

Temperature Effects on Growth of *Alaria marginata*, *Saccharina latissima*, and *Costaria costata*

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Abstract

To understand the long-term effects of climate change, it is essential to understand the short-term effects influenced by abiotic factors such as temperature. My study serves as a step towards understanding the effects of climate change. I used a combination of observational and experimental approaches to analyze the impacts of temperature on the growth of three species of kelp: *Alaria marginata*, *Saccharina latissima*, and *Costaria costata*. I hypothesized that there would be less growth at higher temperatures for all kelp species. Common gardens were created in outdoor seawater tanks under 3 temperature treatments (14 ° Celsius, 18 ° Celsius, and ambient) to measure growth over the experimental period. A Pocket PEA Portable Fluorometer was utilized to determine whether temperature affected chlorophyll fluorescence levels. There was no significant effect of temperature on the overall growth of all the kelp species. However, there was a significant effect of temperature on the meristem growth, suggesting there was a decrease in growth when temperatures increased. The fluorometer indicated no significant difference in chlorophyll fluorescence between each temperature treatment, but there was a suggestive increase in levels of fluorescence after 6 days of growth.

Introduction

The rocky shoreline of the Pacific coast hosts an important ecological group of algae in the phylum Phaeophyta, kelp (order Laminariales). Kelp grow in the intertidal where they are a key habitat-forming group that supports different levels of biodiversity and are a key player in the food web due to their productive nature (Teagle et al 2017). Kelp produce detritus through blade erosion, which settles within kelp beds and surrounding habitats. Detritus is an important food source for filter feeders and especially detritivores, because 82% of annual kelp productivity is used in detrital production (Krumhansl and Scheibling 2012). Kelp are also

capable of sequestering carbon dioxide from the atmosphere and are a vital habitat for a diverse set of species (Duarte 2017). Not only do they play an important role from an ecological standpoint, but they are also economically important with kelp being exploited for alginate production. The demand for alginate has increased over time with its economic importance in store-bought products but is also used for biomedical and bioengineering products (Peteiro 2017).

With kelp forests being an important habitat, they are vulnerable to the effects of climate change. Kelp productivity and abundance have been decimated over the years on the West Coast of North America due to extreme marine heatwaves (Cavanaugh et al 2019) and with kelp deforestation, there has been a decreased rate in detrital production (Krumhansl and Scheibling 2012). Previous studies have shown that kelp habitats are prone to change because of climate change as species relationships and recruitment are shifted. Heatwaves affecting kelp forests had a lower abundance of genetic diversity and affect the control of foundation species and their use in the ecosystem (Wernberg et al 2018, Reed et al 2016).

As we discover more about how kelp are essential to our ecosystem, it is also important to conduct further studies to learn more about their growth patterns and to study which factors may affect them. In this research, I study 3 different kelp species - *Alaria Marginata*, *Saccharina latissima*, and *Costaria costata* - which are grown in the intertidal along the Western Coast of the US and are a crucial ecological habitat in the Salish Sea. This experiment was conducted over the course of 6 days where established methods to measure growth were used for different temperature treatments. With these data, I can ask the question of how temperature affects the growth rate of different types of kelp species and estimate the effects of temperature on photosynthesis.

Methods

Eighteen individuals of each species, *Saccharina latissima*, *Alaria marginata*, and *Costaria costata* were collected from the Friday Harbor Laboratory dock (48°32'42.3" N, 123°00'43.3" W). Six individuals of each species were transferred to 56-gallon outdoor laboratory seawater tanks with three different temperature treatments. The temperature treatments included a control treatment, which was maintained at ambient temperature, a 14° Celsius treatment, and an 18° Celsius treatment. To get tanks to the right temperature, 250-watt Marineland Visi-Therm submersible aquarium heaters were situated on each treatment tank. One aquarium heater was used on the 14° Celsius treatment tank and four aquarium heaters were used on the 18 ° Celsius treatment tank.

Flagging tape was utilized to label each kelp and netting was put on top of kelp secured with tiles in the tanks to prevent kelp from floating and minimize possible sunburn. A hole was punched 4 cm above the stipe and on the blade to measure the intercalary growth (Figure 1). I took measurements of maximum length that included the holdfast and length between the end of the stipe to the 4 cm punched hole of each individual on May 21 (day 0), May 24 (day 3), and May 27 (day 6), which was all measured in centimeters. Observations of temperature were recorded each day to account for weather patterns throughout the week that could affect the growth during the experiment. Chlorophyll fluorescence was measured using a Pocket PEA Portable Fluorometer. The measurement F_v/F_m (variable fluorescence by maximum fluorescence) was utilized for each individual in each temperature condition.

A two-way ANOVA test was created to analyze the significance of temperature on the maximum length measurement of overall growth rate and meristem measurement growth rate from day 0 to day 6 for the 3 different kelp species. To further analyze which temperature

treatment had the most significant growth, a Tukey's HSD test and boxplots were created on R Studio. To find significance in growth rates between each temperature. ANOVA one-way test was created on excel along with a scatterplot to show the patterns of chlorophyll fluorescence.

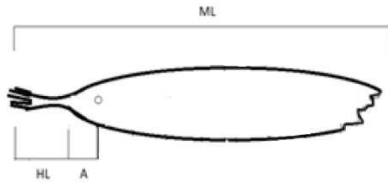
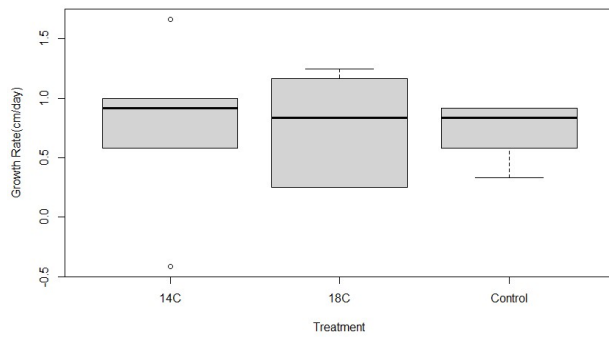


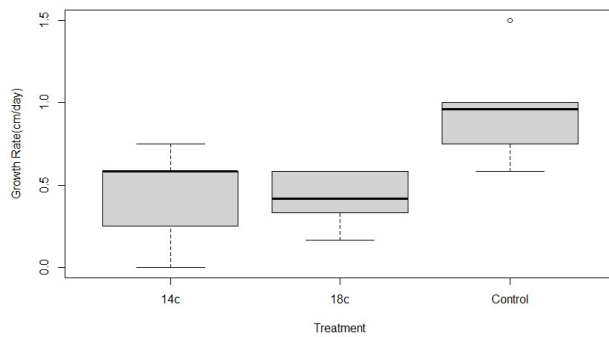
Figure 1. Diagram of where kelp measurements were collected and where the whole punch was made.

Results

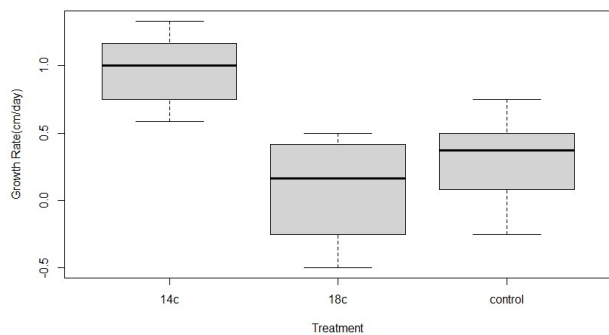
There was no significant difference in overall growth in Table 1 ($P=0.0848$), However Figure 2 suggests there was an increase in growth in certain temperature treatments for *Saccharina latissima* and *Costaria costata*. *Saccharina latissima* had a suggestive increase in overall growth in the control treatment, whereas *Costaria costata* had a suggestive increase in the 14°C . *Alaria marginata* does not show a suggestive increase in growth between each temperature but there was a slight average increase in the 14°C treatment.



(a)



(b)



(c)

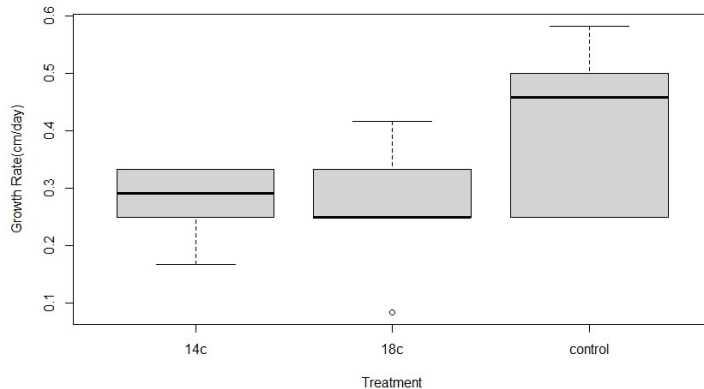
Figure 2. (a) *Alaria marginata* overall growth rate at three temperatures. (b) *Saccharina latissima* overall growth rate at three temperatures. (c) *Costaria costata* overall growth rate at three temperatures.

Table 1. Statistical analysis of the overall growth rate in the 3 temperature treatments

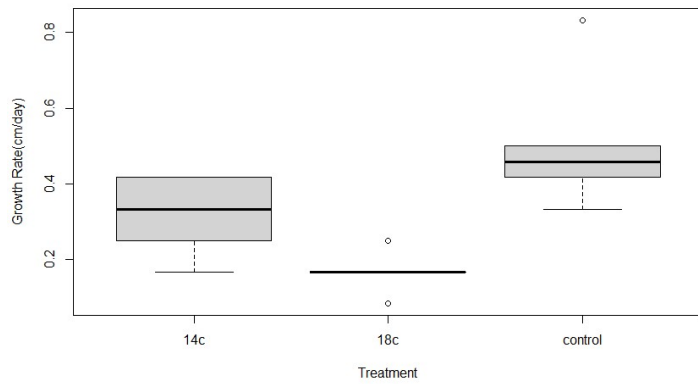
ANOVA					
Source of Variance	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Species	2	0.8405	0.42027	2.2156	0.1199
Treatment	2	0.9848	0.49241	2.596	0.0848
Residuals	49	9.2945	0.18968		

Statistical analysis performed: Two-way ANOVA.

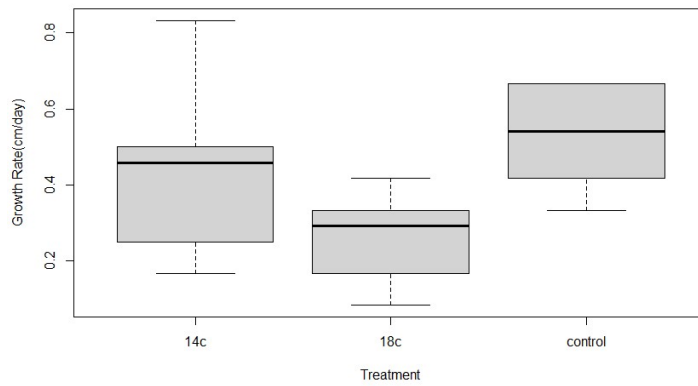
Growth rate proved to be temperature-dependent for *A. marginata*, *S. latissima*, and *C. costata* with a significant difference in meristem growth between the different temperature treatments ($P=8.69E-06$) (Table 2). There was an increased meristem growth rate in the control temperature treatment across all species (Figure 3). *A. marginata* had an average growth rate of 0.42 cm/day, *S. latissima* had an average of 0.5 cm/day and *C. costata* had an average growth rate of 0.53 cm/day for the control treatment. *A. marginata* had an average growth rate of 0.26389 cm/day, *S. latissima* had an average growth rate of 0.167 cm/day, and *C. costata* had an average growth rate of 0.26389 cm/day in the 18°C temperature treatment. Upon conducting a Tukey's HSD (Table 3), I found that growth rates were significantly different between all temperatures, with a suggestive significantly higher difference between control and 18°C ($P = 0.0000047$).



(a)



(b)



(c)

Figure 3. (a) *Alaria marginata* meristem growth rate for three temperatures. (b) *Saccharina latissima* meristem growth rate for three temperatures. (c) *Costaria costata* meristem growth rate from three temperatures. A proposed statistical difference for temperature affecting the growth of meristem.

Table 2. Statistical analysis of the meristem growth rate in the 3 temperature treatments.

ANOVA					
Source of Variance	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Species	2	0.09362	0.046811	2.4789	0.09429
Treatment	2	0.56353	0.281764	14.9213	8.69E-06
Residuals	49	0.952528	0.018883		

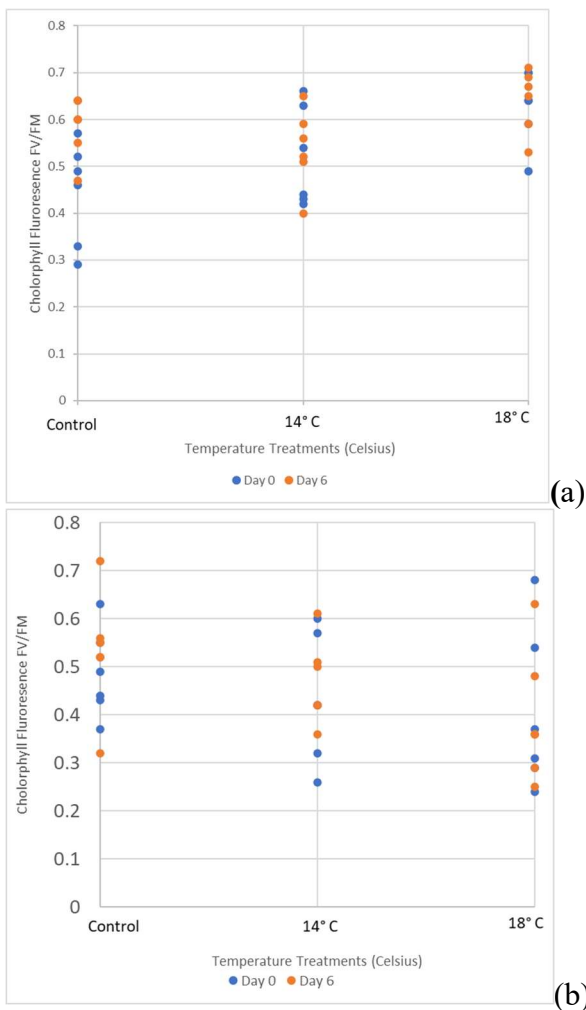
Statistical analysis performed: Two-way ANOVA.

Table 3. Specified statistical analysis of the meristem growth rate in the 3 temperature treatments.

Tukey's HSD				
Source of Variance	<i>diff</i>	<i>lwr</i>	<i>upr</i>	<i>p adj</i>
18c-14c	-0.11574	-0.22645	-0.00503	0.038616
control -14c	0.134259	0.023551	0.244968	0.0139327
control-18c	0.25	0.139292	0.360708	0.0000047

Statistical analysis performed: Tukey's honest significance difference test.

The fluorometry data showed no significant difference between chlorophyll fluorescence levels and different temperature treatments ($P > 0.05$) (Table 4 and Figure 4). However, there is a pattern of increase of photosynthesis across the temperature treatments when observing Figure 4.



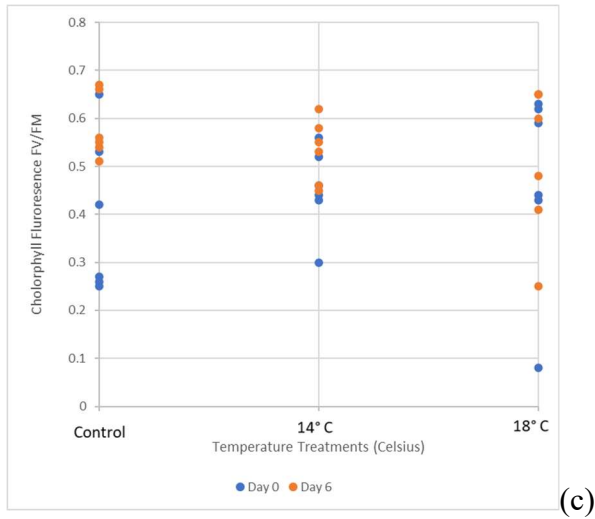


Figure 4. (a) Chlorophyll fluorescence for *Alaria marginata*. (b) Chlorophyll fluorescence for *Saccharina latissima*. (c) Chlorophyll fluorescence for *Costaria costata*. Suggested increase of chlorophyll fluorescence on day 6.

Table 4. Statistical analysis of chlorophyll fluorescence levels in the 3 temperature treatments.

ANOVA	<i>Alaria marginata</i>					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001715	2	0.000858	1.366767	0.284918	3.68232
Within Groups	0.009412	15	0.000627			
Total	0.011127	17				
ANOVA	<i>Saccharina latissima</i>					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000312	2	0.000156	0.199637	0.821169	3.68232
Within Groups	0.011723	15	0.000782			
Total	0.012035	17				
ANOVA	<i>Costaria costata</i>					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001835	2	0.000918	0.635336	0.54343	3.68232
Within Groups	0.021668	15	0.001445			
Total	0.023503	17				

Note: statistical analysis performed: ANOVA one-way factor. No statistical difference for temperature affecting chlorophyll fluorescence.

Discussion

The growth rate of *A. marginata*, *S. latissima*, and *C. costata* responded to temperature in different ways, despite being under experimental conditions, there was a significant decrease in meristem growth when increasing temperatures. A potential conclusion with this information is that the area of growth was weakened due to increasing temperatures. There were damages to the structure of kelp tissue and breaks in the meristoderm due to the increase in temperature (Simonson et al 2015). Contrary to this result, the overall growth rate from measuring the maximum length of kelp had no significant difference in growth rates between each temperature. Even with this suggestive insignificant statistical analysis, I can see an indicative increase in the overall growth rate in the control treatment for *S. latissima* and an indicative increase in the overall growth rate in the 14°C treatment for *C. costata*. *Alaria marginata* seemed to not show any patterns in growth between each temperature treatment (Figure 2). The different growth patterns may have been due to the different thermal tolerance of each kelp species.

Previous studies have found that mortality for *S. latissima* was higher when exposed to 24° C and had optimal growth between 10° C and 15° C. The study also found that when the species was exposed to higher temperatures (18° and 21° Celsius), the performance of growth remained low. This may be due to slower enzyme activities of the plant which affects the metabolism and photosynthesis of the algae, which in turn can reduce carbon acquisition (Nepper-Davidsen et al 2019). The increase in growth rate at the 14°C treatment for *Costaria costata* could suggest that the species can withstand a higher temperature than *Saccharina latissima*. Researchers have found that the photosynthesis maximum for a *Costaria*

costata sporophyte was 14.9° C, whereas *Saccharina Latissima* had an increase of photosynthesis to only 13°C (Borlongan et al 2019). Although *Costaria costata* had a higher overall growth rate in the 14° C treatment, Figure 3 proposes that there was an increase of the meristem growth rate in the control treatment than there was in the 14° C treatment and the 18° C treatment. *Costaria costata* showed a higher variability of growth rates with the maximum in the 14° C treatment being over 0.8 cm/day. This could be potential evidence that *C. costata* had a higher thermal tolerance than *S. latissima*.

Alaria Marginata exhibited very similar growth rates between each temperature suggesting that *Alaria marginata* overall growth rates did not affect each temperature treatment. Opposing this result, the *Alaria marginata* meristem growth rate had an increase of growth rate in the control treatment compared to the higher temperature treatments. A study concluded that *Alaria marginata* had optimum growth at 10-12° Celsius (Kjeldse 1966). Scientists discovered that the family Alariaceae would be the most vulnerable to temperature increase with the algae shifting poleward (Muth et al 2019). I anticipated for *Alaria marginata* to have the same growth rate as the other kelp species, but it is important to note that my study did not consider where they were habituated on the dock and how they were transferred to the seawater tanks. *Alaria marginata* are usually grown in the low to mid intertidal but can be distributed in the higher intertidal if there is enough wave action (Postels and Ruprecht 1840). *Alaria marginata* was habituated on the exposed side of the FHL dock, which allowed them to be exposed to more wave action. On the same depth level, *Saccharina latissima* and *Costaria costata* were habituated on the protected side of the dock where there are no waves and exposed to direct sunlight, which could of potentially affect the growth rate before the experiment that I conducted, thus affecting the results.

The fluorometer measures levels of chlorophyll fluorescence, my data showed no significant difference between chlorophyll fluorescence compared to the different temperature treatments. Although, there was a noticeable increase in chlorophyll fluorescence that occurred across all the treatments. This suggests that the kelp increased their photosynthetic rate in the 6 days they were in the tank. Contrary to this result, studies have shown that rates of photosynthesis increased with increasing temperatures for many different types of kelp including *Alaria marginata* and *Saccharina latissima* (Kjeldse 1966). Acclimatization of each species on the dock could have influenced my results. The kelp species were collected from the higher intertidal, causing them to be more exposed to light constantly. The low chlorophyll fluorescence levels recorded at the beginning of the experiment could have been caused by transfer-induced stress from the docks to the laboratory outdoor seawater tanks. A paper suggests that exposure to high irradiance causes a 40-60% decline in photosynthetic rate in the first hour of exposure (Bruhn and Gerard 1996). This discovery could account for the low photosynthesis levels at the beginning of the experiment where the F_v/F_m measurement was low. Despite the stress, kelp can recover from photoinhibition depending on the repair of the damaged reaction center (Bruhn and Gerard 1996). This could connect to the increase of chlorophyll fluorescence on day 6. If the experiment were conducted for a longer duration, there could have been a difference in chlorophyll levels between each temperature treatment.

To make a full conclusion about how temperature affects growth, a longer duration of growth is suggested. Six days' worth of growth can only give a short-term response to the effects of temperature but could be different in a long-term setting. The manipulated temperature was affected by abiotic factors such as sunlight and rainfall, during the days I experimented on the kelp, but the experiment was designed that way to manipulate kelp natural habitats. As the data

showed *Costaria costata* having the most growth at 14° Celsius, I observed continuous tissue loss at the tip of the blade for every species at all temperature treatments and could have played a factor in my results. As time prolonged, there was a noticeable increase of diatoms that accumulated in the tanks. They attached to the blades of the kelp and the seawater tanks as well. The growth of diatoms could have affected the growth of my kelp with studies shown algal blooms strongly decreasing the growth of kelp (Kavanaugh et al 2009). Even with these factors, by quantifying growth rates of kelp at different temperature treatments, I have shown that temperature does affect the growth of kelp significantly (Table 3).

Creating observations on how different factors affect the health of kelp is an important issue since algae are ecosystem builders in our ocean. Researchers have seen widespread kelp deforestation due to physiological stress which comes with the temperature being a factor (Steneck et al 2002). According to NOAA's 2020 Annual Climate Report, ocean temperature has increased an average rate of 0.08 degrees Celsius per decade since the 1880s with the rate increasing to more than twice the rate since 1981 (Menne et al 2018). With the increasing temperatures, not only can it cause a trophic cascade, but it can affect the morphology of different kelp species. For example, the Northeastern Atlantic, with warmer climates present, causes algae to exhibit a reduced size. Because of the reduced sizes, it diminishes the sequestering of carbon dioxide that can be assimilated and transported through kelp forests. This will ultimately damage the carbon cycle that we know today (Pessarrodona et al 2017). With evidence found from literature, my findings indicate that temperature can have a significant impact on the growth of different kelp species. My work suggests continuous research is needed to monitor the effects of temperature on the growth of other kelp species.

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References

- Alaria marginata* Postels & Ruprecht 1840: *Algaebase*. (n.d.). Retrieved May 30, 2021, from https://www.algaebase.org/search/species/detail/?species_id=3649
- Borlongan, I. A., Matsumoto, K., Nakazaki, Y., Shimada, N., Kozono, J., Nishihara, G. N., Shimada, S., Watanabe, Y., & Terada, R. (2018). Photosynthetic activity of two life history stages of *Costaria costata* (Laminariales, Phaeophyceae) in response to PAR and temperature gradient. *Phycologia*, 57(2), 159–168. <https://doi.org/10.2216/17-70.1>
- Bruhn, J., & Gerard, V. A. (1996). Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. *Marine Biology*, 125(4), 639–648. <https://doi.org/10.1007/BF00349245>
- Duarte, C., Wu, J., Xiao, X., Bruhn, A., & Krause-Jensen, D. (2017). Can Seaweed Farming Play a Role in Climate Change Mitigation and Adaptation? *Frontiers in Marine Science*, 4. <https://doi.org/10.3389/fmars.2017.00100>
- Kavanaugh, M. T., Nielsen, K. J., Chan, F. T., Menge, B. A., Letelier, R. M., & Goodrich, L. M. (2009). Experimental assessment of the effects of shade on an intertidal kelp: Do phytoplankton blooms inhibit growth of open coast macroalgae? *Limnology and Oceanography*, 54(1), 276–288. <https://doi.org/10.4319/lo.2009.54.1.0276>
- Kjeldsen, C. K. (1966.). *Effects of variations in salinity and temperature on some estuarine macroalgae*. Oregon State University.
- Krumhansl, K., & Scheibling, R. (2012). Production and fate of kelp detritus. *Marine Ecology Progress Series*, 467, 281–302. <https://doi.org/10.3354/meps09940>

- Menne, M. J., Williams, C. N., Gleason, B. E., Rennie, J. J., & Lawrimore, J. H. (2018). The Global Historical Climatology Network Monthly Temperature Dataset, Version 4. *Journal of Climate*, 31(24), 9835–9854. <https://doi.org/10.1175/JCLI-D-18-0094.1>
- Muth, A. F., Graham, M. H., Lane, C. E., & Harley, C. D. G. (2019). Recruitment tolerance to increased temperature present across multiple kelp clades. *Ecology*, 100(3), e02594. <https://doi.org/10.1002/ecy.2594>
- Nepper-Davidsen, J., Andersen, D., & Pedersen, M. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Marine Ecology Progress Series*, 630, 25–39. <https://doi.org/10.3354/meps13133>
- Pessarrodona, A., Moore, P. J., Sayer, M. D. J., & Smale, D. A. (2018). Carbon assimilation and transfer through kelp forests in the NE Atlantic is diminished under a warmer ocean climate. *Global Change Biology*, 24(9), 4386–4398. <https://doi.org/10.1111/gcb.14303>
- Peteiro, C. (2018). Alginate Production from Marine Macroalgae, with Emphasis on Kelp Farming. In B. H. A. Rehm & M. F. Moradali (Eds.), *Alginates and Their Biomedical Applications* (pp. 27–66). Springer. https://doi.org/10.1007/978-981-10-6910-9_2
- Reed, D., Washburn, L., Rassweiler, A., Miller, R., Bell, T., & Harrer, S. (2016). Extreme warming challenges sentinel status of kelp forests as indicators of climate change. *Nature Communications*, 7(1), 13757. <https://doi.org/10.1038/ncomms13757>
- Simonson, E. J., Scheibling, R. E., & Metaxas, A. (2015). Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Marine Ecology Progress Series*, 537, 89–104. <https://doi.org/10.3354/meps11438>

- Steneck, R., Graham, M., Bourque, B., Corbett, D., Erlandson, J., & Estes, J. (2002). Kelp Forest Ecosystems: Biodiversity, Stability, Resilience and Future. *Environmental Conservation*, 29, 436–459. <https://doi.org/10.1017/S0376892902000322>
- Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, 492, 81–98. <https://doi.org/10.1016/j.jembe.2017.01.017>
- Wernberg, T., Coleman, M. A., Bennett, S., Thomsen, M. S., Tuya, F., & Kelaher, B. P. (2018). Genetic diversity and kelp forest vulnerability to climatic stress. *Scientific Reports*, 8(1), 1851. <https://doi.org/10.1038/s41598-018-20009-9>