Testing the impacts of a marine heatwave and freshening event on populations of *Z. marina* from the Swedish west coast

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Abstract

Marine heatwaves are increasing in their frequency, intensity, and duration. In the study area, the Skagerrak, it is common for marine heatwaves to co-occur with another stressor, freshening events, referring to a rapid decrease in salinity. Several factors may contribute to a species or population's ability to withstand climatic stressors, such as favourable adaptations, previous exposure to a stressor, or greater genetic diversity. Marine heatwaves have already been linked to devastating losses of marine biodiversity, and freshening can be harmful to organisms, for example by destabilizing the ion equilibrium within the organism. Therefore, it is worth investigating whether previous exposure and genetic diversity may help in increasing a species' resilience and resistance to the combination of these two co-occurring stressors.

A mesocosm experiment, simulating a marine heatwave and a freshening event, was conducted to determine the responses of the foundation species *Zostera marina* (eelgrass). For this, *Zostera marina* plants were sampled from ten different locations in the Kosterhavet archipelago and brought to the Tjärnö Marine Laboratory, located on the west coast of Sweden. It was assumed that these locations had experienced different degrees of variability in both temperature and salinity over the past, and thus, depending on their origin and potential differences in genetics or adaptations, genotypes tested from these locations might respond more robustly to the applied treatment. The measured response variables after the ten-day-long experimental period were growth, number of new leaves, and mortality of *Z. marina*.

Results from the experiment showed a reduction in the growth of leaves between the treatments, but no differences in the number of leaves were found. When testing the combined response of the

Climate change treatment and past exposure (population), no significance was found for any of the measured responses. This could indicate that *Z. marina* is plastic in its response and that its original location, and the conditions the individuals experienced there, do not matter. As no genetic tests were conducted, it remains unclear whether the individuals used in the experiment were distinct populations or if they belonged to the same tolerant population, potentially explaining the lack of effect from exposure history.

This thesis found reduced growth in *Z. marina* individuals exposed to a marine heatwave and a co-occurring freshening event, but whether this effect was due to one or both stressors remains unanswered. Further studies should be conducted on this highly important foundation species to disentangle what stressor or combination of stressors will come to affect *Z. marina* in the future.

Contents

1. Introduction

1.1. Global ocean change, marine heatwaves, and freshening events

As abiotic baselines shift, marine heatwaves will become more likely to occur with progressing climate change (Hobday et al. 2016; Oliver et al. 2018). According to Hobday et al. (2016), a marine heatwave can be defined as a prolonged discrete and anomalously warm-water event, which can be described by its duration, intensity, and spatial extent. More specifically, water temperatures must exceed a threshold value, the 90th percentile, for five or more consecutive days compared to a 30-year historical baseline period (Hobday et al. 2016). Applying this definition, there has been an increase in the frequency (34%) and duration (17%) of marine heatwave events globally between 1925 and 2016 (Oliver et al. 2018). It is important to understand how extreme climatic events of different intensities affect communities or species' performance since marine heatwaves are already known to have affected the structures and functions of marine ecosystems (Wernberg et al. 2016; Pansch et al. 2018).

In nature, stressful events do not occur in isolation. At any given time, there is a combination of stressors co-occurring at varying temporal and spatial scales and ecological levels (Jackson et al. 2021). Many studies, manipulating a single driver, have been conducted to determine the tolerance and sensitivity of a particular organism or community (e.g., Stillman 2003; Hoffman and Todgham 2010), and these studies can be useful to help predict future distributions of species (Somero 2012). These are crucial beneficial assets for a future understanding of the stressors as a combined effect (Boyd et al. 2018) and can provide information about whether the drivers have an additive, antagonistic or synergistic impact (Gunderson et al. 2016).

The timing of different drivers needs to be considered, and whether the stressors are overlapping or sequential (Jackson et al. 2021). Al-Janabi et al. (2019) showed that ocean warming, and ocean acidification reduced the impact of each of the two drivers. They showed that bladderwrack (*Fucus vesiculosus*) performance is positively correlated to ocean warming, and ocean acidification, as well as the combination of both drivers, and nutrient enrichment. Instead, it leaves them vulnerable to hypoxic events. Their argument for positive correlation comes from Sunday et al. (2014), where a positive correlation of

two or more drivers could enhance a positive selection that is robust to change. Negative correlation instead limits adaptive evolution (Etterson et al. 2001). One fitness trait can come at another cost of another evolutionary trade-off (Stearns 1989).

Abrupt fluctuations in salinity, so-called freshening events (Morón 2018), may mostly occur in estuaries (Van Diggelen and Montagna 2016) and in semi-enclosed seas, such as the Baltic Sea, where net precipitation is generally increasing (Gröger et al. 2019). For example, in the Skagerrak, a transition zone between the North Sea and the Baltic Sea, increases or decreases in salinity can be extensive over relatively short temporal scales (Danielssen 1997; Dahl et al. 2005, Morón 2018), due to the water exchange between two water bodies that differ in salinity, i.e., bottom and surface-, or coastal and off-shore waters or over regional areas, for instance, due to periods of heavy river run-off. These freshening events may co-occur with marine heatwaves (Figure 1).
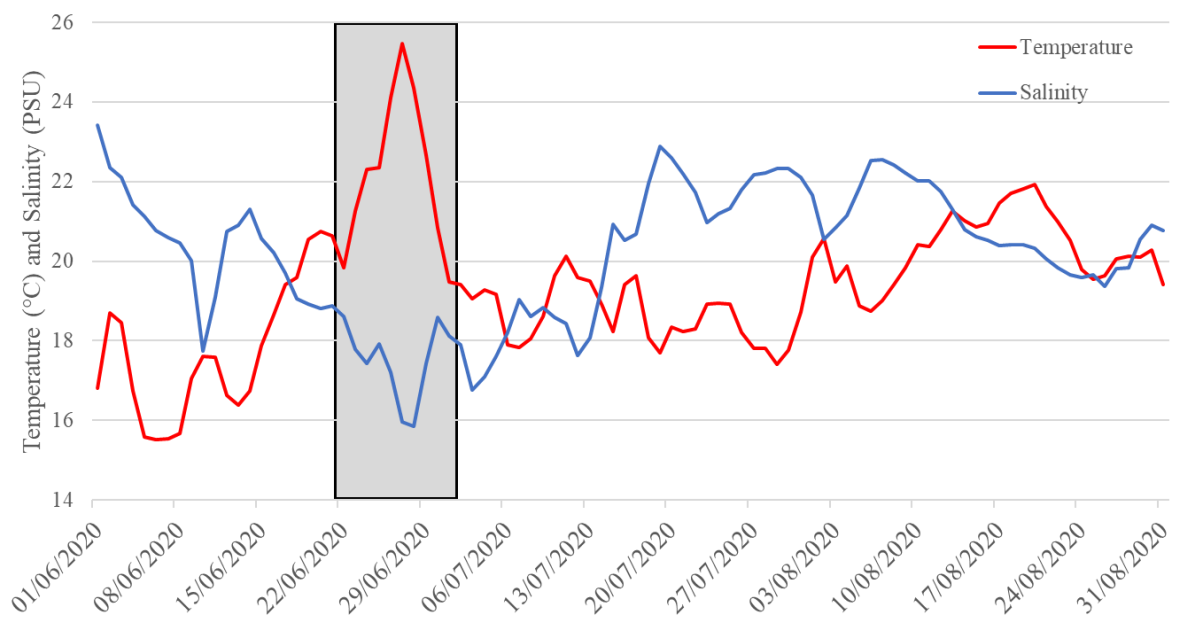
Figure 1. Sea surface temperature and salinity measurements were taken in Tjärnö Bay between the 1st of June and the 31st of August 2020. At the end of June, a marine heatwave coincided with a drop in salinity. The data were collected by the Tjärnö Marine Laboratory.

As marine heatwaves and freshening events will likely co-occur more frequently in the Kosterhavet archipelago (Morón 2018), the combined effects they have on important species and communities should be investigated, as these co-occurring stressors are likely

to become more and more common as global climate change progresses (Pachauri et al. 2014).

1.2. Genotypic diversity

While species biodiversity is widely discussed as a strength in withstanding climate perturbances (Worm and Lotze 2016), less is known about the role of intraspecific diversity, i.e., the most fundamental level of biodiversity. Genotypic diversity can be defined as the clonal diversity in plants (Solé et al. 2004). Historically, the genotype, i.e., the organism's genetic makeup (Lewontin 1970; Reusch and Hughes 2006), was believed to be responsible for generating the majority of differences in the organism's phenotype, which is the observable expression of the genotype (Churchill et al. 1974). The expressions for different phenotypes can be observed through biological appearance, chemical, structural, and behavioral attributes (Churchill et al. 1974). Environmental factors are important in determining the phenotype of an organism and the stress that the environmental factors are mediating. Therefore, individuals in different environments may express differences in their observable traits, i.e., their phenotype (Barboza et al. 2019; Nussinov et al. 2019). Phenotypic diversity is important on the individual, population, species, and community level. For example, a specific phenotype might be more tolerant to a particular driver or disturbance. More tolerant phenotypes can under more frequent stressor events lead to a local adaptation. Pansch et al. (2014) demonstrated the differences between two populations of barnacles (*Balanus (Amphibalanus) improvisus*) regarding the effects of ocean acidification and food availability. The study tested the responses of populations from the Kiel Fjord, Germany, where the variability of pCO$_2$ is higher compared to a population normally experiencing lower environmental (pH and pCO$_2$) variability (Tjärnö, Sweden). The Kiel population performed better facing higher pCO$_2$ levels compared to the Tjärnö population. The study, thus, demonstrates differences between populations that are subjected to different environments.

Understanding intraspecific diversity is highly important when trying to unravel the causes and consequences of disturbances. The potential for populations or an ecosystem's resilience (how fast variables return towards their equilibrium following a perturbation) and resistance (the degree to which a variable is changed, following a perturbation) is of key significance in this context (Pimm 1984). Communities and species have evolved to

be able to survive under certain environmental conditions, but when the environmental conditions start to change, genetic diversity can provide the resilience and resistance for those communities and species, and the functions that they provide will not be lost. Resilience and resistance to environmental change, fluctuations, and their extremes can be provided through genetic diversity within a population (Reusch et al. 2005).

1.3. Climate change impacts coastal ecosystems

Climate change is affecting the structure and functioning of ecosystems at multiple levels of complexity (Gitay et al. 2002; Schroter et al. 2005). Ecosystems of high species diversity are generally more resilient to and better at maintaining ecosystem productivity from stressors exerted by climate change due to higher functional redundancy (García et al. 2018), but the genetic diversity within species may also play a role (Reusch et al. 2005). Seasonal temperature variations that species in temperate regions have adapted to in the long term are mostly predictable, even if these fluctuations may be strong (Bonsdorff et al. 2003). Meanwhile, extreme temperature and freshening events can be unpredictable and possibly outside a species' temperature, or salinity, tolerance and may thus seriously affect an organism's overall performance (Roth et al. 2010; Winters et al. 2011).

Coastal marine environments are among the most exposed to fluctuations (Pansch & Hiebenthal 2019) and may be most vulnerable to increased disturbances (Ehlers et al. 2008). Their resilience and buffering capacity towards environmental stressors have been compromised by the many human activities, such as eutrophication, habitat destruction, over-exploitation, and pollution (Holling 1973; Hughes et al. 2003). Macrophyte-dominated coastal ecosystems are particularly vulnerable to warming (Ehlers et al. 2008) as these ecosystems often lack species redundancy (Micheli and Halpern 2005), as the macrophytes are often the foundation species. Thus, a loss of a certain macrophyte species may affect the entire community (Saha et al. 2019). Yet, in a species-poor coastal community, genotypic diversity can be an important aspect to help communities to recover from extreme climatic events (Reusch et al. 2005).

1.4. Study area and species

1.4.1 The Skagerrak

The Skagerrak is the transitional zone between the Baltic Sea and the North Sea and as such, it is heavily influenced by North Sea - Baltic Sea exchange of water masses (Danielssen et al. 1997). The upper water column is influenced by the outflow of brackish water from the Baltic Sea and local rivers (Gustafsson, 2000), while the lower water column consists of highly saline waters (31–34 PSU) entering the Skagerrak from the North Sea. Projected freshening of the Skagerrak and Kattegat is mainly due to a climate change-driven increase in the outflow of brackish water from the Baltic Sea. Climate change increases precipitation in the Baltic Sea area (HELCOM 2013; Rousi et al. 2013) and is likely to increase the freshwater input to the Baltic Proper (Gröger et al. 2019; Kniebusch et al. 2019; Meier et al. 2021). In addition, the Baltic Sea is one of the hot spots regarding seawater warming (Belkin 2009; Reusch et al. 2018). This increases the stratification of the water column making it less likely to mix with colder, more saline water masses. In addition, the annual cumulative intensity of marine heatwaves has increased by 340%, while the number of marine heatwaves per year has risen from 1.4 to 3.1 between 1989 and 2018 in southern Norway, along the border of the Skagerrak (Filbee-Dexter et al. 2020). An initial analysis has been conducted in Tjärnö on the long-term temperature and salinity data by Morón (2018), which concluded that the number of days and duration of marine heatwaves has increased. For freshening events, there has been a decrease in duration, but an increase in intensity between 1980 and 2016.

1.4.2. Study species *Zostera marina*

This study was conducted with a macrophyte, eelgrass (*Zostera marina,* Linnaeus*)*. This seagrass species inhabits shallow sedimentary shorelines in the northern hemisphere (Green and Short 2003), throughout the Atlantic and the Pacific Ocean, and even in the Arctic circle (den Hartog 1970). As an ecosystem engineer, *Z. marina* provides ecosystem services such as habitat, nutrient cycling, primary production, sediment stabilization, food (Jones et al. 1994, Cole and Moksnes 2016), as well as carbon sequestration (Boström et al. 2014). Since the 1980s, over 60% of *Z. marina* has been lost on the Swedish west coast (Jahnke et al. 2020), likely due to an increase in epiphytic algae caused by reduced light availability and anoxia (Moksnes et al. 2008; Baden et al. 2010). Pihl et al. (2006)

demonstrated that the loss of *Z. marina* on the west coast of Sweden led to a significant reduction in diversity, biomass, and density of fish.

1.4.3. *Zostera marina* – variability in temperature and salinity

In the Southern Baltic Sea, the growth of *Z. marina* ceases at 20 ºC (Rasmussen 1973) and an increase in mortality can be seen at 25 ºC (Greve et al. 2003). However, in the North-East Atlantic along the coasts of Portugal, *Z. marina* is reported to survive water temperatures of up to 27 ºC during the summer season (Cabaço and Santos 2010). *Zostera marina* can be found in the brackish waters of the Baltic Sea in salinities of 5, but also in more saline waters of up to 35 along the Skagerrak and the Atlantic coasts of Norway (Boström et al. 2014). Salinity also plays a role in how well *Z. marina* grows. Populations in the Baltic Sea are constantly stressed by low salinity as seagrasses have to compensate for the loss of ions (Touchette 2007). Due to the low salinity, Salo et al. 2014, demonstrated that *Z. marina* populations from the northern Baltic Sea can cope better when placed in a higher salinity treatment, than populations from the southern Baltic placed in a low salinity treatment. Differences in *Z. marina* populations can be seen between the Baltic Sea and the Skagerrak in the form of clonal and sexual reproduction (Reusch and Boström 2011) as well as in the amount of the stable isotope δ^{34}S, which indicates a lower sulphide intrusion in the plants, determining the health of the plant (Holmer et al. 2009).

There are some knowledge gaps regarding how populations of *Z. marina* in the Skagerrak tolerate a combination of heat and salinity stress. There is already some genetic knowledge regarding connectivity through genetic and biophysical modeling (Jahnke et al. 2018), but there may be local genetic adaptations present depending on how exposed a meadow is to salinity and temperature variability. There are a few studies on how *Z. marina* in this relatively varying environment copes with such abiotic conditions. This is crucial to know, as we are most probably heading towards a more uncertain future with a potentially more varying climate (Bindoff et al. 2019).

1.5. Research questions and hypotheses

RQ1: Do donor sites for seagrass differ in mean sea surface temperature and temperature variability?

> H1: Sheltered sites have a higher mean sea surface temperature and express higher diurnal variability during summer months compared to exposed sites.

RQ2: Does a combined marine heatwave and freshening treatment affect seagrass traits?

> H2: There is a negative impact of the experimental marine heatwave and freshening event on *Z. marina* traits, leading to increased mortality, a reduced number of leaves, and reduced growth of leaves.

RQ3: Do populations of seagrass from different sites differ in their response to marine heatwaves and freshening events?

> H3: *Zostera marina* populations from sites that display greater variability in temperature, and presumably salinity (sheltered sites), will be more tolerant to the applied marine heatwave and freshening event than *Z. marina* populations from sites that vary less in temperature, and presumably salinity (exposed sites).

2.   **Materials and Methods**

The experimental work for this study was conducted in outdoor mesocosms at the Tjärnö Marine Laboratory, Sweden (58.876043º N, 11.146222 °E, Figure 2) between the 16th and the 29th of July, 2021.

2.1. **Sampling of *Zostera marina***

The sampling of *Z. marina* was conducted between the 28th of June and the 2nd of July, 2021, by scuba diving and snorkeling at ten separate meadows in the Kosterhavet archipelago (Table 1, Figure 2). *Zostera marina* was collected from a depth of 1–3 meters. The individuals were collected by first removing the sediment from around the rhizomes and exposing the root system, whereafter two nodes containing one shoot each were removed from the rest of the plant and, as such, the two nodes with the shoots were clonal. The individual plants were then subsequently placed in water-filled zip lock bags underwater and once on the boat stored in a cooling box to avoid damage and stress during the transport to Tjärnö Marine Laboratory. In the laboratory, the plants were placed in 60 L containers provided with a flow-through of surface seawater from the Tjärnö bay to acclimatize for seven days.

Table 1. Sites where eelgrass (*Zostera marina*) was sampled in the Kosterhavet archipelago, Skagerrak, Sweden, and the respective sampling dates. Loggers were placed at these sites above the bottom and the periods of placement are indicated below. For later analysis, the date period was set from the 5th to the 27th of July. The site Ramnekroken was removed from further analysis as the logger had been buried throughout the summer season of 202 and the data were representative.

Sampling site	Latitude (°N)	Longitude (°E)	*Z. marina* sampling date	Max sampling depth m	Logging period
Gåsholmen	58.878628	11.134688	28/06/2021	1.5	01/07/2021 - 01/08/2021
Ramnekroken	58.898535	11.143091	28/06/2021	2.2	03/07/2021 - 02/08/2021
Nycklebykilen	58.888803	11.166456	29/06/2021	2.0	01/07/2021 - 02/08/2021
Tångeholmen	58.896587	11.186828	29/06/2021	1.5	03/07/2021 - 02/08/2021
Tjärnöbo	58.873716	11.191207	29/06/2021	1.5	02/07/2021 - 02/08/2021
Inre Vattenholmen	58.877199	11.115649	30/06/2021	2	30/06/2021 - 02/08/2021
Kockholmen	58.834280	11.140891	30/06/2021	2.2	30/06/2021 - 02/08/2021
Koster	58.882538	10.991989	03/07/2021	1.5	03/07/2021 - 02/08/2021
Flatskär	58.856835	11.140161	01/07/2021	2.4	30/06/2021 - 02/08/2021
Styrsö	58.905591	11.105685	02/07/2021	2.6	01/07/2021 - 02/08/2021

Sediment was also collected from all ten sites (Table 1) and was brought back to Tjärnö Marine Laboratory where all sediment from the different sites was mixed to homogenize

it. After this, the sediment was sieved through a 2 mm sieve to remove larger particles, plant pieces, and other organisms.
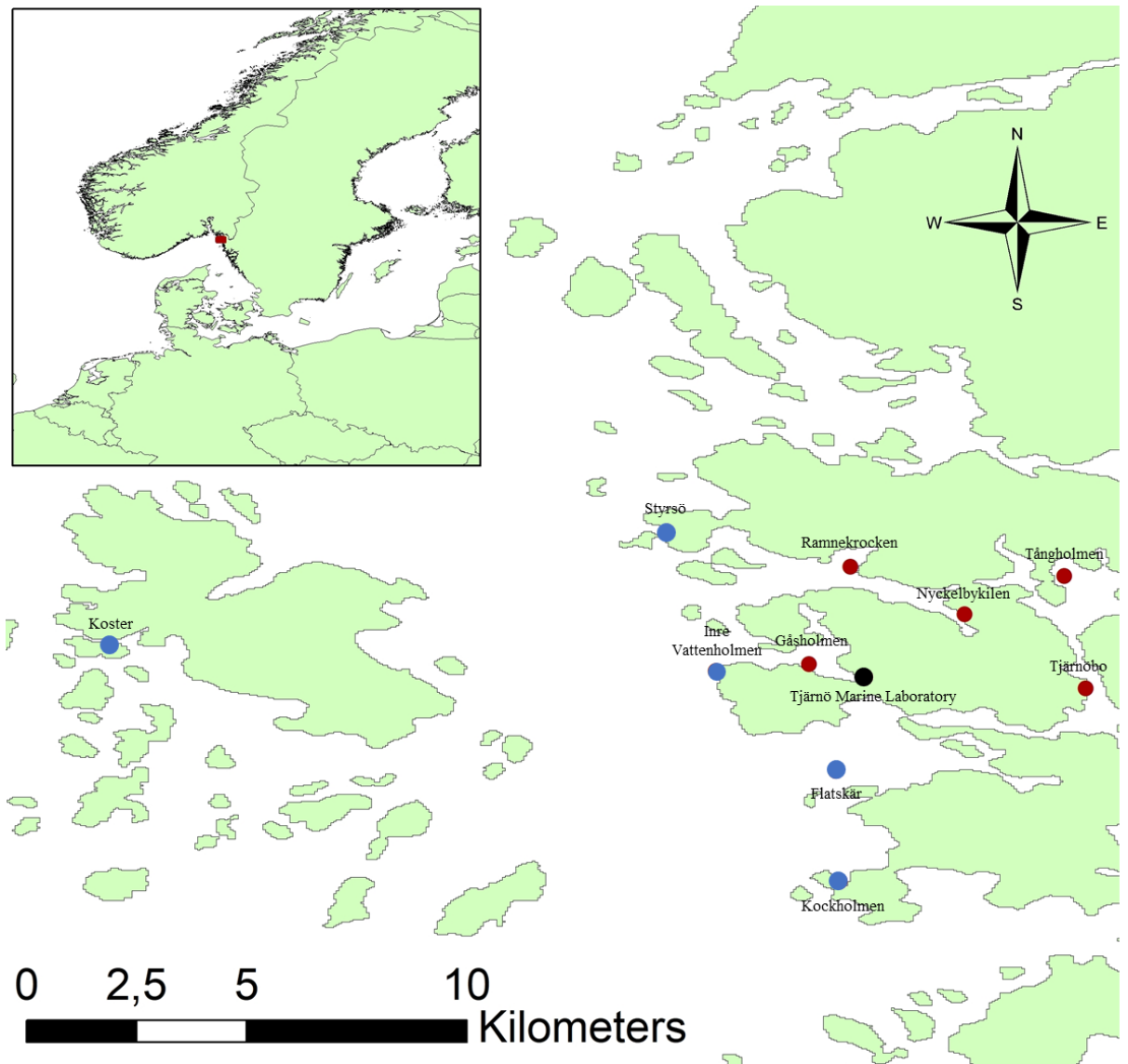

Figure 2. Map showing the location of the ten sampled eelgrass (*Zostera marina*) meadows in the Kosterhavet archipelago. Exposed sites are displayed as blue, while sheltered areas are displayed as red dots. The location of the Tjärnö Marine Laboratory, where the experimental part was conducted, is displayed as a black dot. The map was produced in ArcMap 10.8 GIS.

2.1.1. Environmental variables at donor sites

Sea surface temperature measurements were conducted continuously at the ten donor sites during the experimental period (Table 1, Figure 3) using HOBO loggers (ONSET, USA). This was done to enable comparison between the locations and to establish a baseline on the variability in the different sampling sites throughout the summer season of 2021

(Figure 3). The data collected by the logger placed at Ramnekroken were excluded, due to the logger being buried in the sediment and therefore, not representing relevant environmental seawater conditions. No data are available for salinity variability at the sites during this period (Figure 1).
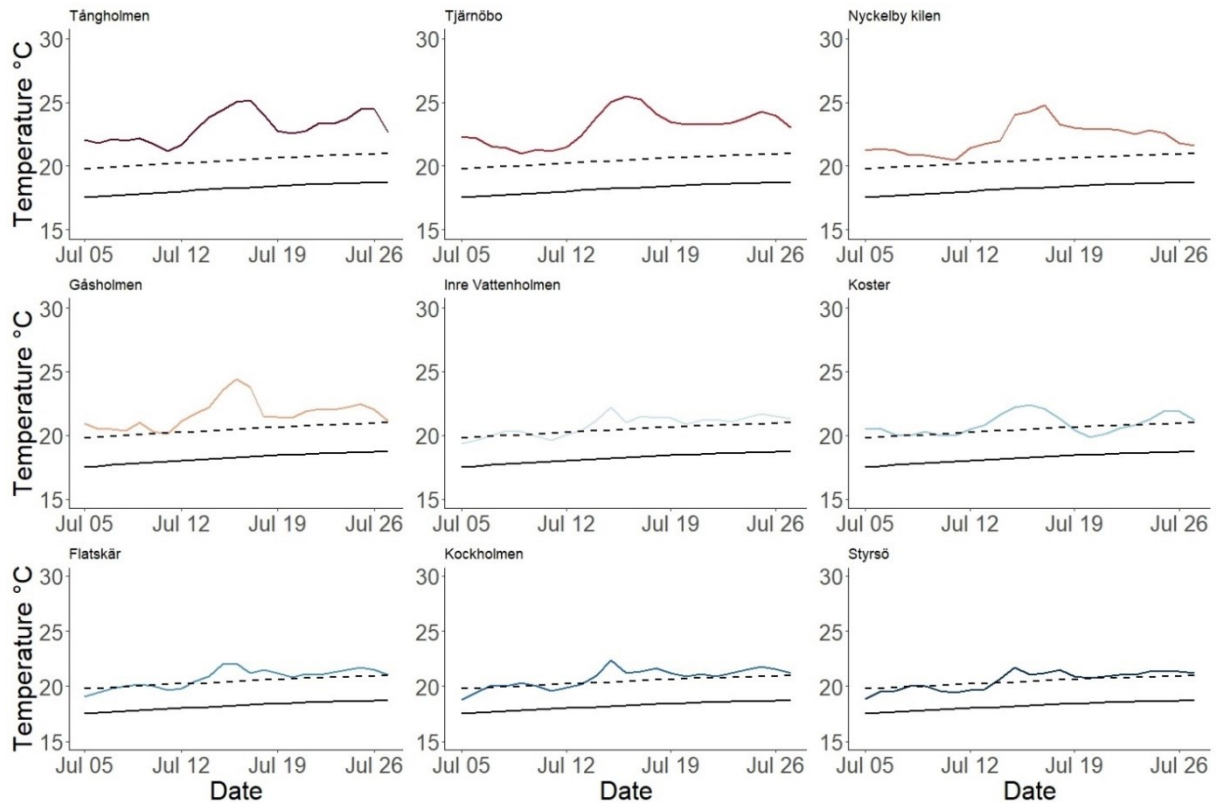

Figure 3. Sea surface temperature (daily means) for the different sampling sites between the 5th and the 27th of July, 2021. The colored lines represent daily mean values for the period of investigation in 2021. The solid black lines represent the climatology, and the dashed lines represent the 90th percentile values, both of which are based on daily temperature data collected by the Tjärnö Marine Laboratory between 1980 and 2014. Values for the climatology and the 90th percentile were identified by Morón (2018) using the "heatwaveR" package (Schlegel and Smith 2018) in R (R Core Team 2021).

The temperature data were further evaluated and plotted according to the maximum, minimum, median, and standard deviation in temperature recorded at the respective sites (Figure 4) over the period from the 5th to the 29th of July. From this evaluation, it was already evident that the sites Tångholmen, Gåsholmen, Nyckeby Kilen, and Tjärnöbo experienced considerably higher (> 3 °C) maximum temperatures during the experimental period (Figure 4A), as well as warmer minimum temperatures, except Gåsholmen (Figure 4B), when compared to the remaining stations (Koster, Inre vattenholmen, Flatskär, Styrsö, and Kockholmen). The median temperature recorded at Tjärnöbo and

Tångholmen is also considerably warmer than for the remaining seven stations (Figure 4C), while the standard deviation in temperature follows the same pattern as for the maximum temperature, with Tjärnöbo, Tångholmen, Gåsholmen, and Nyckelby Kilen showing greater deviations in temperature (Figure 4D).
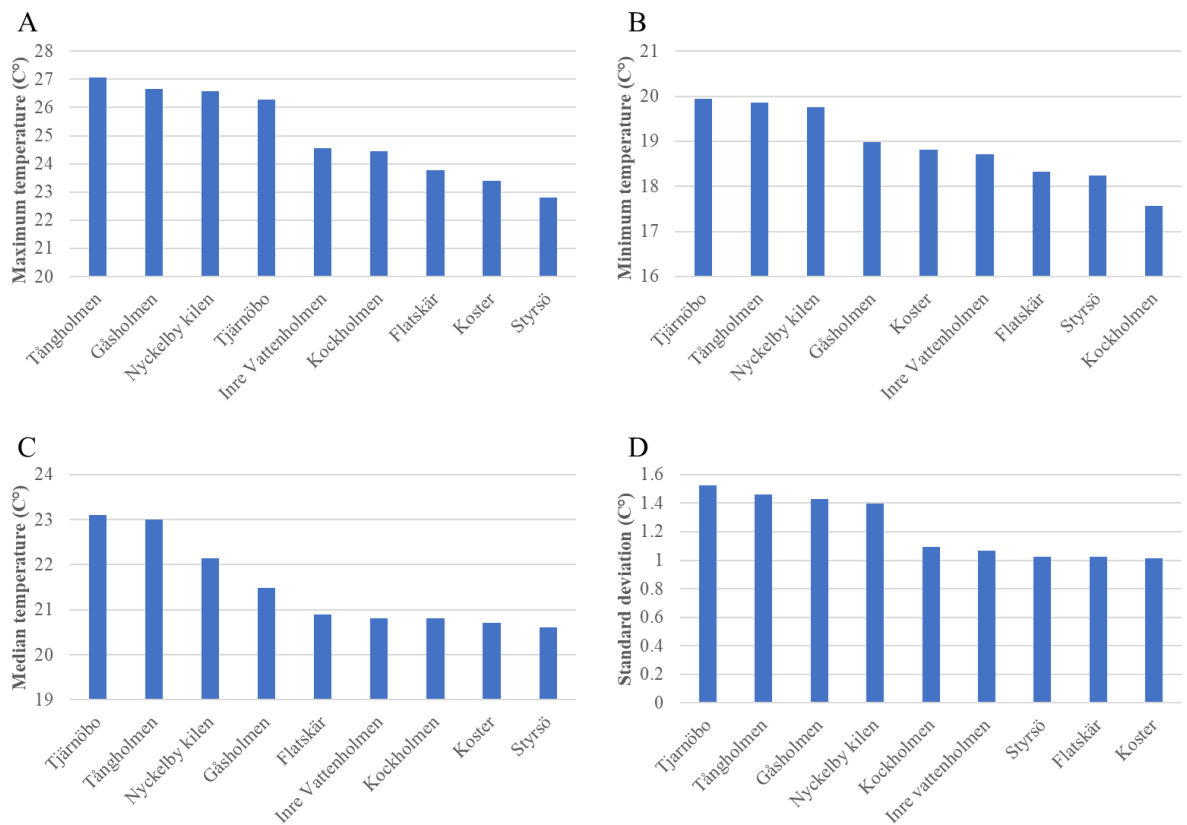

Figure 4. **The temperature attributes** (A) maximum temperature, (B) minimum temperature, (C) median temperature, and (D) standard deviation in temperature at the nine population donor sites, ordered from highest to lowest for each trait. The site Ramnekroken was removed from further analysis as the logger had been buried throughout the summer season of 2021 and the data were representative. The data displayed in the graphs are based on daily means during the experimental period.

The difference observed between the nine sites was further confirmed by plotting the temperatures recorded at the sites as a density plot (Figure 5), which shows the frequency and range of temperatures of the loggers. Figure 5 shows that there is a clear division between sheltered sites, which have a higher mean temperature but also exhibit a lower frequency of these temperatures. The more exposed sites experienced lower temperatures, but the frequency of those temperatures is much higher indicating a more stable temperature variability.
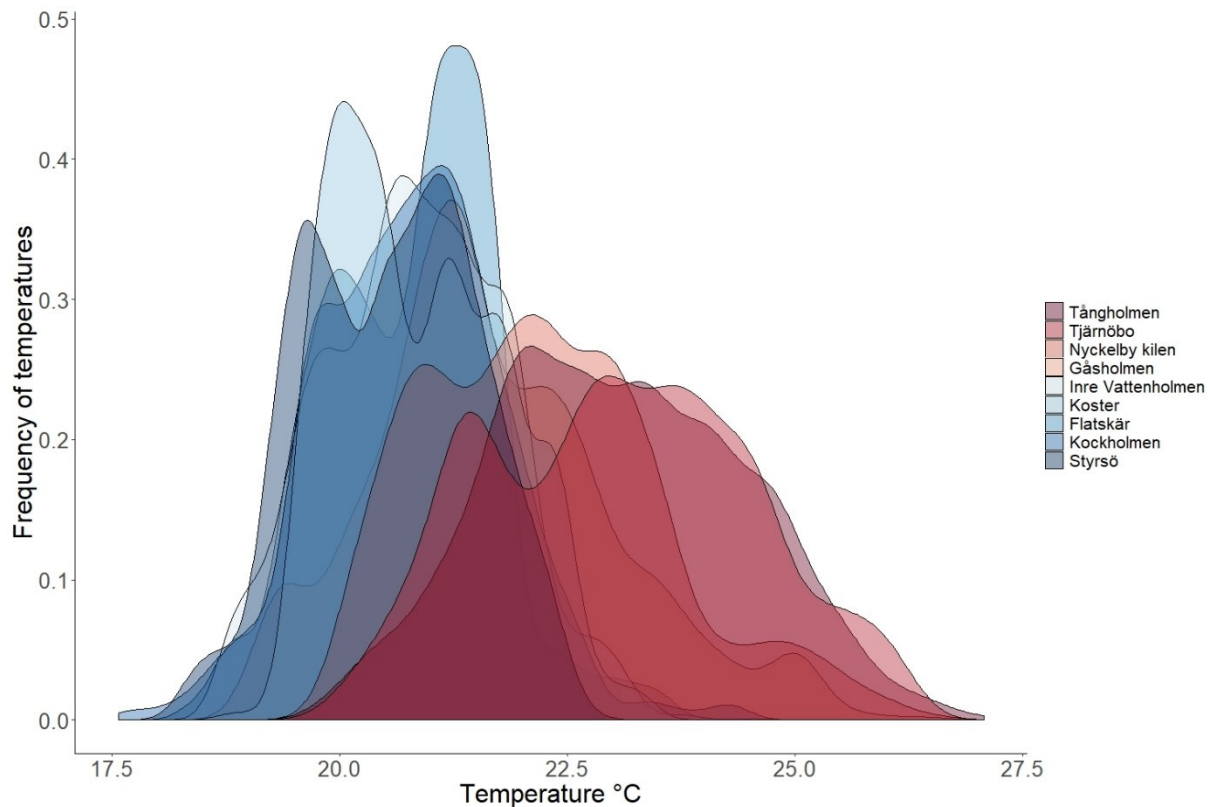
Figure 5. Density plot for temperatures at the different donor sites, illustrating the frequency of measured temperatures. Exposed sites are displayed in blue, while the sheltered sites are displayed in red. The plot is based on high-resolution temperature measurements from the nine donor sites (15-minute intervals) between the 5th and the 27th of July, 2021.

The daily mean temperature at the different sites (Figure 6) also displays the same patterns as seen in Figures 4 and 5. Using this information, the nine sites were divided into two groups: Exposed sites: Inre Vattenholmen, Koster, Flatskär, Kockholmen, and Styrsö, and sheltered sites: Tångholmen, Tjärnöbo, Nyckelby kilen, and Gåsholmen (already presented in blue and red colors respectively in the plots above and below).

Figure 6. Daily mean temperature values at the different sites including climatology and 90th percentile values between the 5th and the 27th of July, 2021. Climatology and 90th percentile based on climatological data from Tjärnö Marine Laboratory, identified by Morón 2018.
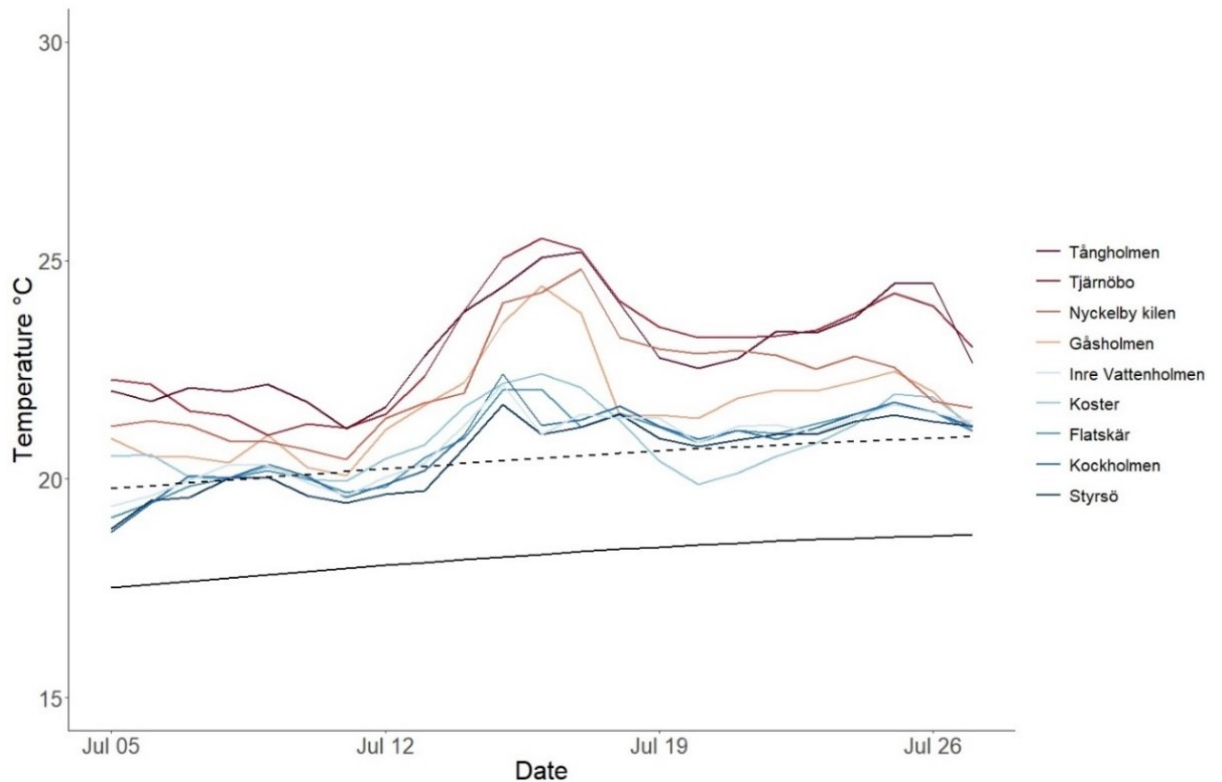
2.2. The Baltotron tank system

The mesocosm system used in the experiment consists of five Baltotron compartments (Figure 7), each consisting of two large open 800 L tanks (250x104x31 cm). In between these tanks, there is a section containing two draining openings, one to recirculate the water and the other to remove water from the system. One tank in each Baltotron compartment functioned as the Ambient treatment, while the other tank functioned as the climate change treatment. Thus, there were two treatments, distributed over ten water baths (Figure 7). Within these ten water baths, a total of 100 four-liter plastic bags (experimental units) were distributed, with each bag containing one shoot from one out of the ten populations of seagrass (Figure 7). The bags were exposed to natural sunlight throughout the experimental period, but due to the harshness of the sunlight, the mesocosm system was covered with a layer of thin shade cloth.

The Ambient treatment received sea surface water from Tjärnö Bay from a depth of 1 meter. This water was directly pumped into a mixing tub in each Baltotron compartment, then into a header tank, which distributed the water into the experimental units from which the water was allowed to overflow into the thermally controlled water baths below. The water that overflowed from the experimental units was never used within the experimental units again but served in the water baths only for maintaining treatment temperatures.
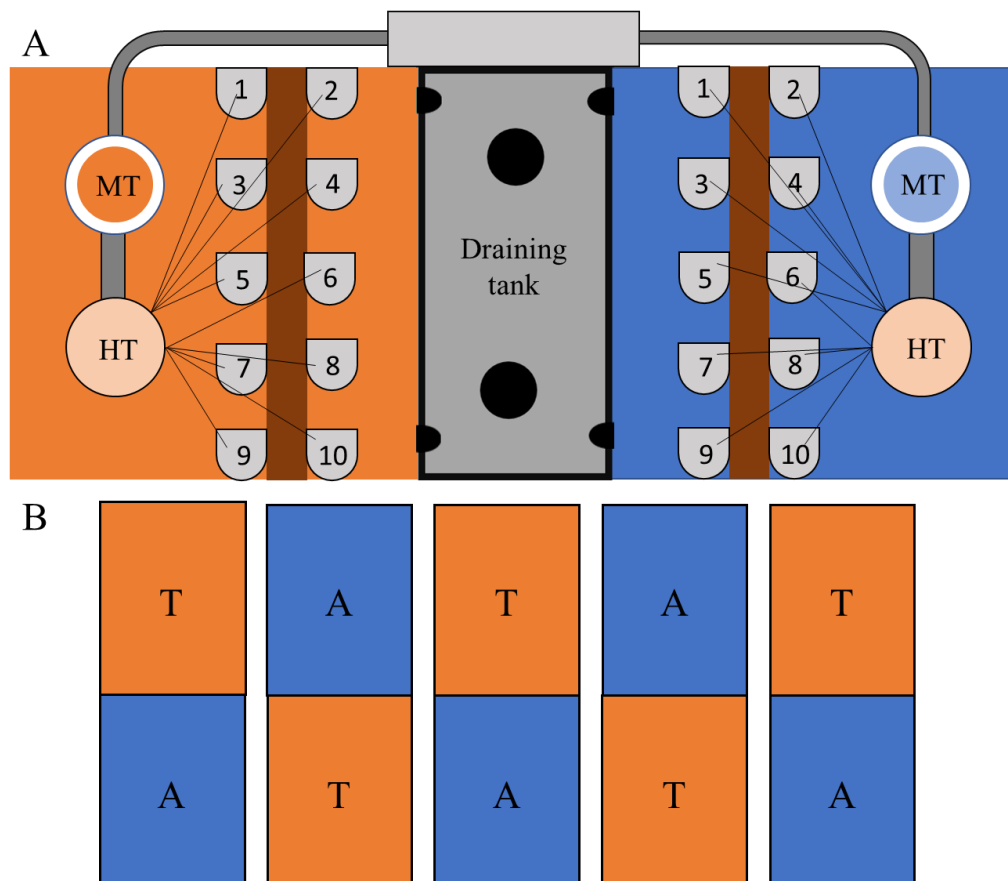
Figure 7. Schematic illustration of the Baltotron system used for the experiment. (A) Detailed illustration of the setup in a one Baltotron compartment. The orange color indicates the Climate change treatment whereas the blue color indicates the Ambient treatment. The mixing tub (MT), header tank (HT), and the experimental units (1–10) are also shown. (B) Shows the placement of the five Baltotron compartments and the Ambient (A) and Climate change treatment (T) in relation to one another during the experiment, leaving 5 independent replicates per treatment.

The water that flowed into the climate change treatment was pumped in from Tjärnö Bay into a mixing tub, where the desired treatment temperature and salinity were implemented continuously (adjusted every 24 hours) using two 600-watt heaters (Schego, Germany) and by pumping fresh water into the mixing tub. To help implement the treatment

temperature and conductivity sondes attached to GHL Profilux 4 computers (Germany) were placed into the mixing tub and programmed to the daily temperature and salinity values by either increasing or decreasing the temperature and salinity in the mixing tub. The water from the mixing tub was pumped (Eheim model 1262, Germany) into a header tank from where the water was distributed into the experimental units and overflowed into the water bath. From here, the water entered the middle section from where it drained into the bay. The water bath around the climate change experimental units received water from the recirculation system, which passed through a heater (Cygnet pool heater), steered by a GHL computer, a pump (Eheim model 1262, Germany), and back into the water bath where it is then drained by the other opening and exited into the sea. Throughout the experimental period, the water flow was kept independent both between the treatments and the experimental units.

The temperature and salinity in both treatments were adjusted daily, based on the water temperature and salinity in Tjärnö Bay, keeping most natural environmental variability. At 09:00 in the morning, the treatments were set to the daily temperature and salinity regimes by checking the temperature and salinity from one of the Ambient treatments and applying the salinity and temperature offsets to all Climate change treatments. The values were changed in the GHL computer that controlled the Climate change treatment.

This experiment was conducted simultaneously with another larger experiment, and this thesis is a side project of that larger experiment. To clarify, both experiments used the same simulated marine heatwave and freshening event treatment, as well as the same ambient conditions. The experimental units in the different experiments received water from the same sources and were kept independent throughout the experimental period.

2.2.1. Experimental units

The experimental units consisted of 100 four-liter plastic bags that were hung from a wooden plank placed horizontally across the water baths. Each water bath had ten plastic bags hung above it, each containing one *Z. marina* individual from one of the sites. The openings of the bags remained above the surface of the water bath. The bags received water from the header tanks through hoses with an inside diameter of four mm. The ends of the hoses reached halfway inside the plastic bags, and to avoid resuspension of sediment inside the bags, the ends were looped upwards (Figure 8). The bags were filled

with 750 mL of sediment two days before the experiment started, to let the sediment settle. Each experimental unit received a clone from one of the 10 populations, where one shoot of each clone was placed in the Ambient treatment, whereas the second shoot of the same clone was placed in the Climate change treatment.

Figure 8. A picture showing the experimental units receiving water from a header tank that was allowed to spill over into the water bath. One bag contained a singular *Zostera marina* plant that was planted in 750 mL of homogenized sediment.

2.3. Experimental treatments – temperature and salinity

The experiment consisted of two treatments with differing temperatures and salinity, each applied in the five independent Baltotron tanks. The "Ambient" treatment served as a

control and implemented the same temperature and salinity regime that occurred in Tjärnö Bay during the experimental period (Figure 9). The second treatment, "Climate change" implemented higher temperatures and lower salinities during the experimental period. More specifically, an offset of +4.7 ºC from the ambient Tjärnö Bay conditions was added to the Climate change treatment. Thus, the temperature in this treatment crosses the local 90th threshold (Morón, 2018) and the experimental period can be classified as a marine heatwave. Additionally, to simulate a freshening event in the Climate change treatment, a -5.5 decrease in salinity compared to the Ambient treatment was implemented (Figure 10). The offsets in temperature and salinity implemented in the Climate change treatment were based on modeling adjusted after Morón (2018), where the treatments were based on deviations in water temperatures that exceed the 90th percentile, and salinity at the 30th percentile. During the experimental period, the Ambient treatment also crossed the 90th percentile threshold which can be seen in Figure 3, Gåsholmen.
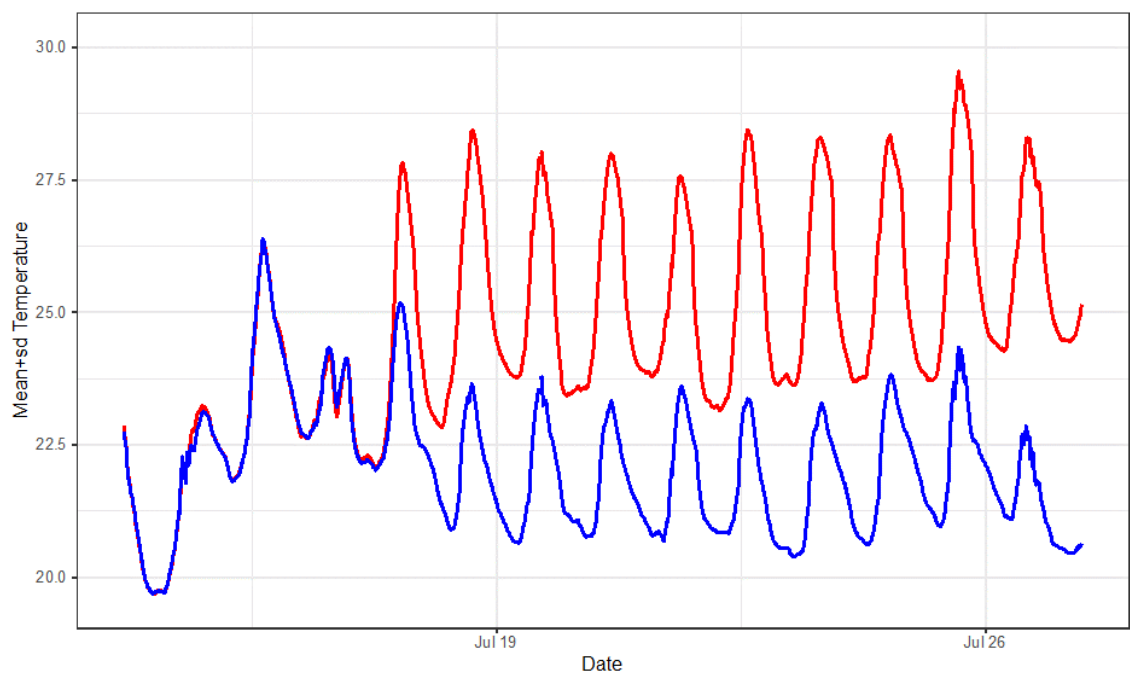
Figure 9. Ambient and Climate change realized diurnal temperatures in all the five independent Baltotron systems. In total, 23 HOBO loggers, logging temperatures every 10 minutes, were placed in the systems to measure temperature throughout the experimental period. The red color indicates the temperature measured in the Climate Change treatment while the blue color indicates the Ambient treatment temperatures. Figure provided by M. Jahnke.
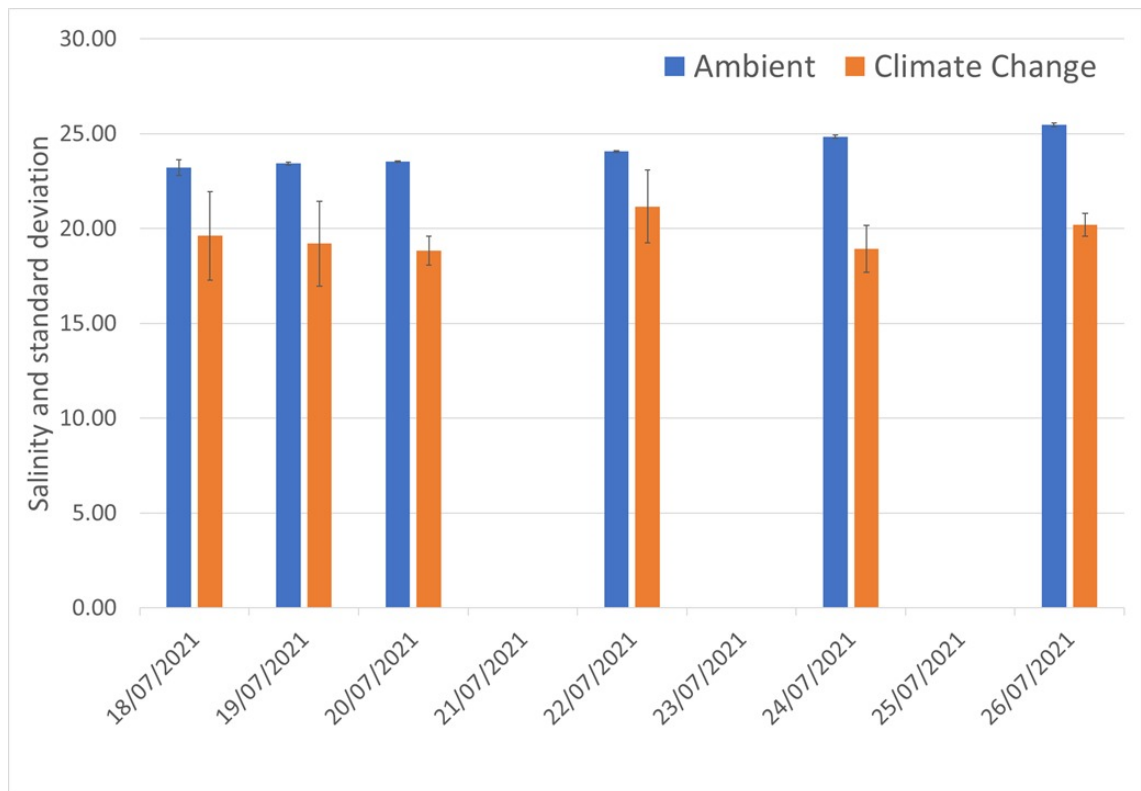
Figure 10. Salinity measurements were taken from the cylinders that functioned as experimental units in the larger experiment that ran in parallel. The project applied the same temperature and salinity treatments. During the experimental period, salinity and temperature measurements were taken every other day, apart from the first three days when the parameters were measured daily.

2.3.1. Maintenance of temperature and salinity in the Baltotron system

The experiment was maintained for 10 days, from the 19th to the 29th of July, 2021. In addition to the 23 HOBO loggers that measured the temperature inside the water baths, temperature and salinity were measured manually every other day at 13:00 by using a temperature and salinity sonde (WTW, Multi 3630 IDS) connected with a temperature and salinity probe (TetraCon 925). This was done to ensure that the daily temperature and salinity implementations were effective inside the mixing tanks. There was an offset of -0.7 in salinity and a -0.3 in °C in the measurements.

2.4. *Zostera marina* response variables

Before *Z. marina* was placed in their experimental units, wet weights (Mettler PC 8800; 0.1 g) of the individual plants were noted (Figure 11), and the number of leaves was counted (Figure 12). Each *Z. marina* individual was photographed (NIKON 1 AW1)

before placement into the experimental units. From these pictures, the initial number of leaves was determined.
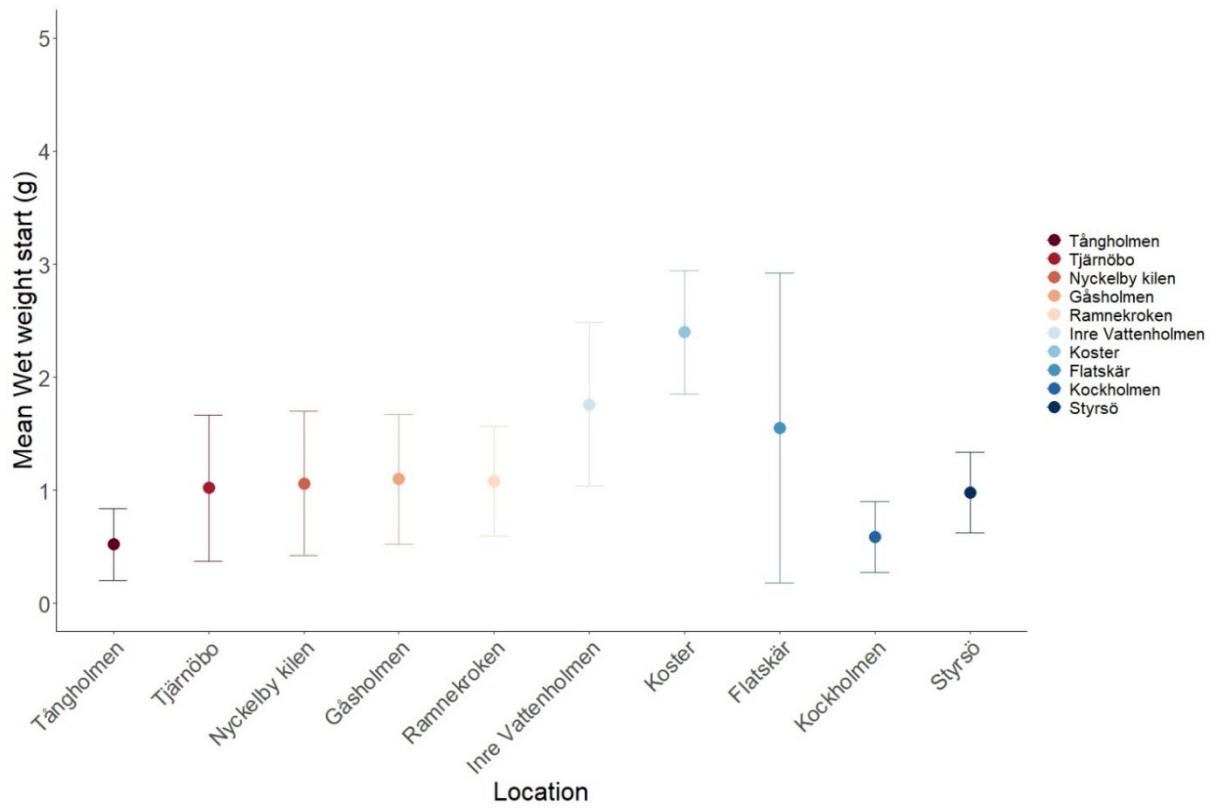

Figure 11. Wet weights per *Zostera marina* population and treatment before the experiment in grams. Weights include the entire plants: leaves, roots, and rhizomes. Presented are medians (n = 10) plus and minus standard deviation (SD).
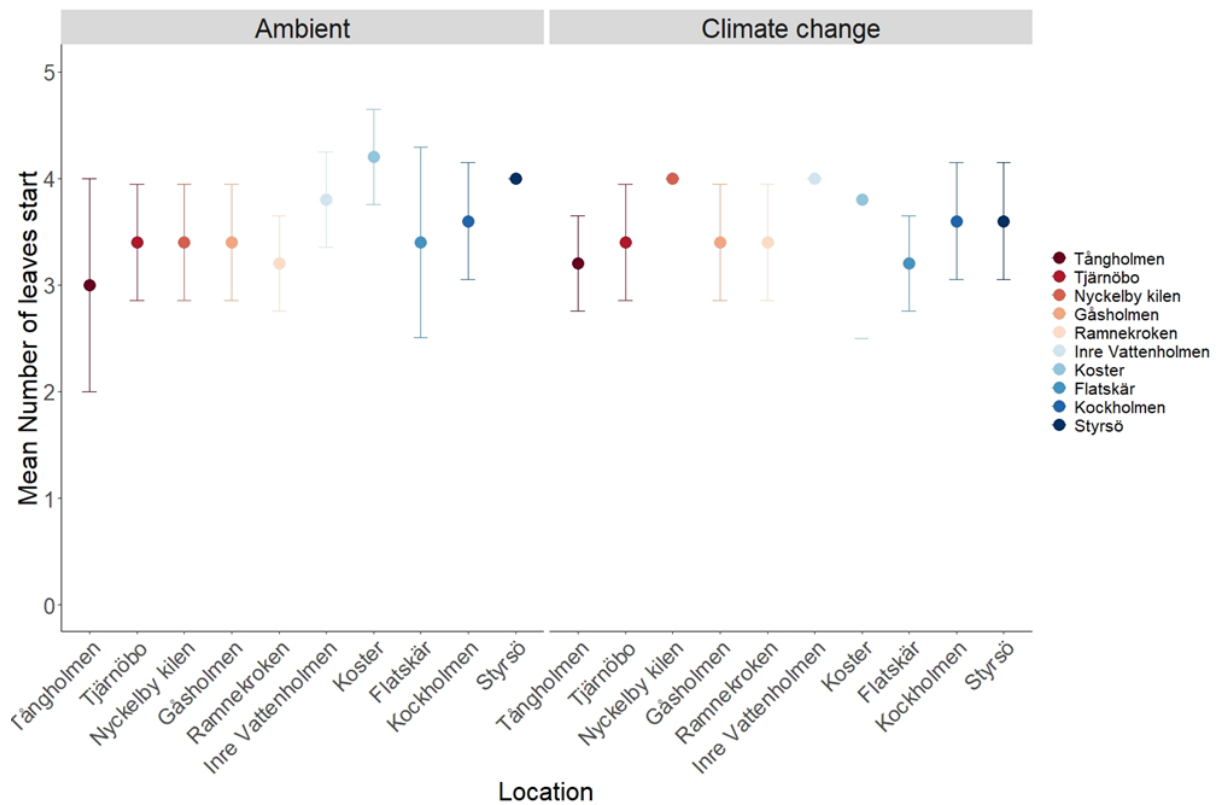
Figure 12. The number of leaves per *Zostera marina* population and treatment was counted before the experiment. Presented are medians (n = 5) plus and minus standard deviation (SD).

After the experimental period of ten days, the individual *Z. marina* plants were separated into roots, which included the rhizomes and roots, and leaves for weighing (Metteler PC 8800; 0.1g) and photographing (NIKON 1 AW1). The number of leaves (Figure A2) and shoots (Figure A3) on each individual plant was documented. The roots and leaves were put into aluminum foil forms and dried for 48 hours at 60 ºC. After drying, the roots and leaves were vacuum sealed. The roots and leaves were then weighed on a high precision scale (Sartorius BP221 S; 0.0001g) and the dry weights were documented (Figure A4). To minimize the absorption of moisture from the air, the roots, and leaves during weighing, the aluminum foils containing the leaves and roots were placed into a desiccator before the measurements.

Pictures of *Z. marina* taken after the experiment were analyzed using the program ImageJ (version 1.53n) to measure the growth of the individual leaf blades of the plants. The growth was measured by piercing the plant with a hypodermic needle 1 cm above the leaf sheath at the start of the experiment. The growth of individual leaves was then measured from the hole down to the leaf tip along the central leaf nerve after the experiment. To get

the plastochrone value the growth is divided by the number of experiment days (Short and Duarte 2002). Dead individuals and individuals whose leaves had not been pierced were removed from further analysis, as their growth could not be determined.

For final analyses in this thesis, only the mortality of plants, the number of leaves (across all shots) and the leaf growth in cm were used. Leaf growth was calculated as summed growth of all pierced leaves per individual. If an individual had piercing marks on all four leaves, all those four leaves were measured, after which all of the measurements were added up to total growth of the leaves.

2.5. Data analyses and statistical procedures

2.5.1. Environmental temperature

The HOBO loggers at each site conducted temperature measurements at 15-minute intervals (Figure A1). Averages of the temperatures from the 5th to the 27th of July were taken from the high-resolution dataset to a single average monthly temperature reading from each location, representing the summer season. A daily average temperature for each location was also determined. The diurnal variability values were determined by first identifying the daily maximum and minimum temperature values for each day and location, and then subtracting the minimum temperature value from the maximum value. In addition, an average diurnal variability value for each location was determined. Finally, the sites were divided into Exposed and Sheltered by looking at different attributes (see Figures 4, 5, and 6). These attributes were: Maximum and minimum overall temperature, highest and lowest recorded temperature, standard deviation within the sites, and diurnal variability (see Table A1).

Before running any of the parametric analyses, the normality of residuals was verified using a Shapiro-Wilk test of normality, and by using graphical tools (qqplots and histograms) in R-studio, and homogeneity of variance was verified using Levene's Test for Homogeneity of Variance. After the homogeneity of variance was confirmed, the statistical tests were used to identify significant differences. A Welch two-sample T-test was performed to compare the average temperature and diurnal variability between sheltered (n = 4) and Exposed (n = 5) sites.

2.5.2. *Zostera marina* **traits**

The experiment finally consisted of a factorial design of two factors with two levels each (Ambient vs. Climate change treatment and Sheltered vs. Exposed). A two-factorial ANOVA was applied to determine differences in how the Ambient and Climate change treatments had affected the total growth of the leaves in relation to the two exposure classifications (Sheltered vs. Exposed).

Data for the number of leaves did not meet the assumption for normal distribution required for a two-factorial ANOVA but followed a Poisson distribution. Thus, a Generalized Linear Model (GLM) was applied to identify differences in the number of leaves between the Ambient (n = 45) and Climate change (n = 39) treatments and between the different exposure classifications, Sheltered (n = 4) and Exposed (n = 5). All data were analyzed using R-studio v.1.4.1 (R Core Team) using the packages Lme4, Car, ggplot2, ggpubr, dplyr, tidyverse, lubridate, and RColorBrewer.

3. Results

3.1. Comparison of temperature data at the donor sites

There was a significant difference between the Exposed (mean = 20.78, df = 7) and Sheltered (mean = 22.51, df = 7) sites concerning average temperature over the 10 days of investigations (Welch two-sample T-test: t = -5.094, p < 0.01), with Exposed sites exhibiting a lower median and variability when compared to Sheltered sites (Figure 13A).
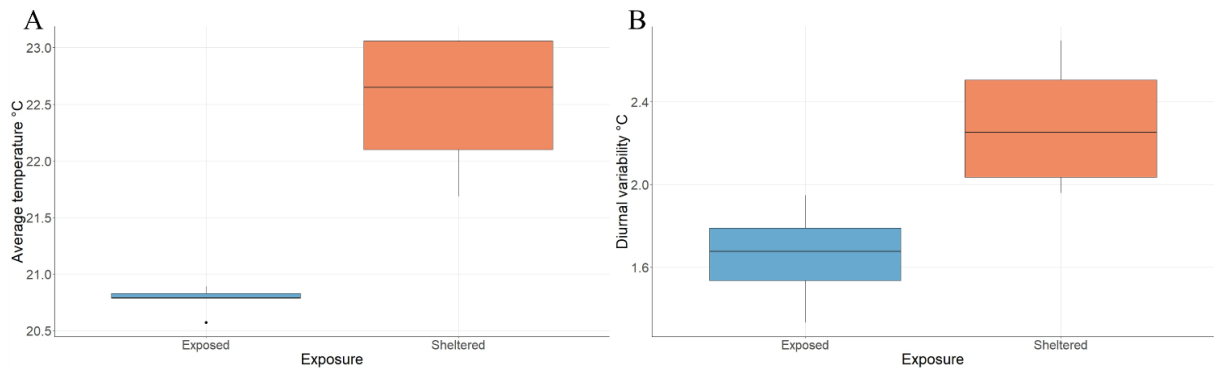

Figure 13. (A) Boxplot showing the average temperature in the Exposed compared to Sheltered sites. (B) Boxplot showing the difference between the average Exposed sites and the Sheltered sites median diurnal variability. Represented are the median as well as the upper and lower quartile ranges, as well as outliers.

A Welch two-sample T-test was also performed to compare the diurnal variability between Sheltered (n = 4) and Exposed (n = 5) sites. There was a significant difference between the Exposed (M = 1.654, df = 7) and Sheltered (M = 2.288, df = 7); t = -3.304, p < 0.01 sites (Figure 13B).

3.2. Effects of the Climate change treatment on *Zostera marina*

3.2.1. Mortality

During the experimental period, a total of eleven individuals died, out of which seven individuals (14%) originated from the Climate change treatment, while the remaining four originated from the Ambient treatment (8%). Only the populations originating from Koster and Ramnekroken showed no mortality while all other populations had either one or two dead individuals at the end of the experiment. Individuals from Exposed areas had lower mortality of 8% in the Ambient treatment (vs 8% for sheltered sites) and 12% in the Climate change treatment (vs 16% for sheltered sites).

3.2.2. Treatments effects on the growth of leaves

A one-way ANOVA was performed to compare the Ambient (n = 45) and Climate change (n = 39) treatments for the total growth of leaves of *Z. marina*, that is to say the summed value (in centimeters) of all pierced leaves per individual (see section 2.4.). The test showed that there was a statistically significant difference between the Ambient and the Climate change treatment on the growth of leaves $p < 0.01$ (Table 2, Figure 14).
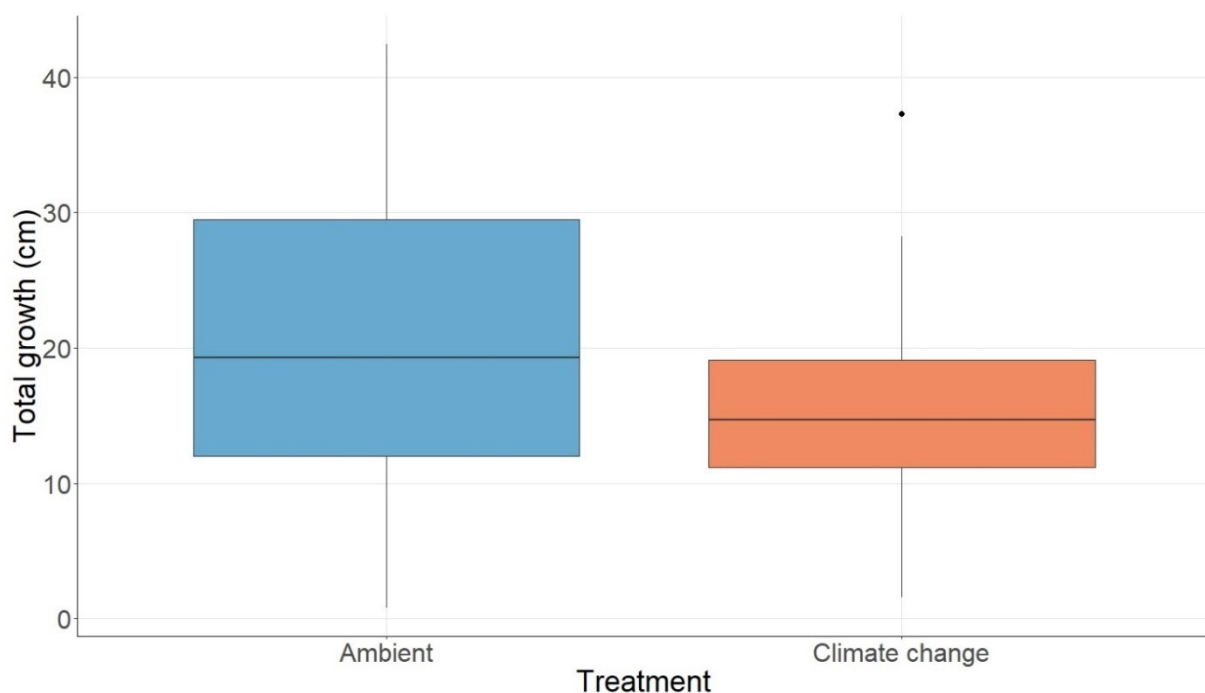
Figure 14. Boxplot showing the difference in the leaf growth of *Zostera marina* between the Ambient and Climate change treatments. Represented are the median as well as the upper and lower quartile ranges, as well as outliers.

Table 2. Output from the one-way ANOVA comparing the growth of leaves of Zostera marina at the end of the experiment between the Ambient and Climate change treatments.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	673.8	673.77	7.803	**0.0065**
Residuals	82	7080.5	86.35		

3.2.3. Treatment effects on the difference in the number of leaves

A one-way ANOVA was performed to compare the Ambient (n = 45) and Climate change (n = 39) treatments for the number of leaves at the end of the experiment. The test showed no significant difference between the two treatments (Table 3, Figure 15).

Table 3. Output from the one-way ANOVA on differences in the number of leaves.

	df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	1.70263	1.70263	2.8716	0.09395
Residuals	82	48.619	0.59291		

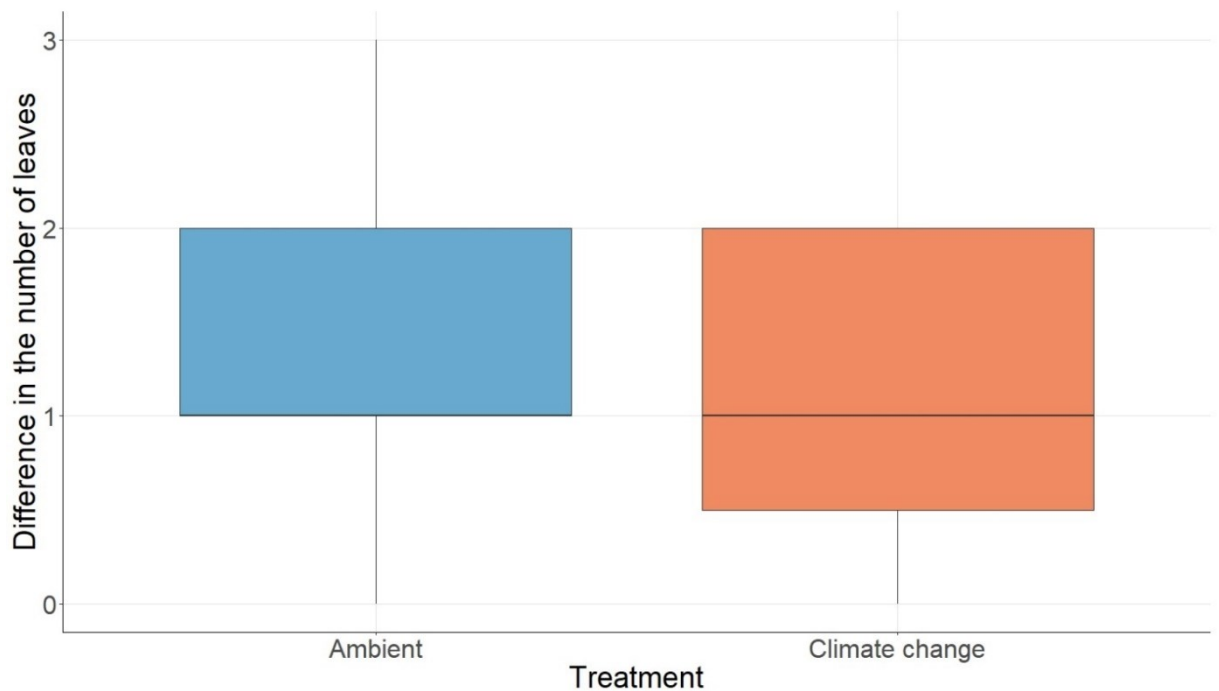**Figure 15. Boxplot showing the difference in the number of leaves** between the two treatments, Ambient and Climate change. Represented are the median as well as the upper and lower quartile ranges. The one-way ANOVA found no significant differences between the treatments.

3.3. Combined impact of the Climate change treatment and exposure history on *Zostera marina*

3.3.1. Treatment and exposure history effects on growth of leaves

A two-way factorial ANOVA was performed to analyze the direct effects of the simulated climate change treatment and the exposure classification (Ambient x Exposed (n = 23), Climate change x Exposed (n = 21), Ambient x Sheltered (n = 22), and Climate change x Sheltered (n = 18)) on the total growth of the leaves. The two-way ANOVA showed that there was a statistically significant effect of the Climate change treatment on leaf growth ($p < 0.01$), but no significant effect of exposure was found ($p = 0.6$; Table 4, Figure 16). The interaction of the two factors was statistically insignificant ($p = 0.6$).
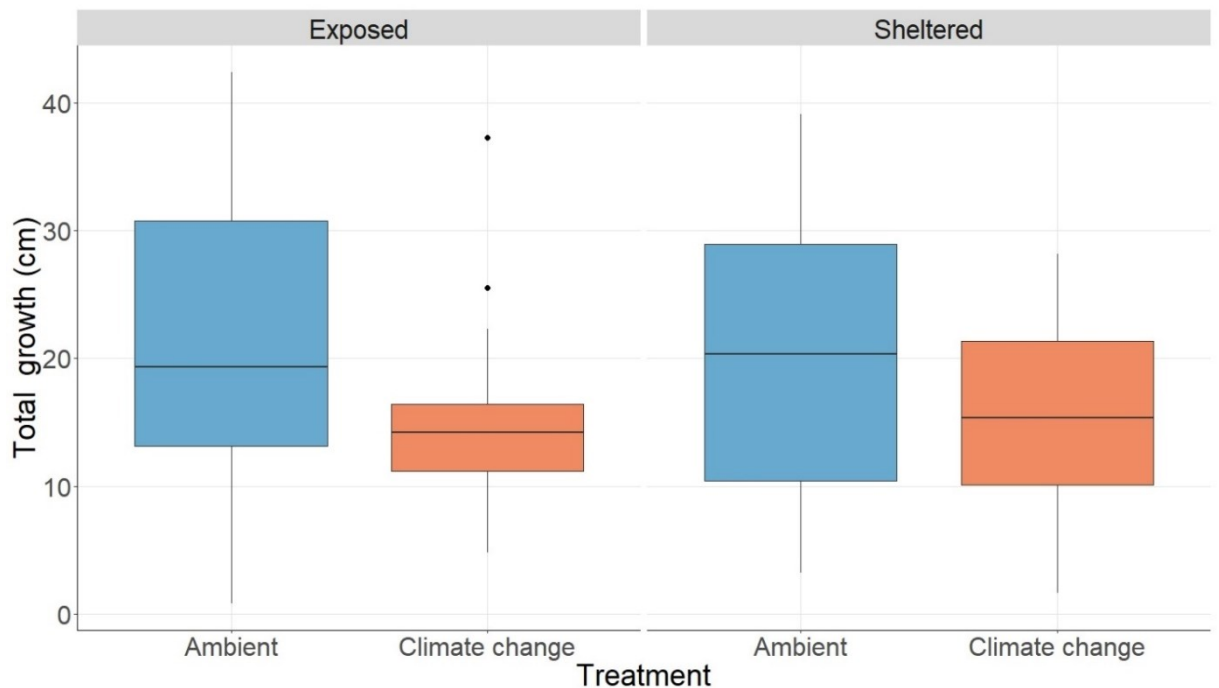

Figure 16. Growth (cm) of *Zostera marina* during the experiment in the Ambient and Climate change treatments and in relation to the two exposure classifications. There was an effect of treatment but there was no significance found between the Sheltered and Exposed in terms of growth during the experiment, and no interaction of the two factors was seen.

Table 4. Output from the two-way ANOVA on the effect of treatment and exposure on the total growth of leaves of *Zostera marina*.

	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	673.8	673.77	7.6617	**0.007**
Exposure	1	19.9	19.93	0.2266	0.6353
Treatment:Exposure	1	25.4	25.38	0.2886	0.5926
Residuals	80	7035.2	87.94		

3.3.2. Difference in the number of leaves between Exposed and Sheltered sites

A Generalized Linear Model (GLM) was applied as the assumption of normal distribution required for a two-way ANOVA was not met, with the number of leaves following a Poisson distribution. The GLM was performed to analyze the effect of treatment Ambient x Exposed (n = 23), Climate change x Exposed (n = 21), Ambient x Sheltered (n = 22), and Climate change x Sheltered (n = 18) on the number of leaves (Figure 17). The test showed no significance in the number of leaves, either from treatment or exposure (p = 0.672).
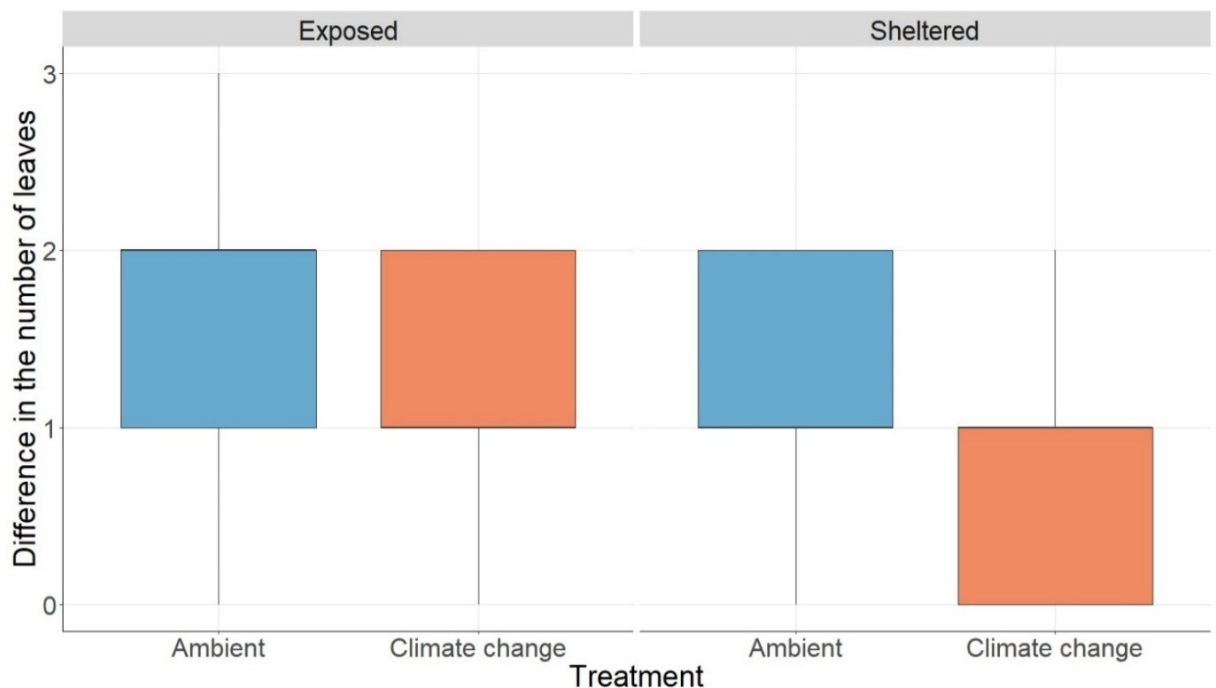

Figure 17. Number *Zostera marina* leaves in Ambient and Climate change treatments and comparing how the two exposure classifications compared to each other. There was no effect found in the treatments or between the two exposure classifications.

Table 5. The Generalized Linear model output table on the effect of treatment and exposure on the number of leaves of *Z. marina*.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.3610	0.1741	2.074	0.0381
TreatmentClimate change	-0.1097	0.2595	-0.423	0.6725
ExposureSheltered	-0.1940	0.2622	-0.740	0.4595
TreatmentClimate change:ExposureSheltered	-0.3828	0.4275	-0.895	0.3705

4. Discussion

4.1. Summary of the main findings

In total, the experimental period lasted for 10 days, of which all 10 days crossed the 90th percentile of temperatures recorded, which exceeds the number of days a marine heatwave needs to be in length (5 days) to be classified as a marine heatwave, according to the definition provided by Hobday et al (2016).

The temperature comparison between the sites showed that there is a significant difference between Sheltered and Exposed eelgrass meadows, in terms of mean temperature and diurnal variability. The Sheltered sites showed more variability in both comparisons and higher mean values than in Exposed sites. The Exposed sites showed a lower mean and, less variability in both, mean temperature and diurnal variability when compared to the Sheltered sites. This indicates that the Exposed sites are more stable in temperature than the Sheltered sites.

Zostera marina showed low mortality in both treatments. In total 11 individuals died during the experiment, four individuals in the Ambient treatment and seven individuals in the Climate change treatment. Only two populations suffered no mortalities while the rest of the populations lost one or two individuals during the experimental period.

Leaf growth of *Z. marina* individuals in the Climate change treatment showed significantly less growth than the individuals in the Ambient treatment. However, there was no interaction between exposure and treatment, which contradicts the initial hypothesis.

The number of leaves was clearly impacted by the Climate change treatment (marine heatwave and freshening event), while exposure history of *Z. marina* did not change the response of the treatment

4.2. Revisiting the Hypotheses

RQ1: For research question and hypothesis 1, the data confirm that there is a significant difference between the two different exposure classifications, supporting the assumption that sheltered sites, and their inhabiting organisms, experience stronger diurnal to seasonal variability in seawater temperature. Yet, it remains to be proven that this pattern also applies to salinity regimes.

RQ2: For research question and hypothesis 2, the data confirm a significant difference in growth of the leaves between the two treatments: Ambient and Climate change. Yet, the data do not support the hypothesis with respect to the difference in the number of leaves.

RQ3: For research question and hypothesis 3, the hypothesis must be rejected as there was no interaction between the treatment and exposure in any of the response variables that were measured for this thesis (i.e., growth and number of leaves). Other traits (not part of the thesis) may reveal different pattern, but this remains to be tested. The expectation of this was that there would have been a clear distinction between individuals taken from the different exposure classifications, and especially as the two exposure classifications had significant differences in average temperature and diurnal variability.

4.3. Field and experimental temperatures in relation to marine heatwave characteristics

The locations Tångholmen, Tjärnöbo and Nyckelby kilen experienced a marine heatwave throughout the 22-day field period 5th to 27th of July, with the locations already having passed the upper threshold for a marine heatwave as defined for Tjärnö bay based on several decades of temperature data (Moron 2018), when the loggers started recording. Gåsholmen also went through a marine heatwave during the field period, but the duration was shorter, the heatwave started on the 12th of July and was still occurring when the loggers were removed. All locations passed the 90th percentile value at some point during the experimental period (Figure 3), thus classifying as a marine heatwave or a heat spike (temperatures exceeding the 90th percentile value for more or less than five consecutive

days, respectively; Hobday et al. 2016). However, the climatology and the 90th percentile values used as a baseline at all stations are based on sea surface temperature values measured at Tjärnö Marine Laboratory, meaning that this climatology does not fully represent the unique conditions experienced at each separate location. The mean temperature and diurnal variability in sites that were classified as exposed, were very comparable to each other and especially the average temperature variation between all such exposed sites. As the logging period was set to start from the 5th of July and run to the 27th of July, 2021, it is unknown whether the individuals used in this experiment have experienced marine heatwaves in a combination with freshening events for certainty but if the temperature logging data gives any indication, they probably have experienced one in their past.

4.4. *Zostera marina* **response in a Climate change scenario**

If the treatments are to give any indication, it seems at least that *Z. marina* populations in Kosterhavet (Skagerrak), can currently withstand the two stressors applied in this study, at least on short timescales. Yet, their performance is reduced because of such temporal events. The duration, frequency and intensity of marine heatwaves are increasing (Hobday et al. 2016). In previous studies *Z. marina* has reacted towards marine heatwaves. Aoki et al. 2021 found that a *Z. marina* meadow in Virginia, east coast of the United States, that was exposed to marine heatwave lost 90% of its shoot density in the inner meadow (middle of the meadow) and lost 20% of the stored carbon. Shoot density started to recover and the re-accumulation of carbon stores in the inner meadow started three years after the event. The outer meadow however remained unchanged in both shoot density and managed to retain the stored carbon in the sediment.

Sawall et al. 2021 conducted a longterm mesocosm study where they increased the sea surface temperatures for nine months (November to August) where *Z. marina* plants were exposed to both natural sea surface temperatures as well as a treatment where sea surface temperatures were increases by 3.6 °C. The study found when *Z. marina* was exposed to higher temperatures, it had a reduced performance on several response measurements such as growth and shoot abundance. They also found that increased temperatures in spring induces an earlier sexual reproduction resulting in a higher mortality.

As *Z. marina* has both sexual and clonal reproduction in Kosterhavet as well as a low allelic richness (Jahnke et al. 2018), the future will show how *Z. marina* manages to endure the changes brought on by climate change. The temperature threshold for *Z. marina* is considered to be > 25° C (Nejrup and Pedersen 2008), but populations that appear at depths of 1.5 to 6 meters have a better chance of surviving marine heatwaves compared to populations appearing above 1.5 meters as the water temperatures have difficulties reaching that high (Aoki et al. 2021). Populations that are found in deeper areas might be able to escape the marine heatwaves as deeper areas as the peak temperatures are not often reached (Aoki et al. 2020).

An unanswered question from this thesis is that the response variables do not give a clear answer why exactly the individuals in the Climate change treatment performed poorer when it came to growth, whether it was the single stressor or the combination of two stressors. *Zostera marina* has a broad salinity tolerance (Nejrup and Pedersen 2008; Boström et al. 2014), and a salinity of 20 is quite normal (Figure 1). Even though this thesis used the correct mean salinity intensity for the summer season, it might not have been low enough to show any meaningful responses.

4.5. The role of genotype or exposure history for the sensitivity to the climate change scenario

While the role of species diversity is well known to help mitigate the effects of climate change (Reid 2006), the role of intraspecific diversity to combat climate change is less known and has been a shortcoming that should be addressed (Pauls et al. 2013). The role of high variability between genotypes can be an effective way for species to combat climate change as it provides the populations with the resilience and resistance to overcome the perturbations that are associated with climate change. According to a study conducted by Reusch et al. 2005, a high genotypic diversity in *Z. marina* can help to increase recovery from a perturbation, as well as its associated communities. The results for this thesis does not include any genetical analyses of the individuals or population that were used in this study, therefore it can only speculate through the two exposures. The populations used in this thesis might be tolerant towards warm sea surface temperature and salinity shifts. According to Morón 2018, Tjärnö archipelago can warm up quickly due to solar irradiation and heavy rains might quickly shift salinity. It could potentially

have made these populations more tolerant to these shifts over time and might be a reason why the treatment in combination with exposure did not find significance in either growth or number of leaves.

As the mean temperatures keep increasing along with extreme events becoming more and more common, the implications from this study regarding mortality show that *Z. marina* is able to withstand marine heatwaves and freshening events. As climate change progresses, the phenotypic plasticity might help in preventing total losses of *Z. marina* through its acclimation capacity (Pazzaglia et al. 2021).

4.6. Applied methods – benefits and drawbacks

It is possible that if the experiment were to have been maintained for a longer duration, clearer differences might have been observed in the responses of *Z. marina*, namely in the number of dead individuals and potentially even in the number of leaves. The exposure categories for the sites (Sheltered and Exposed) are only based on temperature readings from the field period. To identify spatial variability more accurately between the sites more parameters such as salinity, organic content (Bonsdorff et al. 2003), and grain size (Dahl et al. 2020) would be required. Yet, for simply measuring temperatures for within the sites, the data provided by the HOBO loggers was sufficient to give an indication how exposed the sites were. In the Sheltered sites, the loggers were consistently placed at a depth of 1.5 m apart from Ramnekroken and Nyckelby kilen (however, Ramnekroken is removed due to burial of the logger), so the diurnal variability within the Sheltered locations is due to the locations and that locations physical geography.

Several locations were experiencing a marine heatwave during the field period in the Kosterhavet area, which might mean that they were coupled with a simultaneous freshening event. Unfortunately, salinity was not monitored during the field period in the different locations, as salinity loggers were not available. The salinity at the Tjärnö Marine Laboratory was monitored during the experimental period. If a marine heatwave and a freshening event occurred in the occurred at the other locations during the experiment remains speculative, as no salinity measurements are available from this period.

The salinity and temperature sondes occasionally were dislodged and floated up to the surface of the tub. When the sondes were exposed to air, these were unable to measure

the salinity and temperature in the mixing tubs. The dislodged sondes had to be manually resubmerged for the logging of the temperature and salinity measurements to continue. The study managed to implement the temperature increase very well as the five different Baltotron systems had a low standard deviation, the salinity however was more difficult to implement as the standard deviation was higher when comparing the salinities in the different Baltotrons. There was no effect from the Baltotrons identity (position) in any of the measured responses. The study also followed the natural diurnal variability during the experimental period, which might be a benefit for *Z. marina* as the variation in temperature might have provided heat refugia (Salo et al. 2019). In addition to that, it also made the experiment more realistic.

The sediment used in the experiment was mixed from all of the sampling locations in order to homogenize it. Especially for leaf area, length and width, all have positive correlations with sediment ammonium concentrations (Short 1983). The ability to use ammonium more efficiently could also be a factor why some individuals grew more than other individuals used in the experiment.

4.7. Outlook

Future research should start to examine more and more multiple stressor experiments, rather than continue with single stressor experiments. As stated in the introduction section of the thesis, there are always environmental variables that are shifting one way or another. If an experiment is done with two or more stressors, it gives a more realistic picture of what is occurring in nature. The problem with those studies is that they require multiple intensities, durations, and frequencies, that very quickly leads to highly complex experimental setups and designs. These studies however are highly important for us to understand at deeper level what is driving the distribution of species in the future.

What also needs to be considered is that stressors do not have to occur at the same time. Rather than having simultaneous stressors happening at the same time as marine heatwaves and freshening events, stressors can also be sequential (Gundersson et al. 2016). These sequential stressor events are known to happen in nature. One example of this is the study conducted by Wahl et al. 2021, where macrophytes first experienced a marine heatwave followed by a hypoxic upwelling event. These stressors are known to happen in the Kiel bight during the summer months (Wahl et al. 2021), so even if an

organism can survive the first stressor it might leave them sensitive to the second one (Al-Janabi et al. 2019).

Genetic diversity also needs to be taken more and more into consideration in future studies. Genetic diversity can be easily overlooked, but it plays a very big role in maintaining the health of ecosystems, species, and populations. Intraspecific diversity can even have a larger indirect ecological effect than species when interactions alter community composition (Des Rocches et al. 2018).

This study managed to successfully take into consideration two environmental drivers and combine this aspect with how these environmental drivers affect populations from different exposure gradients. Genetic diversity within and across population can vary as intraspecific variation can arise through several mechanisms (Des Roches et al. 2018). When looking at the benefits of conducting studies such as this one, it will provide insights on how genetic diversity can help combat effects of climate change as well as bringing new information on how to better manage and conserve the populations that currently exists.

5. Conclusions and Outlook

The growth of *Zostera marina* was negatively impacted by the two simultaneous stressors, a marine heatwave and a freshening event, lasting for ten days. Though some individuals died during the experiment, the mortality rate remained low across treatments. The number of leaves of *Z. marina* did not vary between the treatments and were not impacted by exposure history. The growth of *Z. marina* showed a significant difference between the treatments, but no interaction between the exposure classifications. The surprising part of this thesis was that there was no interaction between the treatment and exposure classifications. The assumption of previous exposure having a significance in the results of this thesis could not be found, even though the two exposure classifications were significantly different in both mean temperature and diurnal variability.

There are currently programs working on restoration and conservation of *Z. marina* in the region to help the current populations to recover the losses in the area, and this thesis

might help to gain a better understanding for how *Z. marina* will be able to handle future scenarios as well as provide an insight to *Z. marina*'s temperature and salinity tolerance.

6. Acknowledgements

7. Swedish summary – Svensk sammanfattning

Medelvärden för en rad abiotiska variabler, såsom temperatur, salinitet, ljus- samt syremängd förväntas förändras i takt med det fortsatta framskridandet av klimatförändringen (Bindoff et al. 2019). I havet fluktuerar dessa abiotiska förhållanden över tid och rum, exempelvis allt från dagliga fluktuationer i vattentemperatur till den långvariga ökningen av medeltemperaturen över årtionden som är kopplad till klimatförändringen. Nyligen har det även noterats att extrema klimathändelser både till lands och till havs blir vanligare i och med klimatförändringen (Oliver et al. 2018, Bates et al. 2018). I naturen sker förändringar inte i isolation, då flera än en abiotisk variabel kan uppvisa extremvärden samtidigt eller konsekutivt. Det är därför viktigt att utföra studier som ser på kombinationer av två eller flera av dessa abiotiska variabler för att förstå samspelet hur de kan påverka såväl viktiga ekosystem som samhällen i haven (Gunderson et al. 2021).

En av dessa extrema händelser är så kallade marina värmeböljor som av Hobday et al. 2016 definierats som en fem eller flera dagar lång tidsperiod där havsvattentemperaturen överskrider ett tröskelvärde, den 90:e percentilen, baserat på 30 år av lokalt långtids temperaturdata. Mellan åren 1925 och 2016 har marina värmeböljor globalt ökat i intensitet, frekvens och varaktighet (Oliver et al. 2018) och dessa händelser har redan orsakat förändringar hos födovävarna samt strukturer i marina samhällen (Wernberg et al. 2016, Pansch et al. 2018). I samband med marina värmeböljor förekommer i vissa havsområden samtidigt en kraftig minskning av saliniteten, så kallad försötning (eng. freshening). I Skagerrak där studien utfördes, kan saliniteten variera mycket och även sjunka snabbt på en relativt kort tid (Danielssen 1997; Dahl et al. 2005, Morón 2018). De bakomliggande orsakerna till fluktuationer i saliniteten i Skagerrak varierar, men kan påverkas av bland annat lufttryck, vindriktning samt regnmängd. Orsaken till att marina värmeböljor och försötning ofta inträffar samtidigt är ännu oklar, men hur kombinationen av dessa två abiotiska stressfaktorer påverkar enskilda arter har studerats tidigare. Morón (2018) noterade att individer av Vanlig sjöstjärna (*Asteria rubens*) klarade sig bättre då de endast utsattes för extremvärden av den ena abiotiska variabeln än då de utsattes för en kombination av båda extremvärdena.

Ålgräss (*Zostera marina,* Linnaeus) är en makrofyt som kan hittas längs sedimentära botten längs med hela atlantiska kusten över norra halvklotet och kan även i Stilla Havet och Arktis (den Hartog 1970). *Zostera marina* är en viktig art eftersom den fungerar som ett lekområde för flera ekologiskt och ekonomiskt viktiga fiskarter, förhindrar erosion och åter suspension av sediment samt utgör en viktig kolsänka (Jones et al. 1994, Cole och Moksnes 2016). Sedan 1980-talet har över 60 % av *Z. marina* ängarna vid svenska västkusten försvunnit (Jahnke et al. 2020) och en delorsak är mängden epifyttillväxt som i sin tur är starkt knuten till eutrofiering och trofiska kaskader (Moksnes et al 2008, Baden 2010). Denna minskning av *Z. marina* har redan lett till en signifikant minskning av både biodiversitet och biomassa (Cole och Moksnes 2016). Eftersom *Z. marina* är en viktig art med flera viktiga funktioner för kustekosystems välmående är det viktigt att reda ut vilka effekter extrema temperaturer och salinitet har på arten.

Frågeställningarna samt hypoteserna för avhandlingen är:

F1: Förekommer det skillnader i ytvattentemperatur och variabilitet mellan de olika insamlingslokalerna för *Z. marina* individer som användes i detta experiment?

H1: Skyddade lokaler kommer att uppvisa större daglig variation i temperatur samt högre medelvärde jämfört med exponerade lokaler

F2: Påverkar en marin värmebölja kombinerat med försötning karaktärsdragen hos *Z. marina*?

H2: Behandlingen kommer att påverka karaktärsdragen hos *Z. marina* negativt genom ökad mortalitet, mindre mängd blad samt minskad tillväxt

F3: Kommer de olika populationerna uppvisa skillnader i sin respons till en behandling med förhållanden motsvarande dessa under en marin värmebölja kombinerat med försötning?

H3*: Z. marina* individer från de mera skyddade områden kommer vara mera toleranta till behandlingen än individer som härstammar från mera exponerade områden.

Fält- och laboratoriearbetet skedde vid Tjärnö marina laboratorium i Sverige. Fältarbetet bestod av att samla *Z. marina* individer från 10 olika populationer från Kosterhavets Nationalpark. I samband med insamlingen av individerna samlades det också in sediment för att senare kunna plantera individerna i akvarierna. Vid laboratoriet acklimatiserades individerna till ytvatten från Tjärnöbukten i en vecka före experimentet kunde påbörjas.

På varje lokal placerades det dessutom ut en temperaturlogger under juli månad för att i efterhand kunna jämföra lokalerna med varandra samt registrera marina värmeböljor under säsongen.

För att implementera en behandling med förhållanden lik dessa under en marin värmebölja kombinerat med försötning (härefter kallad behandling) och en kontroll för experimentet (Figure 9 och Figure 10), användes Baltotron- bassängsystem med totalt fem replikat av de båda behandlingarna under experimentet. En individ från varje lokal placerades i varje enhet, det vill säga, sammanlagt 100 individer uppdelade över de två behandlingarna (Figur 7). Själva experimentella enheterna bestod av plastpåsar med en volym på fyra liter innehållande en mix av sediment från de olika insamlingslokalerna. Påsarna blev kontinuerligt påfyllda med en lika stor mängd vatten från en huvudtank som placerades ovanför varje bassäng halva. Upprätthållningen av temperatur och sanitetsförhållanden i behandlingen skedde dagligen genom att ställa in akvariesystemet (GHL, Profilux. 4, Tyskland), samt mättes manuellt dagligen för att kontrollera att akvariesystemet registrerade rätt värden.

Experimentet påbörjades den 19 juli 2021. Före *Z. marina* individerna planterades till de experimentella enheterna vägdes (våt vikt) och fotograferades individerna. Även bladen hos de enskilda individerna räknades. Alla individer blev stuckna med en nål 1 cm ovanför bladslidan för att mäta tillväxten av de enskilda bladen hos individerna efter experimentet. Experimentet avslutades den 29 juli och mängden blad samt våt vikten av alla individer mättes, varefter individerna fotograferades. Bladen och rötterna separerades och torkades i en torknings ugn i 48 timmar i 60 °C. Därefter vägdes bladen och rötterna på en precisions våg.

För att kunna identifiera skillnader mellan de skyddade och exponerade områden, användes Welch t-test för att se skillnader i medeltemperatur och dygnsvariation. För *Z. marina* identifierades variationen inom tillväxt och mängden blad mellan behandlingarna med hjälp av envägs ANOVA. Utöver detta utfördes en tvåvägs ANOVA för att se hur exponeringsgraden samt behandligen påverkade tillväxten. En generaliserad linjär modell (GLM) användes för att utreda hur exponeringsgraden samt behandlingen påverkade mängden blad.

Welch t-testet visade att det fanns en signifikant skillnad både i medeltemperatur ($p < 0.01$) samt dygnvariation ($p < 0.01$) mellan skyddade och exponerade områdena.

Inga statiska tester utfördes på mortalitet eftersom mängden döda individer var väldigt låg, 11 individer totalt, varav 4 individer utsattes för kontrollen och 7 för behandlingen. Envägs ANOVAn visade en signifikant skillnad i tillväxten av bladen ($p < 0.01$) men ingen signifikant skillnad mellan mängden blad. Resultaten från tvåvägs ANOVAn visade ingen signifikant interaktion av behandligen samt exponeringsgraden på tillväxt, och GLM analysen visade inte heller någon significant interation av behandligen samt exponeringsgraden på mängden blad.

Resultaten visar att behandlingen hade en negativ inverkan på *Z. marinas* prestationsförmåga, speciellt tillväxten, medan individens ursprung (tidigare exponeringsgrad) inte spelade någon roll, vilket motsäger hypotes 3. Kombinationen av de två stressfaktorerna i behandlingen ledde inte heller till en hög mortalitet bland individerna.

Under sommaren genomgick alla lokaler kortare eller längre perioder av vad som registrerats som marina värmeböljor. Detta tyder på att dessa populationer har upplevt marina värmeböljor tidigare och kan med god sannolikhet vara tåliga mot denna stressfaktor. Även djup utbredningen av de olika populationerna skiljde sig från varandra. Vissa individer samlades in på 3–4 meters djup medan andra samlades från 2–3 meters djup. Temperaturen i behandlingarna kan sålunda ha påverkat individer från djupare ängar kraftigare, eftersom både mängden ljus och temperaturen är lägre ju djupare men rör sig i vattenkolumnen. Även mängden ljus individerna utsattes för i Baltotron systemet kan ha påverkat dem negativt, då ljus mängden under experimentet var betydligt starkare än vad individerna skulle ha upplevt normalt på 2–4 meters djup.

Extremhändelser kan ha kraftigt negativa konsekvenser för en rad vattenlevande organismer, men även för hela samhällen. Resultaten från denna studie tyder på att *Z. marina* är relativt motståndskraftig mot marina värmeböljor av nuvarande intensitet, men framtidens marina värmeböljor kommer troligtvis vara betydligt intensivare (Oliver et al. 2018). De populationer av *Z. marina* som inkluderades i studien är även vana vid fluktuationer i salinitet, vilket förklarar varför saliniteten inte påverkade individerna i behandlingen märkbart. Om experimentet skulle ha pågått en längre tid kunde potentiellt en effekt av försötningen ha synats, men tio dagar är en för kort tidsperiod för att kunna se en effekt. För tillfället är antalet studier om marina värmeböljor lågt i Norden, så även antalet studier som kombinerar flera abiotiska stressfaktorer. Framtida studier kring hur

Z. marina påverkas av extrema förhållanden bör genomföras för att få en klarare bild av hur detta värdefulla ekosystem kommer att klara sig i framtida klimatscenarion.

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9.   Appendix

Table A1. The temperature data were divided into several smaller categories. The categories helped in the classification and ranking of the different sampling sites. These categories are highest overall temperatures, lowest overall temperatures, median temperature, standard deviation in temperatures, highest recorded temperature, and lowest recorded temperature. The data are based on temperatures during the field period from the 5th to the 27th of July, 2021. The site Ramnekroken was removed from further analysis as the logger had been buried throughout the summer season of 2021. The color code indicating from red (sheltered) to blue (exposed). The rank indicating from 9 being the most sheltered location and 1 the most exposed.

| Location	Max overall	Min overall	Median	SD	Diurnal	Higherst recorded	Lowest recorded	Mean	Sum	Rank	
Tångholmen	9	8	8	8	9	9	8	8.4	67.4	9	Sheltered
Tjärnöbo	6	9	9	9	7	8	9	8.1	65.1	8	
Nyckelby kilen	7	7	7	6	8	6	7	6.9	54.9	7	
Gåsholmen	8	6	6	7	5	7	6	6.4	51.4	6	
Inre Vattenholmen	5	4	4	4	3	5	4	4.1	33.1	5	Exposed
Koster	2	5	2	1	6	4	5	3.6	28.6	4	
Flatskär	3	3	5	2	2	3	3	3.0	24.0	3	
Kockholmen	4	1	3	5	4	1	1	2.7	21.7	2	
Styrsö	1	2	1	3	1	2	2	1.7	13.7	1	
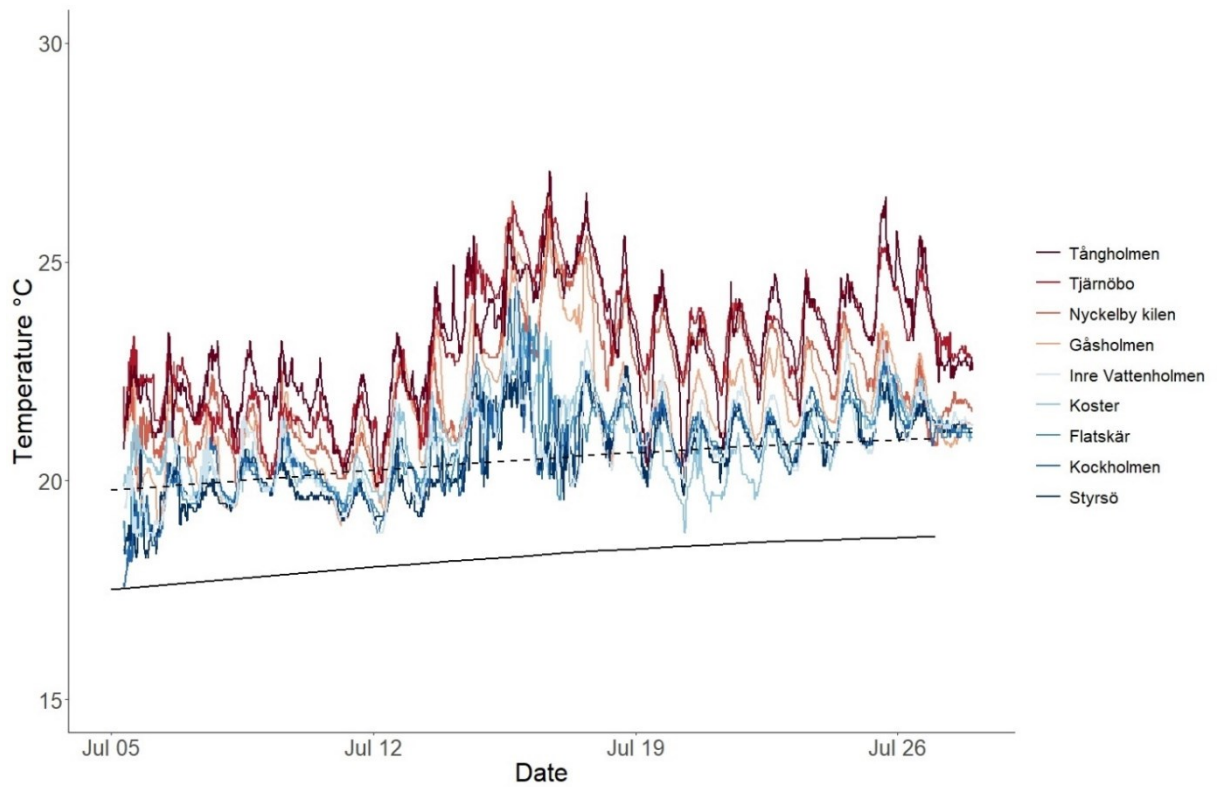
Figure A1. High-resolution temperature data (measurements taken every 15 minutes) showing the different sites from the 5th to the 27th of July, 2021. The solid line represents the climatology from data collected at the Tjärnö Marine Laboratory, the dashed line represents the 90th percentile temperature values.
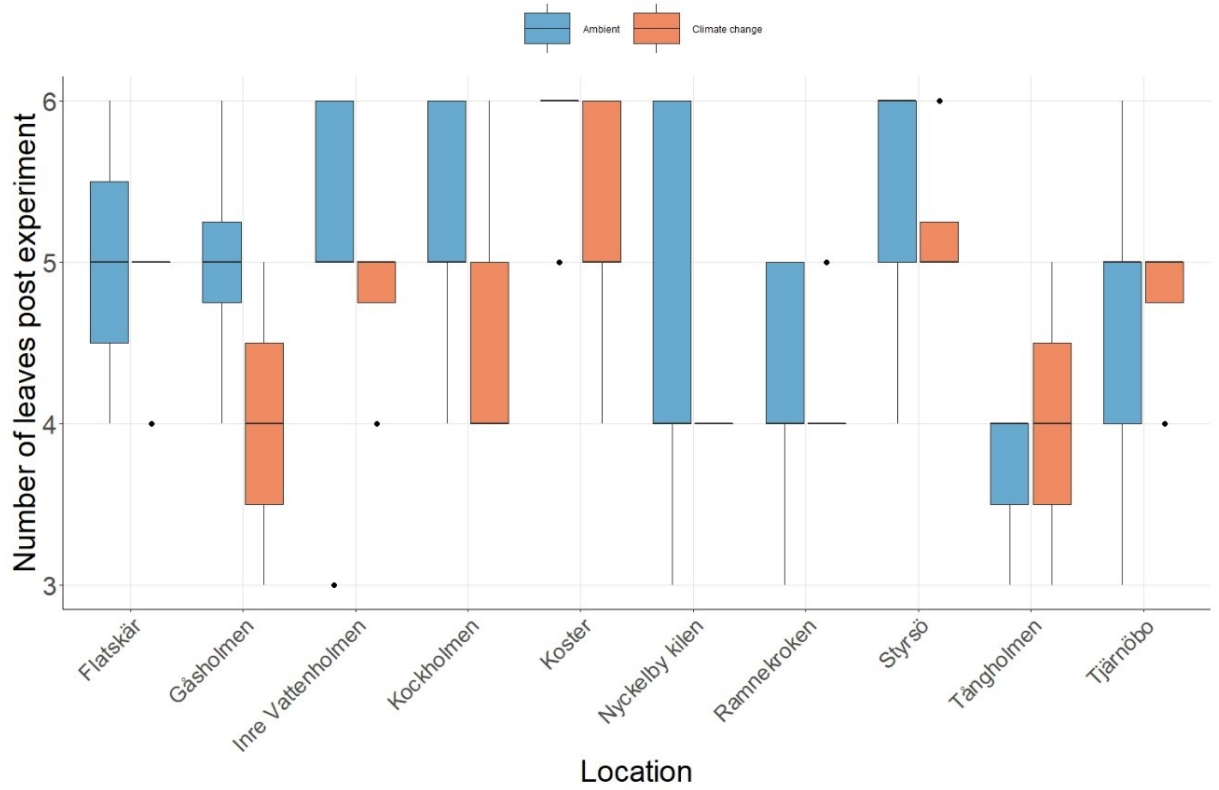
Figure A2. Boxplot showing the difference in the number of leaves of *Zostera marina* at the end of the experiment, between the two treatments, Ambient and Climate change, and across tested *Z. marina* populations (from distinct donor sites). Represented are the median as well as the upper and lower quartile ranges.
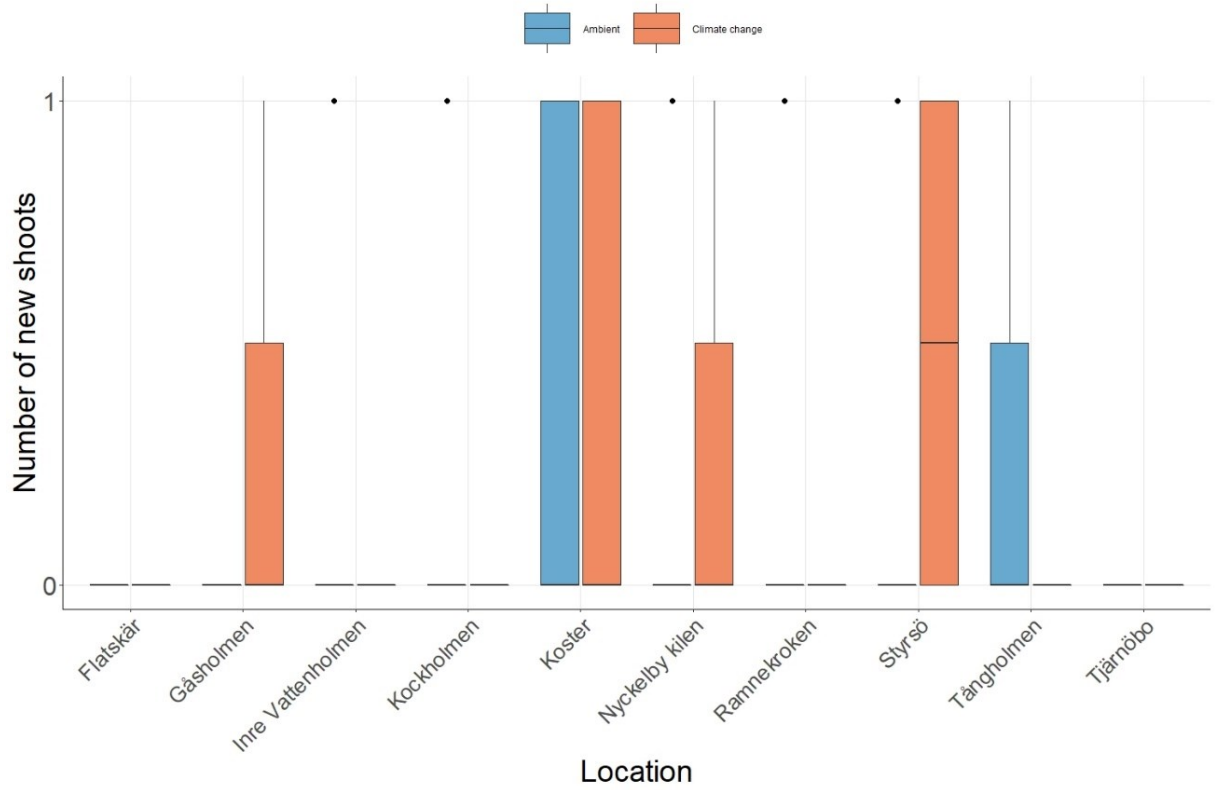
Figure A3. Boxplot indicating the number of new shoots of *Z. marina* that grew during the experimental period in the Ambient and Climate change treatments as well as the different populations (donor sites) Represented are the median, upper and lower quartile ranges.
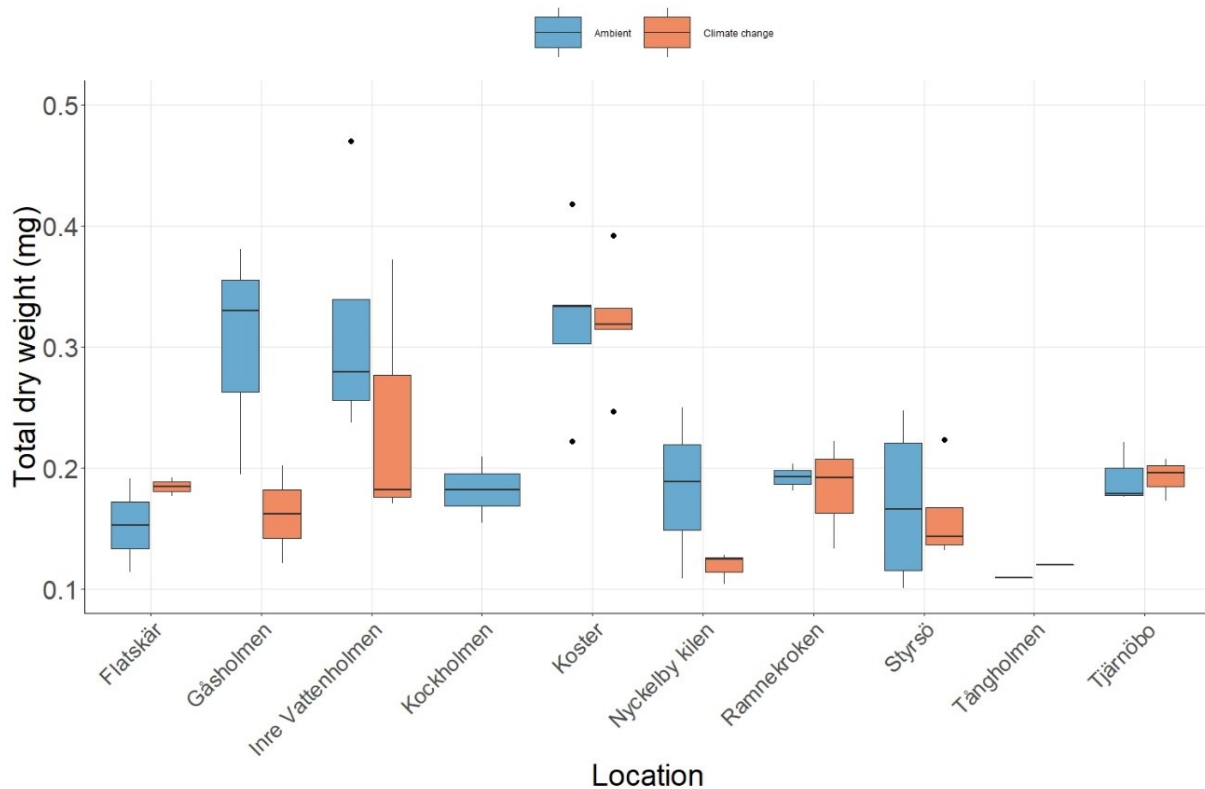
Figure A4. Boxplot indicating the total dry weight measured from the *Z. marina* plants after the experiment across the different locations and treatments (Ambient and Climate change). The dry weight includes the leaves and roots (rhizomes and roots). Represented is the median, as well as the upper and lower quartile ranges.