4.05)	20.03 (6.99)	12.83 (3.79)
Ambient Temp, Ambient pH	12.24 (6.65)	17.55 (5.13)	21.72 (6.14)	10.17 (4.26)
High Temp, Low pH	6.03 (5.17)	8.5 (3.88)	12 (5.26)	6.1 (3.17)
High Temp, Ambient pH	3.17 (2.74)	8.13 (2.21)	12.47 (4.29)	4.17 (2.29)

Table 1: Means and SDs of Count data

Week 3

Treatment	Prop_prod	eggperF	juvperF	E+JperF
Ambient Temp, Low pH	0.68 (0.2)	0.76 (0.4)	0.43 (0.27)	1.2 (0.48)
Ambient Temp, Ambient pH	0.8 (0.15)	0.76 (0.4)	0.66 (0.29)	1.41 (0.41)
High Temp, Low pH	0.89 (0.13)	0.96 (0.55)	0.94 (0.35)	1.9 (0.49)
High Temp, Ambient pH	0.95 (0.08)	0.63 (0.42)	1.26 (0.42)	1.89 (0.53)

Week 4

Treatment	Prop_prod	eggperF	juvperF	E+JperF
Ambient Temp, Low pH	0.89 (0.11)	0.97 (0.53)	1.3 (0.4)	2.26 (0.65)
Ambient Temp, Ambient pH	0.92 (0.1)	0.68 (0.3)	1.29 (0.37)	1.97 (0.42)
High Temp, Low pH	0.99 (0.03)	0.67 (0.48)	1.49 (0.38)	2.16 (0.49)
High Temp, Ambient pH	1 (0)	0.4 (0.36)	1.53 (0.33)	1.93 (0.41)

Table 2: Means and SDs of Productivity Data

Week 3

Treatment	area_um2	gammean
Ambient Temp, Low pH	751.16 (397.51)	26.64 (5.21)
Ambient Temp, Ambient pH	760.97 (372.68)	23.89 (3.89)
High Temp, Low pH	1620.63 (1175.64)	16.21 (3.87)
High Temp, Ambient pH	1510.76 (1142.12)	20.64 (9.85)

Week 4

Treatment	area_um2	gammean
Ambient Temp, Low pH	1561.11 (1101.82)	28.17 (3.97)
Ambient Temp, Ambient pH	1841.37 (1288.6)	28.22 (5.47)
High Temp, Low pH	3088.05 (3079.67)	15.06 (4.16)
High Temp, Ambient pH	3304.37 (2726.05)	11.97 (1.31

Table 3: Means and SDs of sizes and covariates.

Figures:

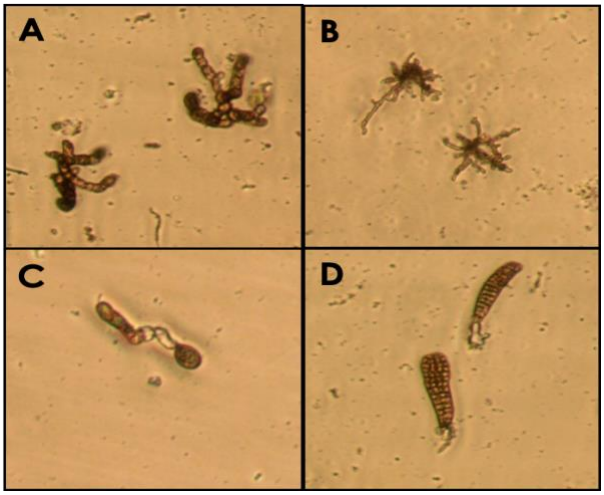

Figure 1: The microscopic stages of bull kelp: A) Female gametophyte (image area =0.065 mm^2); B) Male gametophyte (image area = 0.065 mm^2); C) Female gametophyte producing an egg (image area = 0.077 mm^2); D) Female gametophytes with juvenile sporelings (image area = 0.065 mm^2).

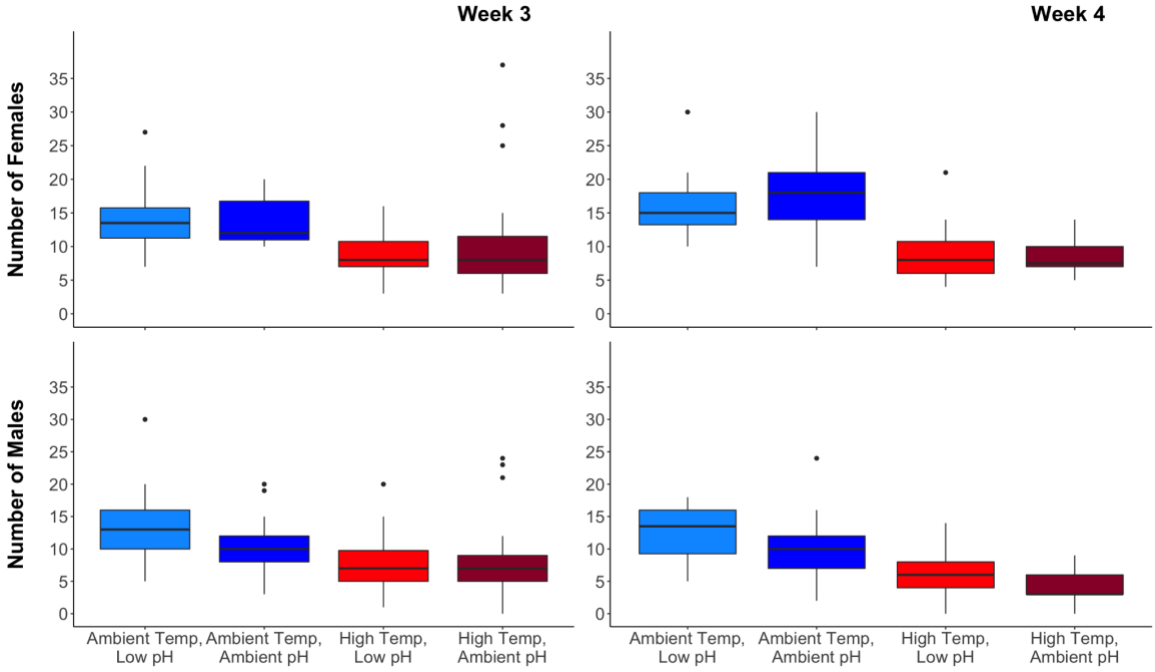

Figure 2: The average number of female and gametophytes present in each photo after 3 and 4 weeks of growth. Error bars represent 1.5 interquartile ranges from the median.

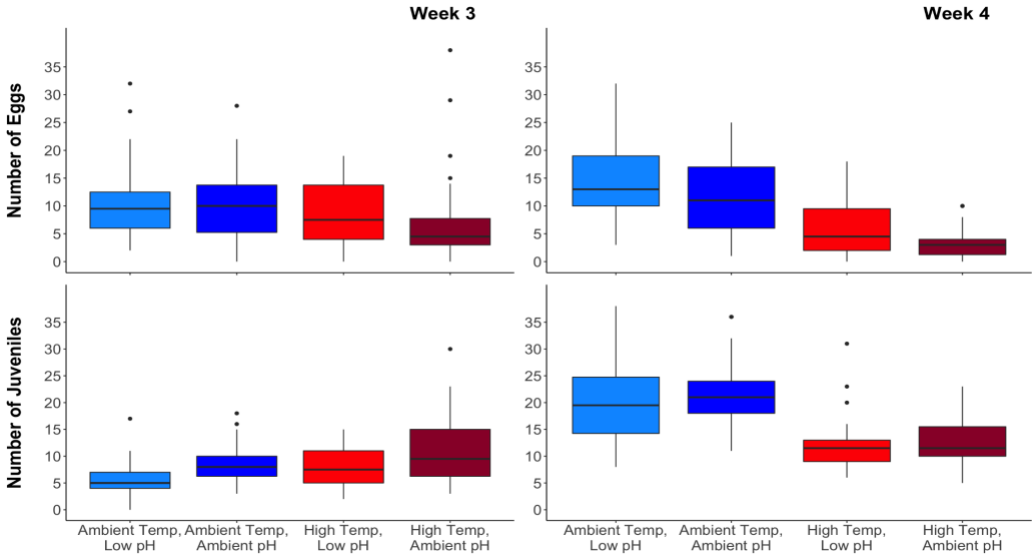

Figure 3: The average number of eggs and juveniles present in each photo after 3 and 4 weeks of growth. Error bars represent 1.5 interquartile ranges from the median.

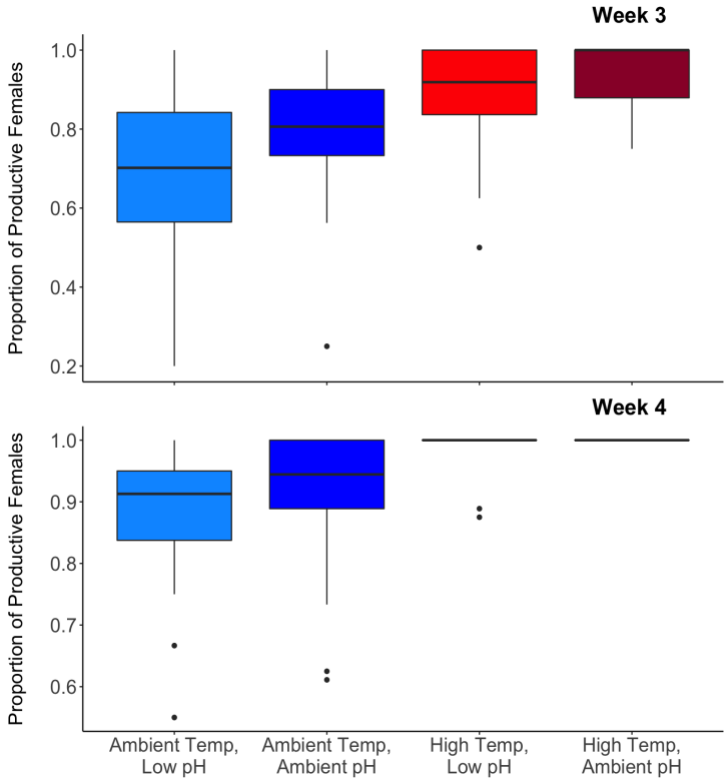

Figure 4: The average proportion of productive female gametophytes after 3 and 4 weeks of growth.

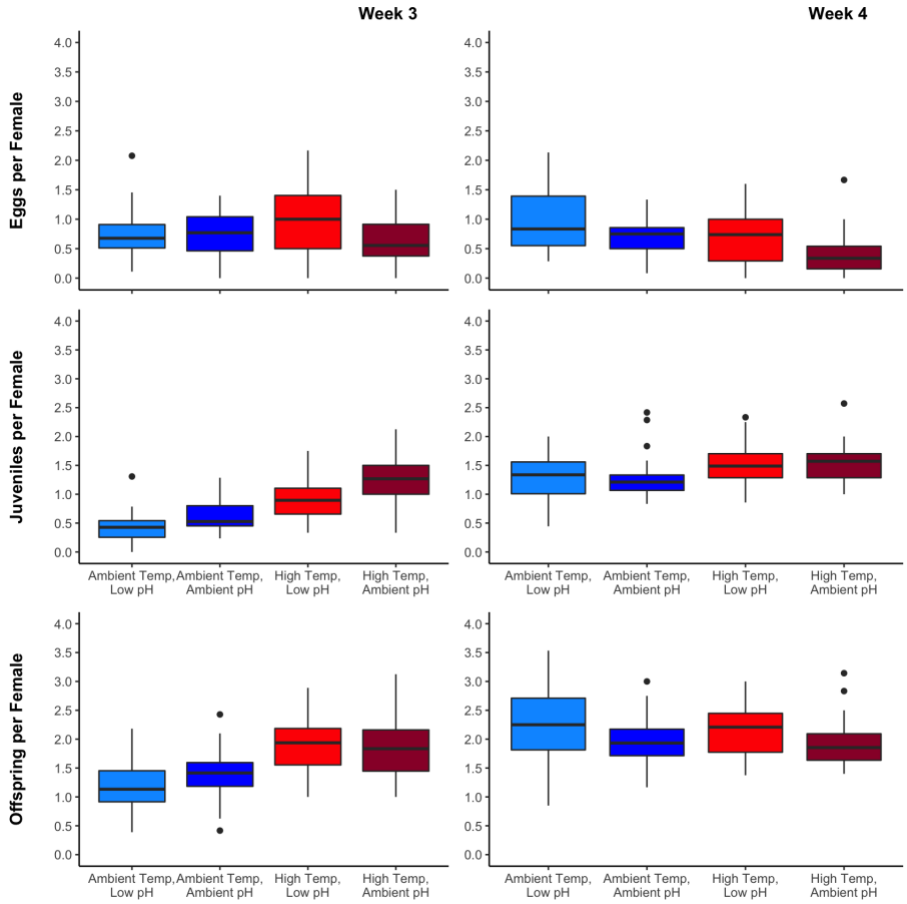

Figure 5: The average number of eggs, juveniles, and total offspring (eggs + juveniles) per female after 3 and 4 weeks of growth. Error bars represent 1.5 interquartile ranges from the median.

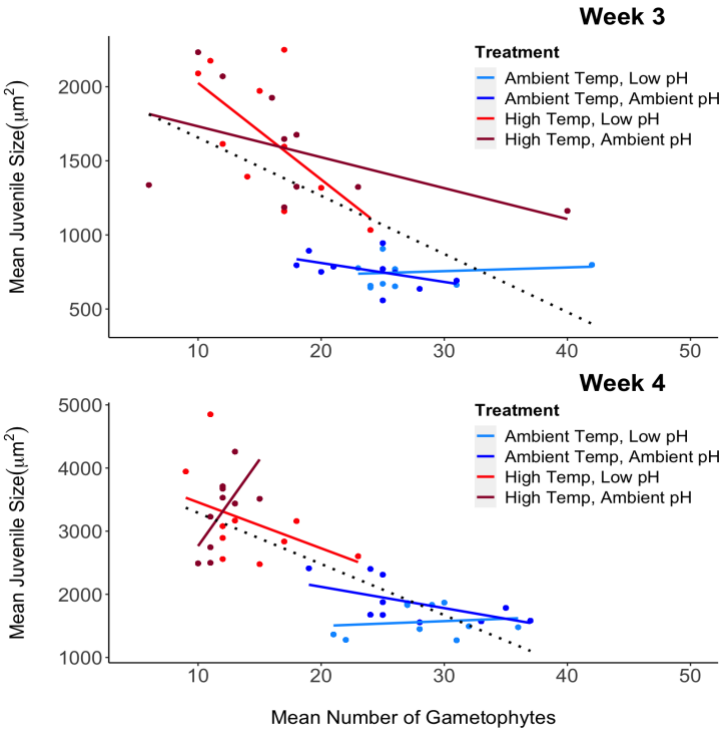

Figure 6: Relationship of the covariate (mean number of gametophytes) to the response variable (mean juvenile size). Tends within groups are represented by colored lines, whereas the trend across all groups is represented by the dotted black line. Heterogenous slopes and different ranges of values for each treatment indicate that the different treatments are confounded with differences in the covariate.

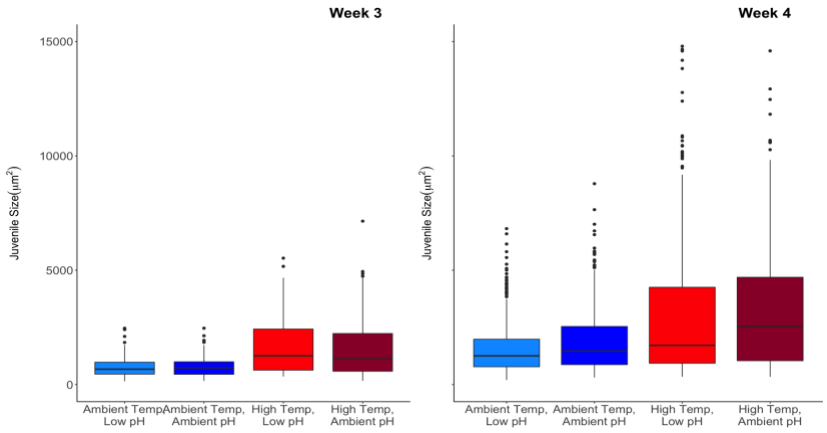

Figure 7: The average size of juveniles after 3 and 4 weeks of growth. Error bars represent 1.5 interquartile ranges from the median.