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National **Environmental Science** Programme

Assessing the feasibility of restoring giant kelp forests in Tasmania

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E7 – Assessing the feasibility of restoring giant kelp beds in eastern Tasmania

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Milestone 5 – Final Report

UNIVERSITY *of* TASMANIA | **IMAS** INSTITUTE FOR MARINE & ANTARCTIC STUDIES

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We acknowledge the Traditional Owners of the lands and waters of lutruwita (Tasmania) where this work was conducted, and pay our respects to Elders past and present.

EXECUTIVE SUMMARY

Dense giant kelp (*Macrocystis pyrifera*) forests were previously a conspicuous and iconic feature of the Tasmanian coast, but loss of ~95% of the species' surface-canopies over recent decades has seen them listed by the Australian Government as an endangered community – the first such listing for a marine community in Australia. The decline of giant kelp forests in eastern Tasmania is associated with increased influence of warm and nutrient-poor East Australian Current water in the region. Active restoration of disappearing giant kelp forests is one potential approach for their conservation. Yet, any restoration effort must overcome the ongoing challenges of climate change and ocean warming that continue to drive giant kelp loss.

This project aimed to investigate whether more warm-tolerant giant kelp existed among remnant Tasmanian giant kelp forests, and if so, assess the feasibility of using these as the foundation to 'future-proof' potential restoration efforts.

We non-destructively collected reproductive tissue from remnant areas of giant kelp across eastern Tasmania, and established a self-replenishing collection of gametophytes (i.e. the microscopic haploid life stage). Using these gametophyte cultures, we also developed an innovative low-temperature low-maintenance storage technique and established one of the world's first giant kelp 'seed banks'. Experiments revealed that ~10% of the collected giant kelp 'family-lines' exhibited significantly above-average tolerance to warm water. Notably however, thermal performance was not related to the region where the kelp were collected, such that those collected from the warmer northern region of Tasmania were typically no more warm-tolerant than those from the cooler south (and vice versa).

The selected warm-tolerant family-lines of 'super kelp' were then used as the foundation for restoration trials in the field. Despite early mixed success, outplanted juvenile *Macrocystis* survived challenging and warm summer conditions, and at two of the three trial sites, several hundred of those giant kelp are now established as juveniles and are expected to continue to develop. Unforeseen setbacks and COVID-delays ensured that some research questions remain unanswered, such as the development of second-generation recruits (i.e. wild juveniles produced by our planted kelp) and the potential for patch expansion. This monitoring is still ongoing.

These results have demonstrated the initial feasibility of restoring giant kelp in Tasmania using future-proofed family-lines. Several critical foci have been identified for a second-generation program, including targeted cross breeding and identification of the mechanisms of warm-tolerance, along with improved understanding of the methods and ecological interactions of outplanting the lab-cultivated kelp. Ultimately, this project has established a solid foundation for selective-breeding of kelp and for continued innovative restoration interventions in our rapidly-changing future.

1. INTRODUCTION

Towering up to 35 m tall, giant kelp (*Macrocystis pyrifera*) form dense underwater forests and create complex and unique habitats that supports a community of other species (Steneck & Johnson 2014; Schiel & Foster 2015). Once a ubiquitous and iconic feature of the Tasmanian coast, coverage of the species' floating surface-canopies have declined in the state by at least 95% over recent decades (Johnson et al. 2011; Butler et al. 2020). These dramatic declines prompted the 2012 listing of giant kelp forests (which also occur to a lesser extent in parts of South Australia and Victoria) as a Threatened Ecological Community under the EPBC Act – the first such listing for a marine community in Australia. The loss of giant kelp forests in Tasmania is associated with ocean-warming and changing oceanography, due to increased influence of the warm, nutrient-poor East Australian Current (EAC) in the region (Johnson et al. 2011; Butler et al. 2020). The behaviour of this poleward flowing boundary current has altered with climate change (Ridgway 2007; Johnson et al. 2011; Oliver & Holbrook 2014) and is a key driver of southeast Australia and Tasmania warming at ~4 times the global average and being a global ocean warming hotspot (Hobday & Pecl 2014).

The strengthening EAC has also facilitated the southerly range-expansion of the large sea urchin *Centrostephanus rodgersii* from NSW into Tasmania (Johnson et al. 2005; 2011; Ling 2008; Ling & Keane 2018). Whilst *Macrocystis* forests were already in decline before the local proliferation of *C. rodgersii*, destructive overgrazing from this urchin is likely to have exacerbated the losses (Ling 2008; Ling & Keane 2018) and be impeding recovery in some areas. Certainly, management of this urchin species is a key consideration for contemporary *Macrocystis* conservation and restoration interventions.

Indeed, restoration of threatened giant kelp forests may present one potential tool for their conservation and management into the future (Eger et al. 2020; Layton et al. 2020; Morris et al. 2020). However, understanding and ameliorating the driver(s) of habitat loss is a critical first step in ecosystem restoration, and failure to do so can lead to ineffective interventions and the waste of valuable resources (Johnson et al. 2017; Layton et al. 2020). Clearly the drivers of *Macrocystis* loss in Tasmania, changing oceanography and ocean-warming, cannot be ameliorated in the short to medium term. Thus, a different approach must be taken to 'future-proof' restoration interventions (Coleman et al. 2019; Wood et al. 2019).

In those areas where giant kelp forests have been lost, they have typically been replaced by either a dense canopy of the much smaller common kelp (*Ecklonia radiata*) and its associated community, or urchin barrens largely devoid of seaweed due to overgrazing by *C. rodgersii* (Johnson et al. 2011; Ling & Keane 2018). However, across those areas – and in the absence of extensive urchin barrens – scattered individuals and small patches of giant kelp remain and seem physiologically and reproductively healthy. Moreover, these scattered remnants are spread along the length of Tasmania from the warmer northern regions to the cooler south (where a handful of more extensive forests also still occur) (Butler et al. 2020). It therefore seems that these scattered, remnant kelp may represent individuals that are relatively tolerant of the modern, warmer, and more nutrient-poor conditions (Raimondi 2004; King et al. 2019; Fernández et al. 2021).

The selection and planting of potentially warm-tolerant *Macrocystis* individuals may help overcome the core drivers of ecosystem loss and provide a foundation for ongoing restoration efforts (Wood et al. 2019; Layton et al. 2020). For these types of interventions however, it is critical to consider the implications of introducing potentially exotic genotypes into novel regions (Coleman et al. 2019; Wood et al. 2019). Crucially, we understand there is high connectivity and little genetic divergence of *Macrocystis* populations across eastern

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Tasmania (Durrant et al. 2015). Furthermore, since there is strong heritability of thermal tolerance and other traits in kelps (including *Macrocystis*) (Camus et al. 2018; Mabin et al. 2019), planting of warm-tolerant lineages should provide ongoing benefits to subsequent 'wild' generations.

2. AIMS

The aim of Project E7 was to assess the feasibility of restoring giant kelp forests in eastern Tasmania using selected warm-tolerant, 'future proofed' *Macrocystis* family-lines. This research necessitated a sequential approach, with the workflow being to collect, identify/select, cultivate, and then plant potentially warm-tolerant genotypes.

We initially aimed to establish a culture collection of individual family-lines of giant kelp derived from reproductive material sampled from remnant *Macrocystis* patches and forests from across eastern Tasmania. Using the first-generation (F1) offspring of the sampled kelp, we aimed to identify whether specific family-line(s) demonstrated increased tolerance to warmer temperatures. This testing would primarily use 'selfed' (i.e. inbred) individuals, to ease identification of the parental lineage, but crosses would also be generated to assess the performance of inbred versus outcrossed genotypes. We then hoped to cultivate the selected family-lines and crosses in large quantities, for outplanting at three restoration trial sites. For that work, we would trial various outplanting methods, and assess the survivorship, growth, and reproductive potential of the selected *Macrocystis* in the field. The ultimate aim was to establish self-sustaining, and hopefully self-expanding, 'seed patches' of warm-tolerant *Macrocystis* at several trial sites in eastern Tasmania.

3. METHODS & RESULTS

3.1 Sampling and establishment of gametophyte cultures

Reproductive material was sampled from six remnant areas of giant kelp across eastern Tasmania in May 2019 (Fig. 1, Table 1). Three sites were located in the warmer northern region and three from the cooler southern region, with the regions separated by ~200 km and a 1–2 °C difference in mean seawater temperature (Table 1). Within each region, the three sites were spread across ~30 km.

At each site, three sporophylls (the specialised spore-bearing blades of *Macrocystis*, Fig. 2A) were collected from 8 haphazardly selected mature *Macrocystis* sporophytes (i.e. the diploid macroscopic life stage of kelps), with each adult separated by at least 10 m. The collected sporophylls (in separate containers for each individual) were stored overnight in seawater within an insulated container, and transported back to the lab the following day. The depth and water temperature at each site was also recorded (Table 1), along with five replicate seawater samples for nutrient analysis (Appendix A).
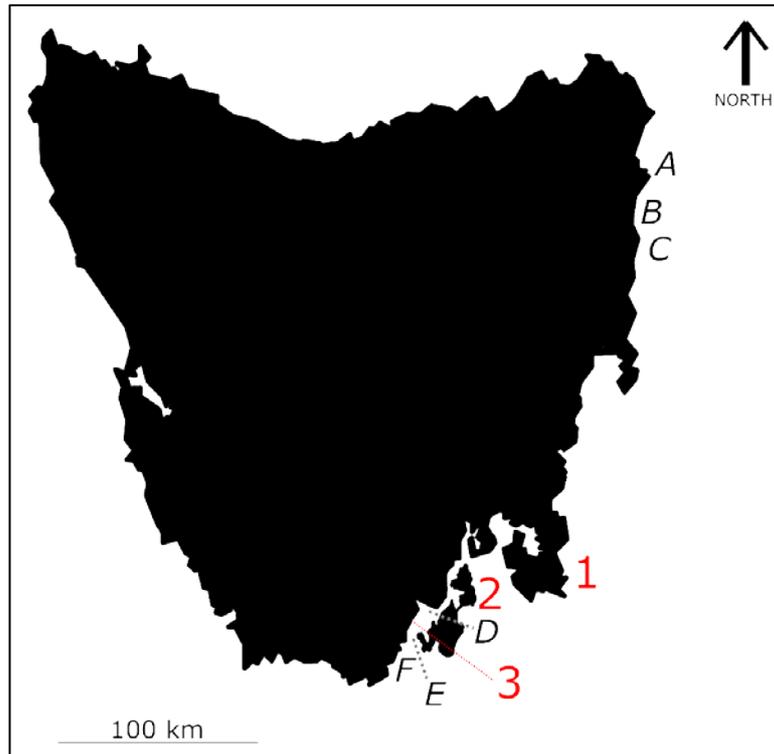
Figure 1. Map of six collection sites (A–F) and three *Macrocystis* restoration sites in eastern Tasmania, Australia. Restoration sites are 1 = Fortescue Bay; 2 = Trumpeter Bay; 3 = Dover. Collection sites are not explicitly identified to protect the potentially sensitive remnant *Macrocystis* patches/forests, but are labelled in order of distribution from north to south.

Table 1. Critical environmental parameters from the collection sites. Sea Surface Temperature (SST) data covers the period 1/1/2000–31/1/2021 (NOAA 2021). Also see Appendix A.

Site (from north to south)	Mean annual SST (°C)	Mean MIN SST (°C)	Mean MAX SST (°C)	*In situ* seawater temperature at time of collection (°C)	Date of collection	Approx. depth (m)
A	15.5	12.6	19.7	14.1	14/5/19	3.0
B	15.5	12.6	19.7	14.3	14/5/19	3.0
C	15.4	12.5	19.6	15.0	21/5/19	4.0
D	14.1	11.8	17.7	13.7	17/5/19	4.0
E	14.0	11.8	17.2	13.7	17/5/19	9.0
F	14.0	11.7	17.2	14.0	22/5/19	7.0

The three sporophylls from each individual were trimmed so that only sori (i.e. the spore-producing tissue) remained (Fig. 2). These trimmed sections were gently wiped with paper towel to remove any mucus or fouling organisms, and then dipped in a solution of 1% iodine in 0.2 μm filtered and sterilised seawater (FSW), then rinsed in FSW, and again wiped clean. After repeating this dipping and rinsing step twice more, the cleaned sori were placed between paper towels in individual foil containers and stored in the dark at 12° C for 18 hours.

The sori from each kelp were then placed into separate containers filled with 400 mL of F/2 culture medium (i.e. FSW and F/2 nutrient solution). The containers were then sealed, gently agitated, and stored under bright light (~120 μmol photon m^{-2} s^{-1}) for 3 hours to stimulate spore-release. The containers were gently agitated every ~45 minutes over this period. Then, 200 mL of each 'spore solution' was transferred to separate 1 L flasks, to which another 700 mL of F/2 culture medium was added. Each of the 48 flasks (i.e. 8 individuals from 6 sites) were then sealed with a conical rubber stopper – which have a filtered air inlet and outlet – and placed into a 12° C temperature-controlled room under red light on 12:12 light/dark cycle and with gentle aeration (Fig. 2F).

Over the next 2–3 weeks, the spores developed into gametophytes (i.e. the haploid microscopic life stage of kelps). Storing the cultures under red light retards fertilisation and development of the gametophytes into the larger sporophyte stage, but still allows gametophyte growth and clonal propagation via fragmentation. In this manner, we established 48 persistent, self-replenishing and 'pure' gametophyte cultures of known provenance. These cultures are the foundation of Project E7, and as of submission of this report, are still maintained at IMAS, Hobart. Every ~6 weeks, 50% of the F/2 media in each flask is replaced, and the rubber stoppers removed, sterilised, and replaced.
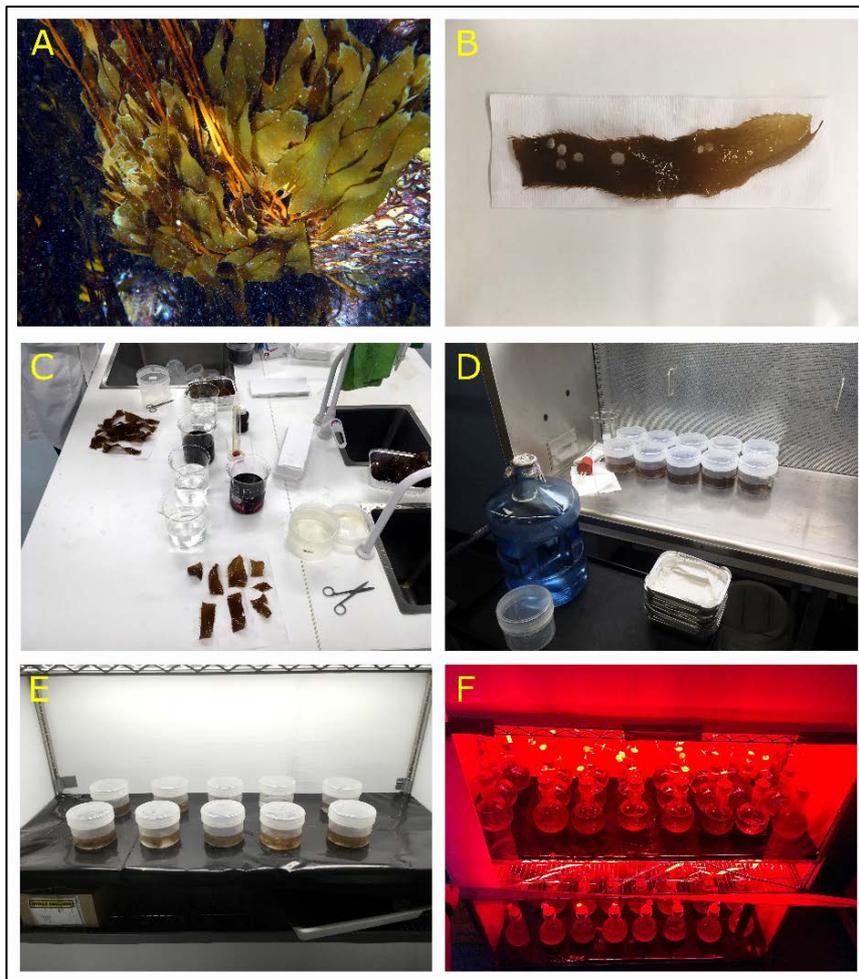
Figure 2. A) spore-bearing 'sporophylls' from an adult *Macrocystis* at one of the collection sites; B) a sporophyll in the lab in preparation for spore release; C) the sporophylls are trimmed and cleaned, and then dried overnight in the dark; D, E) the following day, the sorus tissue is rehydrated and placed under bright lights to stimulate spore release; F) the established gametophyte cultures under red light (which prevents fertilisation).

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3.2 *Macrocystis* seed bank

Given the importance and the effort required to establish the gametophyte cultures, and also the significant declines of *Macrocystis* in Tasmania, we explored the possibility of creating a secondary culture collection for longer term storage of the primary gametophyte cultures. This was not an initial aim of Project E7, but would provide a contingency in case of loss of the primary cultures and also a 'seed bank' for genetic conservation purposes.

For preliminary experiments, which began in December 2019, a 50 mL sample was taken from the 40 densest and healthiest primary gametophyte cultures and added to a common flask. From this, 200 replicate 3 mL samples were placed into individual 5 mL vials and placed into a 4 °C incubation chamber under very dim red light (<5 µmol photon m^{-2} s^{-1}).

To test for any immediate effects of this treatment, 10 randomly-selected vials were withdrawn after only 24 hours of storage, and their gametophytes cultivated to assess their viability and development into sporophytes. For this, the contents of each vial was placed into an individual sealed jar containing 300 mL of F/2 solution and a microscope slide, which acted as the gametophyte settlement substrate. These jars were then placed for 2 weeks under blue light (35 µmol photon m^{-2} s^{-1}), which promotes fertilisation and development to the sporophyte stage. After this period, aeration was added to each jar and additional white lighting provided (total light = 80 µmol photon m^{-2} s^{-1}). The kelp were then allowed to develop for another 2 weeks, after which the microscope slides were photographed for assessment of microscopic sporophyte survivorship (expressed as density) and size. Identical tests of viability also occurred after 1, 2, 4, 8, 26, 40 and 52 weeks after the initial transfer to 4 °C.

3.2.1 RESULTS

The preliminary tests, up to 8 weeks after storage, illustrated that the stored gametophytes readily developed into sporophytes and experienced no reduction in viability. This illustrated the potential of the method for storage over longer periods, and which require much less space and maintenance than the primary cultures. Consequently, in April 2020, we placed 10 replicate samples from each of the 48 gametophyte cultures into low-temperature storage, using the same method as described above. Ongoing testing of the preliminary cultures established in December 2019 revealed that sporophyte development and viability remained unchanged after 26, 40, and 52 weeks in storage.

3.3 Thermal tolerance experiment

The thermal tolerance of the first-generation (F1) sporophytes cultivated from the parental gametophyte cultures was tested using a common garden experiment (Fig. 3). This experimental design tested 49 unique genotypes of *Macrocystis* across four temperature treatments (12, 16, 20, and 24 °C). For testing, the seven densest and healthiest gametophyte cultures from each of the six sites, along with a site-level out cross (i.e. using the 7 pure family-lines from each site) from each of the six sites, and a fully random 'panmictic' cross consisting of an equal-parts mix of the 42 pure family-lines.

Figure 3. The common garden, thermal tolerance setup during the blue light fertilisation stage (upper) and the experimental trials (lower).

The thermal tolerance experiment was conducted on two separate occasions, with the sole difference being the settlement substrate used: twine was used in the first instance and microscope slides in the second. We revised the protocol since the twine proved too complex at the micro-scale to enable easy quantification of the attached microscopic kelp. Thus, overall counts of survivors were far lower during the first round of the experiment. Nevertheless, the results were consistent across both experiments, and the genotypes identified as being more warm-tolerant during the first round of testing were similarly identified during the repeat experiment. Herein, we only describe the second run of the experiment.

For testing, 50 mL of each selected gametophyte culture (or an equivalent equal-parts volume for the outcrosses) was mixed with 650 mL of FSW and placed into a 750 mL container, along with the two microscope slides as the settlement substrate. These containers were sealed except for a seawater inlet, a filtered air inlet, and an outlet. All seawater used throughout the experiment was filtered, sterilised, natural seawater from a common source. No nutrients were added to the seawater.

The common garden experiment consisted of four temperature-controlled water baths (200 x 110 x 12 cm, *L* x *W* x *D*) that each held one container of the 49 unique genotypes. Each bath was randomly allocated a water temperature, and the containers randomly positioned within each bath. All water baths contained a HOBO temperature logger and were situated within the same 12° C temperature-controlled room, with the 16, 20, and 24 °C water baths heated using aquarium heaters. All containers received gentle aeration from a common air source, while each container in each water bath was connected to a common 25 L temperature-controlled seawater header tank. Every ~72 hours the header tanks were opened and each container received ~500 mL of new seawater (at the appropriate temperature), with the excess seawater overflowing from the outlet. Every 7 days the containers were randomly re-positioned within the water bath.

To remove any effect of temperature on fertilisation success, the gametophytes from all temperature treatments were fertilised at the common temperature of 12 °C. During this 'fertilisation' stage (which ran for 28 days), water baths were illuminated on a 12:12 light/dark cycle for 14 days with blue light only (35 µmol photon m^{-2} s^{-1}) (Fig. 3), to promote fertilisation, followed by an additional 14 days under both blue and white light (80 µmol photon m^{-2} s^{-1}). This latter lighting regime remained until the end of the experiment.

Following the 28-day fertilisation period and development to the very early sporophyte stage – based on preliminary experiments and confirmed by assessment of one microscope slide from 4 random jars – the baths were raised to their experimental temperatures at a rate of ~2 °C every 24 hours. After all baths had reached their experimental temperature, the sporophytes were allowed to develop for the next 40 days. This period was based on preliminary experiments. After this time, one slide was removed from each container and the surviving sporophytes quantified and photographed using a compound microscope (at 100X magnification). Sporophytes were censused across a standardised central portion of the microscopic slide (162 mm^2, or half the area of an 18 x 18 mm coverslip), thus providing the density of surviving sporophytes. For each slide, a photograph was taken when a sporophyte was first observed, and then up to four more times whenever sporophytes were observed (each photo typically contained more than one sporophyte, due to clustering). ImageJ analysis software was used to calculate sporophyte size from the photographs.

3.3.1 RESULTS

The overall pattern was that density and size of sporophytes decreased significantly with increasing water temperature (Fig. 4, Tables 2, 3). Nonetheless, sporophytes from several genotypes developed in each of the four temperature treatments, even at 24 °C.

Sporophytes were found at similarly densities in both the 24 and 20 °C treatments, and while those densities were significantly higher at the cooler temperatures, they were no differences between the 16 and 12 °C (Figs. 5). There were also significant differences in sporophyte density between sites (Table 2), however the pattern was less clear than those driven by temperature, and was typically due to a few poorer-performing sites rather than any one site being particularly prolific (Fig. 5). Nonetheless, the lack of a significant interaction between temperature and site (nested within region) (Table 2) indicates the better performing sites did so at all temperatures. Interestingly, despite significant site-level differences there were no differences in sporophyte density when sites were analysed at the level of region (Fig. 6; Table 2); such that sporophytes were found in similar densities regardless of whether the parental lineage came from the warmer northern region or the cooler south.

As with sporophyte density, the final size of the surviving sporophytes also declined significantly with increasing temperature (Fig. 4; Table 3). However, these patterns were less

clear and there was a more variable and gradual decrease in sporophyte size with increasing temperature (Fig. 5). There were significant differences in sporophyte size depending on the site from which their parental lineage was collected (Table 3), but again this was driven largely by a few poorer-performing sites – several of which were consistent with the sites that also typically had lower densities of surviving sporophytes (Fig. 5). Indeed, sporophyte density and size were found to be positively correlated within each temperature treatment (Appendix Fig. 9). Unlike sporophyte density, there were regional-level differences in sporophyte size, and genotypes derived the cooler southern region typically produced larger sporophytes than northern counterparts (Fig. 6; Table 3), even at warmer temperatures, as evidenced by a lack of a significant region by temperature interaction.

Figure 4. Density (upper) and size (low) of first-generation juvenile *Macrocystis pyrifera* sporophytes across four temperature treatments. Density is expressed as per 162 mm² sampling area. Sporophytes are derived from parental material collected from six sites (A–F, ordered from north to south) across eastern Tasmania. For key to sites, refer to Fig. 1 and Table 1.

Table 2. Mixed-model ANOVA of effects of temperature, region, and site (nested within region) on density of *Macrocystis pyrifera* sporophytes after thermal tolerance experiments. Transformation of response variable (*Y*) noted in first column. Significant *P*-values denoted in bold and with *.

Y = sporophyte density (transformation)	factors	*F (df)*	*P*
$(Y+0.1)^{0.15}$	temperature	$F_{(3,12)} = 12.500$	**<0.001***
	region	$F_{(1,12)} = 0.735$	0.408
	region(site)	$F_{(4,12)} = 3.941$	**0.029***
	temperature:region	$F_{(3,12)} = 1.118$	0.380
	temperature:region(site)	$F_{(12,144)} = 1.067$	0.393
$(Y+0.1)^{0.15}$	cross	$F_{(1,188)} = 4.992$	**0.027***
	temperature	$F_{(3,188)} = 14.664$	**<0.001***
	cross:temperature	$F_{(3,188)} = 0.057$	0.987

Table 3. Mixed-model ANOVA tests of effects of temperature, region, and site (nested within region) on size of *Macrocystis pyrifera* sporophytes after thermal tolerance experiments. Transformation of response variable (*Y*) noted in first column. Significant *P*-values denoted in bold and with *.

Y = sporophyte size (transformation)	factors	*F (df)*	*P*
$Y^{-0.35}$	temperature	$F_{(3,12)} = 13.753$	**<0.001***
	region	$F_{(1,12)} = 13.091$	**0.004***
	region(site)	$F_{(4,12)} = 7.948$	**0.002***
	temperature:region	$F_{(3,12)} = 1.031$	0.414
	temperature:region(site)	$F_{(12,106)} = 0.752$	0.697
$Y^{-0.30}$	cross	$F_{(1,147)} = 3.848$	0.051
	temperature	$F_{(3,147)} = 10.841$	**<0.001***
	cross:temperature	$F_{(3,147)} = 0.046$	0.987
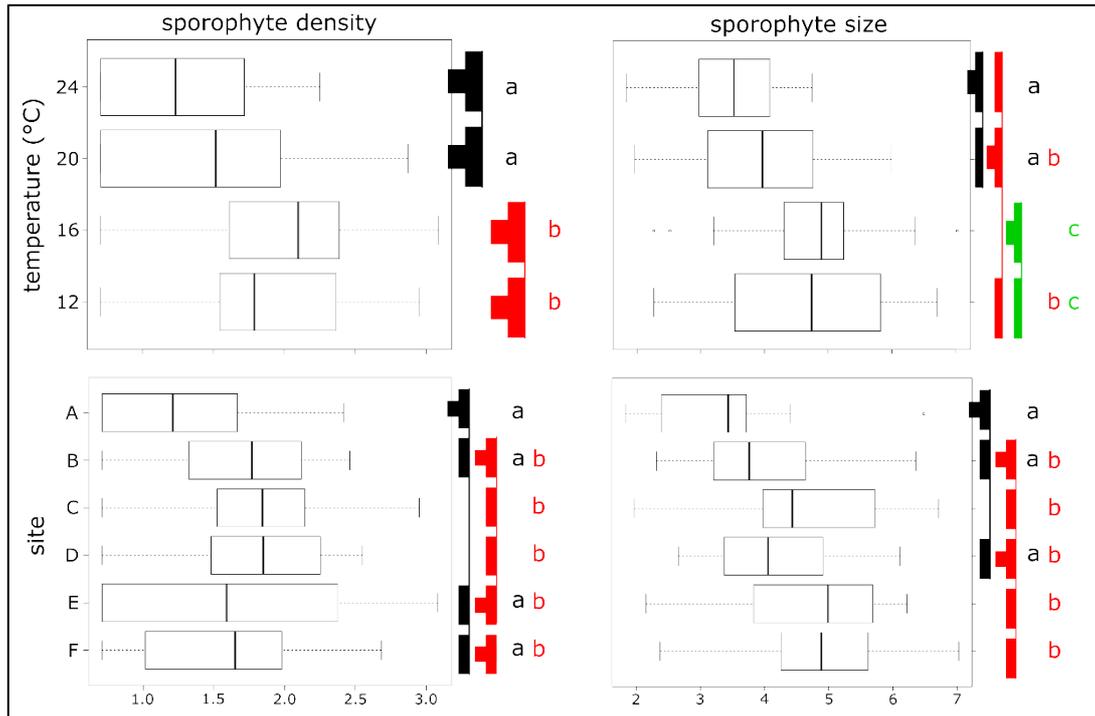
Figure 5. Tukey's HSD post-hoc pairwise comparison tests of density and size of *Macrocystis pyrifera* sporophytes, dependent on site of origin and temperature treatment. Capital letters on left y-axes indicate sites (see Fig. 1 and Table 1), whilst lower case letters on right-hand side denote significant groupings.
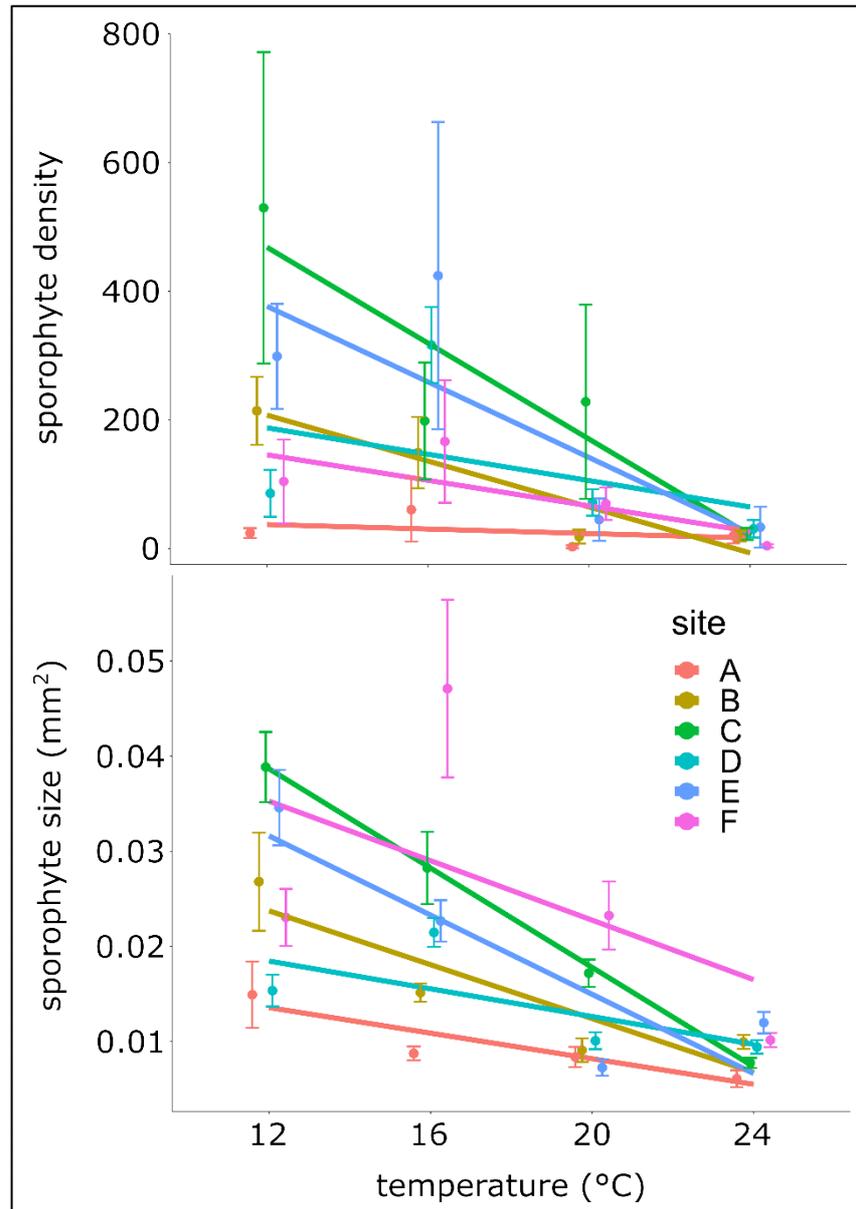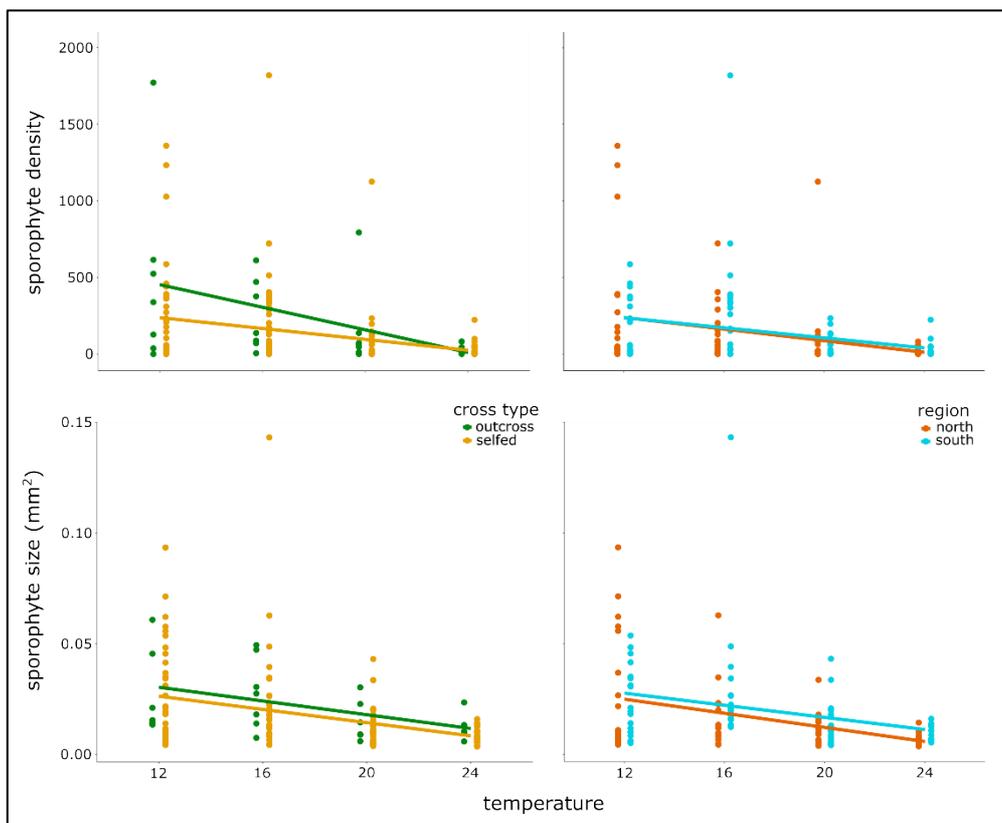

Figure 6. Density (upper pair) and size (lower pair) of first-generation juvenile *Macrocystis pyrifera* sporophytes across four temperature treatments. Sporophytes are grouped according to cross-type (left pair) or region of collection (right pair).

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Outcrossed genotypes produced significantly more sporophytes than selfed genotypes (Fig. 6; Table 2), and while we detected no significant differences in final sporophyte size between outcrossed and selfed genotypes, this was statistically marginal (i.e. *P* = 0.051, Table 3).

Ultimately however, the objective of the testing was to identify whether any specific *individual* genotype(s) exhibited relative tolerance to warmer water. Clearly some family-lines grew larger and more densely than others at warm temperatures (Figs. 4, 6). So, to obtain a composite measure of family-line performance, we multiplied mean sporophyte size by density to generate an index of biomass produced by each genotype at the two warmest temperature treatments (since it is these temperatures that would indicate increased warm-tolerance). We then ranked each genotype based on their sporophyte biomass across the 20 and 24 °C treatments. From those initial top 10 ranked genotypes, two were disregarded since they did not produce any survivors at 24 °C (but did produce high biomass at 20 °C). In their places, we selected the 11th ranked genotype and the genotype with the highest density at 24 °C. Notably, the panmictic cross (i.e. the outcross of all 42 pure family-lines) was identified as one of the best ranked family-lines. These top ten genotypes (see Fig. 7), dubbed 'super kelp', were then subsequently cultivated in large numbers for outplanting trials.

3.4   Outplanting of selected *Macrocystis* genotypes

As with the thermal tolerance experiment, the outplanting trials were also conducted twice. The first occurred immediately after completing the first thermal tolerance experiment, however the results from those trials were poor, and so the methods were refined. Below, we primarily discuss the second outplanting trial, and clarify the revision of the methods.

Three trial sites for outplanting (Dover, Fortescue Bay, Trumpeter Bay, Fig. 1) were selected based on two general criteria: 1) a consistent depth (~7–11 m) and reef profile and 2) have no *Macrocystis* present but have previously supported dense forests of this species. The Dover site was the most sheltered of the three sites, and contained low profile reef amongst a generally soft-sediment 'harbour' environment. The Fortescue Bay and Trumpeter Bay sites were both semi-exposed with high profile slab and boulder reef and more indicative of the open coast. The Trumpeter Bay site was also selected in collaboration with the weetapoona Aboriginal Corporation (wAC). This community has a long history of knowledge of the site and of the local declines of giant kelp, and engaged with our project to facilitate knowledge-sharing and the collaborative restoration of their Sea Country.

At each of the three restoration trial sites, a 12 x 12 m patch of consistent reef profile, substrate, and depth was selected. All macroalgae and *Centrostephanus* sp. and *Heliocidaris* sp. urchins were cleared from this area immediately prior outplanting, to minimise the negative effects of competition and grazing on the planted juvenile *Macrocystis*. Within the cleared patch, the central 10 x 10 m area was to be used for outplanting while the 1 m 'buffer' on each side was intended to limit edge effects and allow assessment of any subsequent *Macrocystis* recruitment and patch expansion. Within that 10 x 10 m area, 100 expansion bolts were drilled into the rock substrate using a Nemo underwater rotary hammer drill. Bolts were separated by ~1m and arranged in a 10 x 10 grid and provided the anchor point to install the planting substrates.

The 10 'super kelp' family-lines were cultivated to the sporophyte stage using the same methods as described above for the thermal tolerance experiment, with 10 replicates of each to be outplanted at each site. One difference however was the settlement substrate used. During the first outplanting trials, AlgaeTwine synthetic twine (~1 mm thickness) was used,

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while polycarbonate plates (40 x 40 x 1.5 mm) were used during the second round of trials. Ultimately, the plate method proved more successful (discussed below). The plates had one hole drilled centrally, for installation using the expansion bolt, and one smaller hole drilled in one corner to allow attachment of a numbered cable tie for identification.

Sporophytes were ready for transplanting after being cultivated in the lab for 11 ± 2 weeks, at which time they were ~1–4 mm in size (Fig. 7A). The containers were transport from the lab to the field, where each plate bolted to a random position on the reef and labelled with a uniquely numbered cable tie.

Outplanting at each site was completed over two days: one for clearing the macroalgae, the other for outplanting the super kelp. Outplanting at all sites occurred from mid-September 2020 until mid-October 2020 (with the staggered timing due to weather constraints). And while some kelp were cultivated in the lab for ~4 weeks longer than others, there were no apparent differences in size or condition of the lab-cultivated kelp between the outplanting dates. Each site was revisited ~3 weeks after outplanting to qualitatively assess kelp survivorship (since the kelp were still too small and delicate to accurately count), followed by quarterly assessments thereafter.

3.4.1   RESULTS

There was rapid sedimentation and growth of turf and understory algae (mostly the foliose green alga *Ulva* sp.) at all sites following the initial clearing of macroalgae (this was especially prolific at the Dover and Trumpeter Bay sites). This meant it was not possible to re-locate every plate during the first follow-up assessments. Nonetheless, the located plates were spread evenly across each patch and were deemed to provide a representative assessment of early outplanting success at each site.

Initial success rates were relatively low, and many plates appeared absent of surviving sporophytes: with 47% failure at Dover, 50% failure at Fortescue Bay, and 53% failure at Trumpeter Bay. With few exceptions, plates either had no visible surviving sporophytes and were heavily fouled with sediments and turf algae, or were only lightly fouled and with abundant remaining sporophytes. At this time, surviving sporophytes typically ranged in size from ~5–40 mm, but a few larger individuals (> 50 mm) were observed at the Fortescue Bay and Trumpeter Bay sites (Fig. 7B). There were no obvious differences in plate position, orientation, or local bathymetry that explained the pattern of success/failure, nor any obvious indication of differences across family-lines (limited count data impeded formal analysis).

Assessment at Dover in early 2021, 96 days after initial outplanting, revealed no surviving sporophytes across the site, which was still heavily fouled with understorey algae, turfing algae, and sediments. At Fortescue Bay, 152 days after outplanting, we located 28 plates spread across the site, which supported 51 healthy juvenile giant kelp (Fig. 7C). The average size of surviving sporophytes was 327 ± 22 mm (SE) however several were >550 mm. At Trumpeter Bay, 196 days after outplanting, we located 67 plates spread across the site, which supported 172 healthy juvenile giant kelp. The average size of those was 404 ± 11 mm (SE), however numerous individuals were >750 mm (Fig. 7D). At the two successful sites, the outplanted kelp had dark pigmentation and appeared in good condition, even despite the slightly-warmer than average summer water temperatures due to *La Niña* (Holbrook et al. 2020; BOM 2021). There were clear indicators that some genotypes survived better than others (Fig. 8), and the best performing kelp (in terms of both density and growth) typically appeared to come from outcrossed, rather than selfed, genotypes.
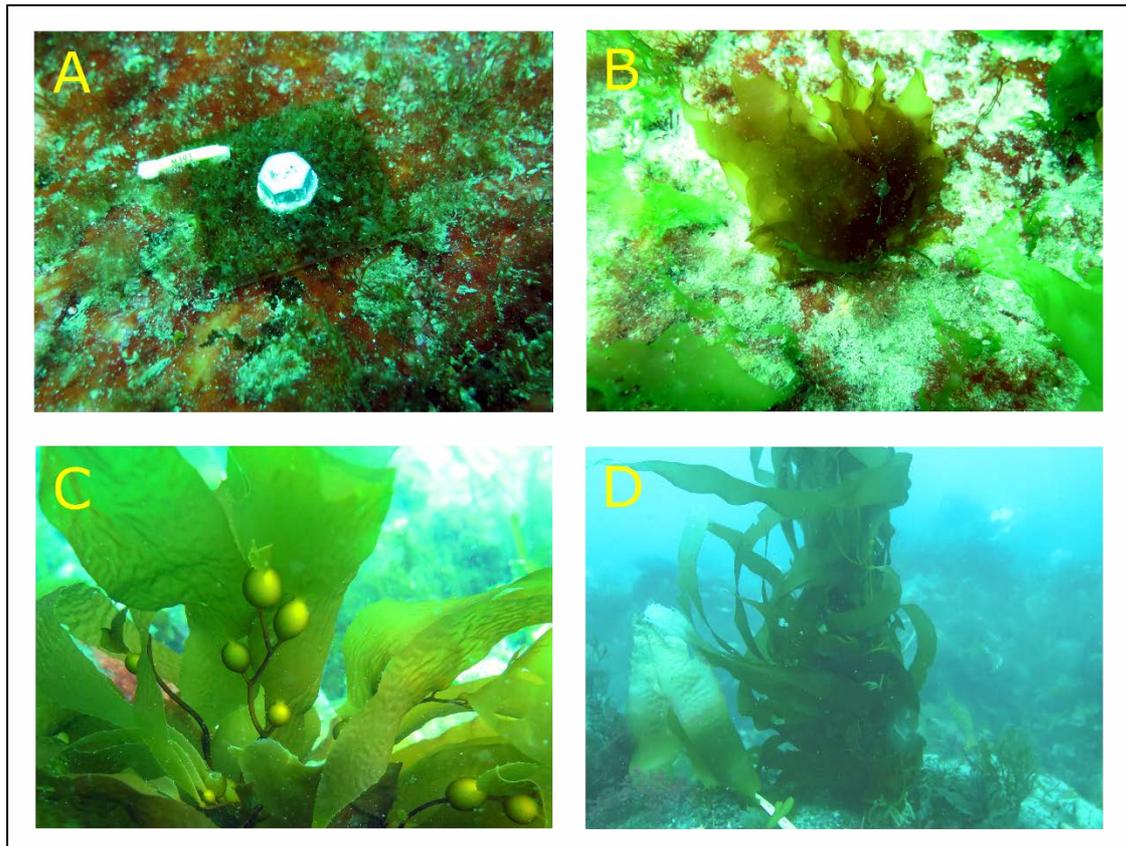
Figure 7. Warm-tolerant *Macrocystis pyrifera* sporophytes outplanted at the Fortescue Bay and Trumpeter Bay restoration trial sites. A) kelp-seeded plate at time of outplanting with hundreds of ~1–4 mm sporophytes; B) surviving juvenile sporophytes ~100 mm in size, 48 days after outplanting; C) a healthy sporophyte at Fortescue Bay in summer 2021, >500 mm in size and 152 days after outplanting; D) a healthy sporophyte at Trumpeter Bay in early autumn 2021, >1 m in size and 196 days after outplanting.

3.5   Setbacks

The sequential nature of the project ensured that some early delays led to setbacks at later stages. Delays to the initial thermal testing experiment led to a late first round of outplanting, which occurred in spring/summer 2019 and is likely the worst possible time for outplanting of juvenile *Macrocystis*. We believe this was a large factor in the lack of survivorship from the first outplanting trials. Nonetheless, we learned from the failures and scheduled a second round of planting for autumn/winter 2020, when conditions would be optimal. Unfortunately, COVID-19 related delays meant that second round of planting was also postponed, until spring 2020 when conditions again would be challenging. The primary consequence of these setbacks was that the project did not have adequate time to assess the maturation and reproduction of the outplanted sporophytes, nor the aim of assessing patch stability and expansion. Despite the end of the project, monitoring of the patches is still continuing, but at a reduced capacity and using other resources.

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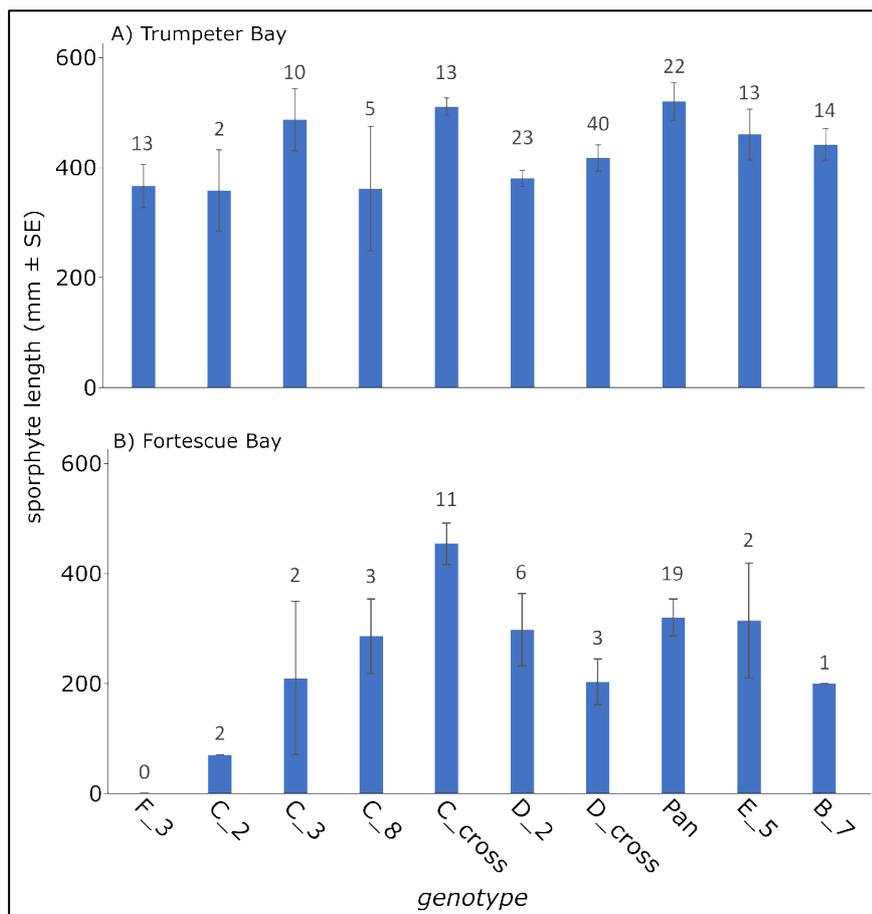
Figure 8. Length and number of survivors (noted above each bar) of ten 'super kelp' genotypes at A) Trumpeter Bay, 196 days after outplanting and; B) Fortescue Bay, 152 days after outplanting. Genotype notation is derived from site name (A–F) and replicate number from each site, while 'cross' denotes site-level outcrosses and 'Pan' the full panmictic cross of all 42 kelp genotypes.

4. DISCUSSION

We created a persistent collection of giant kelp gametophyte cultures derived from spores collected from healthy individuals in remnant patches of *Macrocystis* across eastern Tasmania. Using the first-generation offspring from these cultures, we identified several family-lines of *Macrocystis* that exhibited surprisingly high tolerance to warm water. Because sporophyte density was positively correlated with their size (Appendix Fig. 9), this suggests our results were reliable estimates of performance, and not confounded by density-dependence effects such as self-thinning and stochastic/slowed growth rates (Schiel & Foster 2015). These 'super kelp' provided the subsequent foundation for restoration trials and were outplanted to natural rocky reefs in eastern Tasmania. These trials were successful at two of the three sites, where the outplanted juvenile kelp demonstrated good survival and growth, despite the warm conditions of the 2020-21 summer. The project also developed a method of maintaining the valuable *Macrocystis* gametophyte cultures in long-term low-temperature storage, which not only require less upkeep than the primary cultures, but also acts as a culture backup and foundation for genetic conservation.

4.1   Thermal tolerance of *Macrocystis* in Tasmania

It was not surprising that *Macrocystis* density and size declined with increasing temperature (Schiel & Foster 2015; Mabin et al. 2019). However, it was unexpected that the juvenile sporophytes of several family-lines survived at 24 °C. This is well beyond the maximum water temperatures experienced at any of the field sites where the parental material was collected. While testing suggested that the density (and to lesser extent, size) of the surviving *Macrocystis* were similar at 20 and 24 °C, it was visibly apparent that none of the individuals surviving at 24 °C were thriving. Conversely, many of the surviving juveniles at 20 °C appeared relatively healthy and were well-pigmented. Unfortunately, due to the limited biomass of the microscopic juvenile sporophytes, it was not possible to conduct additional tests to examine finer-scale differences in the physiological performance and condition of the kelp across the temperatures.

24 °C is the thermal-maxima reported for *Macrocystis* globally, experienced by populations in the Baja region of Mexico (Hernández-Carmona et al. 2000; Schiel & Foster 2015). However, those populations from Baja are typically exposed to far higher levels of nutrients than are present in Tasmania/southeast Australia, and it is known that increased nutrients can increase the thermal tolerance and help ameliorate deleterious effects of warm water on *Macrocystis* (Fernandez et al. 2020). This is also why we intentionally provided only ambient (i.e. very low) levels of nutrients during our lab experiment. It is unclear whether the sensitivity of *Macrocystis* to rising water temperatures in southeast Australia is exacerbated by the naturally low nutrient levels (Fernandez et al. 2020), or whether these populations are relatively well-adapted for growth and survival under such conditions (Fernandez et al. 2021).

Elevated nutrient levels at local scales – potentially due to estuarine and/or anthropogenic inputs – may therefore help explain the location of remnant patches of *Macrocystis* in Tasmania, especially in the warmer northern regions. However, there were no obvious sources of external nutrients at most of the northern sites from which we collected the reproductive material (Fig. 10). The exception is the most northerly collection site, where the remnant *Macrocystis* are situated in narrow channel at the mouth of a large embayment that supports shellfish aquaculture and a regional population hub of >2,000 residents. Water samples from this site taken at the time of sampling revealed elevated concentrations of ammonium and phosphate (two important nutrient sources for kelp and other macroalgae) relative to other sites (Fig. 10). Interestingly, the kelp from that site were consistently poor performers in the experimental testing of thermal tolerance: perhaps suggesting the increased local nutrients have allayed selective pressure on the local *Macrocystis* to enhance thermal tolerance. Nonetheless, it is unclear how strong the selective pressure of temperature is on *Macrocystis* in Tasmania, especially given that family-lines collected from warmer northern sites were no more tolerant of warm conditions than the family-lines from the cooler south. Likewise, some of the best performing genotypes at warmer temperatures were derived from parental material collected from cooler southern sites, which also had higher nitrate levels relative to northern sites (Fig. 10).

Although we observed no evidence of consistent regional differences in thermal tolerance, we did detect indications of heterosis and/or inbreeding depression, in which genotypes derived from outcrosses typically performed better than those derived from selfing. Inbreeding depression has been illustrated in *Macrocystis* previously, but typically only after several generations of selfing and/or in the later life-history stages (Raimondi et al. 2004; Westermeier et al. 2010; Camus et al. 2021). Nonetheless, both the thermal tolerance experiment and subsequent outplanting trials indicated better performance by sporophytes derived from outcrossing than those from selfing. It is also noteworthy that the panmictic

cross of all 42 family-lines (from all six sites) produced some of the best performing sporophytes in both the lab and field. This demonstrates the potential influence of increased adaptive capacity and outcrossing. However, it does not diminish the utility of the selfed genotypes that exhibit increased thermal tolerance, since identification of these warm-tolerant family-lines (even despite potential inbreeding depression) provides the foundation for future cross-breeding experiments. We emphasise that it is also highly unlikely that an outcross derived from 42 individuals spread across 6 sites spanning >275 km would occur naturally.

4.2   Successes and failures of outplanting

The first round of outplanting, in spring/summer 2019, utilised twine as the settlement substrate and resulted in no survivors across the three sites. This method involved wrapping the seeded twine around elasticated 'bungee' cord that was strung tightly over the rocky reef. We suspect that the poor survivorship was, in large part, due to the season of outplanting, which was suboptimal for juvenile kelp recruitment. In addition, 'overhandling' of the twine during underwater installation may have also led to abrasion of the microscopic kelp. We note that in a parallel project, outplanting of the same kelp-seeded twine on a kelp aquaculture array yielded extremely high success rates (Layton et al. in prep). The crucial difference being that the twine is handled minimally during installation onto the aquaculture arrays. Subsequent outplanting efforts at restoration trial sites focussed on using plastic plates as the settlement substrate.

Abundant sediments and turf algae are known to inhibit kelp recruitment (Schiel & Foster 2015; Watanabe et al. 2016; Layton et al. 2019a, b), and likely impacted *Macrocystis* survivorship at the Dover site – which demonstrated obvious accumulation of fine sediments. This site is also the most sheltered and inshore of the three sites, which can contribute to increased sedimentation and turf algae formation (Oh et al. 2015; Filbee-Dexter & Wernberg 2018).

Conversely, restoration trials at the two other sites yielded very promising results, with densities of surviving kelp between 0.5–1.7 m^{-2}, which are typical of adult *Macrocystis* densities in natural forests (Schiel & Foster 2015). Although the outplanted kelp at these sites are not yet adults, we expect that most of the surviving juveniles will mature into adults, since they thrived during the warm summer and are now sufficiently large to be beyond the 'danger zone' where they are most susceptible to damage from scour, sediments, and potentially even small grazers (Schiel & Foster 2015; Layton et al. 2019a, b). At the time of writing, water temperatures are now significantly cooler than summer, so these juveniles have now passed the most challenging season for *Macrocystis*.

The initial assessments of transplanting success at each site revealed a sporadic but binary pattern of survivorship, whereby plates typically had either zero surviving kelp or many dozens (or hundreds). This dichotomy of either complete success or failure of a plate suggests that kelp survivorship during these early stages is highly dependent on plate position and processes on the reef that vary at the scale of metres (or less). At a broader scale, seasonality may have also impacted kelp survivorship, since outplanting occurred in late spring, which is not ideal (outplanting was delayed due to COVID-19 restrictions). This is a season of rapid growth of foliose and turf algae on temperate rocky reefs in Australia, each of which are potential competitors and stressors of very small juvenile kelp (Edgar et al. 2004; Tatsumi & Wright 2016; Shelamoff et al. 2019). Moreover, outplanting at this time also meant the very young juvenile kelp had to develop throughout the summer months when temperatures are most challenging.

The significant successes at these two sites, despite a sub-optimal outplanting period and warm summer, provides confidence that the surviving kelp will fulfil the aim of creating a 'seed patch' of reproductive *Macrocystis*. Ongoing monitoring will determine whether the patches can self-maintain and expand through recruitment.

4.3   Future directions

4.3.1   Ecology

Despite the apparent presence of 'super kelp' among remnant *Macrocystis* patches and forests in Tasmania (Fig. 1), it is unclear why these genotypes are not proliferating to re-establish giant kelp forests. While it is possible that at some locations these more tolerant genotypes and remnant patches might be increasing, regional trends (e.g. Butler et al. 2020) and anecdotal observations suggest this is not the case. A more likely explanation is that ecological interactions such as competition and grazing are inhibiting the recovery of *Macrocystis*, regardless of the presence of adapted genotypes.

When *Macrocystis* is lost from an area, it is typically replaced by the more thermally tolerant common kelp, *Ecklonia radiata* (Ling & Keane 2018). Intraspecific positive feedback by *Ecklonia* (Layton et al. 2019b) and subsequent recruitment inhibition of juvenile *Macrocystis*, may be impeding the natural recovery of *Macrocystis,* even in places where it formally thrived. If this is the case, replacement of *Macrocystis* by *Ecklonia* would represent a hysteresis effect and a distinct shift to an alternative stable state. Similarly, degraded areas where even *Ecklonia* has been lost and replaced by expansive urchin barrens or turf-algae habitats also represent a pronounced hysteresis and shift to an alternative stable state (Steneck & Johnson 2014; Ling et al. 2015; Filbee-Dexter & Wernberg 2018). In these circumstances, the alternative state (e.g. dense *Ecklonia*, urchin barren, turf habitat) is self-reinforcing and maintained by positive feedback, and there is low likelihood of *Macrocystis* recovery without additional intervention (also see Marzloff et al. 2011; Johnson et al. 2017; Layton et al. 2020).

Given that we have identified warm-tolerant genotypes and thus the physiological feasibility of restoration, a critical next step is to improve understanding of the ecological interactions of *Macrocystis* with other species, and how these may influence the restoration of giant kelp in these systems. Better knowledge of the function, position, and stability of extant remnant patches would improve selection of restoration sites and optimisation of planting efforts. Key areas of future work include:

- Understanding the abiotic and/or biotic factors that determine the location and size of remnant patches.
- Spatial and temporal assessments of remnant patches to determine patch stability and habitat dynamics.
- Better description of the composition of the community of organisms associated with the remnant *Macrocystis* forests and patches.
- Comparison of the characteristics of *Macrocystis* populations across its range in Australia, spanning South Australia, Victoria, and Tasmania.

4.3.2   Optimising outplanting and other restoration methods

While ecological work is critical to improving the effectiveness and scalability of restoration interventions, there is sufficient current knowledge to continue planting trials and improving the 'the lab-to-reef' process. Priority areas include:

-   Refining current outplanting methods and trialling novel approaches to improve the efficiency and efficacy of establishing 'seed patches' of giant kelp.
-   Improved understanding of site- and environment-specific planting methods, such as the assessment of suitable methods at sheltered versus exposed sites.
-   Assessment and trialling of methods intended to aid 'upscaling' and expanding the spatial coverage of restoration interventions.

4.3.3   The physiology of thermal tolerance

This project has demonstrated the presence of warm-tolerant genotypes and thus a physiological foundation for restoration. However, we are unclear of the specific physiological mechanisms that influence and enhance the thermal tolerance of the identified *Macrocystis* genotypes. This work is likely to be critical in optimising breeding and selection programs in the future. More broadly, improved understanding of the physiology of thermal tolerance will also aid identification of additional populations of *Macrocystis* and other kelp species that may be resilient or threatened by ocean warming and climate change. Specific priority areas include:

-   Expanded thermal tolerance experiments, where sporophytes are grown to larger size to enable testing of key physiological parameters such as photosynthetic rates and respiration, pigmentation, C:N ratios, and fatty acid profiles.
-   In combination with the above, continued testing of 'new' family-lines collected from known patches, novel sites, and mainland populations of *Macrocystis.*
-   Develop reference libraries and utilise '-omics' approaches (i.e. genomics, transcriptomics, and proteomics) to better understand the determinants of thermal tolerance at the genetic level.

4.3.4   *Macrocystis* breeding program

Project E7 has laid a solid foundation for future *Macrocystis* breeding programs. Our work to select for warm-tolerant F1 juveniles from selfed family-lines and non-targeted crossed lines indicates exciting potential to improve thermal performance via selective breeding techniques. Additional traits, such as increased growth or improved resilience to grazing, may also prove worthy targets for selective breeding programs. Moreover, the indications of heterosis from our work illustrates the potential for optimised outcomes to be achieved via targeted crosses of the identified warm-tolerant genotypes. Ongoing breeding programs also present unique opportunities for synergy and collaboration between restoration efforts and the burgeoning kelp aquaculture industry in Australia. Specific areas for future works include:

-   Expand the current first-generation breeding program by focussing on targeted out-crossing of identified warm-tolerant genotypes, selecting for further performance in temperature tolerance and growth rates, particularly in low nutrient environments. One aim of this work is to increase performance (thermal tolerance and growth) whilst

improving the genetic diversity and adaptive capacity of planted kelp. Targeted cross-breeding will also enable quantitative estimates of trait heredity and family-level variance in *Macrocystis*.

- Continued sampling of *Macrocystis* in Tasmania and novel sampling from mainland Australian populations to identify new wild-type family-lines that also manifest improved thermal tolerance. The aim is to optimise genetic diversity and performance, and provide a strong foundation for genetic conservation of the species in Australia, for ongoing restoration and development of commercial giant kelp farming in Australia.

4.4   Conclusion

This research has illustrated the physiological potential that may be harnessed to 'future-proof' *Macrocystis* restoration efforts, and that selective screening of adaptive genotypes can be utilised where drivers of habitat loss (such as ocean warming) cannot be remediated prior to restoration interventions. We created a long-term seed bank and a living collection of *Macrocystis* gametophytes derived from reproductive material collected from across Tasmania. The warm-tolerant 'super kelp' selected from these cultures were then used as the foundation for restoration trials in the field. Our outplanted selected juvenile *Macrocystis* survived and thrived during a challenging and warm summer. Unforeseen setbacks meant that is was not possible to achieve some goals in the time frame of the project, specifically, assessment of the capacity of our 'seed patches' to reproduce and expand through second-generation recruitment. These elements are being monitored beyond the life of this project.

Restoration science is best advanced when both successes *and* failures are used as the foundation for future work. While some aspects of the project realised mixed results, the overall outcomes have been highly successful at demonstrating the feasibility of restoring giant kelp in Tasmania. Nonetheless, there remains work to be done to refine existing processes and extend the work into wholly novel areas, each of which will aid further development of innovative approaches to restoration of kelp in Australia and globally.

5.   DATA MANAGEMENT AND ACCESSIBILITY

This work is currently being prepared for publication as a peer-reviewed paper in the international literature. All project outputs will be publicly available in accordance with the NESP Data Management and Accessibility Guidelines. All data generated by the project are available from the Australian Ocean Data Network (AODN) via the IMAS portal as open data at:

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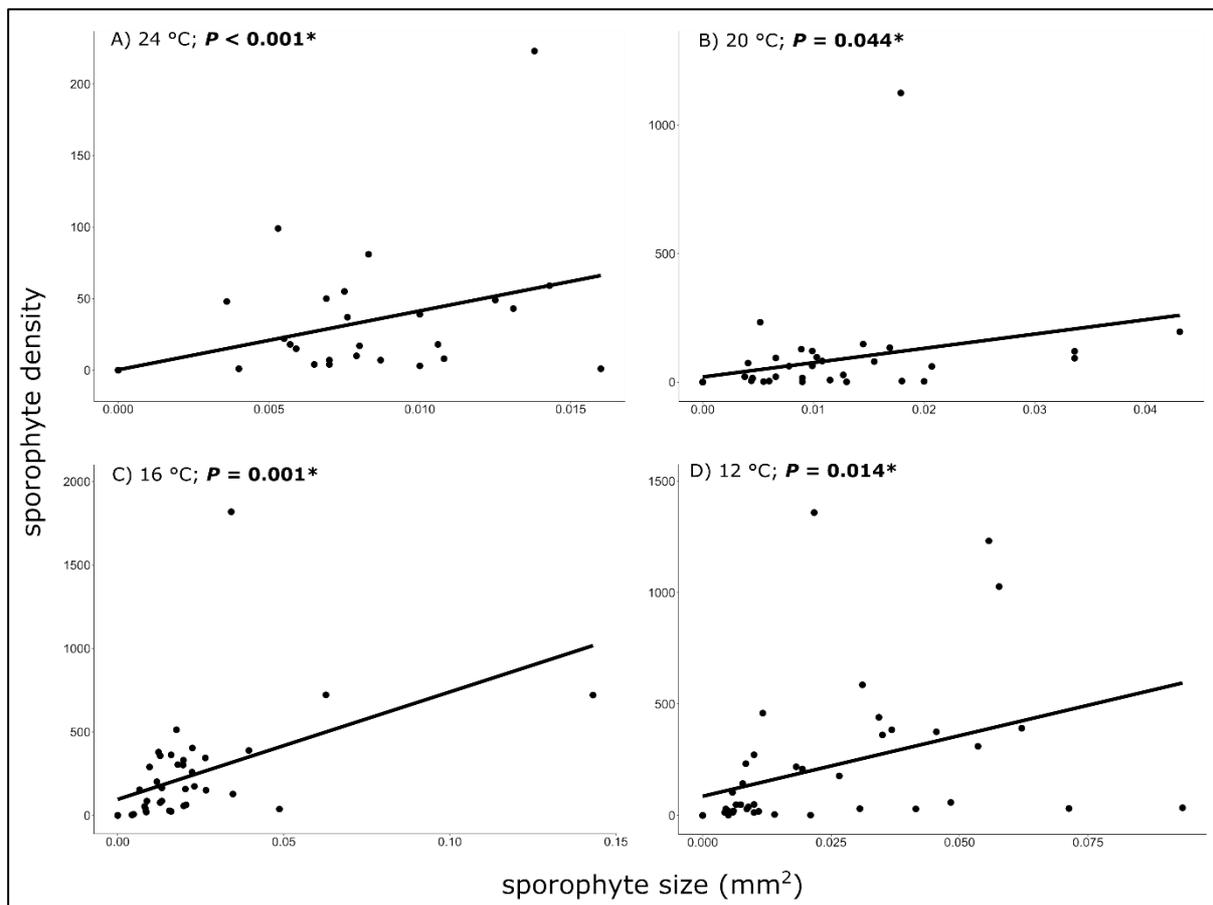
APPENDIX

Figure 9. Relationship between sporophyte size and density from the thermal tolerance experiment, showing significantly positive relationships between the two response variables within each temperature treatment. Note the different axis scales.
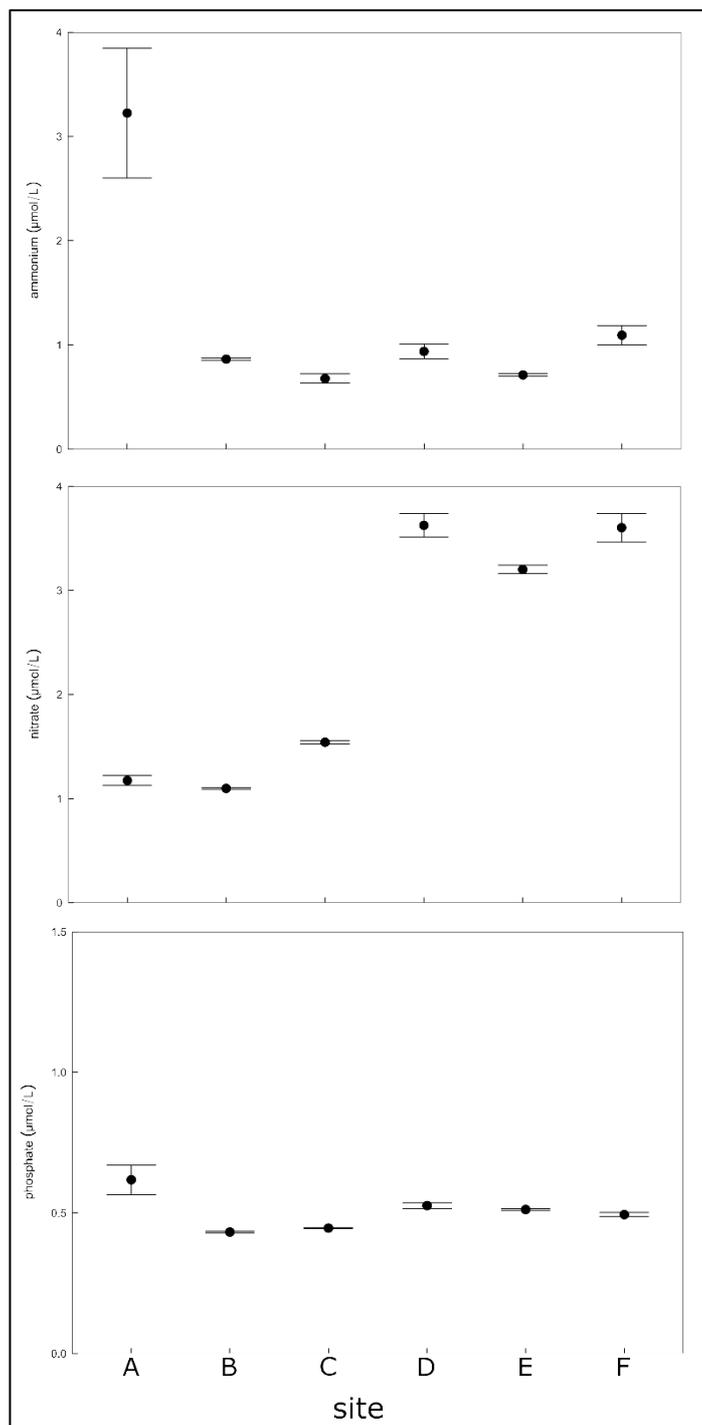
Figure 10. Nutrient concentrations from seawater samples taken during collection of *Macrocystis* reproductive material from 6 sites across Tasmania in May 2019. Sites are labelled A–F in order of their north-south distribution, with sites A–C in the warmer northern region and sites D–F in the cooler southern region.

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