# Effects of sori incubation temperature on *Nereocystis luetkeana* gametophyte and sporophyte development

Naomi Hi‘iaka Vliet [1,2], Sadie L. Small [1], Brooke L. Weigel [1]

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[1] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

[2] Department of Education, Montana State University, Bozeman, MT 59715

Contact Information:

Naomi Hi‘iaka Vliet

Department of Education

Montana State University

Bozeman, MT 59715

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**Abstract**

Climate change is affecting marine ecosystems around the world, including those in the Northeastern Pacific Ocean. Warming ocean temperatures have been linked to kelp forest declines, including bull kelp (*Nereocystis luetkeana*) forests in the Salish Sea. However, the temperature tolerances of different stages of the *N. luetkeana* life cycle are not well established. This project examined the effects of a short-term marine heatwave on *N. luetkeana* sori and subsequent life stages, including gametophytes and embryonic sporophytes. We incubated mature sori at temperatures of 18°C, 20°C, and 21°C for 3.75 days, and grew the gametophytes from each sori treatment at both 10°C and 16°C for 40 days. Gametophytes were able to develop normally and produce sporophytes from sori incubated at all temperatures, but gametophyte incubation temperature had significant impacts on the life cycle. Gametophytes grew larger and at a faster rate at 16°C compared to 10°C; however, sporophytes developed more quickly and were more abundant when gametophytes were grown at 10°C. Sorus incubation temperature affected the life cycle as well; when sori were incubated at 21°C, gametophytes were more numerous but fewer sporophytes developed, regardless of gametophyte incubation temperature. Our findings suggest that sori can withstand temporary high temperatures and produce zoospores that develop into gametophytes and sporophytes, completing the life cycle, but if gametophytes experience high temperatures the development of sporophytes may be hindered. A decrease in sporophyte development, i.e. fewer individuals completing their life cycle, could lead to loss of important kelp forest habitat. However, it is important to note that all temperature treatments did produce sporophytes, indicating that it is possible

for life cycle completion to occur. Further research should identify the impacts of prolonged temperature stress (>3 days) on sorus development and microscopic life stages.

## Introduction

Climate change, fueled in large part by post-industrial human activities (Nordell 2003; Seinfeld 2011; IPCC 2022), is affecting marine ecosystems around the world. Ocean acidification, already an issue in the Northeastern Pacific Ocean, has been increasing due to anthropogenic $CO_2$ emissions (Feely et al. 2008; Feely et al. 2010). In the Salish Sea, sea surface temperatures are increasing by about 0.56°C per decade (Amos et al. 2015), making the environment less hospitable for cold-adapted organisms that are native to the region.

There are 22 species of kelp (brown algae in the order *Laminariales*) in the Salish Sea. While many of these species provide important understory habitat as well as food sources, there are only two species that are canopy-forming: giant kelp (*Macrocystis pyrifera*), found in the Strait of Juan de Fuca and on the outer coast of Washington, and bull kelp (*Nereocystis luetkeana*), found throughout the region (Mumford 2007). *N. luetkeana* has a geographic range from central California through the Aleutian Islands (Smale 2020). As the only canopy-forming kelp in most of the Salish Sea, bull kelp creates crucial kelp forest habitat, offering shelter from their blades at the surface and their stipes extending up to 30 meters to the ocean bottom (Shaffer et al. 2020). Kelp is also an important food source for marine ecosystems, with kelp-derived carbon found in fish, birds, and mammals (von Biela et al. 2016).

Bull kelp exhibit an annual biphasic lifecycle (Figure 1). Adult sporophyte blades produce reproductive tissue called sori. The sori release haploid zoospores, which settle and develop into haploid female and male gametophytes. Female gametophytes are fertilized by sperm from male gametophytes, after which they develop into diploid sporophytes. Sporophytes typically develop in the spring, with rapid blade growth rates of up to 6 cm a day (Maxwell & Miller 1996) and reach reproductive maturity by late spring to early summer. Most adult bull kelp are washed out by winter storms and do not survive for a second season (Springer et al. 2007).

Bull kelp prefer cold and wave-exposed waters (Lunning & Freshwater 1988; Starko et al. 2022). Warming ocean temperatures have been linked to kelp loss around the world (Krumhansl et al. 2016), which is expected to be exacerbated as temperatures continue to increase (Riahi et al. 2022). In the Salish Sea, bull kelp forests have declined, particularly in southern Puget Sound (the southernmost part of the Salish Sea), due in part to the higher ocean temperatures found in this region (Berry at al. 2021).

Previous research has explored the effects of warming on different parts of the kelp life cycle, including the effect of temperature on zoospore germination (Schiltroth 2021), juvenile sporophyte recruitement (Muth et al. 2019), juvenile sporophyte development (Mabin et al. 2019), and blade morphology and growth of adult sporophytes (Supratya et al. 2020). However, little is known about how the exposure of reproductive sori to warming ocean temperatures might affect zoospore germination and subsequent gametophyte growth and sporophyte development, or whether there is any correlation between sori incubation temperature and the thermal tolerance of microscopic life stages. This is important to consider as the blades of bull kelp, including those with sori, sit at

the top of the water column and are exposed to warmer temperatures than the microscopic life stages, which live at the bottom of the ocean.

**Methods**

Bull kelp (*Nereocystis luetkeana*) blades with mature sori patches were collected from approximately 55 individual kelp at Turn Rock (GPS: 48.535394, -122.964596) in the San Juan Islands on July 1, 2022. Blades with sori were collected and placed in buckets of seawater, then transferred to 12-14°C flow-through seawater tanks within 1 hour of collection. The blades were cut to a uniform length of 40 cm and randomly divided into six temperature-controlled tanks. Each tank contained two bins, between which 12 sori were distributed, for a total of 72 sori in the experiment. An initial pulse amplitude modulated (PAM) fluorometry reading was taken from 5 blades from each temperature treatment. PAM fluorometry measures the photosynthetic efficiency of photosystem II as a proxy for photosynthetic performance. The tanks were all initially set to the ambient incoming seawater temperature (12-14°C). Two tanks remained at 12°C as cold water controls, with a total of 24 sori at this temperature. The remaining four tanks were gradually raised by about two degrees every 6 hours to their final temperatures of 16°C, 18°C, 20°C, and 22°C, with 12 sori at each temperature. Tanks had flow-through seawater, but because the ambient water was very cold, the tanks set to 22°C remained around 21°C. The blades were then allowed to incubate for 90 hours (3.75 days) at their final temperatures, with about 50.66 μmol $m^{-2}$ $s^{-1}$ of light for 14:10 light:dark cycles. Two to four blades were removed from each tank during the experiment as they began releasing their zoospores early, leaving 8-10 sori per tank at the end.

After sorus temperature incubations, final PAM fluorometry readings were taken from five blades from each temperature treatment. To prepare sori for zoospore release, one circle of approximately 3 cm radius was cut from each sorus (circles were sometimes incomplete when the sorus was less than 6 cm wide.) The circles were gently wiped with a clean paper towel, rinsed in a beaker of betadine iodine solution for 30 seconds, rinsed in a beaker of filtered seawater for 2 minutes, then placed between layers of clean paper towels dampened with filtered seawater and left to desiccate in the refrigerator for 4-6 hours. To ensure genetic diversity, 8-10 replicate sori per treatment were combined prior to zoospore release. The sorus circles were then immersed in sterile seawater (autoclaved and filtered) overnight for about 16 hours at 10°C, with sori from each treatment released into different beakers. The solution was given a final stir, then left for 20 minutes to settle. About 20 mL of zoospore solution from the top of the beaker was transferred to a clean falcon tube and gently shaken before counting the zoospore density of each treatment using a hemocytometer.

To prepare gametophyte cultures, the zoospore solutions were pipetted into the wells of two sets of three culture plates, with six replicate wells from each sorus incubation temperature. Zoospore density was calculated for each treatment using a hemocytometer to ensure that each well was populated at roughly the same density (500 spores/ml). The culture plates were placed in a temperature-controlled room, with three left at the ambient temperature of 10°C and three placed in a water bath at 16°C. They received 24.81 umol $m^2$ $s^{-1}$ of light for 15:9 light:dark cycles and were allowed to germinate.

After 14 days and again after 21 days, the wells were photographed to document gametophyte size with an AmScope microscope camera (MU1003B) at 10x (day 14) and 4x (day 21) magnification using a Nikon Eclipse TE2000-U inverted microscope. Gametophyte photos were analyzed in ImageJ for area using the wand tool to select individual gametophytes, and growth rate was calculated between time intervals using the equation: (final size – initial size) / days. At 26 days, the total number of sporophytes in each well were counted, at 29 days the number of gametophytes were counted in three random fields of view per well, and at 40 days the number of sporophytes were counted using three random fields of view per well.

**Results**

Sori health and zoospore release

We used PAM fluorometry to assess photosynthetic efficiency of the blades containing sori before and after the temperature incubation period. There was a significant increase in photosynthetic performance between the first and last date (ANOVA, df=1, f=6.099, P=0.02), but no significant difference between temperature treatments (ANOVA, df=4, f=1.993, P=0.11), and no significant interaction between temperature and date (ANOVA, df=4, f=1.346, P=0.27). This indicates that temperature did not affect photosynthetic performance, and sori were still in good condition at the end of the short-term warming. After sori incubation and zoospore release, zoospore counts were performed using a hemocytometer (Table 2). The ripeness of individual sori varied at the beginning of the trial, and zoospore counts did not indicate any major differences in

zoospore release between treatments. Zoospore counts were primarily used to settle the zoospores at relatively similar densities in each treatment.

Gametophyte development

Gametophyte size did not differ significantly with sori incubation temperature (ANOVA, df=2, f=1.855, P=0.158). However, there was a significant difference in the size between gametophytes grown at 16°C and those grown at 10°C (ANOVA, df=1, f=123.444, P < 0.001), with larger gametophytes in the warmer (16°C) treatment (Figures 3 and 4). By week 3, the mean gametophyte size at 10°C was 4,122 +/- 233 $um^2$ (*n*=194) and the mean gametophyte size at 16°C was 11,680 +/- 716 $um^2$ (*n*=145). On average, gametophytes grown at 16°C were almost three times as large as those grown at 10°C.

There was a significant effect of gametophyte incubation temperature on the growth rate of gametophytes (ANOVA, df=1, f=89.88, P < 0.001), and gametophytes incubated at 16°C grew faster than those incubated at 10°C (Figure 5). There was a marginally significant difference in growth rate between gametophytes grown from sori incubated at different temperatures (ANOVA, df=2, f=3.14, P=0.0575), but none of the sori treatments were significantly different from one another (Tukey HSD pairwise comparisons, P>0.05). There was also a significant effect of gametophyte incubation temperature on the number of gametophytes that developed. While gametophytes were larger at 16°C, more gametophytes developed at 10°C (ANOVA, df=5, f=50.94; Tukey HSD pairwise comparisons, P=0.026 for the 21°C sori treatment, P < 0.001 for the 20°C sori treatment) (Figure 6, Table 3).

In addition, sori incubation temperature had a significant effect on the number of gametophytes that developed. More gametophytes developed from sori incubated at 21°C than at 20°C, regardless of gametophyte incubation temperature (ANOVA, df=5, f=50.94; Tukey HSD pairwise comparisons, P=0.006 for the 10°C gametophytes, P<0.001 for the 16°C gametophytes) (Figure 6, Table 3). There were significantly fewer gametophytes from the 18°C sori treatment, and no gametophytes developed from the 12°C and 14°C sori treatments, all three of which were most likely due to errors when the gametophyte culture plates were prepared from zoospore solutions. Hereafter, results will only be discussed from the 18°C, 20°C and 22°C sori incubation treatments.

Sporophyte Development

After 26 days, we found that significantly more sporophytes developed in the 10°C gametophyte incubation than in the 16°C incubation (ANOVA, df=1, f=46.19, P < 0.001), regardless of sori incubation temperature (Table 4, Figure 7). We also found a significant effect of sori incubation temperature on the number of sporophytes (ANOVA, df=2, f=12.23, P< 0.001). There were significantly more sporophytes from sori incubated at 20°C and 21°C than at 18°C (Tukey HSD pairwise comparisons, P<0.001), but this was likely due to the erroneously low gametophyte density at 18°C. After 26 days, there were significantly more sporophytes from the sori incubated at 21°C than at 20°C when grown at 10°C (Tukey HSD pairwise comparisons, P=0.002).

After 40 days, there were still a significantly higher number of sporophytes in the 10°C gametophyte treatments than in the 16°C treatments (ANOVA, df=5, f=50.64; Tukey HSD pairwise comparisons, P< 0.001) across sori incubation temperatures (Table 5, Figure 8). There was also a significant effect of sori incubation temperature on the

number of sporophytes (ANOVA, df=5, f=50.64), with more sporophytes developing from the 20°C sori treatment than the 21°C (Tukey HSD pairwise comparisons, 16°C gametophyte treatment, P=0.022; 10°C gametophyte treatment, P=0.026).

## Discussion

### Sori condition and zoospore release

After incubating sori at temperatures of up to 21°C for 3.75 days, there was no significant difference in photosynthetic efficiency between incubation temperatures, nor was there a significant difference in zoospore release. This indicates that short-term high-temperature events do not negatively impact the health of mature sori or their ability to complete a critical step in the kelp life cycle. A previous study found that sea surface temperatures greater than 17°C had negative impacts on the release and germination of bull kelp zoospores (Schiltroth 2021). However, that study used sori that had been exposed to warm temperatures for most of their development, while our study focused on short-term warming events. The effects from long-term warm temperature exposure are likely different than those from short-term warming.

### Gametophyte development

Gametophyte development was strongly affected by the temperature at which gametophytes were grown, with the gametophytes incubated at the cooler temperature of 10°C growing smaller and slower than those at 16°C. However, despite larger gametophytes at 16°C, more gametophytes developed at 10°C than at 16°C, regardless of sorus incubation temperature.

There was a significant correlation between sori incubation temperature and gametophyte density, with the most gametophytes developing from sori incubated at 21°C. A similar study found that gametophytes grown from sori that experienced both warm (17°C -20°C) and cold (10°C -14°C) temperatures were able to germinate when grown at 10°C and 15°C, correlating the finding that germination and gametophyte growth is possible from sori that experience high temperatures (Schiltroth 2021). However, our study had errors in the plating density of at least some of the samples, with nothing developing from sori incubated at 12°C and 14°C and very few gametophytes from sori incubated at 18°C. It is possible that we erroneously plated the zoospores from the 21°C sori treatment at a higher density than the 20°C sori treatment, causing our results to be skewed.

Sporophyte development

Significantly more sporophytes developed in the 10°C gametophyte treatments than the 16°C treatments. While the gametophytes grew larger at 16°C, they developed fewer sporophytes, thus leaving fewer adult sporophytes for the next generation. This is similar to a previous study on sporophyte recruitment, where gametophytes failed to develop sporophytes at 18°C (Muth et al. 2019). This is a particularly important metric, as it demonstrates whether the kelp will be able to complete their life cycle. This has important ramifications for kelp forest conservation, because even if their previous life stages (zoospores, gametophytes) are undeterred by warming events, the failure of sporophyte recruitment will prevent the continuity of annual bull kelp forests.

There was also a significant difference in the development of sporophytes between sori incubations. The 18°C sori treatment had much fewer sporophytes than the

20°C and 21°C. However, this was likely due to a plating error, as the 18°C sori incubation wells had very few gametophytes. The 21°C sori treatment had significantly fewer sporophytes than the 20°C treatment, which could indicate a negative impact of the higher temperature experienced by the sori. However, as there were density errors in other treatments and the 21°C sori treatments had higher gametophyte densities than the 20°C sori treatments, it is possible that this was caused by the higher gametophyte density. It has previously been found that fewer sporophytes develop at high gametophyte densities (Tatsumi et al. 2022).

Effects of sori incubation temperature

For the most part, exposure of mature sori to short-term high temperatures caused little to no negative impacts on the kelp life cycle. There may be a decline in sporophyte production when sori are exposed to temperatures of 21°C and above, but further research is warranted to examine this relationship more closely. Even if the 21°C sori incubation lessened the number of sporophytes produced, it is important to note that there were still sporophytes that developed from this treatment. This is a positive result for kelp forests because it means that brief surface-temperature marine heatwaves may not prevent kelp from completing their life cycle. However, this may not hold true for long-term high-temperature exposure. A sorus that developed at 20°C in a related experiment appeared very different than a sorus developed at a more typical temperature of 12°C (Figure 9) and released virtually no zoospores using the same methods as in this experiment, indicating that high temperature impacts may be greater when experienced for the entire development of the sorus.

Future work

While this experiment examined the effects of a short-term heat event on the development of gametophytes and sporophytes from mature sori, it would be interesting to investigate the effects of sustained high temperature on the development of sori, which would likely have different impacts. As ocean temperatures rise, sori will be exposed to higher temperatures for more of their development, which could have more drastic impacts on their development than short-term ocean warming events.

**Tables:**

| Life cycle stage | Treatment temperature (°C) | Measured temperature (°C) |
|---|---|---|
| Sorus | 12 | 12.07 ± 0.12 |
| | 16 | 15.93 ± 0.10 |
| | 18 | 17.66 ± 0.37 |
| | 20 | 19.92 ± 0.06 |
| | 21 | 20.56 ± 0.42 |
| Gametophyte | 10 | 8.86 ± 0.62 |
| | 16 | 15.90 ± 0.29 |

**Table 1**. Treatment temperatures and measured temperatures with standard deviation for sori incubation and gametophyte incubation.

| Sorus treatment (°C) | Zoospore density (spores/ml) |
|---|---|
| 12 | 2,165,000 |
| 16 | 1,885,000 |
| 18 | 4,390,000 |
| 20 | 2,515,000 |
| 21 | 2,855,000 |

**Table 2**. Zoospore density, counted with a hemocytometer, before starting gametophyte cultures.

| Sorus incubation temperature (°C) | Gametophyte incubation temperature (°C) | Average gametophytes per field of view | Standard error |
|---|---|---|---|
| 18 | 10 | 0.778 | 0.268 |
| 18 | 16 | 0.500 | 0.075 |
| 20 | 10 | 11.333 | 1.330 |
| 20 | 16 | 4.778 | 0.569 |
| 21 | 10 | 16.444 | 1.426 |
| 21 | 16 | 12.111 | 0.957 |

**Table 3**. Average number of gametophytes per field of view at 4x zoom for each treatment on day 29; averages are from three fields of view per well and six wells per treatment.

| Sorus incubation temperature (°C) | Gametophyte incubation temperature (°C) | Average sporophytes per well | Standard error |
|---|---|---|---|
| 18 | 10 | 3.167 | 0.477 |
| 18 | 16 | 0.167 | 0.167 |
| 20 | 10 | 115.0 | 9.252 |
| 20 | 16 | 2.333 | 0.494 |
| 21 | 10 | 167.0 | 19.043 |
| 21 | 16 | 0.0 | 0.0 |

**Table 4**. Average number of sporophytes per well for each treatment on day 26; averages are from total counts for each well and six wells per treatment.

| Sorus incubation temperature (°C) | Gametophyte incubation temperature (°C) | Average sporophytes per field of view | Standard error |
|---|---|---|---|
| 18 | 10 | 5.50 | 1.039 |
| 18 | 16 | 0.778 | 0.436 |
| 20 | 10 | 20.833 | 0.588 |
| 20 | 16 | 6.722 | 0.641 |
| 21 | 10 | 15.500 | 2.369 |
| 21 | 16 | 1.278 | 0.250 |

**Table 5**. Average number of sporophytes per field of view at 4x zoom for each treatment on day 40; averages are from three fields of view per well and six wells per treatment.

**Figures:**

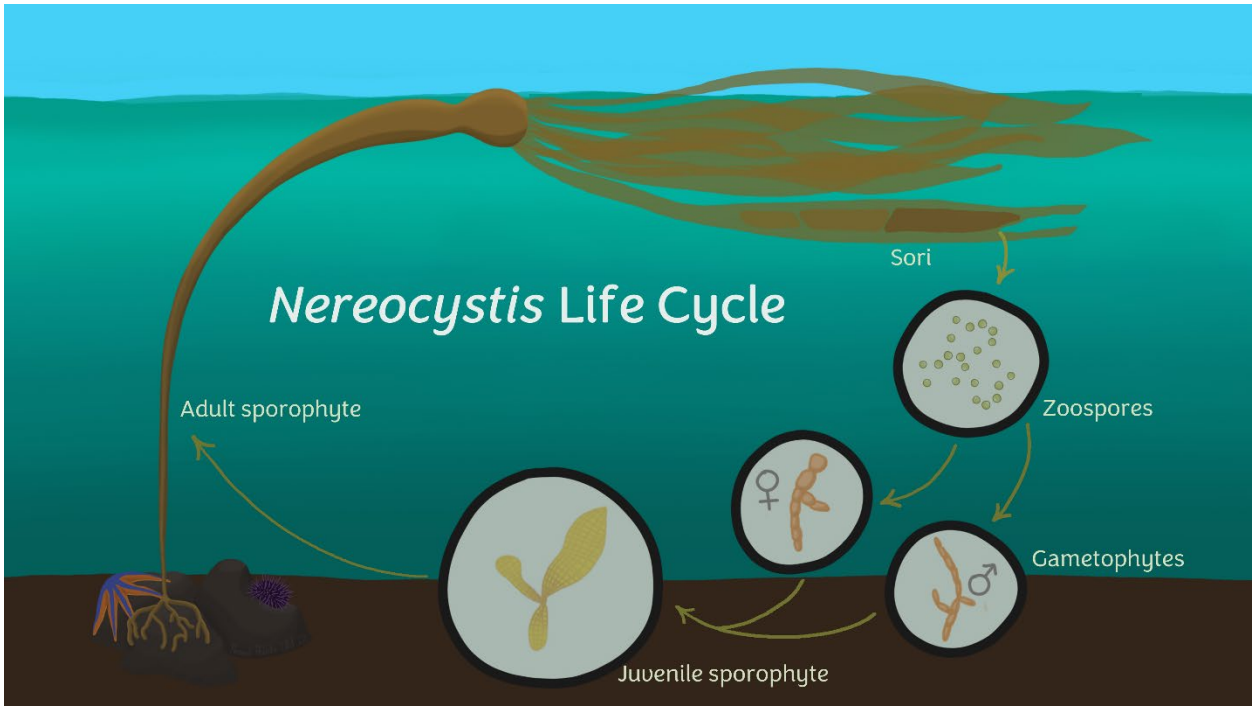


**Figure 1**. Life cycle of *Nereocystis luetkeana*, illustrated by Naomi Hi‘iaka Vliet.

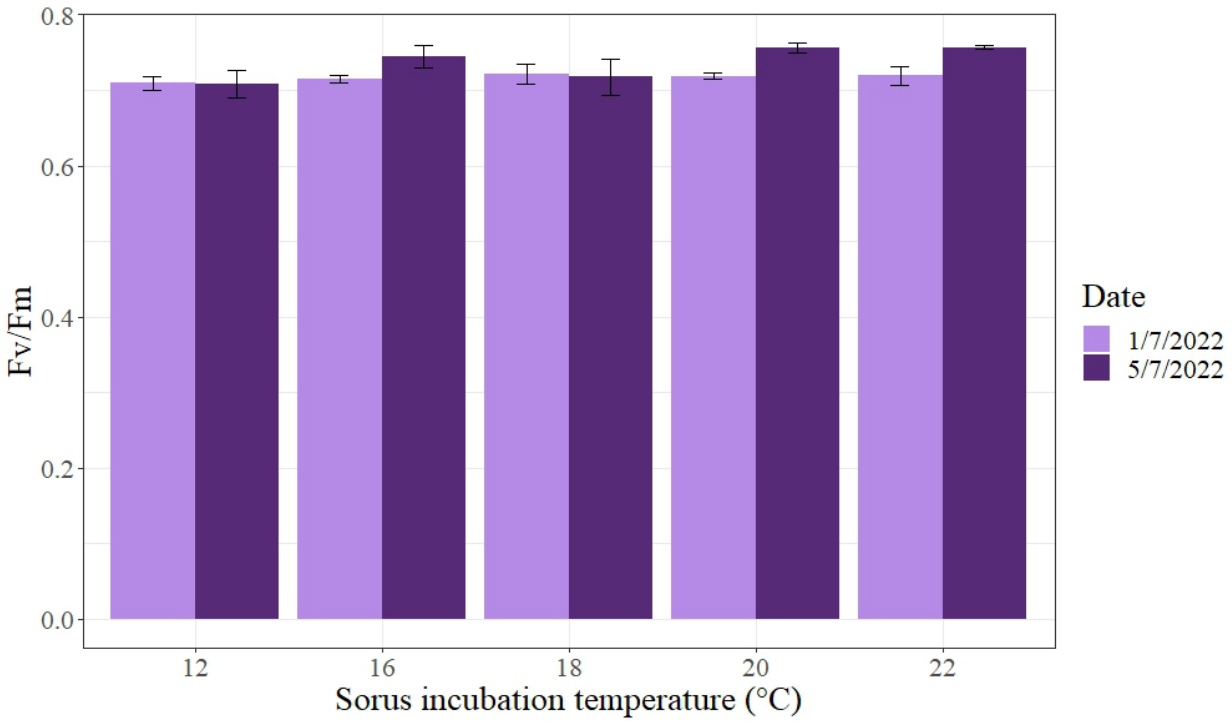


**Figure 2**. PAM measurements at beginning (1/7/22) and end (5/7/22) of sori temperature incubations.

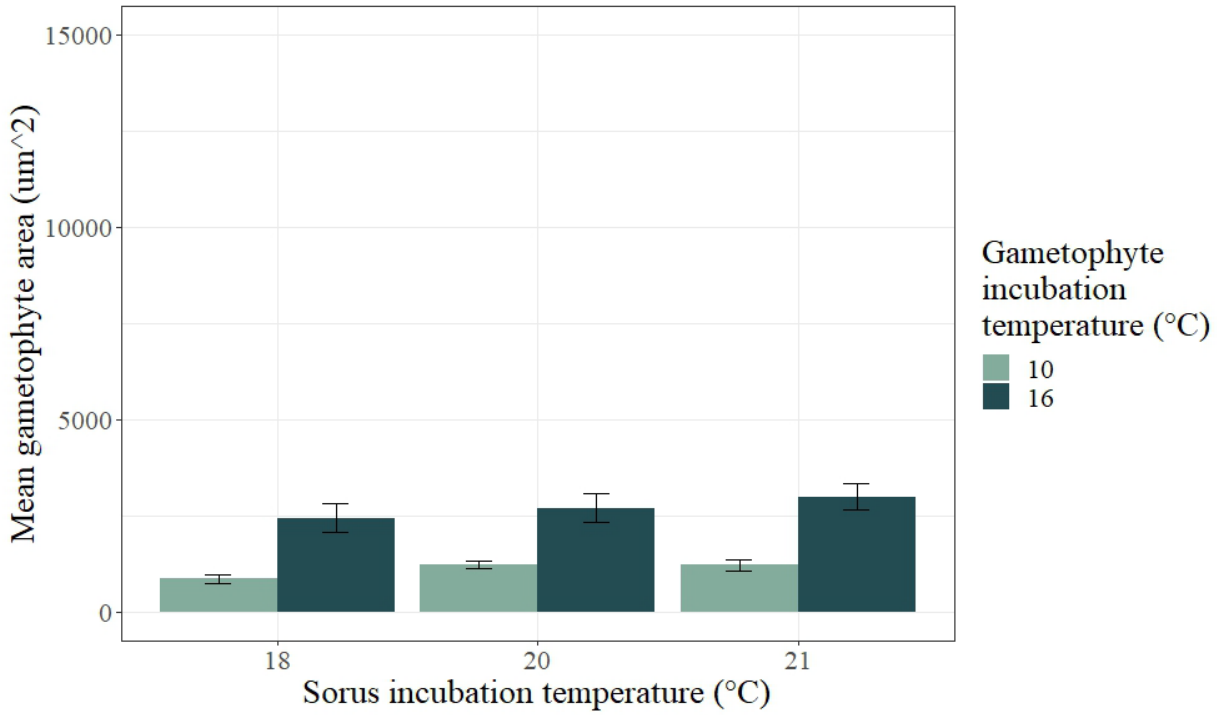


**Figure 3**. Average area of gametophytes by treatment, week 2. Error bars represent standard error from the mean.

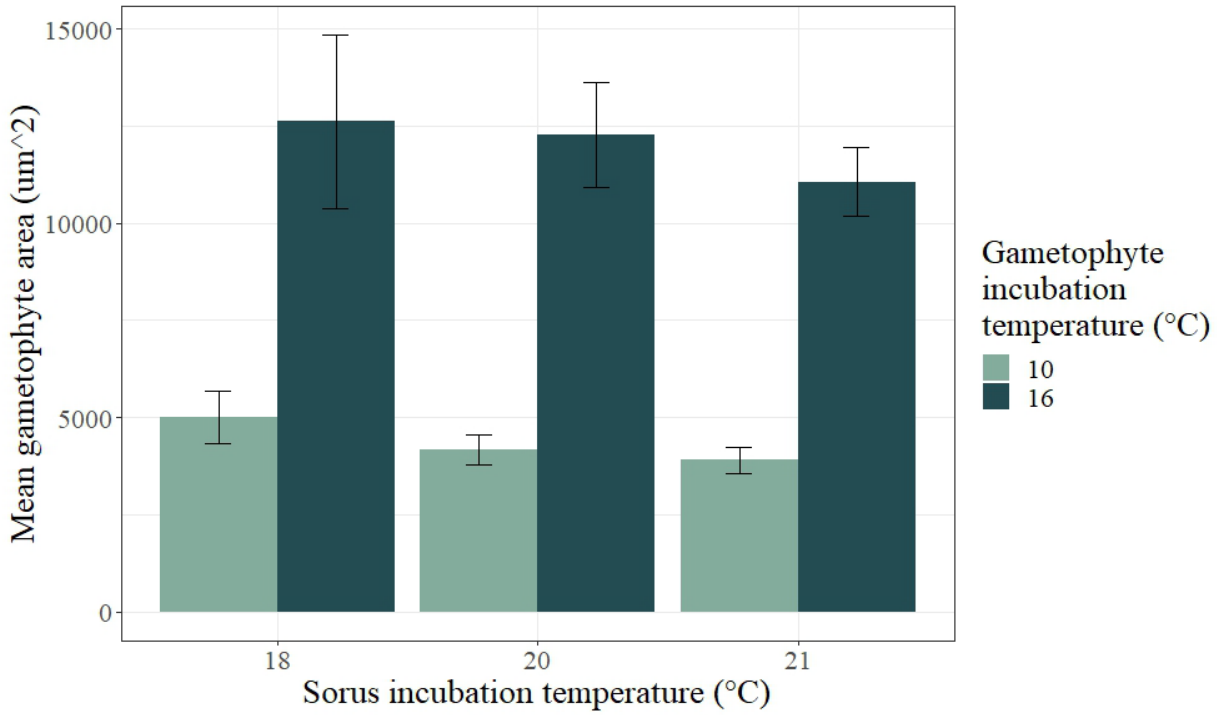


**Figure 4**. Average area of gametophytes by treatment, week 3. Error bars represent standard error from the mean.

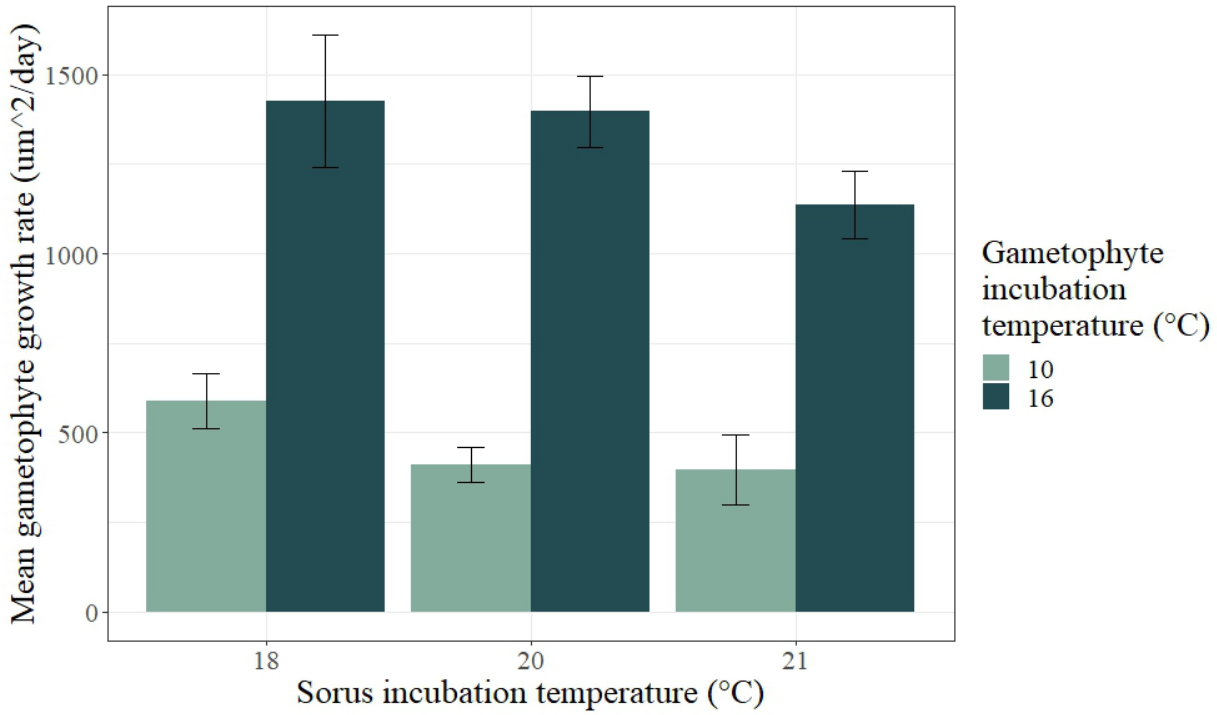


**Figure 5.** Growth rate of gametophytes between weeks 2 and 3. Error bars represent standard error from the mean.

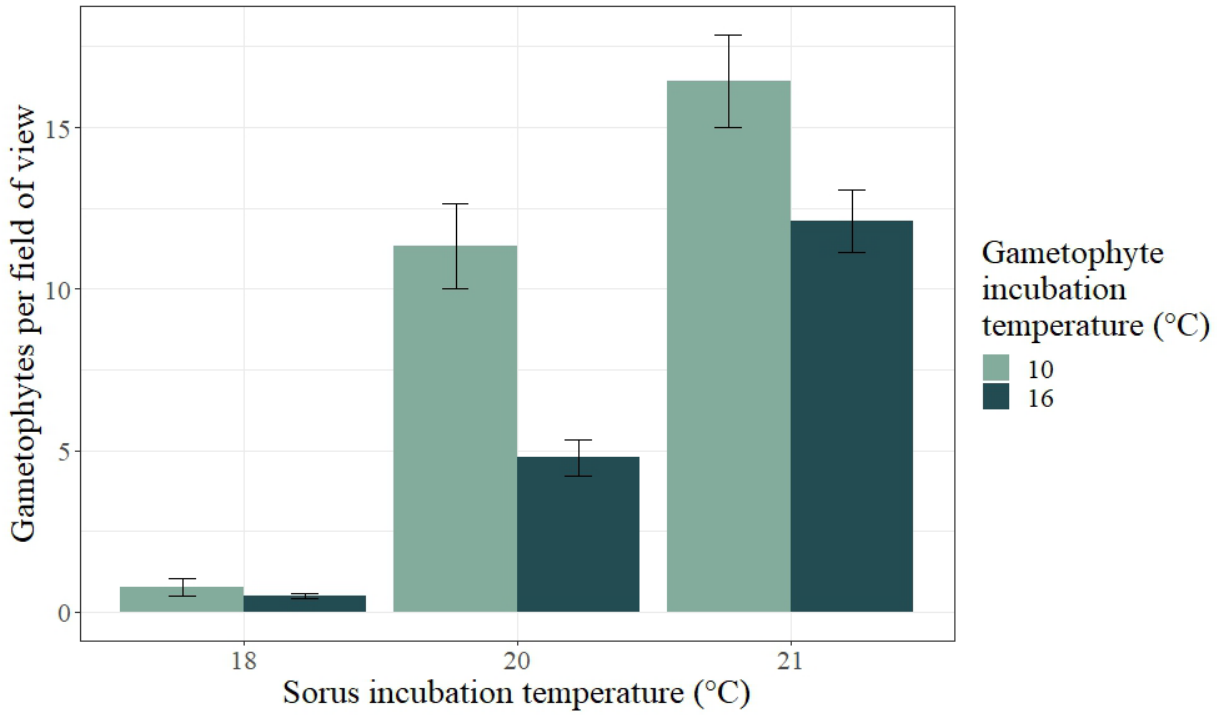


**Figure 6**. Number of gametophytes per field of view for each treatment at 4x zoom on day 29. Error bars represent standard error from the mean.

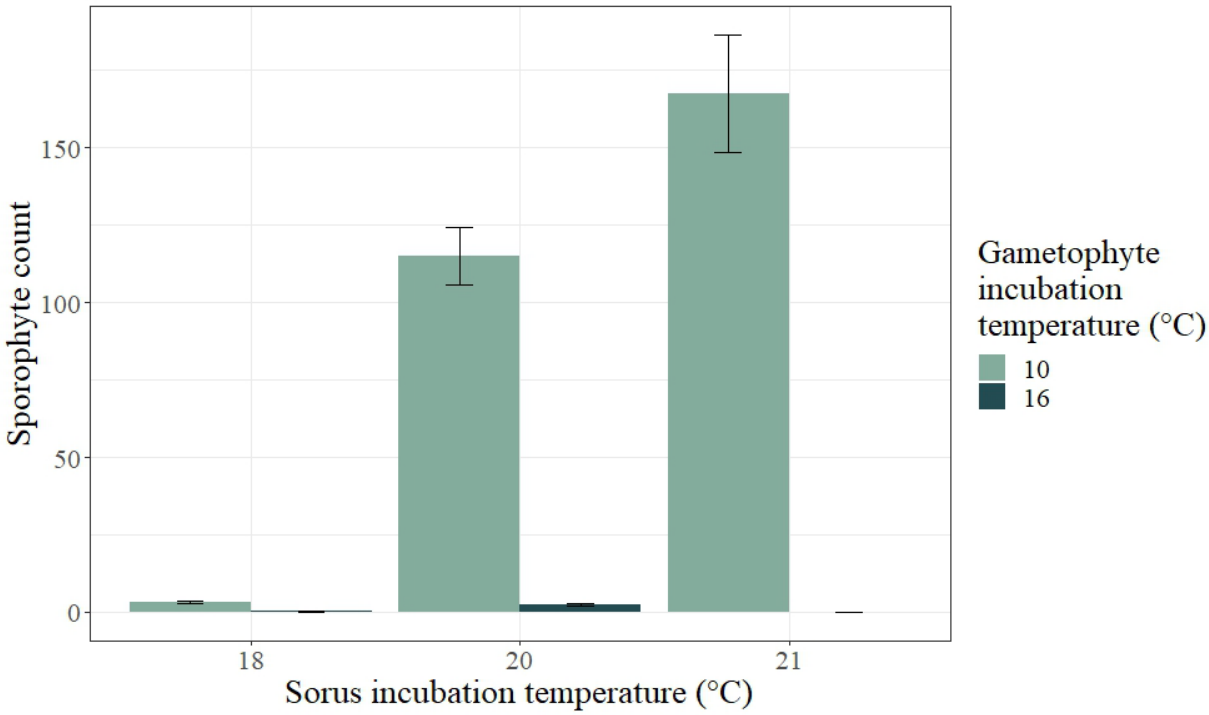


**Figure 7**. Average total number of sporophytes counted in each well of each treatment on day 26. Error bars represent standard error from the mean.

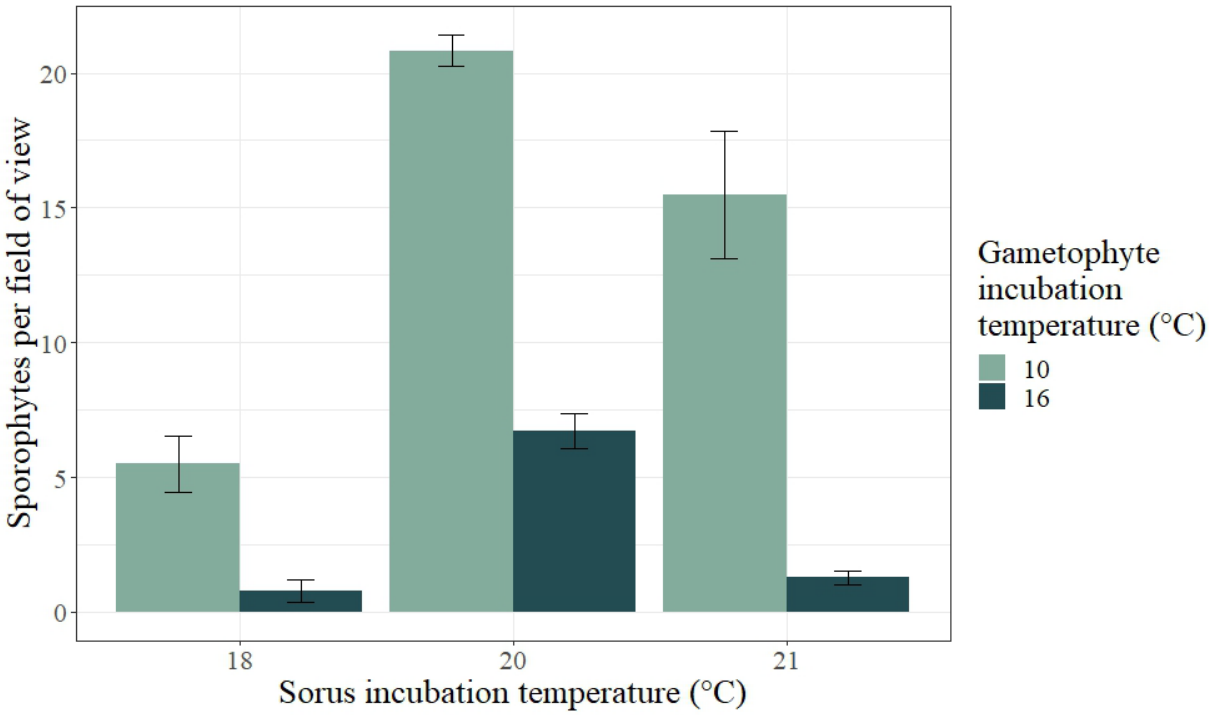


**Figure 8**. Number of sporophytes per treatment, average per field of view at 4x zoom on day 40. Error bars represent standard error from the mean.

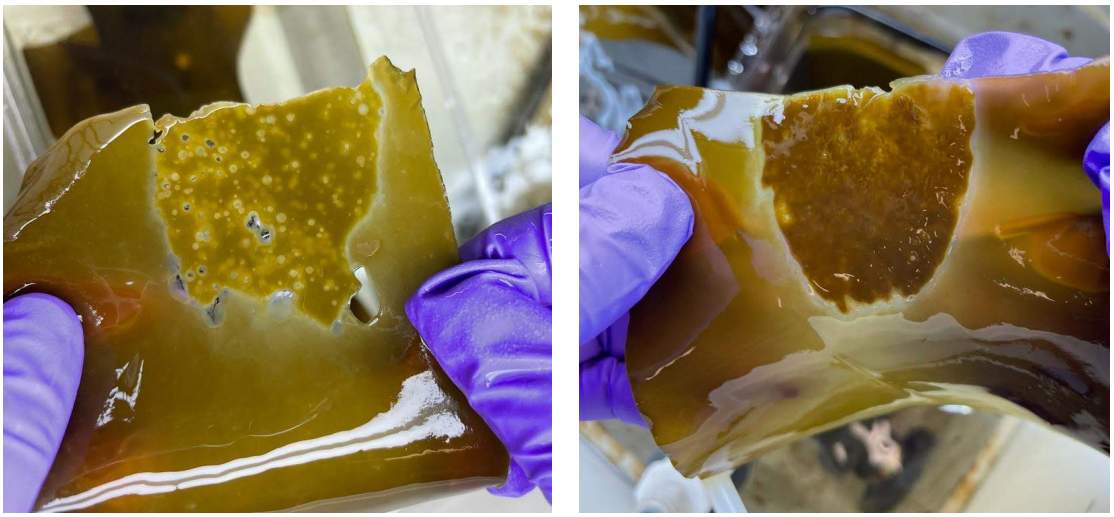

**Figure 9**. Bull kelp sorus that fully developed at 20°C (left) and 12°C (right) over 1.5 weeks in a related ocean warming experiment at Friday Harbor Labs (Weigel et al., *in prep*).

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