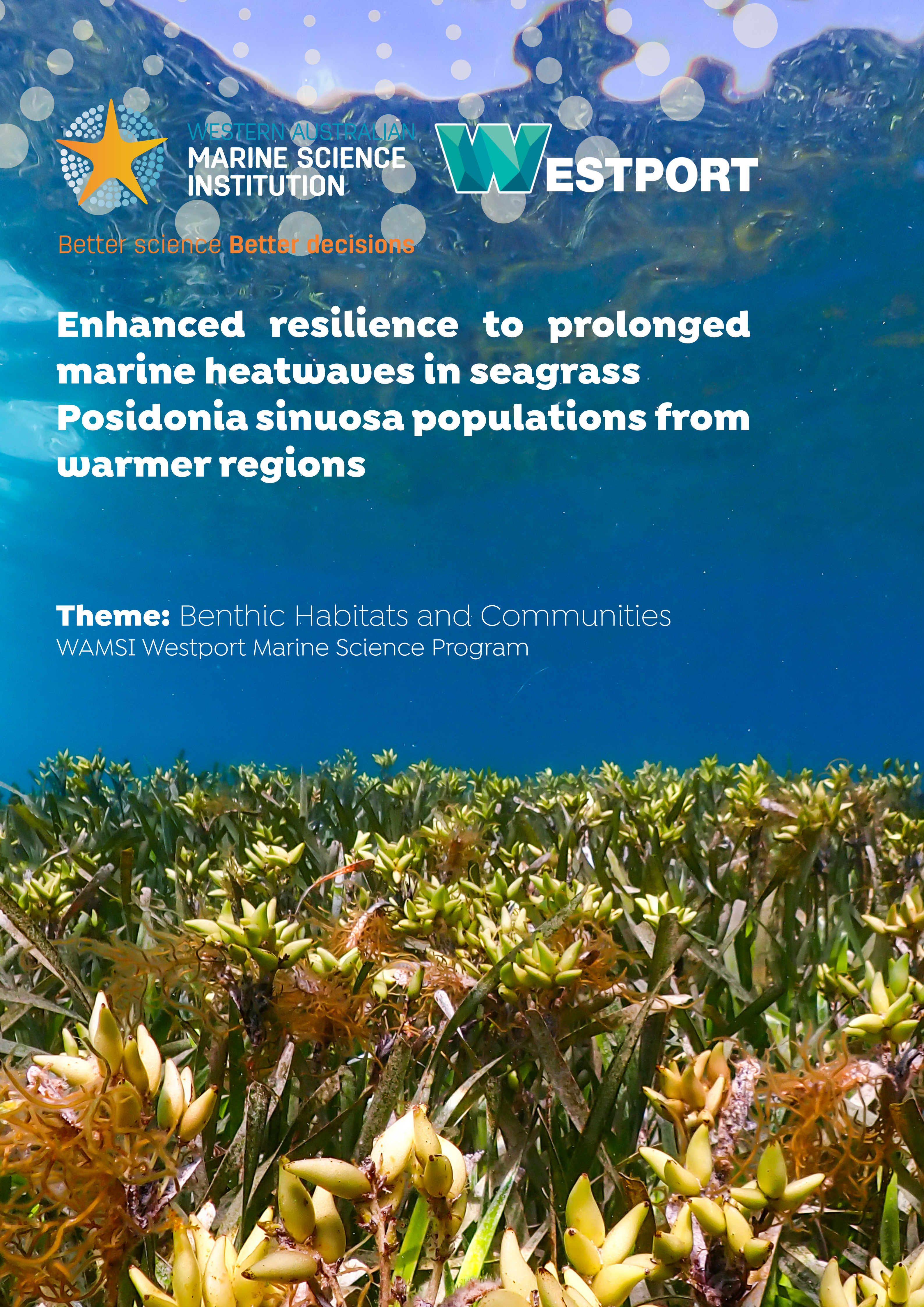

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Enhanced resilience to prolonged marine heatwaves in seagrass Posidonia sinuosa populations from warmer regions

Theme: Benthic Habitats and Communities
WAMSI Westport Marine Science Program

WAMSI WESTPORT MARINE SCIENCE PROGRAM

ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a $13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.

FUNDING SOURCES
The $13.5 million WAMSI Westport Marine Science Program was funded by the Western Australian Government, Department of Transport. WAMSI partners provided significant in-kind funding to the program to increase the value to >$22 million.

DATA
Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).

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FRONT COVER IMAGE
Theme: Benthic habitats and communities
Front cover image: Seagrass (*Posidonia australis*) in Cockburn Sound. Photo courtesy of Rachel Austin (The University of Western Australia).

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Supplementary Tables

The WAMSI Westport Marine Science Program is a $13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.

Enhanced resilience to prolonged marine heatwaves in *Posidonia sinuosa* seagrass populations from warmer regions

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Project

Project 2.2: Pressure-response relationships, building resilience and future proofing seagrass meadows (Appendix B)

Executive Summary

Climate change is driving the intensification of marine heatwaves (MHWs), which pose a significant threat to seagrass ecosystems globally. Understanding the response of seagrass species to these extreme thermal events is crucial for predicting their resilience and informing conservation strategies. In this study, we evaluated the impacts of prolonged MHW conditions (30 °C) on the photophysiology, metabolism, and growth of three populations of *Posidonia sinuosa* from Western Australia (Shoalwater, Garden Island, and Jurien Bay). We aimed to determine whether populations with higher thermal optima exhibit higher levels of resilience to MHWs compared to populations with lower thermal optima.

Key findings relevant to the resilience of *P. sinuosa* under future warming scenarios include:

- This study demonstrates that all *P. sinuosa* populations assessed were negatively impacted by the simulated MHW of 30 °C over four weeks. This negative impact and limited recovery highlight potential long-term impacts of MHWs beyond 30 °C on *P. sinuosa* health. However, population differences in the response and recovery of the simulated MHW were observed at specific biological levels, highlighting that some populations are more resilient to these extreme events.
- This study accepts the null hypothesis that populations with lower T_{opt} (e.g. Shoalwater) are less tolerant to MHWs and exhibited greater and earlier impacts across multiple biological levels, with significant reductions in photosynthetic activity, growth, and metabolic performance under MHW temperatures. In contrast, populations from warmer regions (which also had a higher T_{opt}), like Jurien Bay, showed greater resilience to MHW temperatures, maintaining higher levels of photophysiological functions and growth under this simulated extreme event.
- Our results indicate that the thermal thresholds developed for photosynthesis using short term experiments (Said et al., 2024) can be used to predict the resilience of seagrass to MHW events and identify vulnerable species/populations.
- The findings suggest that management strategies should take into account population-specific responses to thermal stress. For instance, selective translocation or assisted gene flow from more resilient populations (e.g., Jurien Bay) to vulnerable areas (e.g., Shoalwater) could enhance the resilience of seagrass meadows to future warming events.

- The intermediate performance of the Garden Island population (greater resilience to MHW than Shoalwater but lower than Jurien Bay) highlights the complexity of other factors influencing resilience (e.g. genetic diversity). The greater resilience of the Garden Island population compared to the Shoalwater population may be attributed to its higher thermal optima, despite both populations being from a similar environmental region and separated by only 10 km, while the lower resilience compared to the Jurien Bay population cannot be justified through their thermal optima, as they are similar. This highlights the need for exploring further how thermal optima and resilience to MHW can vary among populations within the same environmental region and what factors, such as genetic diversity, might explain these variations.

1 Introduction

Human activities have increased greenhouse gases in the atmosphere, leading to hotter summers and more frequent extreme climatic events including heatwaves. In coastal habitats, marine heatwaves (MHWs) are increasing in intensity, frequency and duration and this pattern is predicted to continue in the future (Oliver et al., 2018, 2019). Marine heatwaves are defined as five or more days of sea surface temperature above the regional 90th percentile (Hobday et al., 2016) and have caused widespread impacts on marine species and ecosystems globally (IPCC, 2013; Pecl et al., 2017). Seagrasses, marine flowering plants, are an ecosystem that has been impacted by MHWs (Hobday et al., 2018; Lefcheck et al., 2016; Diaz-Almela et al., 2007; Marbà and Duarte, 2010; Thomson et al., 2015; Carlson et al., 2018; Moore and Jarvis, 2008; Moore et al., 2014). Such impacts are a concern as seagrass habitats are among the Earth's most productive ecosystems (Duarte and Chiscano, 1999) and provide critical habitat (e.g. for economically important fish species), so their decline can have cascading effects on associated species (Hughes et al., 2009). Seagrass ecosystems are declining worldwide, driven not only by extreme events but also by human activities like coastal modification (Orth et al., 2006), with higher summer temperatures and MHWs increasingly contributing to significant seagrass loss globally (e.g. Strydom et al., 2020).

As summer temperatures and MHWs increase in intensity and frequency, it is crucial to evaluate the resilience or mortality risk of seagrasses due to warming and predict their future distribution and potential loss of ecosystem functions (Frölicher et al., 2018; Kendrick et al., 2019; Smith et al., 2023; Unsworth et al., 2015). Seagrass vulnerability to future warming can be predicted using their thermal tolerance, which is defined as the temperature range within which a species can operate, where biological activity follows an exponential increase, reaching a maximum at the optimum temperature, followed by a steep decline to reach the upper thermal lethal limit (Dell et al., 2011). Seagrass thermal tolerance varies between species(Lee et al., 2007) and among populations of a single species, often reflecting local adaptation to the climate they experience (Donelson et al., 2019; Said et al., 2024; Wesselmann et al., 2020; Wesselmann et al., 2024), as well as differences within a species and population at different biological levels (e.g. the thermal tolerance for survival is usually broader than for other essential processes like reproduction or growth; Andrews et al., 2014; Ling et al., 2008; Wesselmann et al., 2020). Therefore, the resilience or vulnerability of seagrass to MHWs is likely to vary between species, populations, and biological levels, but will also depend on the characteristics of these events; factors such as the duration, and the frequency and magnitude of MHWs have led to different effects on the survival and performance of seagrasses (Hatum et al., 2024).

Australia hosts some of the largest areas of seagrass, estimated as 9.3–12.8 million hectares, comprising 15–43 % of global seagrass meadows, with the greater range based on percentage reflecting the larger uncertainties in global estimates (30–60 Mha) (Serrano et al., 2019). Western Australia is a key region for studying the impacts of ocean warming on seagrasses due to its vast (20 % of Australia's total seagrass area: Serrano et al., 2019) and diverse seagrass meadows (19 of the 60 known seagrass species worldwide, Waycott et al., 2014) and the fact that seagrasses in this region have already experienced significant temperature increases, leading to substantial losses (Arias-Ortiz et al., 2018). For instance, a heatwave that persisted about 2 months with warming anomalies of 2-4 °C in the austral summer 2010/2011 in the West coast of Australia affected the world Heritage Area of Shark Bay (Wernberg et al., 2012), causing a loss of >1300 km^2 of seagrass meadows (Strydom et al., 2020) and impacting green turtles and dugongs and leading to the closure of scallop and blue swimmer crab fisheries (Kendrick et al., 2019).

As part of the WWSP, physiological thermal tolerance for oxygen production (thermal optima; T_{opt}) were developed for six seagrass species (*Posidonia sinuosa*, *P. australis*, *Amphibolis antarctica*, *A. griffithii*, *Zostera nigricaulis*, *Halophila ovalis*) in different regions across Western Australia (WA) in order to predict the long-term impacts of rising temperatures. This research identified that *Z. nigricaulis* in Perth was the most vulnerable species and populations to warming, which are already experiencing SST above their thermal optima (Said et al., 2024). However, this study also indicated that most *Posidonia* and *Amphibolis* seagrass populations assessed are vulnerable to future heatwaves, as their thermal optima were equal to or less than 2010/11 heatwave conditions, suggesting that a slightly more intense MHW than in the past (e.g. exceeding 1 °C the 2010/11 MHW; Strydom et al., 2020) could lead to negative impacts on seagrass health and result in mortality (Said et al., 2024). This finding is concerning, as *Posidonia* and *Amphibolis* species form dense and productive meadows providing essential habitats for other species and support key ecosystem functions and services in Cockburn Sound and other regions in WA (Nordlund et al., 2016); however, their capacity to recover from disturbances such as MHWs is limited due to their long lifespans and slow growth rates (Kilminster et al., 2015; Marba and Walker, 1999). Fortunately, some populations of these vulnerable seagrass species have displayed higher physiological thermal tolerances (T_{opt}) and might, therefore, be more resilient to future MHW or warming conditions. If these populations are more resilient to MHWs, active management approaches to build resilience to future warming conditions could be explored. For instance, if the physiological thermal tolerance observed is heritable, assisted migration (also known as assisted gene flow) could be implemented by translocating seagrass populations with higher thermal tolerance into vulnerable populations in colder regions (Coleman & Bragg 2021). This approach was largely explored in terrestrial ecosystems (Twardek et al., 2023) and several studies have experimentally demonstrated the potential success of assisted migration in marine ecosystems (mainly on corals: Boström-Einarsson et al., 2020; Humanes et al., 2021; Palumbi et al., 2014; Van Oppen et al., 2015), although very few have explicitly implemented this intervention as part of an official management effort (Twardek et al., 2023). However, to date, the potential of assisted migration has not yet been explored in seagrasses.

The persistent seagrass *Posidonia sinuosa*, has a broad distribution along the west coast of Western Australia, from Geraldton to Busselton (Cambridge and Kuo, 1979) and has been identified as a promising candidate for assisted migration trials (Said et al., 2024). *P. sinuosa* populations in warmer regions, such as Jurien Bay, have higher thermal optima (T_{opt}: 31 °C) compared to populations from cooler regions like Perth, where the thermal optima is lower (T_{opt}: 26 °C). Transplanting thermally tolerant populations from warmer regions (e.g., Jurien Bay) to areas with lower thermal tolerance (e.g., Perth) could enhance resilience to rising temperatures in these vulnerable populations. However, while this thermal optima data represents an important step in understanding seagrass thermal tolerance at the physiological scale, this data was obtained from short-term experiments (one day) which do not represent the duration of MHWs in nature that can last for weeks to months (e.g. Hobday et al., 2018). Therefore, it is crucial to further investigate the effects of prolonged exposure to elevated temperatures on *P. sinuosa* to understand whether populations with higher thermal optima have greater capacity to cope with extended MHWs compared to those with lower thermal optima, and to assess this at multiple biological scales (physiological, plant and meadow scale). This will enable us to evaluate resilience of seagrass to MHW events, as well the likelihood of success of climate mitigation measures such as assisted migration.

This study aims to evaluate the performance of two populations of *Posidonia sinuosa* from a cooler region (Shoalwater and Garden Island, Perth) and one population from a warmer region (Jurien Bay) across multiple biological levels (physiological, plant-scale) under temperatures (T) representative of a MHW (T: 30 °C) over a duration of one month. The two populations from Perth were selected as they have different thermal tolerances, Shoalwater with a lower thermal optima (T_{opt}: 26 °C) and Garden Island with a higher thermal optima (T_{opt}: 30 °C; Said, unpublished data). The Jurien Bay population that exists in warmer temperatures had the highest thermal optima (T_{opt}: 31 °C). The null hypothesis being tested is that populations with lower T_{opt} (e.g. Shoalwater) are less tolerant to MHWs and will experience greater and earlier impacts across multiple biological levels (physiological, plant-scale) compared to populations with higher T_{opt} (Garden Island and Jurien Bay), which may exhibit greater resilience to MHW conditions.

2 Materials and Methods

2.1 Experimental design

The response and recovery of *P. sinuosa* from three populations (Shoalwater, Garden Island, and Jurien Bay) to a prolonged MHW was assessed through a two-phase experiment comprising an impact and a recovery phase. The impact phase was designed to test the effects of temperature and time (duration of the experiment) on the resilience of the three populations, selected based on their thermal optima, Shoalwater (26 °C), Garden Island (30 °C) and Jurien Bay 31 °C (Said et al., 2024, Said unpublished data; Figure 1 and Table 1). To assess the effect of temperature (T), we used two levels (Control T at 24 °C and MHW T at 30 °C) and for the effect of time, we used four levels (Week 0, Week 1, Week 2 and Week 4). The control temperature treatment represents Perth's and Jurien's summer average seawater temperature, while the MHW temperature simulates seawater temperatures reached following the 2010/2011 MHW in the most northern distribution of the species (Jurien Bay – Geraldton; Pearce and Gaughan, 2011). We used four replicate aquaria for each population (n=3) and temperature (n=2) treatment combination, 24 tanks in total. The recovery phase was designed to test the recovery potential of the three populations of *P. sinuosa* from the MHW temperatures. At the end of the impact phase (four weeks), the MHW temperature treatments (30 °C) were converted into "recovery temperature treatments" by decreasing the temperature from 30 °C to the control temperature of 24 °C. To assess recovery, we compared MHW treatments (*P. sinuosa* from all the three populations recovering for two weeks at 24 °C after being exposed to 30 °C during the impact phase) with the control treatment (*P. sinuosa* from all three populations) continuously exposed to 24 °C during both the impact and recovery phases after two weeks.

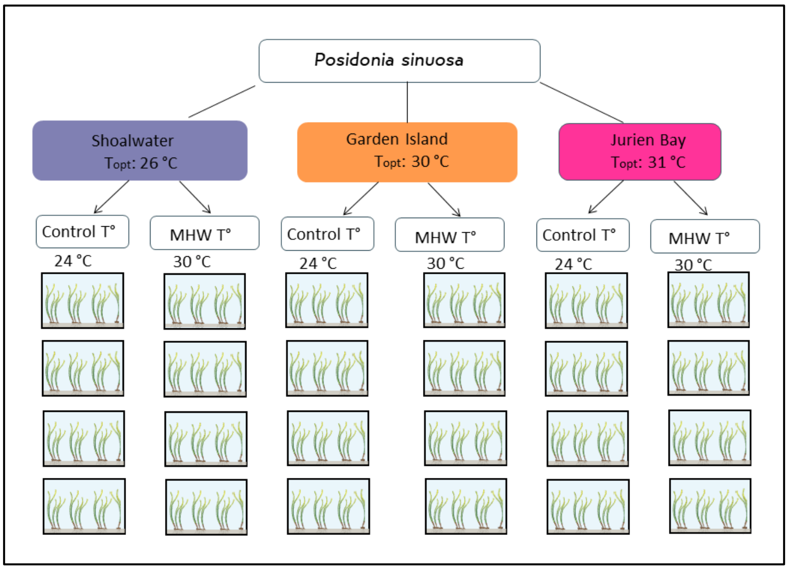

Figure 1: Experimental design for the impact phase for measuring the effects temperature (control treatment at 24 °C and MHW temperature treatment at 30 °C) over four weeks on seagrass (*Posidonia sinuosa*) sourced from Shoalwater, Garden Island and Jurien Bay

2.2 Sampling

Whole plant ramets of *P. sinuosa* were collected in May 2024 from the meadow edge at approximately 2-5 m depth at Shoalwater, Garden Island and Jurien Bay in Western Australia (Table 1). Ramets comprised a minimum of four shoots, rhizomes, and roots. Plants were transported in aerated insulated containers with seawater collected *in situ* back to Edith Cowan University, WA. At the time of collection, salinity and temperature were 35 ppt and 19.6 – 20.9 °C and had an average mean density of 191 ± 52 – 395 ± 58 shoots/m^2 and 152 ± 51 – 268 ± 67 g/m^2, respectively, with Shoalwater showing the highest shoot density and biomass per area and Jurien Bay the longest leaf length and lowest leaf width (Table 1).

Table 1: Information on sampling site of *Posidonia sinuosa* plants for thermal tolerance experiment, *in situ* environmental conditions (e.g. Temperature; T) and mean shoot density and biomass of the meadow sampled.

Sampling site	Coordinates	Sampling date	Depth (m)	T (°C)	Salinity (ppt)	Mean Density (shoots/m^2)	Mean Biomass (g/m^2)	Leaf length (cm)	Leaf width (cm)
Shoalwater (T_{opt}: 26 °C)	-32.2724, 115.69050E	6/05/2024	2	19.6	35.3	395 ± 58	268 ± 67	34 ± 4	0.71 ± 0.03
Garden Island (T_{opt}: 30 °C)	-32.1568, 115.68445E	6/05/2024	4	20.6	35.1	191 ± 52	167 ± 83	36 ± 7	0.70 ± 0.02
Jurien Bay (T_{opt}: 31 °C)	-30.3301, 115.0314E	7/05/2024	5	20.9	35.3	200 ± 64	152 ± 51	48± 4	0.56 ± 0.01

1 T: Temperature (°C)

2.3 Experimental set-up

Upon arrival at the experimental facilities, plants were transferred into 500 L aerated seawater tanks. Plants were gently cleaned to remove epiphytes before being planted into 50 L tanks filled with 10 cm layer of washed, quartz river sand covered by aerated seawater, and left to acclimate for four days at average *in situ* temperature of 21 °C (Table 1). During plant acclimation and throughout the entire experiment (including both impact and recovery phases), plants were provided with 180 ± 20 µmol photons m^{-2} s^{-1} of light using marine aquarium Light Emitting Diode (LED) modules with a full spectrum light (MarinTech™ Pty Ltd) on a photoperiod of 12 h in light and 12 h in dark cycle. Light was measured at the base of the canopy using a micro-PAR sensor (In-Situ Marine Optics™) and adjusted with a 50 % shade cloth. Salinity was maintained at 36 (± 1) ppt, and the seawater in the tanks was refreshed every four days with seawater pre-heated to the treatment temperature (Figure 1). Following acclimation, temperature in all tanks was increased from 21 °C by 1 °C per day, reaching 24 °C in the control treatment tanks over three days and reaching 30 °C in the MHW treatment tanks over nine days. Once target temperatures were reached, plants were maintained at these temperatures for an additional week, to ensure a minimum exposure of one week at their target temperature before the start of the experiment.

In each 50 L aquaria, 20 ramets from one of the three *P. sinuosa* populations was planted (Table 2; Figure 2). Each aquaria was independent and comprised a main tank (volume: 50 L) and sump tank (volume: 20 L) with heaters (300-600 W) to maintain the target water temperature ± 1 °C and a pump recirculating the water from the sump tank to the main tank with a pump, inside temperature-controlled rooms. The 24 tanks were randomly distributed across two temperature-controlled rooms, both set to a room temperature of 21 °C, with 16 tanks in one room and 8 in the other. Due to limitations on the electrical load, all tanks could not be housed within a single aquaria room. The 20 ramets per tank enabled periodic harvests to assess the response over time. Sampling occurred at the start of the experiment (30th of May 2024: Week 0), after one week (Week 1; 6th of June 2024), after two weeks (13th of June 2024: Week 2) and after four weeks (26th of June 2024; Week 4; Table 2*).*

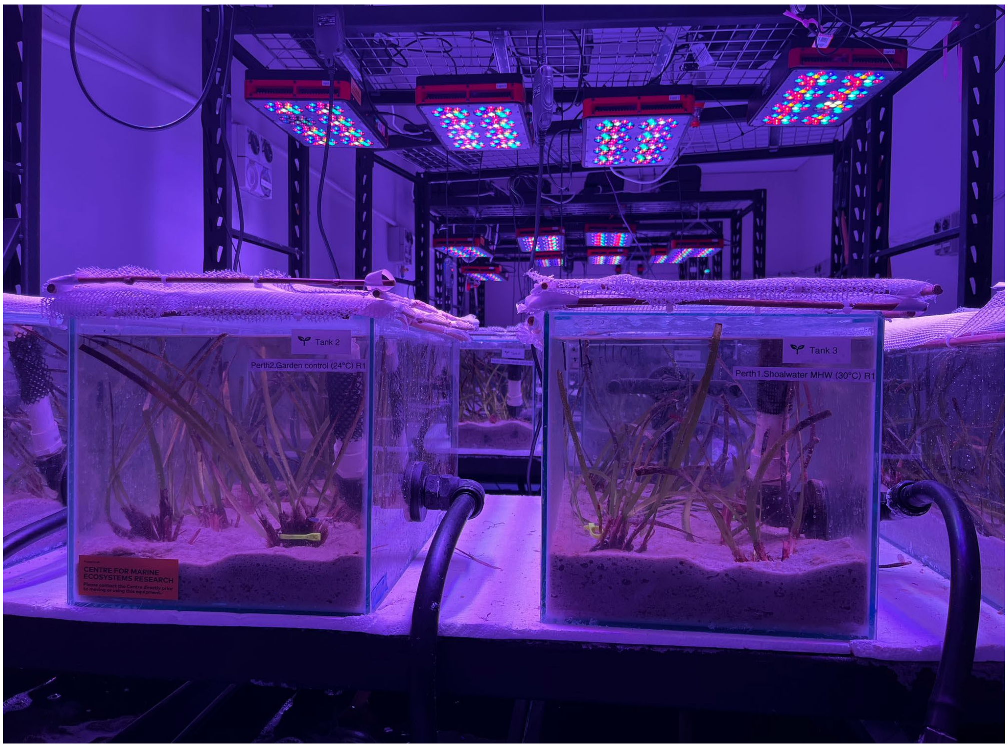

Figure 2: *Posidonia sinuosa* planted in aquarium tanks to test the effects of temperature and duration on the resilience of three different *P. sinuosa* populations: Shoalwater, Garden Island and Jurien Bay.

At the end of the impact phase (Week 4), temperatures in the MHW treatment were decreased from 30 °C to the control temperatures of 24 °C over six days, at a rate of 1 °C per day. Plants in all treatments were maintained at 24 °C for an additional two days and then the recovery phase began (3rd of July2024: Week 5) to assess the recovery potential of *P. sinuosa*. Recovery was assessed after two weeks (17th of July 2024: Week 7) by comparing the condition of the plants in the recovery treatments (exposed to 30 °C during the impact phase and 24 °C during the recovery phase) with plants from the control treatment (exposed to 24 °C during the impact and recovery phases; Table 2 and Figure 3).

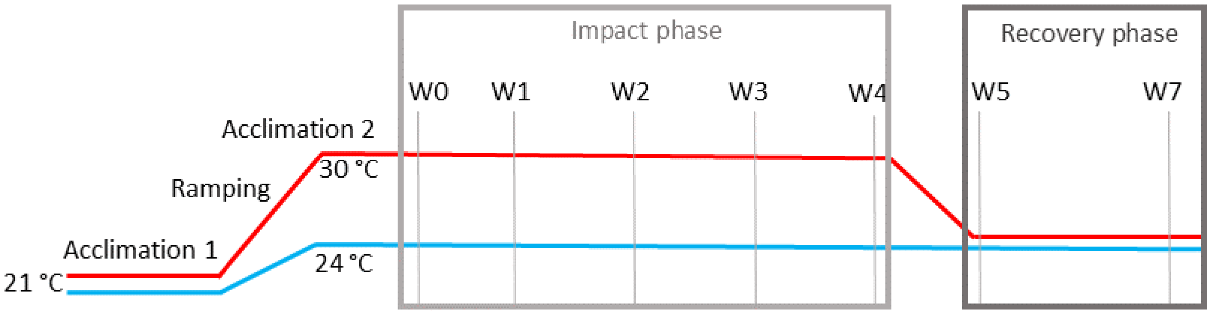

Figure 3: Conceptual diagram of the experiment timeline (impact phase in light grey and recovery phase in dark grey). The control treatment at 24 °C is indicated with the blue line and the MHW temperature treatment at 30 °C with the red line. The experiment started with an initial four days of acclimatization at *in situ* temperatures of 21 °C (Acclimation 1), followed by eight days of ramp-up to achieve target temperatures, then by eight days of acclimating plants at the target temperatures (24 °C and 30 °C) (Acclimation 2) and then the impact phase for 28 days (four weeks). The impact phase was followed by six days of decreasing temperatures to achieve recovery temperatures of 24 °C, two days of acclimation of plants to the recovery temperatures and finally 14 days (two weeks) of the recovery phase of the experiment.

2.4 Response variables

Several response variables were measured over time, with the frequency of measurements varying depending on the response variable assessed (Table 2). For instance, photophysiology measures were taken at the beginning (Week 0), at the first weeks (Week 1 and Week 2) and at the end of the impact (Week 4) and recovery phase (Week 7) as this variable is expected to respond quickly to the experimental temperatures (from days to weeks). Conversely, responses at the organismal (plant) and meadow scale, usually take longer (from weeks to months). Therefore, the response variable of leaf elongation, for example, was measured only at the end of the impact (Week 4) and recovery phase (Week 7; Table 2).

***Table 2**: Response variables and frequency of measurement used to understand the effects of temperature and time (in weeks and days) on three different populations (Shoalwater, Garden Island and Jurien Bay) of the seagrass Posidonia sinuosa*

Biological level	**Indicator grouping**	**Measurement**	**Measurement intervals**					
			Impact phase				**Recovery phase**	
			Week 0 (day 0)	Week 1 (day 7)	Week 2 (day 14)	Week 4 (Day 28)	Week 5 (Day 35)	Week 7 (day 49)
Physiological	Photophysiology (PAM)	Yield and ETR	✓	✓		✓	✓	✓
	Carbohydrates reserves	Rhizome soluble sugars and starch	✓			✓	✓	✓
	Metabolic rates	GPP, NP and R			✓	✓	✓	✓
Plant-scale	Leaf density (% change)	Nº leaves	✓		✓	✓	✓	✓
	Leaf elongation (cm/day -1)	Leaf growth	✓			✓	✓	✓
	Necrosis	Necrosis	✓		✓	✓	✓	✓

2.4.1 Photophysiology

In each aquarium, Chlorophyll *a* fluorescence, parameters were measured in three randomly selected shoots from three independent ramets using a diving Pulse Amplitude Modulated fluorometer (PAM, Walz). The maximum quantum yield (F_v/F_m) of PSII measurements were obtained by placing the fibre optic cable approximately 3 cm above the sheath and using the saturation pulse method after an overnight dark adaptation enabling all reaction centres to be open and oxidisation of all primary electron acceptors (Beer et al., 2001). After being exposed to illumination conditions for one hour, yield measurements for calculating the effective quantum yield (AF/F_m; which is the maximum fluorescence measured during a saturating pulse) and electron transport rate (ETR) was taken after approximately two hours of illumination. ETR represents a measure of productivity relative to the absorbed irradiance and was calculated as per the following formula: ETR = Y x Ei x AF x 0.5, where Y is the yield in light after two hour of illumination, Ei is the incident irradiance (µmol photons m^{-2} s^{-1}), AF is the absorption factor, 0.5 is the fraction of photons absorbed by PSII in plant and data was expressed as µmol electrons m^{-2} s^{-1} (Beer et al., 2014). The absorption factor (AF) was determined following Beer and Bjork (2000) by measuring light in the water column near the leaf (measure 1) and again after placing the same leaf over the light sensor (measure 2) and calculated as a proportion: (measure 1 - measure 2)/ measure 1. The multiple measures of AF within a tank were averaged for each tank at each time of measurement and ranged from 0.62 to 0.98 over the course of the experiment.

2.4.2 Metabolic rates (Net production, Respiration and Gross Primary Production)

Metabolic rates (Net production, Respiration and Gross Primary Production: see Wesselmann et al., 2020 for details) were measured at Week 0, Week 2 and Week 4 (Table 2). At each time point, metabolism was measured using 30-minute incubations (15 minutes dark, 15 minutes light) in four sealed transparent acrylic chambers (1.9 L) with plant material from each of the four tanks from each temperature (n=2) and population (n=3). The chambers were filled with seawater and plant material (two shoots randomly selected within a tank) from their respective population and temperature, along with an additional blank chamber (containing no plant material) and chambers were submerged in a temperature-controlled tank (~300 L) filled with seawater. First, metabolic rates were measured at the control temperature of 24 °C for the three populations and, following this, the temperature was increased to 30 °C and new plants from the MHW treatment from each population were placed in chambers and metabolism was measured at the MHW temperature. FireSting sensors were inserted through the chamber walls to continuously monitor dissolved oxygen (mg O_2) and connected through a four-channel meter to a computer recording mg of O_2 and the internal temperature of the chamber was also measured using a submersible temperature sensor (accuracy ± 0.5 °C) connected to the FireSting O_2 meter. To ensure adequate mixing, water within each chamber was circulated using pumps connected to piping. In order to express metabolic rates per biomass, plants from each incubation were sorted into above-ground (leaves and new sheath) and below-ground biomass (rhizome and roots) and dried in a 60 °C oven for 48 hours and weighed (g) to obtain a dry weight estimate of biomass.

2.4.3 Carbohydrates reserves

Seagrass resilience to the experimental temperatures was measured by analysing carbohydrate levels in rhizomes, which serve as the primary storage for essential soluble carbohydrates (starch and sucrose). These carbohydrates are crucial for sustaining growth and preserving photosynthetic tissues during periods of reduced photosynthesis. The initial carbohydrate levels (Week 0) were compared with those at the end of the impact phase of the experiment (Week 4; Table 2). Rhizome samples from one ramet from each tank was collected (24 samples at the start, Week 0, and 24 samples at the end, Week 4). Samples were dried for 48 h at 60 °C in an oven and grounded into a fine powder for the extraction of non-structural carbohydrates (sucrose and starch) using hot ethanol, followed by analysis using anthrone. Samples of a portion of the dried rhizome material (~5 g) were sent to the University of Queensland and analysed for soluble sugar and starch concentrations and total carbohydrates were calculated as the sum of soluble sugar and starch and expressed as a %.

2.4.4 Organismal responses (plant-meadow scale)

At the beginning (Week 0), middle (Week 2) and end (Week 4) of the impact phase of the experiment and at the beginning (Week 5) and end (Week 7) of the recovery phase, the relative proportion of leaf surface area with necrotic tissue was assessed and expressed as the proportion of necrotic photosynthetic tissue per shoot (Table 2). At the beginning of the experiment (Week 0), the leaves and number of shoots were counted, then summed to derive a total number per pot (total at start). At the beginning, middle and end of the impact phase and at the beginning and end of the recovery phase, leaves and shoots were recounted and then the relative change for both variables were calculated separately (the difference in the totals at the time of harvest and start expressed as a percentage relative to the total at the start). Shoot growth was estimated using the hole punch method (Short and Duarte, 2001). At the beginning of the experiment (Week 0), seagrass shoots were pierced just below the ligule with a needle and shoot growth was estimated as the elongation of leaf tissue in between the ligule and the mark position of all leaves in a shoot at the end of the impact phase of the experiment (Week 4), divided by the experimental duration (28 days). At the beginning of the recovery phase (Week 5), seagrass shoots were pierced again and shoot growth was estimated at the end of the recovery phase (Week 7).

2.4.5 Statistical analysis

Two datasets were created for each experimental phase (impact, recovery) and analysed separately. The impact dataset comprised data collected from three populations (Levels = Shoalwater, Garden Island and Jurien Bay) exposed to two different temperatures (Levels = 24 °C and 30 °C) over different times (Levels = Week 0, Week 1, Week 2 and Week 4). Not all response variables had the same number of duration levels (Table 2). The recovery dataset comprised data collected from three populations (Levels = Shoalwater, Garden Island and Jurien Bay) exposed to 24 °C water temperatures following the impact phase. As only the end phase was included there was no duration factor for this analysis. PERMANOVA analyses were conducted on the impact dataset to test the effects of population, temperature, time, and their interactions on the response of *Posidonia sinuosa*, using a similarity matrix constructed from Euclidean distances between samples. For the recovery dataset, PERMANOVA was applied to examine the effects of population, temperature, and their interaction on the response of *P. sinuosa*, also using a Euclidean distance-based similarity matrix. The experimental units were the replicate tanks (n=4) for each combination of temperature, population, and time. The value for each response variable is the sum or the average for this variable obtained from each tank. Separate univariate PERMANOVA analyses were carried out on each response variable. Primer v7 statistical package and PERMANOVA+ were used for all analyses.

3 Results

3.1 Photophysiology

Electron transport rate (ETR) ranged from 19 to 58 µmol electrons m^{-2} s^{-1} and there was a significant interaction between population, temperature and Time (Weeks; Table 3, Figure 4.1). The ETR declined over time but only under elevated MHW temperatures (30 °C), and the decline was greater in the Shoalwater population (T × W × P, $p < 0.05$, Table 3; Table *1*). Specifically, the Shoalwater population exposed to 30 °C at the end of the experiment (Week 4) exhibited significantly lower ETR values compared to those at 24 °C, as well as compared to the beginning of the experiment (Week 0) at 30 °C. Additionally, the ETR values for Shoalwater at Week 4 were significantly lower than those for the Garden Island and Jurien Bay populations at the same Time point (Week 4; Figure 4.1). Maximum quantum yield (F_v/F_m) ranged from 0.46 to 0.73 and there was a significant effect of both Time ($p<0.05$) and Temperature ($p<0.05$, Table 3). The maximum quantum yield was significantly lower at the end of the experiment (Week 4) compared to the beginning (Week 0) and also lower under MHW temperatures (Figure 4.2).

At the end of the recovery phase of the experiment, the electron transport rate was significantly lower in the plants that had been exposed to the elevated MHW ($p<0.05$) and there were no differences between populations. In fact, compared to the impact phase, the Garden Island and Jurien Bay populations declined further so that their ETRs were similar to those of the Shoalwater population. In contrast, for the maximum quantum yield (Fv/Fm), there was a significant interaction between Temperature and Population ($p<0.05$) at the end of the recovery period. The maximum quantum yield was significantly lower, but only for the Shoalwater population for plants that had been exposed to the MHW. Compared to the impact phase, there was a decline in maximum quantum yield in the control temperature treatments for Garden Island and Jurien Bay but an increase for these same populations in the plants that had been exposed to the MHW.

3.2 Metabolic rates

Metabolic rates of *P. sinuosa* during the impact phase of the experiment were also affected by the experimental factors. Net Production (NP) ranged between -0.7 to 0.8 mg O_2 g DW^{-1} h^{-1} and there was a significant interaction between Time (Weeks) and Temperature (W X T, $p<0.05$; Table 3) and Population and Temperature (P X T, $p<0.05$; Table 3). Net production (NP) was significantly lower after two weeks (Week 2) and by the end of the experiment (Week 4), declined further, but only under MHW temperatures (30 °C; Figure 5.1). Additionally, NP for the Shoalwater and Jurien Bay populations was significantly reduced under MHW conditions (30 °C) compared to control temperatures, but this reduction was not observed in the Garden Island population (Figure 5.1). This decrease in NP for the Shoalwater and Jurien Bay population in the MHW treatment corresponds with a decline over the Time in the below-ground to above-ground ratio of plant biomass used to measure the metabolic rates (Supplementary Figure *1*, Supplementary Figure 2 and Supplementary Table 1).

Respiration (R) ranged between -0.05 to -1.6 mg O_2 DW^{-1} h^{-1} with a significant effect of Time (Weeks) and Temperature ($p<0.05$) (Figure 5.2). Plants exposed to MHW conditions (30 °C) consumed more oxygen than those kept at control temperatures, while plants in the middle (Week 2) and end (Week 4) of the experiment consumed more oxygen than at the beginning of the experiment (Week 0; Figure 5.2).

During the recovery phase, the NP of *P. sinuosa* was significantly affected by Population and Temperature ($p<0.05$, Table 4). NP was still significantly lower after decreasing the temperature of the MHW treatments (30 °C) to the recovery temperatures (24 °C) for two weeks, indicating that plants previously exposed to MHW did not fully recover to the NP levels of plants exposed to control temperatures. Moreover, the population of Shoalwater and Garden Island presented significantly lower NP at both control and recovery temperatures compared to the population of Jurien Bay (Figure 5.4 and Table 4). Despite differences at the end of the recovery phase in respiration rates, there were no significant differences between control temperatures and recovery temperatures for any of the three populations (Figure 5.5, Table 4).

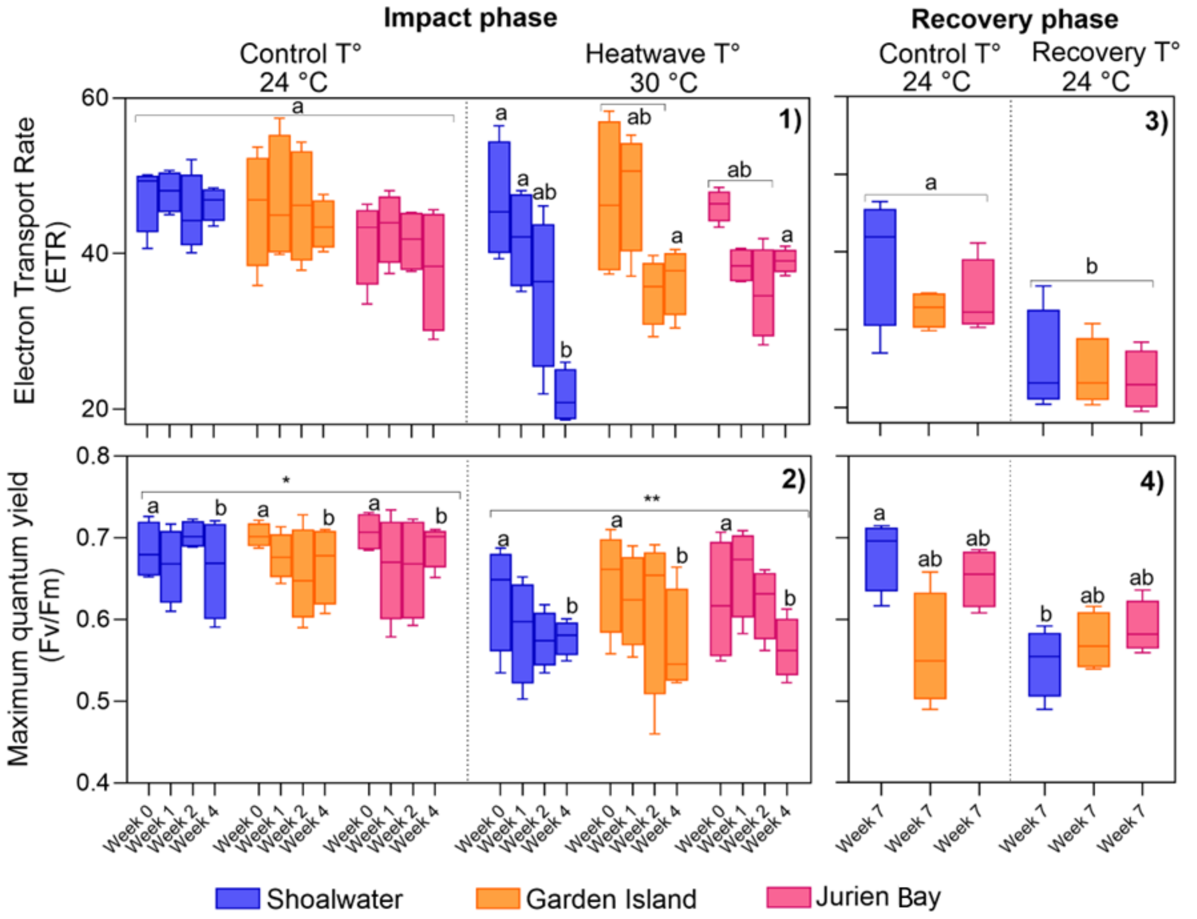

Figure 4: Effects of population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; MHW at 30 °C) tested on the photophysiology of *Posidonia sinuosa* for the impact and recovery phase (which were analysed separately). Numbers correspond to response variables: (1) electron transport rate (ETR) and (2) maximum quantum yield (Fv/Fm) for the impact phase, and (3, 4) for the recovery phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA for the impact phase and two-way PERMANOVA for the recovery phase.

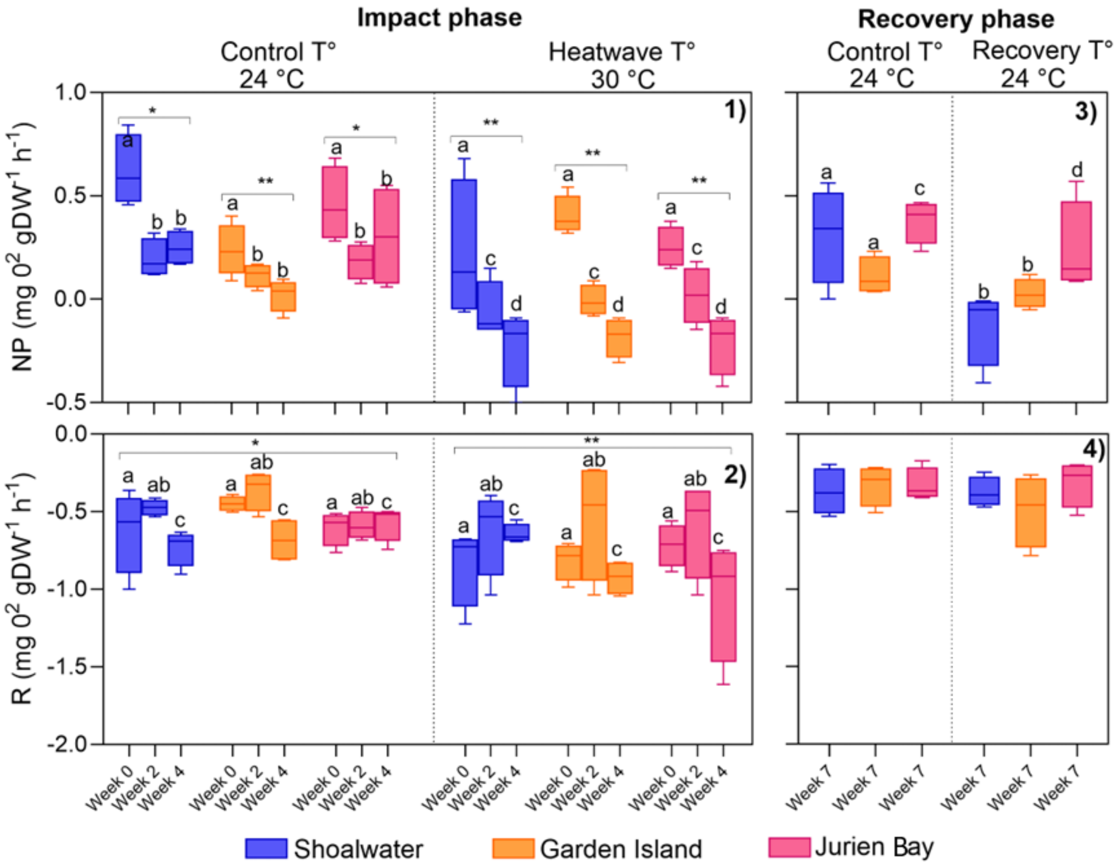

Figure 5: Effects of population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; MHW at 30 °C) tested on the metabolic rates of *Posidonia sinuosa* for the impact and recovery phase (which were analysed separately). Numbers correspond to response variables: (1) Net Production (NP) and (2) Respiration (R) for the impact phase, and (3, 4) for the recovery phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA for the impact phase and two-way PERMANOVA for the recovery phase.

3.3 Carbohydrates reserves

Carbohydrate reserves of *P. sinuosa* during the impact phase of the experiment was affected by the experimental factors, either Time (in weeks) only, or Temperature only, or interactions of these factors with population (Table 3). Total carbohydrates ranged between 25 and 55 % DW with a significant interaction between Time (in weeks) and Temperature (W x T, $p<0.05$, Table 3). Total carbohydrates under MHW temperatures were significantly higher at the end of the experiment (Week 4) than at the beginning (Week 0; Figure 6.3; Table 3). Sugars ranged between 21 and 42 (±2) % DW and was affected by a significant interaction between Time (Weeks) and Temperature (W X T, $p<0.05$, Table 3), with a significant increase in sugars at the end of the experiment (Week 4) than at the beginning (Week 0) under MHW conditions (Supplementary Figure 2 and Supplementary Table 1). Starch ranged between 1 and 19 % with a significant single effect for the factor Population, Time (in weeks) and Temperature ($p<0.05$, Table 3, Supplementary Figure 2 and Supplementary Table 1). Starch was significantly higher for the population of Shoalwater than for the population of Garden Island and Jurien Bay. Further, the amount of starch was significantly higher under MHW temperatures than under control temperatures and higher at the end of the experiment than at the beginning (Supplementary Figure 2: Effects of

Population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; Heatwave at 30 °C) tested on the carbohydrate reserves of Posidonia sinuosa for the impact phase. Numbers correspond to response variables: (1) Sugars and (2) Starch for the impact phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA (Supplementary Table 1 and Supplementary Figure 2).

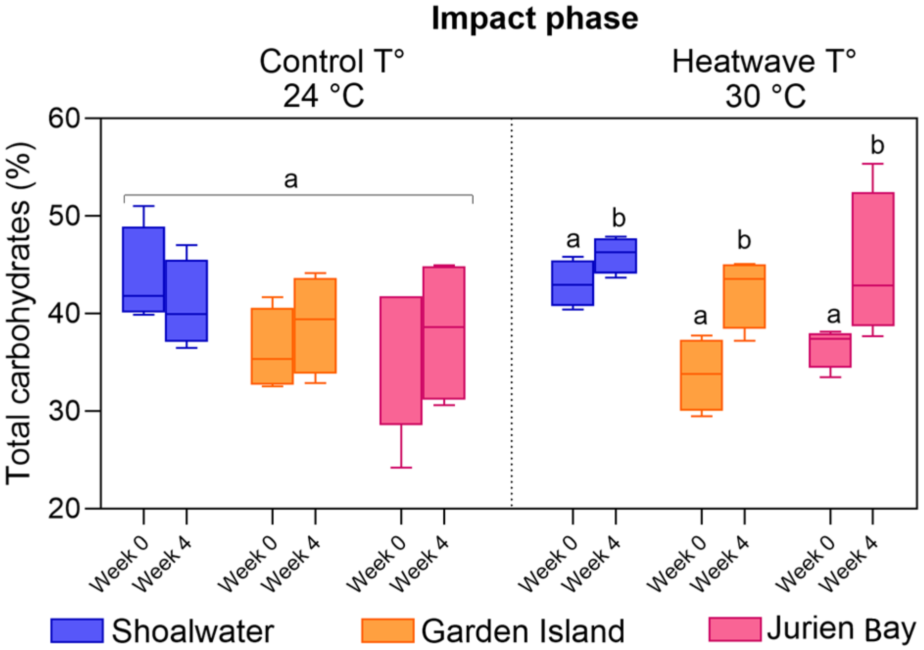

Figure 6: Effects of Population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1 2 and 4), and Temperature (Control at 24 °C; MHW at 30 °C) tested on the total carbohydrate reserved of *Posidonia sinuosa* for the impact phase. Letters on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA

3.4 Plant-scale responses

Plant-scale responses of *P. sinuosa* during the impact phase of the experiment were affected by the experimental factors, either Time (in weeks) only or Temperature only, or interactions of these factors with Population (Table 3). Necrosis ranged between 5 to 58 % and increased significantly over Time, with necrosis significantly increasing over Time from Week 0 to Week 2 and again in Week 4 ($p<0.05$, Table 3). In addition, necrosis was also significantly affected by an interaction between Population and Temperature (P X T, $p<0.05$, Table 3), with Garden Island having lower necrosis under control temperatures than Shoalwater and Jurien Bay (Figure 7.1). Leaf density (% change) ranged between 58 to 117 % and was significantly reduced under MHW temperatures compared to control temperatures (Figure 7.2). Growth measured as leaf elongation ranged between 0.1 to 0.3 cm day^{-1} with a significant interaction between Population and Temperature (P x T, $p<0.05$, Table 3). Shoalwater and Garden Island had significantly lower leaf elongation rates under MHW temperatures (30 °C) than under control temperatures. In addition, under MHW conditions, Shoalwater had lower leaf elongation rates than Garden Island and Jurien Bay (Figure 7.3 and Table 3).

During the recovery phase, no significant differences were found between control temperatures and recovery temperatures for any of the three populations for necrosis (Figure 7.4) and leaf density (Figure 7.5, Table 4). Conversely, Leaf elongation was still significantly lower after decreasing MHW treatments (30 °C) to recovery temperatures (24 °C) for 2 weeks (p<0.05), indicating that plants previously exposed to MHW treatments did not fully recover to the leaf elongation levels of plants exposed to control temperatures (Figure 7.6, Table 4).

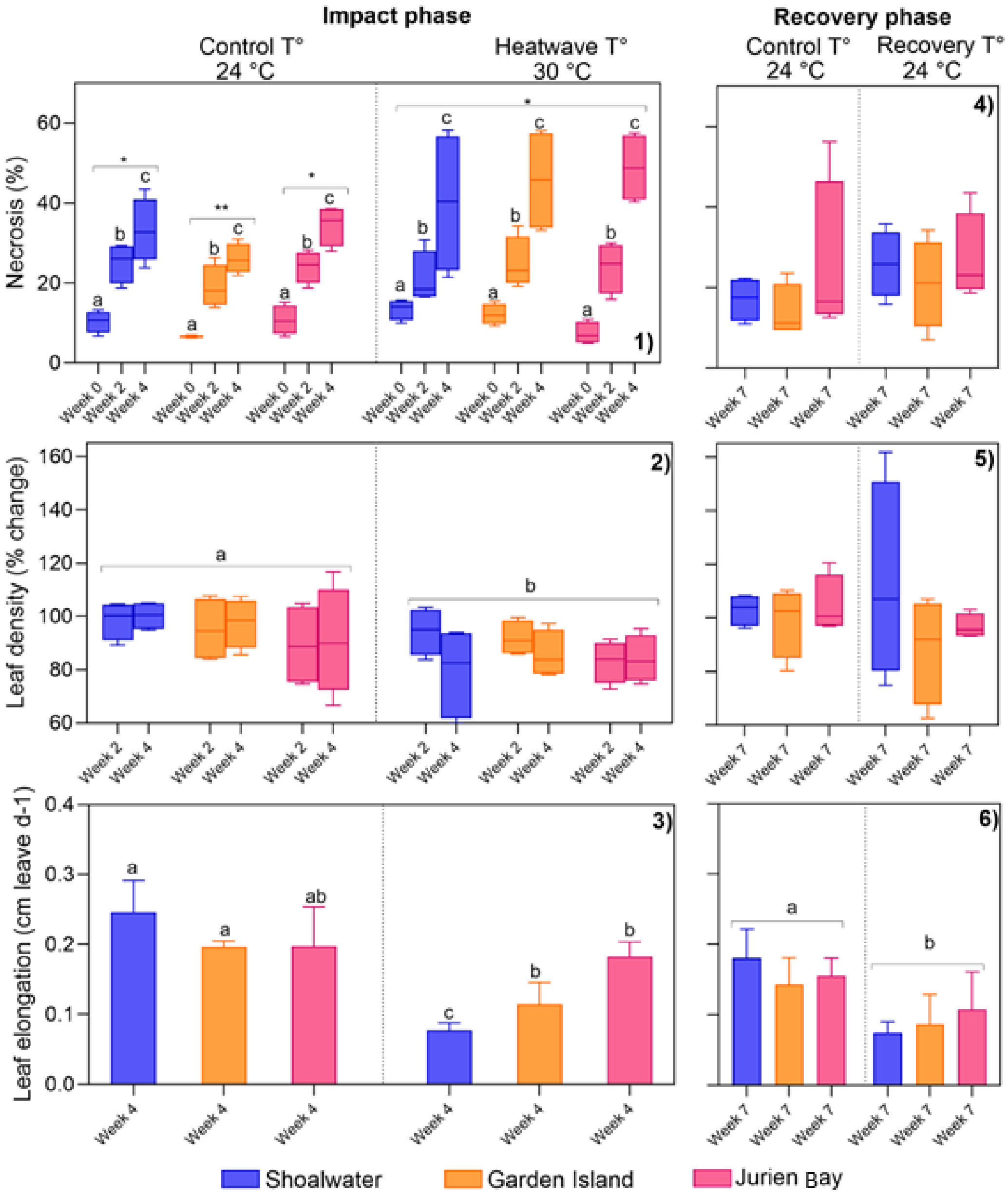

Figure 7: Effects of Population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; MHW at 30 °C) on organismal/meadow-scale responses of *Posidonia sinuosa* for the impact and recovery phase (which were analysed separately). Numbers correspond to response variables: (1) Necrosis (%), (2) Leaf density (% change), (3) Leaf elongation (cm day^{-1}) for the impact phase, and (4, 5, 6) for the recovery phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA for the impact phase and two-way PERMANOVA for the recovery phase.

Table 3: Summary of PERMANOVA mains test outcomes examining the effects of Population (Shoalwater, Garden Island and Jurien Bay), Time (Week 0, 1, 2 and 4) and Temperature (24 °C and 30 °C) on response variables of adult *Posidonia sinuosa* during the impact phase of the experiment. Significant comparisons are indicated in bold text ($p < 0.05$), n.s. = not significant.

	Max. yield (F_v/F_m)			**ETR**			**NP**		
Source	df	SS	p-value	df	SS	p-value	df	SS	p-value
Population (P)	2	0.0029	n.s.	2	183.22	n.s.	2	0.00661	n.s
Week (W)	3	0.0233	**0.0484**	3	1098.6	**0.0001**	2	0.27569	**0.0001**
Temperature (T)	1	0.1199	**0.0001**	1	662.78	**0.0001**	1	0.18127	**0.0001**
P x W	6	0.0041	n.s.	6	226.7	n.s.	4	0.00369	n.s
P x T	2	0.0029	n.s.	2	343.19	**0.01**	2	0.04722	**0.004**
W x T	3	0.0103	n.s.	3	559.42	**0.0021**	2	0.0578	**0.001**
P x W x T	6	0.0163	n.s.	6	484.83	**0.0403**	4	0.01226	n.s
Residual	72	0.2035		72	2507.4		54	0.20303	
Total	95	0.3836		95	6066.2		71	0.78757	

	R			**Total carbohydrates**			**Necrosis**		
Source	df	SS	p-value	df	SS	p-value	df	SS	p-value
Population (P)	2	0.044991	n.s	2	0.69123	n.s.	2	0.0309	n.s.
Week (W)	2	0.56151	**0.0035**	1	18.109	n.s.	2	4.1605	**0.0001**
Temperature (T)	1	0.53714	**0.0009**	1	1.6956	n.s.	1	0.10442	**0.0062**
P x W	4	0.19348	n.s	2	46.879	n.s.	4	0.09053	n.s.
P x T	2	0.13701	n.s	2	22.183	n.s.	2	0.15478	**0.0052**
W x T	2	0.08746	n.s	1	83.774	**0.0266**	2	0.05718	n.s.
P x W x T	4	0.28425	n.s	2	10.467	n.s.	4	0.10685	n.s.
Residual	54	2.5246		36	561.76		54	0.70773	
Total	71	4.3704		47	745.56		71	5.4129	

	Leaf density			**Leaf elongation**		
Source	df	SS	p-value	df	SS	p-value
Population (P)	2	394.69	n.s.	2	0.00538	0.1165
Week (W)	1	71.103	n.s.			
Temperature (T)	1	940.62	**0.0104**	1	0.04697	**0.0001**
P x W	2	127.72	n.s.			
P x T	2	84.01	n.s.	2	0.02388	**0.001**
W x T	1	215.91	n.s.			
P x W x T	2	126.18	n.s.			
Residual	36	4759.6		18	0.02055	
Total	47	6719.8		23	0.09678	

Table 4: Summary of PERMANOVA mains test outcomes examining the effects of Population (Shoalwater, Garden Island and Jurien Bay) and Temperature (24 °C and 30 °C) on response variables of adult *Posidonia sinuosa* during the recovery phase of the experiment. Significant comparisons are indicated in bold text ($p < 0.05$), n.s. = not significant.

	Max. yield (F_v/F_m)			**Effect. Yield (AF/Fm')**			**NP**		
Source	df	SS	p-value	df	SS	p-value	df	SS	p-value
Population (P)	2	0.01369	n.s.	2	79.631	n.s.	2	0.28079	**0.0137**
Temperature (T)	1	0.02243	**0.0041**	1	707.43	**0.0003**	1	0.29669	**0.0044**
P x T	2	0.02088	**0.0203**	2	30.57	n.s.	2	0.14604	n.s.
Residual	18	0.03721		18	549.3		18	0.49584	
Total	23	0.0942		23	1366.9		23	1.2194	

	R			**Necrosis**			**Leaf density**		
Source	df	SS	p-value	df	SS	p-value	df	SS	p-value
Population (P)	2	0.03092	n.s.	2	316.29	n.s.	2	801.8	n.s.
Temperature (T)	1	0.01599	n.s.	1	202.57	n.s.	1	50.774	n.s.
P x T	2	0.03774	n.s.	2	55.082	n.s.	2	473.79	n.s.
Residual	18	0.41795		18	2449.5		18	6344.4	
Total	23	0.5026		23	3023.4		23	7670.8	

	Leaf elongation		
Source	df	SS	p-value
Population (P)	2	0.00123	n.s
Temperature (T)	1	0.02944	**0.0009**
P x T	2	0.00391	n.s.
Residual	18	0.02622	
Total	23	0.06081	

4 Discussion

This study demonstrates population differences in the response and recovery of *Posidonia sinuos*a to a prolonged Marine Heatwave (MHW) of 30 °C over four weeks. Among the three populations studied, the population growing in the warmer waters of Jurien Bay, with the highest thermal optima (31 °C), exhibited greater capacity to cope with the MHW, maintaining physiological and growth functions more effectively under MHW conditions. In contrast, the Shoalwater population from the cooler region of Perth, with the lowest thermal optima (26 °C), was more vulnerable to the MHW, as indicated by reductions in photosynthetic performance and growth rates. The Garden Island population, also from the cooler region of Perth but with a higher thermal optimum than Shoalwater (30 °C), displayed intermediate resilience, with greater capacity to cope with the prolonged MHW event than Shoalwater but not as robust as Jurien Bay, highlighting that traits other than thermal optima may be influencing the resilience to MHW (e.g. genetic diversity). These findings demonstrate that the thermal thresholds (thermal optima and maxima) obtained for photosynthesis from short term experiments (one day; Said et al., 2024) can be used as a predictive tool to forecast the resilience or vulnerability of seagrass species and populations to future MHW and warming conditions. This improved understanding can inform targeted management strategies, such as assisted migration of thermally resilient populations, like Jurien Bay, to vulnerable areas, to support the long-term sustainability of seagrass meadows ensuring they will be able to withstand future warming conditions.

4.1 Effect of marine heatwave differs between *P. sinuosa* population

The population specific responses of *Posidonia sinuosa* to simulated MHW conditions (30 °C) for four weeks were evidenced in the photophysiology, metabolism and growth, in the impact and the recovery phase. At the end of the 4-week MHW treatment, the population with the lowest thermal optima, Shoalwater (T_{opt}: 26 °C) had a significantly lower electron transport rate (ETR), a proxy for photosynthesis, than the other two populations, as well as a lower growth rate. The greater tolerance in Jurien Bay (T_{opt}: 31 °C) was due to no observed declines in leaf growth under the MHW conditions compared to the control. Interestingly, the Garden Island population with a similar thermal optima (Topt: 30° C) to Jurien Bay, but close to the Shoalwater population in Perth waters, did show significantly lower leaf elongation rates under MHW conditions compared to controls. Leaf elongation is a critical measure of seagrass health, and it reflects the plant's ability to maintain tissue growth and regenerative capacity under stress (Short & Duarte, 2001). These differences suggest that populations coming from cooler environments may be more susceptible to impacts from a MHW event. But the relationship is more complex, as tolerance was also explained by the difference in photosynthetic thermal optima for the two populations in Perth waters, where the Garden Island population (Topt: 30 °C) was not as impacted as Shoalwater (Topt: 26 °C). Although there were population specific responses to the MHW impact from some variables, this was not always the case, with other response variables showing a consistent decline under simulated MHW conditions (e.g. decrease in maximum quantum yield, net production, necrosis, leaf density and increase in respiration and carbohydrate stores), indicating a negative impact on *P. sinuosa* plants.

At the end of the recovery phase some variables either showed a return to control conditions, were on a trajectory of recovery similar to the control group, showed no improvement from the end of the impact phase or continued to decline. Net production was the only response variable showing an increase in condition with duration, with population specific differences. The Jurien Bay population showed the greatest improvement, there was some recovery in the Garden Island and none in the Shoalwater population. The other response variables that had population specific differences were photosynthetic measures that continued to decline or showed no change during the recovery phase.

There was a greater reduction in the maximum quantum yield at Shoalwater and no improvement at the other two locations. In contrast, for the electron transport rate, there was a lag effect, with ETRs at Garden Island and Jurien Bay declining further during the recovery phase. These responses continue to support the hypothesis that Shoalwater, with the lowest thermal optima, is less resilient (less resistant and lower recovery) to MHWs compared to Jurien Bay (more resistant, greater recovery) and Garden Island is intermediate to the two. Although the response variable of leaf growth did not show population specific differences following the recovery phase, growth rates were lower in all populations, showing a continued negative effect of the MHW. However, there were no continued negative effects as measured by respiration, necrosis or leaf loss.

The greater resilience and recovery capacity of the warmer population of Jurien Bay compared to the colder population of Shoalwater under MHW conditions is consistent with previous studies showing that seagrass populations from warmer environments generally tolerate better elevated temperatures than those from colder environments (Beca Carretero et al., 2018: Franssen et al., 2014; Marin-Guirao et al., 2018; Said et al., 2024), following the hotter is better hypothesis (Knies et al., 2009). For instance, *Zostera marina* populations from Spain, in warmer environments, showed a higher and stronger activation of heat expression genes than populations from colder environments, reflecting higher thermal tolerance (Franssen et al., 2014). Similarly, *Halophila stipulacea* in the warmer environment of the Red Sea has been found to grow better at higher seawater temperatures than populations of this seagrass species living in cooler environments in the Mediterranean Sea (Wesselmann et al., 2020). *Posidonia oceanica* and *Cymodocea nodosa* from warmer environments exhibited a greater capacity to maintain the carbon balance, photosynthesis to respiration ratio (Marin-Guirao et al., 2018) and adjust their lipid components in response to elevated temperatures (Beca-Carretero et al., 2018) compared to plants from cooler environments.

Therefore, the results of this study, together with the above-mentioned outcomes of previous studies, demonstrates that seagrass populations within warmer environments are generally more resilient to elevated temperature conditions (e.g. MHW), which is a pattern that has been associated with the higher expression of heat-responsive genes in other marine organisms such as corals (Barshis et al., 2013). However, some contrasting results have been observed with some seagrass species, such as *P. oceanica* (Bennet et al., 2022a, 2022b), suggesting that other factors beyond exposure to local SST may influence seagrass thermal tolerance. This is reinforced by the fact that this study found that two *P. sinuosa* populations (Shoalwater and Garden Island) from a similar thermal regime responded differently to the simulated MHW, although this response was partly explained by photosynthetic thermal optima, other factors such as genetic diversity of the population might explain the resilience of specific seagrass populations (Williams, 2001: Hughes and Stachowicz, 2004). Even though the populations collected in this study were taken from slightly different depths (2m for Shoalwater, 4-5 m for Garden Island and Jurien Bay), we do not expect depth to have signifficantly influenced their resilience to the simulated MHW conditions. According to the literature, seagrass plants from shallower populations usually show greater resilience to elevated temperatures than deeper populations due to their exposure to higher and more variable temperatures (as observed for *P. oceanica* meadows from 5m vs 25 m depth; Marin-Guirao et al., 2016). However, our results indicate the opposite scenario, as the shallowest population (Shoalwater) showed lower resilience to MHW conditions, suggesting that depth was not a major factor in their response to MHW conditions.

4.2 Thermal optima reflect seagrass resilience to prolonged marine heatwaves

This study accepts the null hypothesis that seagrass populations with lower T_{opt} (e.g. Shoalwater) are less resilient to MHW events and will experience greater and earlier impacts and slower recovery across multiple biological levels (physiological, plant-scale) compared to populations with higher T_{opt} (Garden Island and Jurien Bay). This indicates that short-term experiments used to obtain the thermal optima of oxygen production across species and locations (Said et al., 2024) can be used to predict the resilience of seagrass to MHW events and identify the most vulnerable populations/species. However, the thermal optima obtained may reflect the optimum temperature the plants can handle over a short period of time, while plant overall resilience is determined over a longer period at their optimum temperature (after more than 2-4 weeks), as indicated by a decrease in the photosynthetic efficiency, oxygen production, increase in carbohydrate storage and decrease in growth. Moreover, the thermal optima generated the Said et al. (2024) study were for oxygen production, where photosynthesis has a higher thermal tolerance than other processes such as growth (Lee et al., 2007).

4.3 Effect of marine heatwave on *P. sinuosa* differs between biological parameters

The response of *Posidonia sinuosa* to MHW conditions varied across biological parameters (e.g., physiological vs. growth responses) and the duration of exposure (e.g., Week 2 vs Week 4). After two weeks of elevated temperature exposure (30 °C), *P. sinuosa* from all populations were less productive, as indicated by lower net oxygen production. This reflects a decrease in the oxygen plants are producing relative to the oxygen they are consuming, as mitochondrial respiration rates increase at higher temperatures (Atkin & Tjoelker, 2003). Necrosis of leaves also increased significantly after two weeks in all populations, meaning that the amount of photosynthetic material decreased, which combined with an increase in the respiration, could further explain the decrease in the net oxygen production. After four weeks at 30 °C, persistent damage to the photosynthetic apparatus of *P. sinuosa* from all populations became evident through a significant decline in maximum quantum yield, supporting prior findings that MHW conditions impact photosystem II (PSII) in the long term (Gao et al., 2019: Beer, Björk, & Beardall, 2014). Net production continued to decrease, and respiration rates were significantly higher than at the beginning of the experiments. However, respiration rates increased under both control and MHW conditions, possibly due to the oxygen consumed by epiphytes growing on seagrass leaves due to the experimental conditions. Moreover, respiration involves the breakdown of complex molecules such as carbohydrates to support consumption of oxygen (Williams et al., 2002). As carbohydrates reserves increased at the end of the experiment for all populations, *P. sinuosa* may have increased respiration to access soluble sugars to obtain energy, carbon dioxide and water during stress events, such as MHW's (Longstaff and Dennison, 1999). Although this somewhat contradicts the increase in carbohydrate stores observed in the rhizomes, it does align with previous research where the slow growing seagrass *P. oceanica* increased carbohydrate storage after a MHW at 27-29 °C for six weeks and reduced leaf growth, as the carbon fraction stored as carbohydrate reserves came from the fraction of photosynthetically fixed carbon that was no longer invested in growth (Marin-Guirao et al., 2018). Increase in the carbohydrate storage is linked to an increased metabolic consumption of plant resources to cope with elevated temperature (Sala et al., 2012). In this way, it can be considered a response to allocate photosynthetic production into rhizome carbohydrate stores to prepare for future stressful conditions.

The exposure to MHW temperatures also led to an increase in the necrotic tissue from 30 % to 50 % (compared to control treatments) and reductions in leaf density, aligning with previous research showing that high temperatures have a stronger effect on above-ground parts of seagrasses (George et al., 2018). The observed decline in the photophysiological performance and oxygen production of plants exposed to MHW conditions likely reduced internal oxygen transport to non-photosynthetic tissues, causing degradation basal leaf meristems (Nagel 2007) and may likely account for the increased leaf loss under MHW temperatures. In fact, shoot and leaf loss is a survival strategy to reduce respiratory load when photosynthesis can no longer sustain a positive carbon balance and has been frequently observed during seagrass mortality events (Borum et al., 2007). Therefore, even though some populations during the MHW simulated in this study approached their thermal optima (T_{opt}: 30 °C for Garden Island; T_{opt}: 31 °C for Jurien Bay; Said et al., 2024), the decline in photosynthetic efficiency, oxygen production, leaf loss and the increase in the carbohydrate reserves (specially starch) suggest that plants were unable to tolerate these temperatures beyond two to four weeks.

Recovery of plants was measured after exposing plants to ambient temperatures (24 °C) for two weeks after the marine MHW of 30 °C for four weeks. Respiration, leaf density, and tissue necrosis after this recovery period at 24 °C were comparable to those of control plants that had not previously been exposed to the MHW. However, *P. sinuosa* plants did not fully recover as measured by photosynthetic performance (as indicated by lower electron transport rates), net oxygen production, and leaf elongation rates. This lack of recovery suggests an extended impact on plants two weeks following exposure to MHW conditions (30 °C) for one month. The results indicate that *P. sinuosa* populations may either require longer recovery periods following a prolonged MHW or may not fully recover from such stress. Similarly, *P. australis* did not recover when exposed to ambient temperatures for one week following a simulated MHW (31 °C) for two weeks, as demonstrated by the relative metabolite abundance that did not return to control levels (Jung et al., 2023). The persistence of the observed reduced growth rates of *P. sinuosa* even after two weeks at ambient temperatures after MHW exposure aligns with previous research indicating that shoot and leaf recovery of slow growing seagrass species occur over longer scales (Kilminster et al., 2015; Kendrick et al., 2019) and implies potential long-term effects on seagrass persistence and meadow-scale processes, particularly as MHW events become more frequent and intense in the future (Oliver et al., 2018; 2019). However, we cannot rule out that *P. sinuosa* would not recover after a MHW of 30 °C for a month with a longer recovery period (> 2 weeks), as recovery of *P. oceanica* was observed after a recovery period of six weeks following a marine MHW of 27-29 °C (Marin-Guirao et al., 2018).

The exposure and recovery from extended MHW conditions (30 °C for four weeks) was detrimental for all three *P. sinuosa* populations at several biological levels. Even the more resilient population from Jurien Bay, demonstrated clear signs of physiological stress. Specifically, this population experienced declines in photosynthetic performance (evidenced by a lower maximum quantum yield), reduced net oxygen production, decreased leaf density, and increased respiration after four weeks of exposure. These findings suggest that prolonged exposure to this simulated MHW, even at temperatures near its thermal optimum, had detrimental effects on *P. sinuosa* and aligns with prior studies showing increased respiration rates and a shift in carbon balance as temperatures approach or exceed thermal optima for seagrass species (Wesselmann et al., 2020).

4.4 Management implications

The findings of this study confirmed that populations from warmer regions with higher thermal optima (e.g. Jurien Bay) are more resilient to cope with MHWs than populations from cooler regions with lower thermal optima (e.g. Shoalwater). Therefore, conceptually, Jurien Bay plants could serve as source populations for translocations to Cockburn Sound, enhancing gene flow for increased thermal tolerance under future warming conditions. The translocation of adults with warm adapted genotypes should be sourced from existing genetic boundaries to maintain a variety of locally adapted genotypes and preserving genetic diversity (Sera, 2017). Overall, this study has led to a deeper understanding of MHW impacts on *P. sinuosa* populations and enables the development of evidence-based solutions to enhance the resilience of seagrass meadows. By identifying which populations will be vulnerable or resilient to future warming conditions, this work informs efforts to future proof seagrass ecosystems, increasing their resilience to future warming conditions (McLeod et al. 2019).

5 Conclusions/recommendations

This study demonstrates that the resilience of *Posidonia sinuosa* populations to MHW is closely linked to their thermal optima obtained for photosynthesis, with significant implications for their capacity to withstand and recover from MHW. Populations from warmer regions, such as Jurien Bay, exhibited higher resilience and recovery potential compared to those from cooler areas, like Shoalwater. However, the intermediate performance of the Garden Island population highlights the complexity of other factors influencing resilience (e.g. genetic diversity). The findings of this study improve our understanding of MHW impacts on *P. sinuosa* and provides opportunities to implement proactive conservation management strategies by identifying populations with greater resilience to MHWs (Jurien Bay) and undertaking 'assisted migration' to Cockburn Sound to future proof seagrass meadows and enhance their capacity to withstand future warming conditions. As MHWs are expected to increase in frequency and intensity in the future, proactive measures based on scientific knowledge, like this study, will be crucial for preserving seagrass ecosystem functions and services. While the data generated from this study progresses our understanding of the resilience of different populations of seagrass to MHWs, it is recommended to explore further additional factors that may contribute seagrass resilience to MHW (e.g. genetic diversity) and how seagrass resilience may vary between individuals of the same population.

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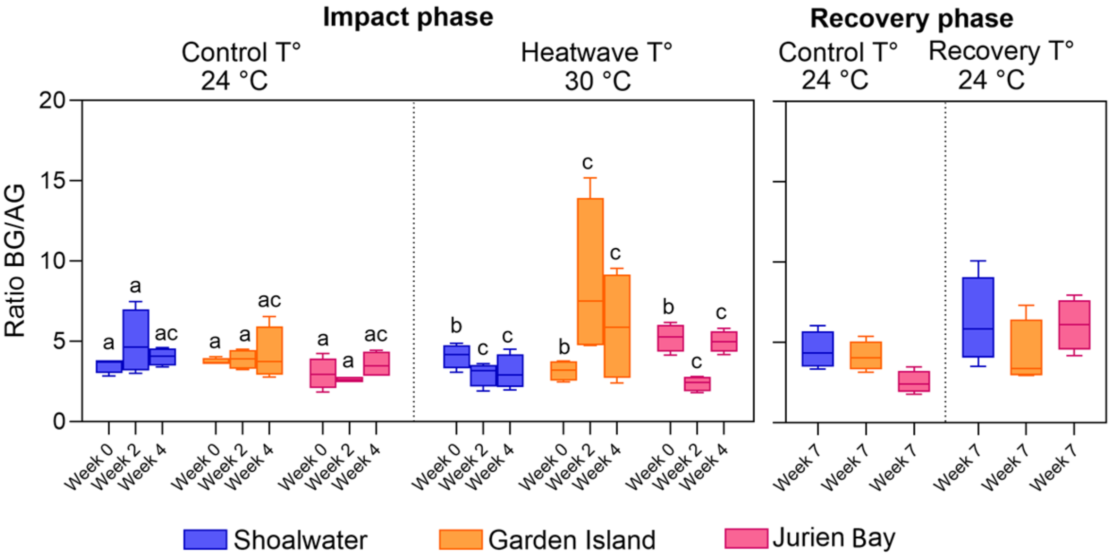
7 Appendices

Supplementary Table 1: Summary of PERMANOVA mains test outcomes examining the effects of Population (Shoalwater, Garden Island and Jurien Bay), Time (Week 0, 1, 2 and 4) and Temperature (24 °C and 30 °C) on the ratio AG:BG used during metabolic incubations of adult *Posidonia sinuosa*, and sugars and starch reserves of rhizomes during the impact phase of the experiment. Significant comparisons are indicated in bold text ($p < 0.05$), n.s. = not significant.

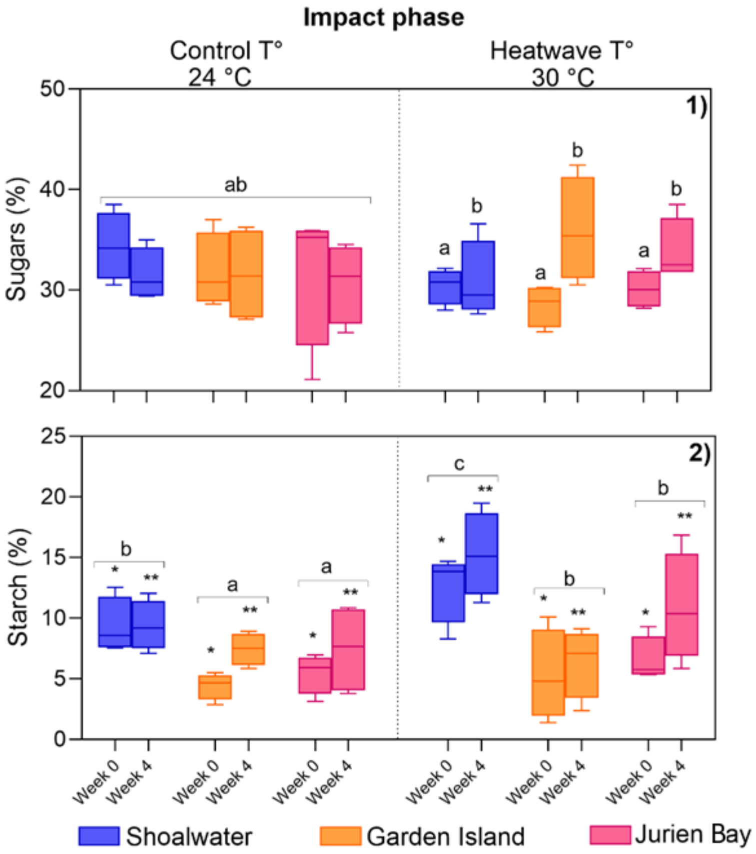
	Ratio BG: AG			**Sugars**			**Starch**		
Source	df	SS	p-value	df	SS	p-value	df	SS	p-value
Population (P)	2	0.0407	**0.0653**	2	269.34	**0.011**	2	279.24	**0.0001**
Week (W)	2	0.1885	**0.0002**	1	140.31	**0.0246**	1	57.605	**0.0117**
Temperature (T)	1	0.0029	n.s	1	41.948	n.s.	1	60.511	**0.0089**
P x W	4	0.0047	n.s	2	69.17	n.s.	2	6.9084	n.s.
P x T	2	0.0010	n.s	2	13.019	n.s.	2	44.235	n.s.
W x T	2	0.1422	**0.0002**	1	118.44	**0.043**	1	2.9937	n.s.
P x W x T	4	0.0307	n.s	2	1.2571	n.s.	2	12.459	n.s.
Residual	54	0.3816		36	964.78		36	286.88	
Total	71	0.7923		47	1618.3		47	750.83	

Supplementary Table 2: Summary of PERMANOVA mains test outcomes examining the effects of population (Shoalwater, Garden Island and Jurien Bay) and temperature (24 °C and 30 °C) on the ratio AG:BG used during metabolic incubations of adult *Posidonia sinuosa* during the recovery phase of the experiment. Significant comparisons are indicated in bold text ($p < 0.05$), n.s. = not significant.

		Ratio BG: AG	
Source	df	SS	p-value
Population (P)	2	1239.7	n.s.
Temperature (T)	1	2243.2	n.s.
P x T	2	1035.5	n.s.
Residual	18	22700	
Total	23	27219	



Supplementary Figure 1: Effects of Population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; Heatwave at 30 °C) tested on the below-ground to above-ground ratio of *Posidonia sinuosa* during the metabolic incubations for the impact and recovery phase (which were analysed separately). Numbers correspond to response variables: (1) electron transport rate (ETR) and (2) maximum quantum yield (Fv/Fm) for the impact phase, and (3, 4) for the recovery phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA for the impact phase and two-way PERMANOVA for the recovery phase.



***Supplementary Figure 2**: Effects of Population (Shoalwater, Garden Island and Jurien Bay), Time of experiment (Week 0, 1, 2 and 4), and Temperature (Control at 24 °C; Heatwave at 30 °C) tested on the carbohydrate reserves of Posidonia sinuosa for the impact phase. Numbers correspond to response variables: (1) Sugars and (2) Starch for the impact phase. Letters and * on graphs represent the significant pairwise comparisons for factor(s) or interactions determined by three-way PERMANOVA*

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