Hyposaline conditions impact the early life-stages of commercially important high-latitude kelp species.

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ABSTRACT:

This study examines how hyposaline stress impacts the early life-stages of commercial kelp species from Alaska. Kelp are important species both ecologically and commercially, and are likely to experience significant impacts due to ongoing climate change. Climate-driven glacial melt and changing rainfall patterns globally will release large amounts of freshwater into coastal systems in the coming decades. Both bull kelp (*Nereocystis luetkeana*) and ribbon kelp (*Alaria marginata*) are high-latitude species of commercial and ecological importance. These species inhabit very different environments: while bull kelp is a subtidal, canopy-forming species, ribbon kelp is an intertidal subcanopy species. In this study, fertile specimens of both were collected from various locations in Alaska and induced to release spores. These were cultivated for 30 days in four salinity treatments: 32 ppt, 25 ppt, 20 ppt, and 13 ppt. Both species grew and produced gametophytes in salinities down to 20 ppt, although *A. marginata* seems to be better adapted to hyposaline conditions. Below 20 ppt, we observed several impacts on progression between life-stages. The response of gametophyte growth and the production of eggs and sporophytes to different salinities varied both by species and by population. Gametophytes of *N. luetkeana* grew fastest at 32 ppt, while those of *A. marginata* grew fastest between 20 and 25 ppt (Juneau) or 25 and 32 ppt (Kodiak). In terms of egg production, *A. marginata* displayed significant population-level variation. Juneau individuals produced the same number of eggs regardless of salinity. Kodiak individuals produced fewer eggs in hyposaline conditions. The production of sporophytes from eggs for both species from all locations was unaffected by salinities above 20 ppt; however, no sporophytes at all were produced at 13 ppt. All of this has implications for commercial production in the hatchery phase, as hyposaline stress may induce *N. luetkeana* to produce sporophytes faster than in full oceanic salinity. In terms of wild populations, the observed population-level and species-level differences in adaptation to hyposaline conditions suggest that decreased salinities in coastal areas are likely to impact the distribution of these two species over the coming decades.

1 Introduction

The ongoing process of climate change is altering the world's oceans in profound and complex ways. Some of these impacts are well-known and their effects are extensively documented, particularly the global rise in sea surface temperatures and increase in frequency and duration of marine heatwaves (Leathers et al., 2023; Hobday et al., 2016; Smith et al., 2024). At high latitudes, climate change may have additional impacts related to the seasonality of low-salinity events, a reduction in glacial coverage and release of glacial melt into coastal waters (Bliss et al., 2014). Glacial runoff introduces increased amounts of freshwater and glacial sediment into the coastal environment, decreasing salinities and altering light attenuation (Arimitsu et al., 2016). We will also likely see an increase in stressor synergies, including an increase in nutrient and pollutant runoff (Schoenrock et al., 2018). Localized decreases in coastal water temperatures due to glacial runoff are also likely (Schoenrock et al., 2018). This is likely to have significant impacts for marine benthos, particularly primary producers and foundation species.

Foundation species are fundamental to the physical and ecological structure of the ecosystems in which they occur, and so climate change impacts on these species are likely to have cascading effects on ecosystems as a whole (summarized in Wernberg et al., 2024). Kelps are a group of foundation species and primary producers of the Order Laminariales which dominate temperate, subpolar, and polar rocky coastlines worldwide (Steneck et al., 2003). They form vast underwater forests which support a network of associated species and may themselves be of commercial importance. A number of kelp species are farmed or harvested globally and are processed and used for a variety of applications (Kim et al., 2019). Kelp are consequently of tremendous importance to human populations both ecologically and economically.

There has been increased interest recently in the impact of stressors, including hyposaline stress, on kelp physiology, particularly reproduction and development during the early life-stages. Overall, a decrease in environmental salinity has been associated with reduced photosynthetic capacity and a loss of photosynthetic pigments (Karsten, 2007; Li et al., 2020; Monteiro et al., 2019; Spurkland and Iken, 2011). Studies have also noted declines in sporophyte and gametophyte growth rates and spore settlement densities (Buschmann et al. 2004; Lind and Konar, 2017; Monteiro et al., 2021; Muth et al., 2021). However, most existing research on this topic has focused on sugar kelp (*Saccharina latissima*) or winged kelp (*Alaria esculenta*), as both of these are of great commercial importance in the North Atlantic. In terms

of other seaweed groups, hyposaline stress has been shown to cause oxidative damage (Wang et al., 2020), change metabolite expression (Siddiqui et al., 2022), and decrease photosynthetic capacity (Marambio et al., 2022) in rhodophytes. In chlorophytes, hyposaline stress has been shown to induce the accumulation of polyamines in tissues (Lee, 1998). Chen et al. (2023) investigated the impacts of hyposaline stress on five common intertidal seaweed species from Fujian Province, China, including reds, greens, and browns, and found that decreased salinity universally increased the release of dissolved organic carbon and decreased photosynthesis. This has been corroborated in subsequent studies (Bennett et al., 2024).

Very few studies thus far have investigated the impacts of hyposaline stress on bull kelp (*Nereocystis luetkeana*) or ribbon kelp (*Alaria marginata*). Both of these species are of major ecological and commercial importance in the northeast Pacific. *N. luetkeana* is a primary canopy-forming species in this region and is generally wild-harvested for commercial use (Springer et al., 2010; Stekoll et al., 2006; Stekoll, 2019), although there has been significant interest in its commercial cultivation (Stekoll et al., 2024). This species has suffered extensive declines along parts of its range in recent years, which have prompted numerous research and conservation initiatives (Supratya and Martone, 2023). On the other hand, *Alaria* spp. is an intertidal subcanopy genus which is extensively farmed in the subpolar and cold-temperate Pacific and Atlantic regions. Most research involving the genus *Alaria* has focused on *A. esculenta*, but even these studies are limited in number (Farrugia Drakard et al., 2023). Both of these species are annuals, experiencing massive spore production in the late summer and early autumn, followed by the persistence of microscopic life-stages (spores, gametophytes, and juvenile sporophytes) on the benthos until around late spring (McConnico and Foster, 2005). Juvenile sporophytes undergo rapid maturation in mid-to-late spring, resulting in the persistence of populations from year to year (McConnico and Foster, 2005).

Gametophytes of both species are present on the benthos in late fall through winter and into spring (Weigel et al., 2023; McConnico and Foster, 2005), when heavy rainfall and snowfall in the intertidal zone are likely to contribute to hyposaline conditions in coastal areas. Freshwater influx during these periods generally results in a less dense surface layer of freshwater lying atop denser seawater in coastal areas (Brown et al., 2019). However, while *N. luetkeana* is subtidal (Carney et al., 2005), *A. marginata* occurs in the lower intertidal (McConnico and Foster, 2005). With this in mind, we can expect gametophytes of the latter species to be more frequently exposed to oscillations in salinity due to their relative proximity both to the surface freshwater layer and to coastal precipitation runoff. Additionally, much of

Alaska is subject to high levels of precipitation in the form of rain or snow year-round, which would expose intertidal species directly to freshwater influx.

There are significant biological and ecological differences between the two species which have the potential to impact tolerance and resilience of the microscopic stages to specific stressors. For example, *A. marginata* develops dedicated sporophylls (sorus-bearing structures) close to the base of the thallus just above the holdfast, while in *N. luetkeana* the sorus tissue develops in patches along the blades. Therefore, spores in *A. marginata* are released relatively close to the benthos, while spores from *N. luetkeana* are released at the surface. While both species experience peak spore production during summer, sorus tissue of *A. marginata* tends to mature earlier and can be induced to release spores as early as May. On the other hand, sorus tissue of *N. luetkeana* persists for longer, and spores are released well into autumn. As has already been discussed, while *N. luetkeana* is a subtidal, canopy-forming species, *A. marginata* is an intertidal sub-canopy species. Therefore, *A. marginata* is exposed to frequent hyposaline stress due to freshwater influx from rainfall and riverine input, while *N. luetkeana* is exposed to hyposaline stress only infrequently and likely only at the canopy level.

The aim of this study was therefore to investigate the impacts of hyposaline stress on the microstages of bull kelp (*N. luetkeana*) and ribbon kelp (*A. marginata*), with a view to determining the impacts of ongoing climate change in cold-temperate, subpolar, and polar regions on both commercial cultivation and the ecology of wild populations. We hypothesize here that spore germination, gametophyte growth, egg and sporophyte production, and sporophyte growth of both species decrease with decreasing salinity.

2 Methodology

2.1 Sorus collection and sporulation

We collected fertile specimens of *Alaria marginata* and *Nereocystis luetkeana* from two locations each in July 2023 (Figure 1). *A. marginata* was collected from Juneau, Alaska, and Kodiak, Alaska, while *N. luetkeana* was collected from Little Port Walter, Alaska, and Kodiak, Alaska. Juneau is located among the straits and passages of the North American Fjordland, and experiences significant glacial influence from the glaciers of the Juneau Icefield (Ziemen et al., 2016). The closest of these to the collection site, Mendenhall Glacier, terminates in the Mendenhall Lake and discharges into expansive estuarine wetlands (Siegela, 1988). The coastline in this region is greatly influenced by these wetlands, and kelp populations established here are likely to experience more frequent hyposaline events. The Juneau coastline is subject

to freshwater input from glacial melt, rainfall, and snowmelt. Little Port Walter is on the south end of Baranof Island, closer to the exterior of the North American Fjordland. It has no glacial influence but is still subject to significant freshwater input from snowmelt and rainfall. Kodiak Island lies across the Gulf of Alaska just east of the Aleutian Peninsula, and constitutes a much more oceanic environment with no glacial influence. Changes in salinity along the Kodiak coastline are likely to be rapidly equalized with the open ocean due to the higher exposure of this environment. We would expect the adaptations of kelp populations established in these locations to reflect surrounding environmental conditions.

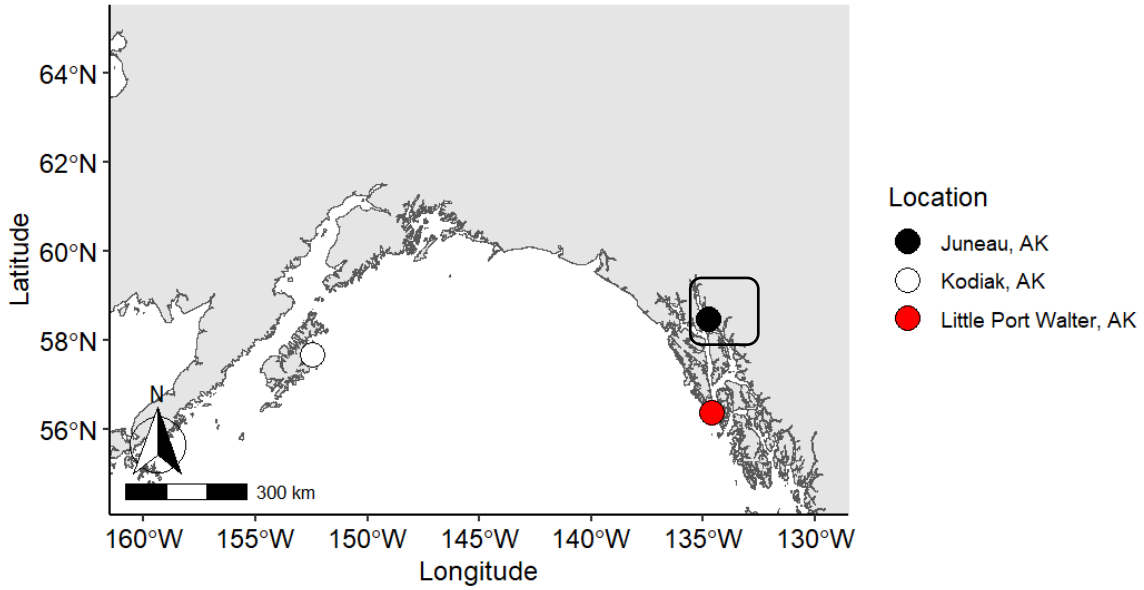

Figure 1: Map of the study sites. *Alaria marginata* was collected from Juneau (JNU) and Kodiak (KOD), while *Nereocystis luetkeana* was collected from Juneau (JNU) and Little Port Walter (LPW). Black square represents the approximate location and extent of the Juneau Icefield.

Sporophylls from 10 individuals from each location were cleaned in 10% iodine solution (Betadine®) in freshwater, dried with paper towels, and stored for 24 hours in a cold (4 °C), dark, dry environment. Sporophylls from each location were then separately placed in filtered, UV-sterilized seawater at 12 °C under fluorescent lighting (40 – 60 µmol m^{-2} s^{-1}) for one hour to induce sporulation. We filtered the resultant spore solutions (one solution for each population, consisting of spores pooled from 10 individuals) through a 46 µm sieve and determined zoospore densities using a hemocytometer with an Improved Neubauer grid. Subsequently, the spore solutions were diluted with UV-sterilized seawater to a density of 2000 zoospores mL^{-1}.

2.2 Experimental set-up and design

The experimental design consisted of four salinity treatments: 32 ppt, 25 ppt, 20 ppt, and 13 ppt. 32 ppt is a fully oceanic treatment, and 13 ppt is the approximate lower tolerance threshold for Arctic kelp species (Karsten, 2007). Intervals between these two treatments were selected based on ease of dilution from full oceanic salinity. For each treatment, we filled 5 petri dishes of surface area 23.76 cm^2 with 15 mL of the 2000 zoospores mL^{-1} spore solution, for a total of 20 petri dishes per location/species combination and 80 petri dishes for the whole experiment. These were stored in the dark at 12 °C for 48 hours to allow zoospore settlement.

After 48 hours, microphotographs were taken at 200x phase contrast magnification of 5 haphazardly selected fields of view per petri dish using a Leica DMi8 S inverted microscope. These were used to determine the average initial settled zoospore count. Separate culture media were prepared for each of the salinities to be tested as follows: for 1000 mL of a) 32 ppt – 990 mL UV-sterilised seawater, 10 mL Provasoli's enriched seawater medium with iodine (PESI) working solution (Provasoli, 1968; Tatewaki, 1966), b) 25 ppt – 800 mL UV-sterilised seawater, 190 mL Milli-Q water, 10 mL PESI working solution c) 20ppt – 600 mL UV-sterilised seawater, 390 mL Milli-Q water, 10 mL PESI working solution d) 13ppt – 400 mL UV-sterilised seawater, 590 mL Milli-Q water, 10 mL PESI working solution. Salinities were checked after preparation of the culture media using a handheld refractometer. The solution in each petri dish was decanted out and replaced with 15 mL of the appropriate culture medium – 5 petri dishes per salinity treatment within each location/species combination.

We then placed the petri dishes in incubators set at 12 °C, light intensity 40 – 60 μmol m^{-2} s^{-1} and a L:D regime of 12h:12h. Microphotographs at 200x magnification of 5 fields of view per petri dish were taken after 5 days and 10 days. These were used to determine the average number of zoospores and average number of germinated zoospores per location and temperature treatment. Subsequently, microphotographs of 10 gametophytes per petri dish were taken at 15 days, 20 days, 25 days, and 30 days. These were used to determine the average gametophyte size, average number of eggs produced per female, average number of sporophytes produced per female, and average sporophyte size per location and salinity treatment. Medium changes were conducted every 5 days until the conclusion of the experiment after 30 days.

2.3 Data analysis

All statistical analyses were carried out using R version 4.0.2 in RStudio version 2024.04.2 Build 764 (R Core Team, 2024). All analyses were carried out for *A. marginata* and *N. luetkeana* separately.

For each sampling point up to 10 days, the average number of zoospores and the average number of germinated zoospores per petri dish (N = 5 fields of view) were used to calculate the average proportion of zoospores germinated for each location and each salinity treatment (N = 5 petri dishes). These data were arcsine-transformed and analyzed using a three-way mixed ANOVA with Time (0DAY, 5DAY, 10DAY) as a within-subjects factor and Location (JNU vs KOD or LPW vs KOD) and Salinity (32PPT, 25PPT, 20PPT, 13PPT) as between-subjects factors. *Post-hoc* pairwise ANOVAs with Bonferroni correction were performed to explore significant effects.

For the 30 day sampling point, average gametophyte size per location and salinity treatment (N = 5 petri dishes) was calculated as the average of the lengths of each photographed gametophyte measured along the longest axis (N = up to 10 gametophytes). Both male and female gametophytes were included. These data were analyzed using two-way ANOVAs with Location (JNU vs KOD or LPW vs KOD) and Salinity (32PPT, 25PPT, 20PPT, 13PPT) as between-subjects factors. *Post-hoc* pairwise ANOVAs with Bonferroni correction were performed to explore significant effects.

The average numbers of eggs and sporophytes produced per female were calculated for sampling points from 15 to 30 days and used to obtain averages per location and salinity treatment. These data were analyzed using three-way mixed ANOVAs as described above. *Post-hoc* two-way ANOVAs split by Location (JNU and KOD or LPW and KOD) and pairwise ANOVAs with Bonferroni correction were performed to explore significant effects.

At 30 days, the average sporophyte size per location and salinity treatment was calculated as the average of the lengths of up to 10 sporophytes per petri dish (using no more than 3 sporophytes per female gametophyte) measured along the longest axis. These data were analyzed using two-way ANOVAs with Location (JNU vs KOD or LPW vs KOD) and Salinity (32PPT, 25PPT, 20PPT, 13PPT) as between-subjects factors. *Post-hoc* pairwise ANOVAs with Bonferroni correction were performed to explore significant effects.

3 Results

3.1 Zoospore survival and germination

For *Alaria marginata*, a three-way interaction between Location, Time, and Salinity determined the proportion of zoospores germinated ($F_{6,64}$ = 3.288, $p < 0.05$; Supplementary Material Table 1). Specifically, the lowest salinity tested had a negative effect on the proportion of *A. marginata* zoospores germinated from the Juneau population at the 10 day timepoint (Figure 2). There was no effect of salinity on the Kodiak population or at any other timepoint (Figure 2).

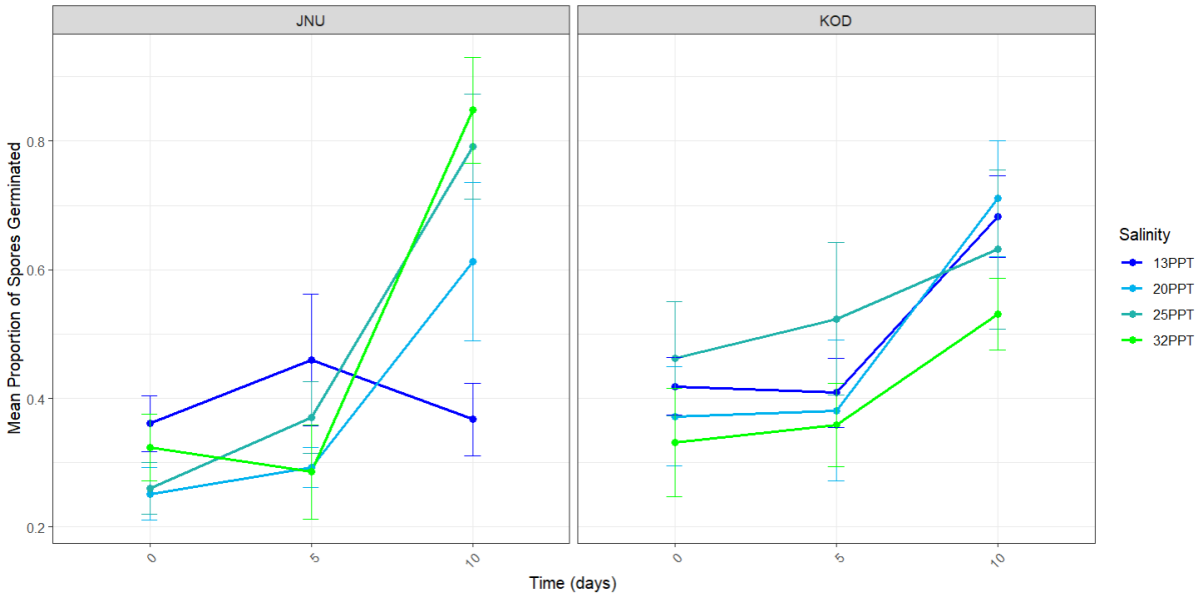

Figure 2: Mean proportion of *Alaria marginata* zoospores germinated for specimens from Juneau (JNU) and Kodiak (KOD) at 32 ppt, 25 ppt, 20 ppt and 13 ppt. Error bars show standard error.

For *Nereocystis luetkeana*, we observed significant two-way interactions between Time and Salinity ($F_{6,64}$ = 2.498, $p < 0.05$; Supplementary Material Table 2) and between Location and Salinity ($F_{3,32}$ = 3.903, $p < 0.05$; Supplementary Material Table 2). Specifically, the proportion of spores germinated was higher in the 13 ppt treatment than in the 32 ppt treatment at KOD at 5 days, and at LPW at all timepoints (pairwise *t*-test within 5DAY across LPW and KOD: 13PPT v 32PPT t = 3.41, $p < 0.05$; pairwise *t*-test within LPW across 0DAY, 5DAY and 10DAY: t = 4.14, $p < 0.05$; Figure 3).

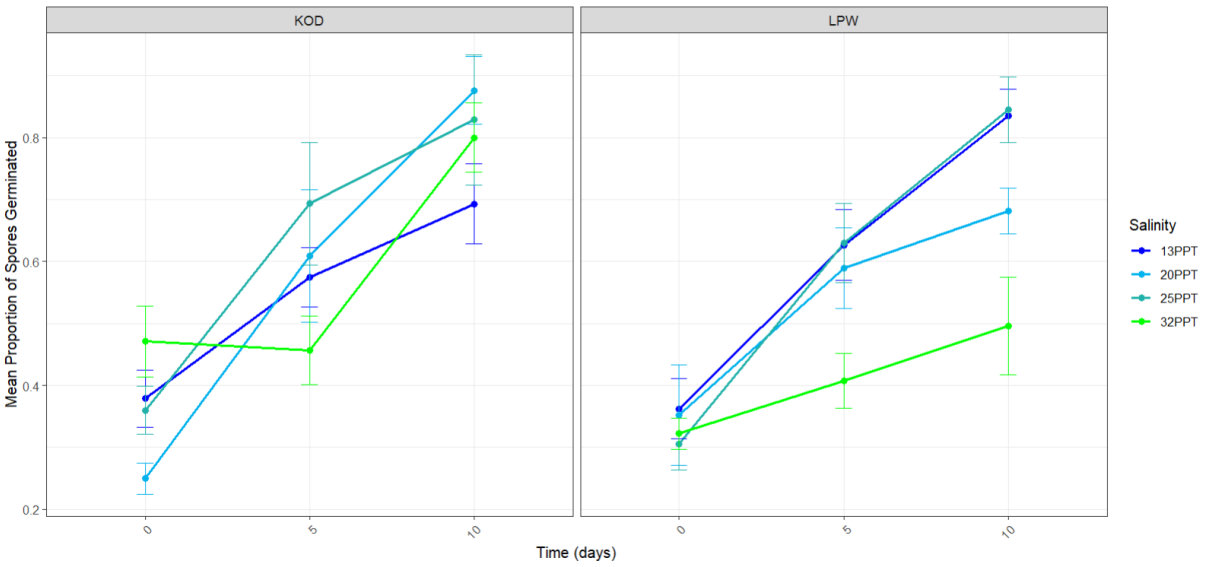

Figure 3: Mean proportion of *Nereocystis luetkeana* zoospores germinated for specimens from Kodiak (KOD) and Little Port Walter (LPW) at 32 ppt, 25 ppt, 20 ppt and 13 ppt. Error bars show standard error.

3.2 Gametophyte size

In terms of gametophyte size at 30 days, there was a significant two-way interaction between Location and Salinity for both *A. marginata* ($F_{3,32}$ = 3.639, $p < 0.05$; Supplementary Material Table 3) and *N. luetkeana* ($F_{3,32}$ = 4.051, $p < 0.05$; Supplementary Material Table 3). Salinity had an effect on gametophyte size for gametophytes of both species and from both locations within each species (JNU and KOD, or LPW and KOD) (Supplementary Material Table 4).

Overall, *A. marginata* gametophytes from Kodiak grew faster in the 25 ppt treatment compared to the 13 ppt and 20 ppt treatments (pairwise *t*-test at 30DAY: 13PPT v 25PPT t = -12.3, $p < 0.05$, 20PPT v 25PPT t = -8.85, $p < 0.05$; Figure 4). Pairwise testing could not distinguish differences between treatments for gametophytes from Juneau, but we observed a trend of higher growth rates at 20 ppt and 25 ppt (Figure 4). *N. luetkeana* gametophytes from both locations grew faster in higher salinity treatments. For *N. luetkeana* from Little Port Walter, gametophyte size increased as salinity increased up to 25 ppt (pairwise *t*-test 13PPT v 20PPT t = -6.80, $p < 0.05$; 13PPT v 25PPT t = -9.46, $p < 0.05$; Figure 4). A similar pattern was observed for *N. luetkeana* gametophytes from Kodiak, wherein gametophyte size increased with salinity up to 32 ppt (pairwise *t*-test 13PPT v 20PPT t = -9.33, $p < 0.05$; 13PPT v 25PPT t = -11.2, $p < 0.05$; 13PPT v 32PPT t = -10.6, $p < 0.05$; 20PPT v 32PPT t = -5.21, $p < 0.05$; Figure 4).

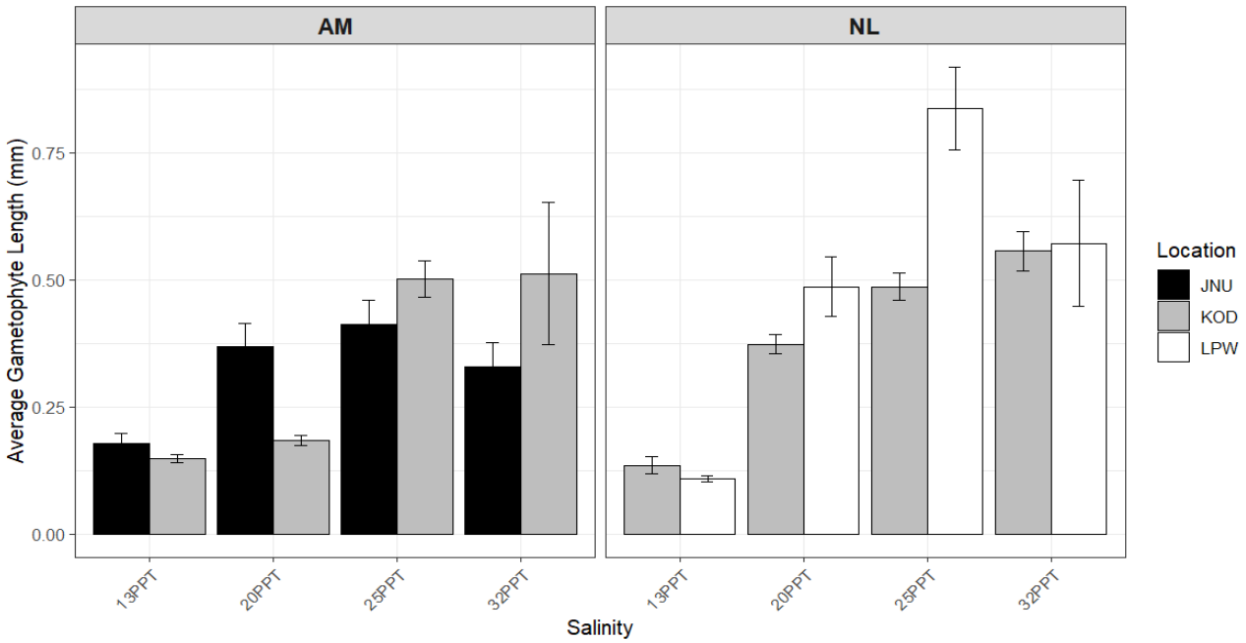

Figure 4: Mean gametophyte length for *Alaria marginata* (AM) and *Nereocystis luetkeana* (NL) specimens from Juneau (JNU), Kodiak (KOD), and Little Port Walter (LPW) at 30 days. Error bars showing standard error.

3.3 Egg and sporophyte production

This study investigated female fecundity in terms of egg production and sporophyte production per female of each species. Data is presented here for the 30 day timepoint for sporophyte production, but for the 25 and 30 day timepoint for egg production. This is because the number of eggs produced is expected to peak between 20 – 25 days and then decline as eggs develop into sporophytes. Presenting both the 25 and 30 day egg production data is expected to provide a more representative picture of egg production overall. *A. marginata* females from Kodiak produced more eggs compared to Juneau females, and salinity had a significant effect on egg production only in Kodiak, where fewer eggs were produced at lower salinities (Supplementary Material Table 5; pairwise *t*-test within KOD: 13PPT v 25PPT $t = 4.23$, $p < 0.05$, 13PPT v 32PPT $t = 3.59$, $p < 0.05$; Figure 5).

The development of sporophytes from eggs varied significantly between Locations ($F_{1,32} = 4.661$, $p < 0.05$) and between Salinities($F_{3,32} = 3.048$, $p < 0.05$; Supplementary Material Table 6). Fewer sporophytes per gametophyte were produced in Juneau cultures compared to Kodiak cultures, and fewer sporophytes per gametophyte were produced at lower salinities overall (Figure 6). Pairwise testing could not distinguish differences between treatments for either location, but we observed a trend of higher sporophyte production at 25 and 32 ppt for Kodiak females, and at 20, 25 and 32 ppt for Juneau females (Figure 6).

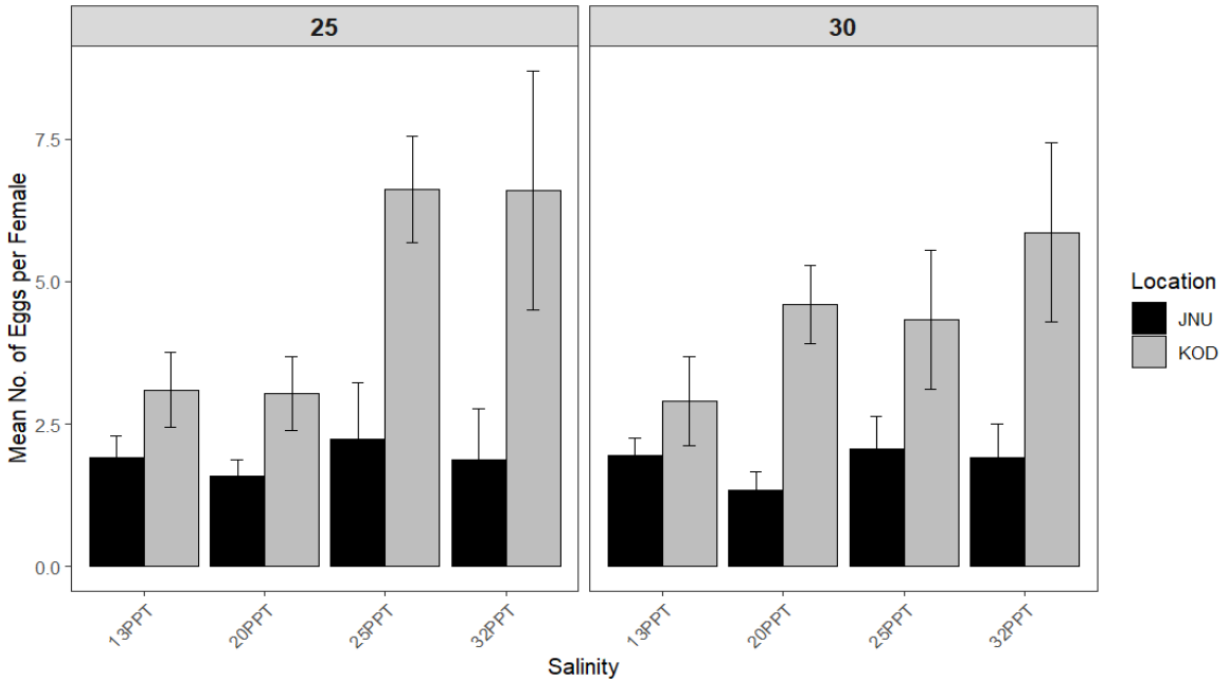

Figure 5: Mean number of eggs produced per female across salinity treatments for *Alaria marginata* specimens from Juneau (JNU) and Kodiak (KOD) at 25 days (left) and 30 days (right). Error bars showing standard error.

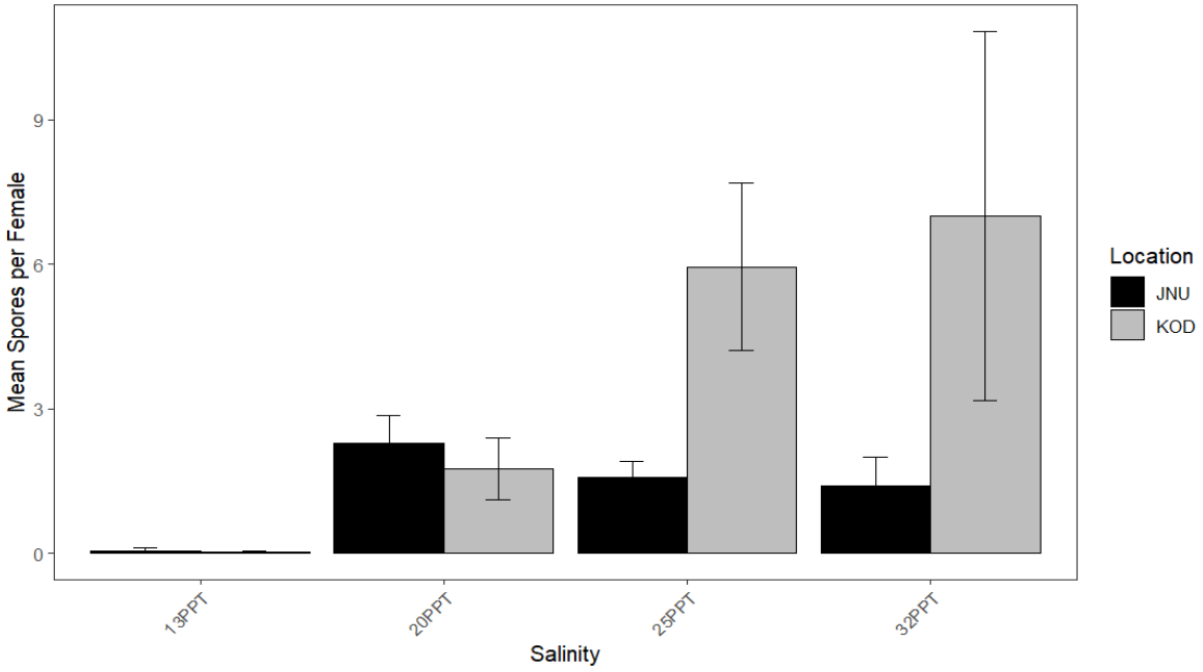

Figure 6: Mean number of sporophytes produced per female at 30 days across salinity treatments for *Alaria marginata* specimens from Juneau (JNU) and Kodiak (KOD). Error bars showing standard error.

In terms of egg production in *N. luetkeana*, we observed a significant Location*Salinity*Time interaction ($F_{4.11,43.88} = 4.062$, $p < 0.05$; Supplementary Material Table 7). Splitting this dataset by Location indicated a significant Salinity*Time interaction for Little Port Walter ($F_{4.17,22.24} = 3.737$, $p < 0.05$; Supplementary Material Table 8). Time ($F_{1.35,21.57} = 36.767$, $p < 0.05$) and

Salinity ($F_{3,16}$ = 12.663, p < 0.05) individually had a significant effect on egg production for specimens from Kodiak (Supplementary Material Table 8). Individuals from Little Port Walter were subject to a significant effect of Salinity only at the 25 day timepoint: overall, more eggs were produced in 20 ppt at this timepoint than in 32 ppt (Figure 7). For individuals from Kodiak, at both 25 and 30 days more eggs were produced in 20 ppt and 25 ppt than in 13 or 32 ppt (pairwise *t*-test within KOD: 25PPT v 32PPT t = 2.95, p < 0.05; Figure 7).

Sporophyte production in *N. luetkeana* was influenced by Salinity, but was not significantly different between locations ($F_{3,32}$ = 24.758, p < 0.05; Supplementary Material Table 9). *Post-hoc* pairwise tests indicated the existence of three disparate groupings: a) 13 ppt, b) 20 ppt and 25 ppt, and c) 32 ppt (pairwise *t*-test within 30DAY: 13PPT v 20PPT t = -6.30, p < 0.05; 13PPT v 25PPT t = -7.12, p < 0.05; 13PPT v 32PPT t = 4.54, p < 0.05; 20PPT v 32PPT t = 4.62, p < 0.05; 25PPT v 32PPT t = 4.86, p < 0.05). Overall, no sporophytes were produced at 13 ppt, very few at 32 ppt, and most at 20 ppt and 25 ppt (Figure 8).

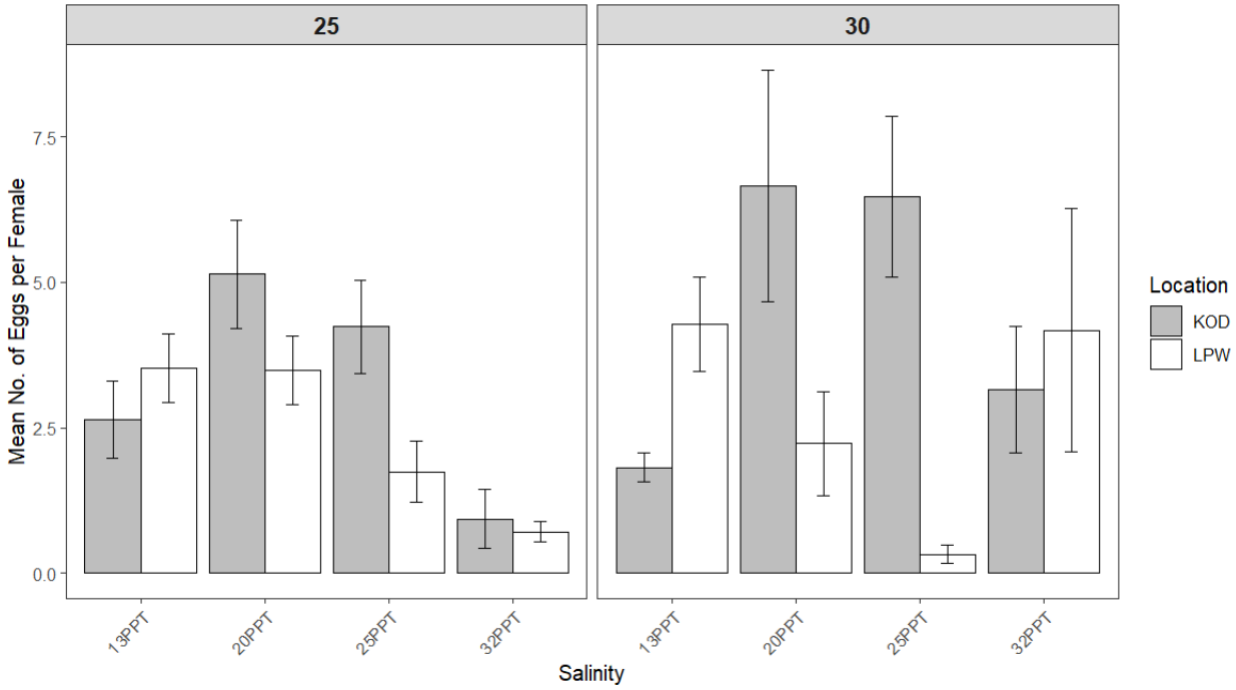

Figure 7: Mean number of eggs produced per female across salinity treatments for *Nereocystis luetkeana* specimens from Little Port Walter (LPW) and Kodiak (KOD) at 25 days (left) and 30 days (right). Error bars showing standard error.

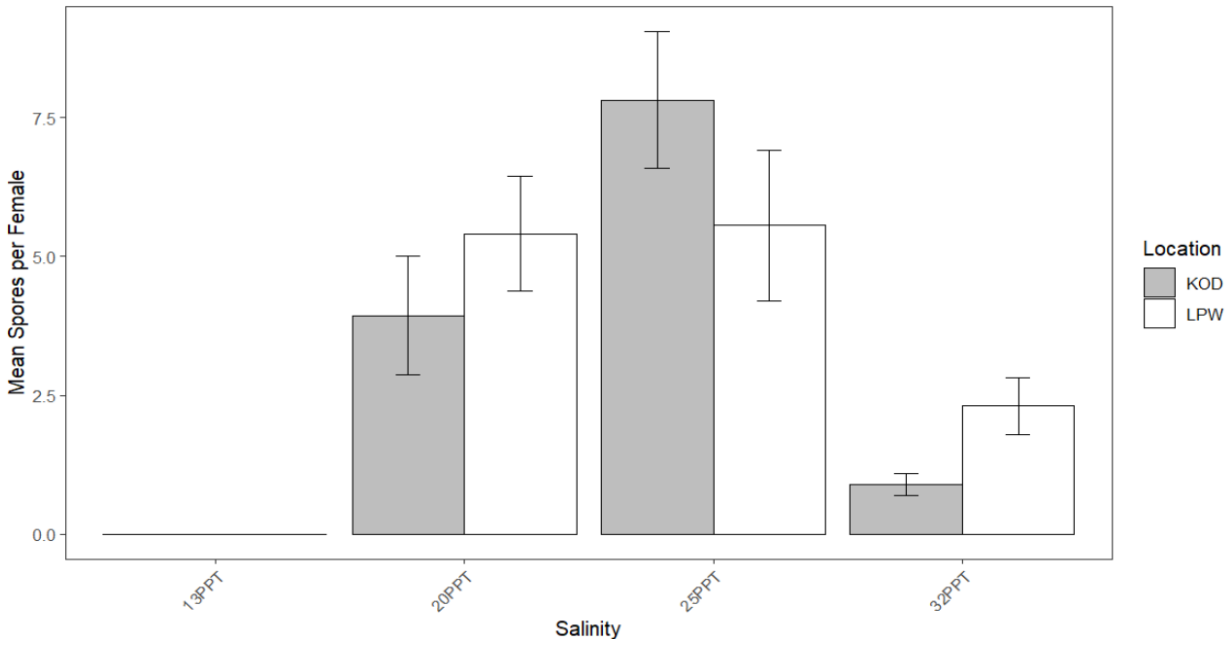

Figure 8: Mean number of sporophytes produced per female at 30 days across salinity treatments for *Nereocystis luetkeana* specimens from Little Port Walter (LPW) and Kodiak (KOD). Error bars showing standard error.

In summary, *A. marginata* individuals from Kodiak produced more eggs and more sporophytes than those from Juneau and appeared to be more reproductive at higher salinities (25 ppt and 32 ppt). Egg production in *N. luetkeana* individuals from Little Port Walter was affected by salinity only up to 25 days and peaked at 20ppt at these timepoints. On the other hand, individuals from Kodiak produced most eggs consistently at 20 ppt and 25 ppt. Most *N. luetkeana* sporophytes were produced at 20 ppt and 25 ppt. No sporophytes were produced for either species at 13 ppt.

3.4 Sporophyte size

For *A. marginata* at 30 days, we observed a significant two-way Location*Salinity interaction ($F_{3,32}$ = 7.464, $p < 0.05$; Supplementary Material Table 10), and Salinity appeared to be a significant factor at both locations (JNU: $F_{3,16}$ = 4.50, $p < 0.05$, KOD: $F_{3,16}$ = 12.60, $p < 0.05$; Supplementary Material Table 11). Juneau sporophytes grew faster at 20 ppt than in any other salinity (pairwise *t*-test within JNU: 13PPT v 20PPT t = -8.10, $p < 0.05$; Figure 9) – growth was not observed at 13 ppt as no sporophytes were produced in this treatment. Kodiak sporophytes appeared to be split into two distinct groups: a) the 13 ppt and 20 ppt treatments and b) the 25 ppt and 32 ppt treatments (pairwise *t*-test within KOD: 13PPT v 25PPT t = -9.85, $p < 0.05$, 20PPT v 25PPT t = -6.92, $p < 0.05$; Figure 9). Overall, Kodiak sporophytes grew larger at 25 ppt and 32 ppt within the experimental period (Figure 9).

For sporophytes of *N. luetkeana*, Location ($F_{1,32}$ = 10.737, p < 0.05) and Salinity ($F_{3,32}$ = 10.975, p < 0.05) were significant as main effects (Supplementary Material Table 11). Overall, sporophytes from Little Port Walter grew larger than those from Kodiak within the experimental period (Figure 9). Although sporophytes from Little Port Walter trended towards a larger size at 25 ppt compared to other salinities, there was no significant difference between the 20 ppt, 25 ppt and 32 ppt treatments (pairwise *t*-test within 30DAY: 13PPT v 20PPT t = -6.76, p < 0.05, 13PPT v 25PPT t = -5.09, p < 0.05; Figure 9). Once again, growth was not observed at 13 ppt as no sporophytes were produced in this treatment.

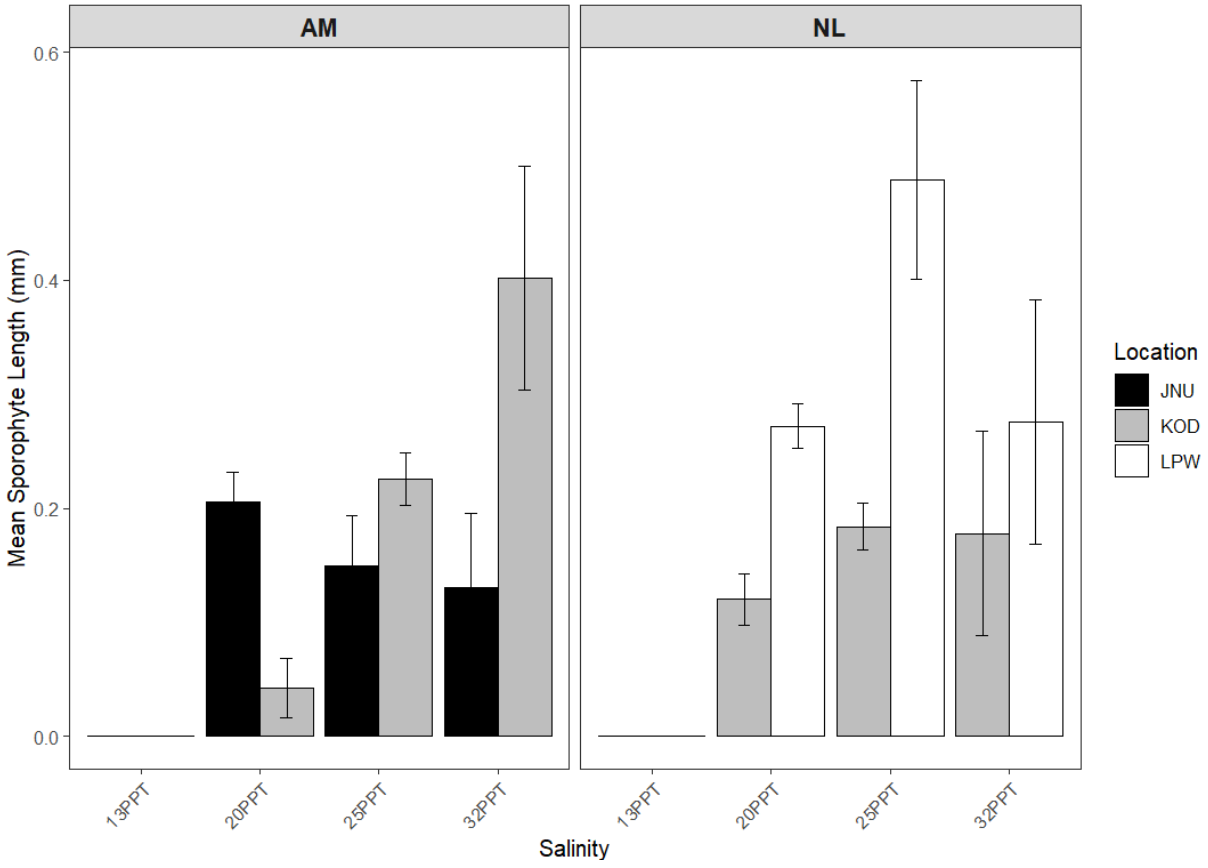

Figure 9: Mean sporophyte length at 30 days for *Alaria marginata* (AM) and *Nereocystis luetkeana* (NL) cultures from Juneau (JNU), Kodiak (KOD), and Little Port Walter (LPW) across salinity treatments. Error bars showing standard error.

4 Discussion

This study investigated the impact of hyposaline conditions on the early life-stages of *Alaria marginata* and *Nereocystis luetkeana*. Both species were resilient to some extent to decreased salinities down to 20 ppt. Below this point, we observed several impacts on reproduction and progression between life-stages. The response of gametophyte growth and the production of eggs and sporophytes to changes in salinity varied both by species and by population.

Of the two species considered here, *A. marginata* seems to be markedly more resilient to hyposaline conditions. Gametophytes of *N. luetkeana* displayed a mostly linear response to salinity in terms of growth: as conditions became less saline, gametophytes grew slower. On the other hand, gametophytes of *A. marginata* from Kodiak grew equally well at 25 ppt and 32 ppt, and specimens from Juneau actually grew faster at 20 ppt and 25 ppt compared to fully oceanic conditions. This indicates that gametophytes of *A. marginata* have wider salinity tolerance margins and may have lower salinity optima than gametophytes of *N. luetkeana*. This may be due to the different ecological niches of these two species, as previously described. The relatively high tolerance of *A. marginata* gametophytes to hyposaline conditions is therefore likely to be adaptive. While no studies have considered the response of *A. marginata* to salinity, gametophytes of *N. luetkeana* have been shown to experience declines in growth rate under hyposaline conditions (Lind and Konar, 2017).

In addition to being overall more tolerant of hyposaline conditions, gametophytes of *A. marginata* also appeared to vary in their response to differences in salinity depending on their population of origin. Specifically, gametophytes from Juneau exhibited a lower salinity optimum than gametophytes from Kodiak. Gametophytes from Juneau grew fastest at 20 ppt and 25 ppt, and actually displayed decreased growth rates at 32 ppt. Once again, this is likely to have an ecological explanation. As previously described, Juneau is a more glaciated region than Kodiak and kelp populations established there are therefore more likely to experience frequent hyposaline events. Consequently, we would expect *A. marginata* populations in Juneau to be exposed to a constant slightly hyposaline environment compared to populations in Kodiak. Therefore, the lower salinity optimum for gametophytes from Juneau is likely to be adaptive. Indeed, studies have shown that in general, responses to salinity in high-latitude kelp species tend to be population-specific (Buschmann et al., 2004; Monteiro et al., 2019). This is not unexpected, as coastal salinity is influenced extensively by point sources of freshwater such as glacial outflows and rivers and is likely to be more variable than other environmental stressors of interest (Farrugia Drakard et al., 2023). All of this indicates that within a given species, certain populations may be more resilient to climate-induced changes in salinity than others.

The same patterns are evident in terms of the production of eggs and sporophytes. Once again, *A. marginata* displayed significant population-level variation. Gametophytes from Kodiak produced more eggs overall than those from Juneau. However, gametophytes from Juneau produced the same number of eggs regardless of salinity, while those from Kodiak produced

slightly more eggs at 25 ppt and 32 ppt. This suggests a tolerance to hyposaline conditions in this species; Juneau *A. marginata* will produce the same number of eggs regardless of salinity, and Kodiak *A. marginata* will produce the same number of eggs down to 25PPT. Conversely, *N. luetkeana* gametophytes had uniformly produced more eggs at salinities below 32 ppt by Day 25, and only began to produce significant numbers of eggs in 32 ppt towards the end of the experiment at Day 30. This may be a stress response rather than an environmental adaptation, as it appears to be related to timing of egg production rather than overall number of eggs produced. It is likely that *N. luetkeana* held at 32PPT would have continued to produce an equivalent or higher number of eggs than those held at lower salinities had the experiment been extended for a further week. Strasser et al. (2022) showed that gametophytes of *Laminaria ochroleuca* exhibited higher levels of reproductive success after a simulated marine heatwave of 27°C compared to those maintained under control conditions of 17°C. The authors attributed this to a stress-induced survival mechanism promoting gametogenesis (Strasser et al., 2022). Similarly, Dethier et al. (2005) showed that moderate stress resulted in earlier reproduction and increased reproductive parameters in *Fucus gardneri*. The relationship between abiotic stress and reproduction in seaweeds is not well understood. However, it is possible that in the case of this experiment, moderate hyposaline stress makes the allocation of energy to vegetative growth unfavorable, and the gametophytes in question switch to reproductive allocation in order to ensure successful reproduction prior to mortality. This does have implications for the commercial production of *N. luetkeana*, as it suggests that lowering salinities to between 20–25 ppt will induce the production of eggs – and consequently juvenile sporophytes – up to a week earlier than culturing at full oceanic salinity. This could significantly reduce hatchery costs in commercial operations.

Conversely, the germination response of *N. luetkeana* spores is likely to be due to adaptation. While spores of this species from Kodiak germinated in equivalent proportions at all salinities, a higher proportion of spores from Little Port Walter germinated at salinities below 32 ppt. *N. luetkeana* populations in Kodiak experience fully marine conditions almost exclusively, whereas populations at Little Port Walter are likely to experience influxes of freshwater during outflow events from the nearby creek. In *A. marginata*, the opposite is true: while spores from Kodiak once again germinated in equivalent proportions at all salinities, a higher proportion of spores from Juneau germinated at salinities above 13 ppt. Although we can only speculate based on the data presented here, it is possible that successful spore germination is controlled in large part by population-level genetic differences.

At this point, it is important to put these results in the context of the genetic environment. The genetic diversity of most Alaskan kelp species, as well as the genetic structure of their populations, is not particularly well understood. The phylogeography of both split kelp (*Hedophyllum nigripes*) and sugar kelp (*Saccharina latissima*) in the Gulf of Alaska has been resolved (Grant et al., 2020; Grant and Chenoweth, 2021; Mao et al., 2020). However, while genetic differentiation of *N. luetkeana* has been studied range-wide, the specific population genetics within the Gulf of Alaska have not been resolved (Gierke et al., 2023), and no studies have considered *A. marginata*. As genetic diversity and range-wide genetic differentiation among populations is likely to vary significantly across species, we consider this to be a high priority for future research.

Although both species produced eggs at all salinities, no sporophytes of either species were produced at 13 ppt. This could be the result of either failed fertilization or a high mortality rate for embryonic sporophytes at low salinities. Gametophytes of both species were confirmed to be present in an approximately 50:50 male:female ratio at all salinities, and so if this is a case of failed fertilization, it is likely to be due either to failure of the males to produce sperm or failure of the sperm to reach and successfully fertilize the eggs. Both sperm release and chemotactic orientation towards the egg are induced by pheromones secreted by the eggs (Maier et al., 2001), but these secretions are complex and the mechanism of chemotaxis in kelp gametes is not well understood. Further research into the effects of a hyposaline state on the pheromonal secretions of eggs and chemotaxis by sperm is recommended.

N. luetkeana produced approximately equal numbers of sporophytes per female at all salinities above 13 ppt, and these sporophytes did not vary significantly in size between salinities. On the other hand, *A. marginata* once again exhibited a degree of population-level variation. Females from Juneau produced approximately equal numbers of sporophytes at all salinities above 13 ppt, and these sporophytes were of approximately equal size across salinities. However, females from Kodiak produced more sporophytes at 25 ppt and 32 ppt, and these sporophytes were also larger than those at 20 ppt. Once again, it is likely that sporophytes from Juneau populations are adapted to relatively lower salinities, and are able to persist and grow at 20 ppt as well as at 25 ppt and 32 ppt, whereas sporophytes from Kodiak populations have a narrower range of tolerance.

In this study, we utilized fixed levels of salinity as stressor treatments. This is not necessarily representative of the natural environment, where salinity can fluctuate at a much finer temporal scale due to stochastic events. Conducting a study of this nature *in-situ* would be challenging

due to the microscopic nature of the life-stages under consideration. However, we would recommend that future studies consider including an element of stochastic variation in their stressor conditions. Additionally, it would be very interesting to consider the impacts of magnitude and duration of stress events on kelp early life-stages. For example, how do gametophytes respond to an acute, severe stress event as opposed to a chronic stress conditions?

There is a general lack of information regarding the responses of high-latitude kelp species to major environmental and climate-related stressors. The results presented here show that the responses to salinity of gametophytes and sporophytes in *N. luetkeana* (a subtidal canopy-former) and *A. marginata* (an intertidal subcanopy species) seem to be determined largely by environmental adaptation. *A. marginata* showed significant adaptation to hyposaline conditions and population-level variation in response to salinity at all the life-stages considered here. *N. luetkeana* was particularly sensitive to hyposaline conditions and may be induced to produce juvenile sporophytes earlier at lower salinities.

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6 References

Arimitsu, M. L., Platt, J. F. and Mueter, F. (2016). Influence of glacier runoff on ecosystem structure in Gulf of Alaska fjords. *Marine Ecology Progress Series, 560,* pp. 19 – 40.

Bennett, E., Paine, E.R., Hovenden, M., Smith, G., Fitzgibbon Q. and Hurd, C.L. (2024). Short-term hyposalinity stress increases dissolved organic carbon (DOC) release by the macroalga *Sargassum fallax* (Ochrophyta). *Journal of Phycology, 60*(5), pp. 1210 – 1219.

Bliss, A., Hock, R. and Radić, V. (2014). Global response of glacier runoff to twenty-first century climate change. *JGR Earth Surface, 119*(4), pp. 717 – 730.

Brown, N. J., Nilsson, J. and Pemberton, P. (2019). Arctic ocean freshwater dynamics: transient response to increasing river runoff and precipitation. *JGR Oceans, 124*(7), pp 5205 – 5219.

Buschmann, A. H., Vásquez, J. A., Osorio, P., Reyes, E., Filún, L., … and Vega, A. (2004). The effect of water movement, temperature and salinity on abundance and reproductive patterns of *Macrocystis* spp. (Phaeophyta) at different latitudes in Chile. *Marine Biology, 145,* pp. 849 – 862.

Carney, L. T., Waaland, J. R., Klinger, T. and Ewing, K. (2005). Restoration of the bull kelp *Nereocystis luetkeana* in nearshore rocky habitats. *Marine Ecology Progress Series, 302*, pp. 49 – 61.

Chen, J., Ji, D., Xu, Y., Chen, C., Wang, W., … and Xu, K. (2023). Effect of hyposaline stress on the release of dissolved organic carbon from five common macroalgal species. *Frontiers in Marine Science, 9,* DOI: https://doi.org/10.3389/fmars.2022.1106703.

Dethier, M. N., Williams, S. L. and Freeman, A. (2005). Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. *Ecological Monographs, 75*(3), pp. 403 – 418.

Farrugia Drakard, V., Hollarsmith, J. A. and Stekoll, M. S. (2023). High-latitude kelps and future oceans: a review of multiple stressor impacts in a changing world. *Ecology and Evolution, 13*(7), e10277.

Gierke, L., Coelho, N.C., Khangaonkar, T., Mumford, T. and Alberto, F. (2023). Range wide genetic differentiation in the bull kelp *Nereocystis luetkeana* with a seascape genetic

focus on the Salish Sea. *Frontiers in Marine Science*, DOI: https://doi.org/10.3389/fmars.2023.1275905

Grant, W.S. and Chenoweth, E. (2021). Phylogeography of sugar kelp: northern ice-age refugia in the Gulf of Alaska. *Ecology and Evolution*, *11*, pp. 4670 – 4687.

Grant, W.S., Lydon, A. and Bringloe, T.T. (2020). Phylogeography of split kelp *Hedophyllum nigripes*: northern ice-age refugia and trans-Arctic dispersal. *Polar Biology*, *43*, pp. 1829 – 1841.

Hobday, A.J., Alexander, L.V., Perkins, S.E., Smale, D.A., Straub, S.C., … and Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography*, *141*, pp. 227 – 238.

Karsten, U. (2007). Research note: Salinity tolerance of Arctic kelps from Spitsbergen. *Phycological Research*, 55(4), 257–262. https://doi.org/10.1111/j.1440-1835.2007.00468.x

Kim, J. K., Stekoll, M. S. and Yarish, C. (2019). Opportunities, challenges and future directions of open water seaweed aquaculture in the United States. *Phycologia, 58*, pp. 446-461.

Leathers, T., King, N. G., Foggo, A. and Smale, D. A. (2023). Marine heatwave duration and intensity interact to reduce physiological tipping points of kelp species with contrasting thermal affinities. *Annals of Botany*, *133*(1), pp. 51 – 60.

Lee, T. (1998). Investigations of some intertidal green macroalgae to hyposaline stress: Detrimental role of putrescine under extreme hyposaline conditions. *Plant Science*, *138*(1), pp. 1 – 8.

Li, H., Monteiro, C., Heinrich, S., Bartsch, I., Valentin, K., … and Bischof, K. (2020). Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiologia Plantarum*, 168(1), 5–26. https://doi.org/10.1111/ppl.13009

Lind, A. C. and Konar, B. (2017). Effects of abiotic stressors on kelp early life-history stages. *Algae*, 32(3), 223–233. https://doi.org/10.4490/algae.2017.32.8.7

Maier, I., Hertweck, C. and Boland, W. (2001). Stereochemical specificity of lamoxirene, the sperm-releasing pheromone in kelp (Laminariales, Phaeophyceae). *The Biological Bulletin*, *201*(2), pp. 121 – 125.

Mao, X., Augyte, S., Huang, M., Hare, M.P., Bailey, D., … and Jannink, J-L. (2020). Population genetics of sugar kelp throughout the Northeastern United States using genome-wide markers. *Frontiers in Marine Science,* DOI: https://doi.org/10.3389/fmars.2020.00694

Marambio, J., Rosenfeld, S. and Bischof, K. (2022). Hyposalinity affects diurnal photoacclimation patterns in the rhodophyte *Palmaria palmata* under mimicked Arctic summer conditions. *Journal of Photochemistry and Photobiology*, *11,* DOI: https://doi.org/10.1016/j.jpap.2022.100124.

McConnico, L. A. and Foster, M. S. (2005). Population biology of the intertidal kelp, *Alaria marginata* Postels and Ruprech: a non-fugitive annual. *Journal of Experimental Marine Biology and Ecology*, *324*(1), pp. 61 – 75.

Monteiro, C., Li, H., Bischof, K., Bartsch, I., Valentin, K. U., … and Heinrich, S. (2019). Is geographical variation driving the transcriptomic responses to multiple stressors in the kelp *Saccharina latissima*? *BMC Plant Biology*, *19*(1), pp. 1–15. https://doi.org/10.1186/s12870-019-2124-0

Monteiro, C., Li, H., Diehl, N., Collén, J., Heinrich, S., … and Bartsch, I. (2021). Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycological Research*, 69(1), 48–57. https://doi.org/10.1111/pre.12443

Muth, A. F., Bonsell, C. and Dunton, K. H. (2021). Inherent tolerance of extreme seasonal variability in light and salinity in an Arctic endemic kelp (*Laminaria solidungula*). *Journal of Phycology*, 57(5), 1554–1562. https://doi.org/10.1111/jpy.13187

Provasoli, L. (1968). Media and prospects for the cultivation of marine algae. Paper presented at the Proceedings of the US-Japan Conference, Hakone, 12 – 15 September 1968, 63 – 75.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schoenrock, K.M., Bacquet, M., Pearce, D., Rea, B. R., Schofield, J.E., … and Kamenos, N. (2018). Influences of salinity on the physiology and distribution of the Arctic coralline algae, *Lithothamnion glaciale* (Corallinales, Rhodophyta).

549 Siddiqui, S.A., Agrawal, S., Brahmbhatt, H. and Rathore, M.S. (2022). Metabolite expression
550 changes in *Kappaphycus alvarezii* (a red alga) under hypo- and hyper-saline conditions.
551 *Algal Research, 63,* DOI: https://doi.org/10.1016/j.algal.2022.102650.

552 Siegela, D. I. (1988). The recharge-discharge function of wetlands near Juneau, Alaska: Part I.
553 Hydrogeological Investigations. *Groundwater, 26,* pp. 427 – 434.

554

555 Smith, K. E., Aubin, M., Burrows, M. T., Filbee-Dexter, K., Hobday, A. J., … and Smale, D.
556 A. (2024). Global impacts of marine heatwaves on coastal foundation species. *Nature*
557 *Communications, 15,* pp. 5052.

558 Springer, Y. P., Hays, C. G., Carr, M. H., and Mackey, M. R. (2010). Toward ecosystem-based
559 management of marine macroalgae—The bull kelp, *Nereocystis luetkeana*.
560 *Oceanography and Marine Biology: An Annual Review*, 48, 1–41.
561 https://doi.org/10.1201/EBK1439821169

562 Spurkland, T. and Iken, K. (2011). Salinity and irradiance effects on growth and maximum
563 photosynthetic quantum yield in subarctic *Saccharina latissima* (Laminariales,
564 Laminariaceae). *Botanica Marina*, 54(4), 355–365.
565 https://doi.org/10.1515/BOT.2011.042

566 Stekoll, M. S., Deysher, L. E., and Hess, M. (2006). A remote sensing approach to estimating
567 harvestable kelp biomass. *Journal of Applied Phycology*, 18(3–5), 323–334.
568 https://doi.org/10.1007/s10811-006-9029-7

569 Stekoll, M. S. (2019). The seaweed resources of Alaska. *Botanica Marina, 62*(3), pp. 227 –
570 235.

571 Stekoll, M. S., Pryor, A., Meyer, A., Kite-Powell, H. L., Bailey, D., … and Yarish, C. (2024).
572 Optimizing seaweed biomass production – a two kelp solution. *Journal of Applied*
573 *Phycology*, DOI: 10.1007/s10811-024-03296-w

574 Steneck, R.S., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., … and Tegner, M.J.
575 (2003). Kelp forest ecosystems: biodiversity, stability, resilience and future.
576 *Environmental Conservation, 29*(4), pp. 436 – 459.

577 Strasser, F., Barreto, L. M., Kaidi, S., Sabour, B., Serrao, E., … and Martins, N. (2022).
578 Population level variation in reproductive development and output in the golden kelp

Laminaria ochroleuca under marine heat wave scenarios. *Frontiers in Marine Science, 9,* DOI: https://doi.org/10.3389/fmars.2022.943511

Supratya, V. P. and Martone, P. T. (2023). Kelps on demand: Closed-system protocols for culturing large bull kelp sporophytes for research and restoration. *Journal of Phycology,* 60, 73 – 82.

Tatewaki, M. (1966). Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. *Phycologia*, *6*, pp 62 – 66.

Wang, W., Chen, T., Xu, T., Xu, K., Xu, Y., … and Xie, C. (2020). Investigating the mechanisms underlying the hyposaline tolerance of intertidal seaweed, *Pyropia haitanensis*. *Algal Research, 47,* DOI: https://doi.org/10.1016/j.algal.2020.101886.

Weigel, B. L., Small, S. L., Berry, H. D. and Dethier, M. N. (2023). Effects of temperature and nutrients on microscopic stages of the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *Journal of Phycology, 59*(5), pp. 893 – 907.

Wernberg, T., Thomsen, M. S., Baum, J. K., Bishop, M. J., Bruno, J. F., … and Vanderklift, M. A. (2024). Impacts of climate change on marine foundation species. *Annual Review of Marine Science, 16,* pp. 247 – 282.

Ziemen, F. A., Hock, R., Aschwanden, A., Khroulev, C., Kienholz, C., … and Zhang, J. (2016). Modeling the evolution of the Juneau Icefield between 1971 and 2100 using the Parallel Ice Sheet Model (PISM). *Journal of Glaciology, 62(231),* pp. 199 – 214.