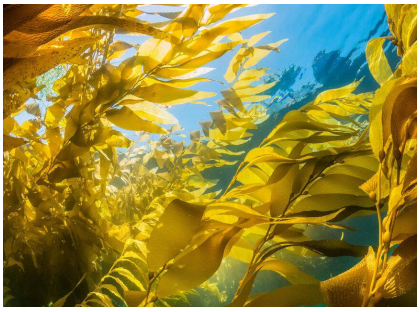
California Sea Grant Kelp Recovery Research Program Final Report 2023

SCALING A NEW COST-EFFECTIVE INTERVENTION TOOL TO RESTORE AND FUTURE-PROOF COASTAL KELP FORESTS





University of California, Irvine

Assistant Professor Joleah Lamb (UCI PI), Professor Matthew Bracken (UCI co-PI),
Phoebe Dawkins (UCI Graduate Student), Andrea Paz-Lacavex (UCI-UCSC Graduate Internship Student),
Evan Fiorenza (Assistant Research Specialist I, UCI Graduate Student), Min Han (UCI CA-SURE Student)

PROJECT PRINCIPLE OBJECTIVES

Key outcomes of this proposal include strong local and global alliances to scale up kelp restoration and will lead to practical solutions to future-proof kelp forests against climate and anthropogenic stress.

AIM 1 Applying new *Green Gravel* restoration techniques in California

Goal 1 Produce reproducible protocols for the canopy-forming kelp, *Macrocystis pyrifera*
Goal 2 Experimentally test deployment strategies to maximize retention on reefs in California

AIM 2 Create anticipatory management strategies by incorporating assisted evolution approaches

Goal 3 Acclimatize *Green Gravel* to assess temperature tolerant outplants
Goal 4 Assess translocation of genetic populations into a temperature stressed region

Local & International Benefits

This project directly aligns with efforts to develop rapid and cost-effective restoration strategies for kelp forests worldwide. Outcomes will contribute practical protocols needed to manage and restore kelp forests in California and inform global-scale techniques as part of the *Green Gravel* restoration consortium. This project will directly engage with the local stakeholders by providing (1) an accessible restoration tool that harnesses citizen science efforts at the management scale, and (2) a multi-faceted educational outreach tool that simultaneously elucidates critical stages of kelp biology as well as current conservation methods.

Project Partnerships

OVERVIEW OF FINAL PRODUCT OUTPUTS

Visualized experimental protocol for giant kelp restoration (AIM 1)

Kelp forests along the California coast are facing unprecedented loss due to climate-driven ecological stressors necessitating innovative restoration strategies. Given global declines and documented 95% loss in California, scalability of these strategies is critical. However, there remains a lack of resources for researchers, both technical and non-technical, that provide accessible guidelines for kelp restoration.

A **culturing handbook** (**Goal 1 & Goal 2**), titled 'Green Gravel Restoration Handbook for Giant Kelp (*Macrocystis pyrifera*),' is a shared product with the University of California, Irvine and University of California, Santa Cruz. This 43-page culturing handbook provides critical background and introductory information to giant kelp restoration in Southern California, including considerations for restoration site selection, facility requirements and material preparation, methods for collection, sporulation, seeding, rearing, and deployment of *M. pyrifera* for restoration. We have included a range of figures (see examples in **Figure 1**) to help elucidate different culturing processes for technicians, managers and researchers that are new to kelp restoration. Additionally, we have included an excel sheet that acts as a laboratory aid to support growers with the following **tools / templates**: 1) a comprehensive, itemized list of expenses and links to products, 2) checklists of field work materials, 3) a tool to calculate spore density for gravel inoculation, 4) a calendar to guide adjustments to rearing conditions and expectations for giant kelp culture development, 5) a calendar to help schedule weekly lab responsibilities for a technician, 6) a log for general notes and kelp culture progress with examples.

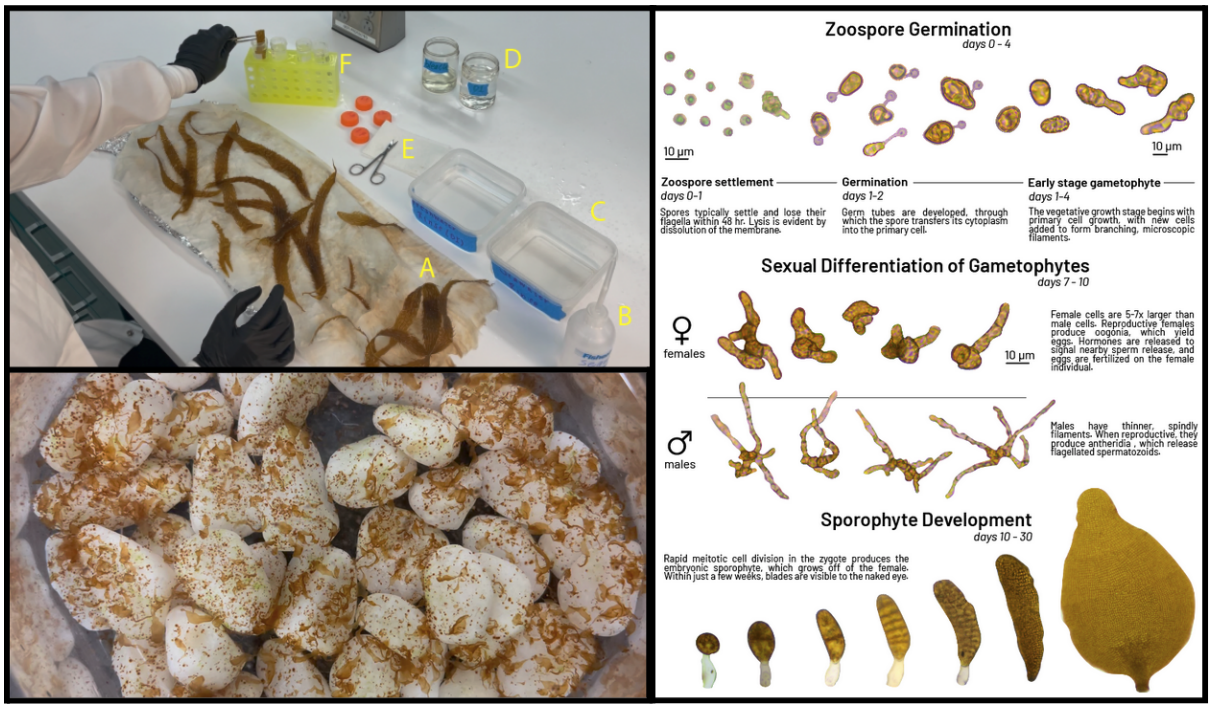


Figure 1 Visual aids included in the culturing handbook. Sori selection and sporulation (top left). Green Gravel cultures with 1 - 2 cm sporophytes, ready for deployment (bottom left). Developmental life history stages from laboratory growth trials (right).

A **video protocol and succinct protocol** (**Goal 1**), titled 'Field collection and laboratory maintenance of giant kelp for coastal restoration,' has been developed as a product of Green Gravel deployment trials in San Diego by the University of California, Irvine and in in Baja California, Mexico by the University of California, Santa Cruz. This video protocol helps to make scientific and technical information about giant kelp restoration available and accessible to a diversity of end-users. This product is in its final draft stages (see **Figure 2**) for an invited submission to the peer-reviewed *Journal of Visualized Experiments*.

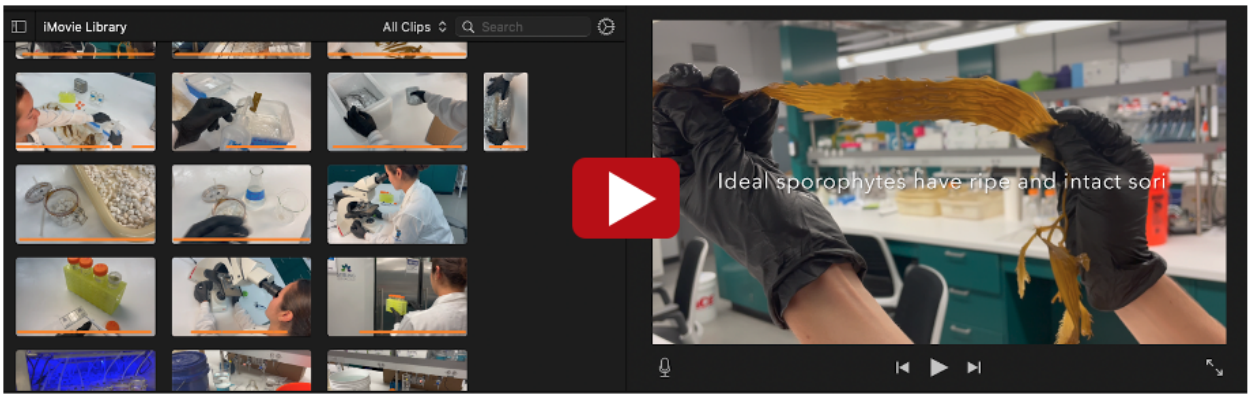


Figure 2 Snapshot of the development of a Green Gravel video protocol.

Thermal acclimatization to future-proof giant kelp restoration (AIM 2)

As critical marine habitats continue to deteriorate at unprecedented rates, it has been suggested that traditional techniques of reviving populations should be elevated to incorporate concepts of assisted evolution such as genetic reinforcement to anticipate future environmental stressors. Failure to integrate such methods may result in failure of restoration efforts. Assisted evolution has an exciting possible application with kelp restoration through translocation of beneficial genotypes into threatened areas or acclimatization of sporophytes to forecasted environmental stressors.

Exposing plants to stressful conditions is known to lead to increased tolerance to stress later in life, also known as hardening or priming. Acclimatizing plants to stress has also been shown to yield enhanced stress tolerance beyond the sexual generation as well. In **thermal experiments**, we acclimatize early stage kelp individuals (**Goal 3**) originating from different source populations (**Goal 4**) post-germination to assess stress-tolerance later in the kelp life history. Studies suggest that under projected climate-change conditions, increased temperature is particularly detrimental for development of kelps beyond the germling stage. With artificial treatments during cultivation and rearing, we will test whether exposure to temperature stressors affects the fitness of reared kelp originating from different populations (compared the local population) and improves subsequent transplantation success.

Outcomes

In this stage of data collection, kelp density and sex ratio has been estimated at 24 days for two different source populations and at two different temperatures: Santa Cruz (cooler K1 population) and San Diego (warmer K4 population) at 12℃ (standard for aquaculture, winter sea surface temperature of K1) and 20℃ (summer sea surface temperature of K4, heatwave conditions for K1). Current image analysis of survivorship, germination rate, reproduction, and female fecundity of individuals through time (**Figure 3**) and in response to a simulated marine heatwave will further investigate the effects of local thermal adaptation and inform rearing practices that support effective kelp restoration.

Response to thermal variability was shown to be significantly different among K1 and K4 differentiated populations, where temperature did not have an effect for the warmer K4 population, and did have an effect for the cooler K1 population (**Figure 4**).

Implications for Restoration

These preliminary results suggest a possible adaptive divergence in thermal tolerance traits of different source populations. Kelp gametophytes are often depicted as a resistance stage, suggesting they produce an all-purpose phenotype relatively insensitive to environmental variability. However, these results indicate that thermal variability imposes a significant pressure at this early stage. Researchers and managers are exploring techniques to boost kelp resilience against increasing temperatures and other climate change impacts. Future-proofing strategies, such as genetic rescue, assisted gene flow, and selective breeding, may be necessary to restore regions affected by severe kelp declines. Experimental manipulation of source populations and experimental hardening of restoration efforts will inform management strategies that will maximize survival and retention of transplanted kelps under projections of future climate stressors.

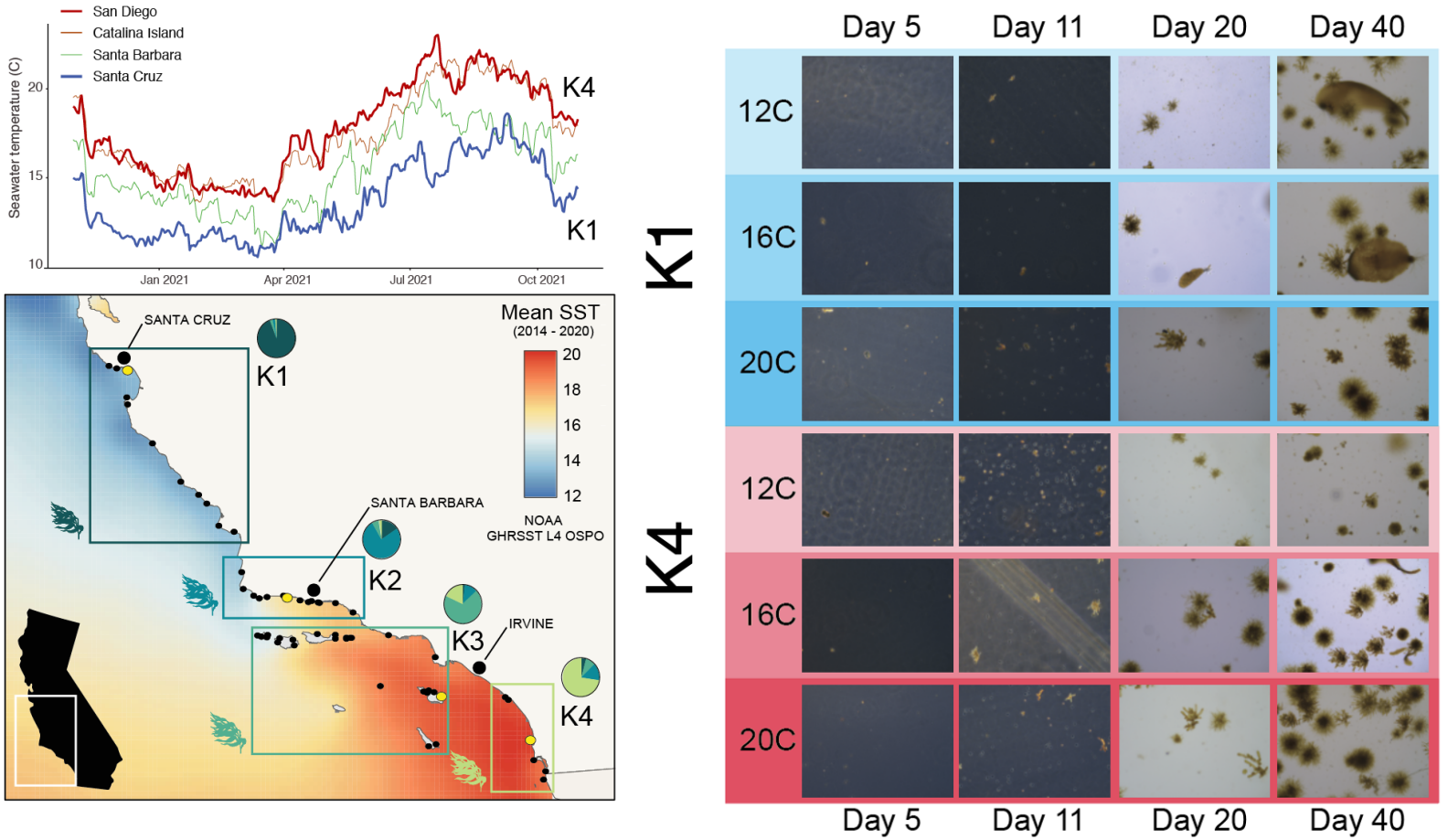


Figure 3 Seawater temperature monitoring from November 2020 to November 2021 (left side, top panel) and genetic continuity and geographic clusters of *Macrocystis* in California overlaid on average sea surface temperature (SST) data from June 2014 to May 2020 (left side, bottom panel). Black filled circles represent sampling locations obtained from Johansson et al. (2015) and are enclosed by colored boxes that represent the predominant genetic cluster (K1-K4 regions). Pie charts represent the average *Macrocystis* genotype for each region. Mean SST was generated using datasets from the National Oceanic and Atmospheric Administration's Office of Satellite and Product Operations (OSPO). Map generated using R v4.1.2. Example image time series following the growth and development of gametophytes and sporophytes originating from K1 and K4 populations cultured at three different temperatures (right side). K1 = Santa Cruz, K4 = San Diego.

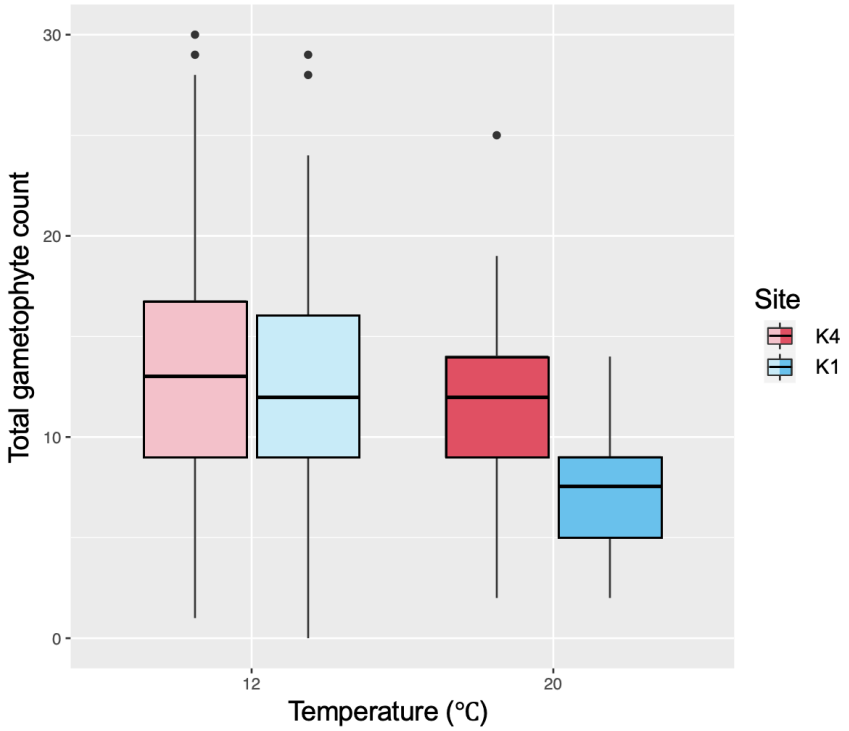


Figure 4 Boxplot illustrating total gametophyte count of two populations cultured at two different temperatures (N = 300 images from 60 samples). K1 = Santa Cruz, K4 = San Diego.

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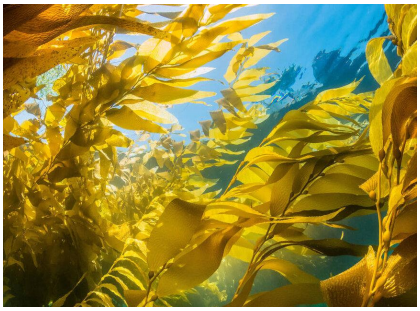

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A **video protocol and succinct protocol** (**Goal 1**), titled 'Field collection and laboratory maintenance of giant kelp for coastal restoration,' will be submitted to the peer-reviewed Journal of Visualized Experiments by the end of June 2023. The video protocol illustrates key methods to giant kelp culturing outlined in the handbook paired with a written protocol.

Thermal acclimatization to future-proof giant kelp restoration (AIM 2)

As critical marine habitats continue to deteriorate at unprecedented rates, it has been suggested that traditional techniques of reviving populations should be elevated to incorporate concepts of assisted evolution such as genetic reinforcement to anticipate future environmental stressors (Coleman et al. 2020). Failure to integrate such methods may result in failure of restoration efforts. Assisted evolution has an exciting possible application with kelp restoration through translocation of beneficial genotypes into threatened areas or acclimatization of sporophytes to forecasted environmental stressors.

Exposing plants to stressful conditions is known to lead to increased tolerance to stress later in life, also known as hardening or priming (Boyko et al. 2010; Boyko & Kovalchuk 2011). Acclimatizing plants to stress has also been shown to yield enhanced stress tolerance beyond the sexual generation as well (Coleman-Derr & Tringe 2014). In **thermal experiments**, we acclimatize early stage kelp individuals (**Goal 3**) originating from different source populations (**Goal 4**) post-germination to assess stress-tolerance later in the kelp life history. Studies suggest that under projected climate-change conditions, increased temperature is particularly detrimental for development of kelps beyond the germling stage (Shukla & Edwards 2017). With artificial treatments during cultivation and rearing, we will test whether exposure to temperature stressors affects the fitness of reared kelp originating from different populations (compared the local population) and improves subsequent transplantation success. Experimental manipulation of source populations and experimental hardening of restoration efforts will inform management strategies that will maximize survival and retention of transplanted kelps under projections of future climate stressors.

AIM 1 – METHODS, RESULTS & OUTCOMES

Visualized experimental protocol for giant kelp restoration

In preparation for submission at the *Journal of Visualized Experiments*

Phoebe D. Dawkins[1], Andrea Paz-Lacavex[2], Evan A. Fiorenza[1], Makena Rush[1], Matthew Bracken[1], and Joleah B. Lamb[1]

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Additional Resources

Draft Video Protocol, Supplementary Appendix of Tools, Draft Management Protocol

OVERVIEW

Kelp forests are facing unprecedented loss due to climate-driven ecological stressors necessitating innovative restoration strategies. Effective application of these strategies will greatly benefit from the ability to harness citizen science efforts at the management scale. However, there remains a lack of resources for non-technical researchers that provide accessible guidelines for citizen scientist-led kelp restoration. To this end, we have produced two separate restoration outputs that are crafted for different audiences. The first is a JoVe publication, which will be accompanied by a supplementary video protocol (**Figure 1**), detailing methods for sample collection, chemical preparation, seeding substrata, and rearing, with an extensive appendix of functional tools to support restoration efforts in practice. These tools include a calculator for density calculations for zoospore release, volume calculations for media enrichment, a comprehensive list of expenses, templates for logging progress, and a calendar of expectations for life stage development. The second product is a longer managerial protocol including specific information about California-based restoration and permitting. Our visualized protocols act as critical visual aids, democratizing restoration practices and increasing engagement. Links to the video protocol, the appendix of tools, and the full managerial protocol are listed above.

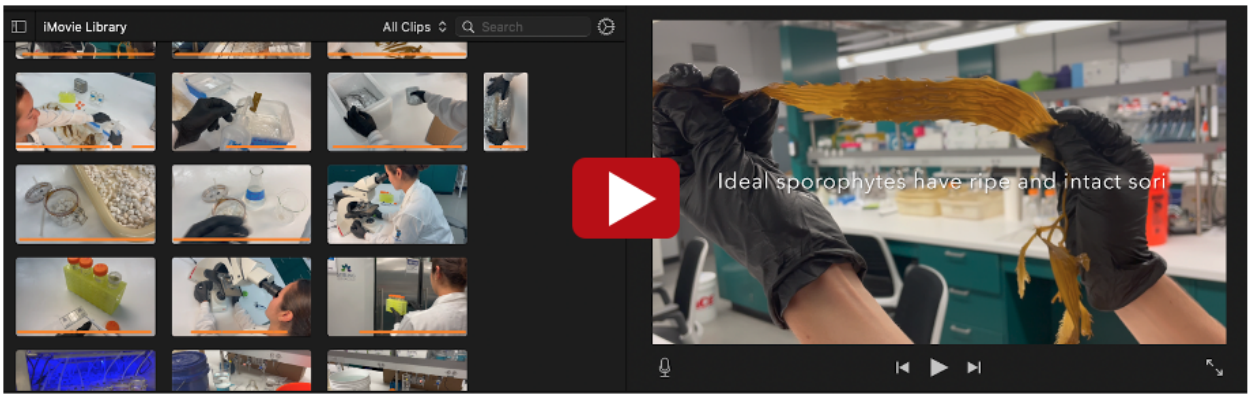

Figure 1 Snapshot of the development of our Green Gravel video protocol in iMovie. **Click** on the image to view the draft video protocol.

INTRODUCTION

Canopy-forming kelps across California – particularly bull kelp (*Nereocystis luetkeana*) and giant kelp (*Macrocystis pyrifera*) – have suffered major declines associated with with dramatic shifts in trophic dynamics and environmental stress events, resulting in up to 95% canopy loss (e.g., Rogers-Bennett & Catton, 2019). Persistence of the alternative community states associated with kelp loss necessitate application of scalable interventions that restore underwater kelp forests from the bottom up. Success of assisted recovery techniques is often hampered by resource limitations (e.g., Tracey et al., 2015), while active restoration efforts are limited to small scales due to reliance on continual transplantation of kelp (Layton et al. 2020). These efforts often require the destructive harvest of large quantities of whole adult donor plants, which may compromise closely associated and vulnerable populations.

Green Gravel overcomes important limitations that currently constrain kelp restoration. Principally, this tool has shown to be cost-effective and efficient, with great potential for upscaling to restore large areas. The cost of this tool (~$7 US m−2) is comparable to other methods of transplantation and recruitment enhancement (Carney et al. 2005), while also being methodologically efficient. *Green Gravel* does not require manipulation of adult kelps or installation of artificial structures. Due to the sizes of individual gravel units, this tool is manageably transported and handled. Moreover, handling did not prevent eventual successful growth of plants onto the gravel, suggesting this method is robust to partial kelp mortality from rough treatment during deployment (Fredriksen et al. 2020). Implementation of this tool also does not require diving, circumventing intensive underwater labor by skilled divers and strict health and safety considerations. Unlike classic restoration methods, *Green Gravel* does not require destructive collections of donor plants, reducing impacts to associated organisms in these highly diverse ecosystems. Reproductive tissue from adult plants must be harvested for zoospore release, but this can be achieved through partial, non-destructive removal of sporophylls or sori without damaging the primary meristem of adult plants (Fredriksen et al. 2020). *Green Gravel* represents a promising kelp restoration tool due to cost-effectiveness, scalability, and feasibility.

As this is a newly developed technique, the efficacy of this tool has only been exhibited with sugar kelp (*Saccharina latissima*) in Norwegian fjords. While *Green Gravel* is a promising restoration tool for canopy-forming giant kelp in California, modifications must be made to the published protocols and tested under a range of environmental conditions. For example, there are a number of fundamental differences between *Saccharina* and canopy-forming kelps like *Macrocystis* and *Nereocystis* that necessitate adjustments of rearing and deployment (**Table 1**), particularly the ultimate size of mature individuals. Average *Saccharina* plants grow to be no larger than 3m tall, whereas *Macrocystis* plants grow up to 50m. This order-of-magnitude size difference requires the reassessment of current outlined methods and experimental testing of a suite of deployment strategies.

Table 1. Differences in species-specific life history characteristics of sugar kelp (*Saccharina latissma*) versus canopy-forming species, including giant kelp *(Macrocystis pyrifera)* and bull kelp (*Nereocystis luetkeana*). References from: (Vadas 1972; North et al. 1986; Denny et al. 1997; White & Marshall 2007; Redmond 2013).

Life history characteristic	*Saccharina latissima*	*Macrocystis pyrifera*	*Nereocystis luetkeana*
Maximum Growth Rate	5 cm day^{-1}	60 cm day^{-1}	6 cm day^{-1}
Thermal Limits	< 20 ºC	< 20 ºC	< 17 ºC
Depth Range	0 - 30 m	2 - 30 m	4 - 22 m
Lifespan	2 - 5 years	6 - 8 years	1 year
Reproductive Season	Annual (primarily winter)	Perennial	Annual (primarily autumn)
Maximum Size	< 3 m	< 50 m	< 45 m
Holdfast Size	< 10 cm	< 1 m	< 40 cm

DEVELOPMENT OF GIANT KELP SYSTEMS

Experimental Laboratory Incubation System (Goal 1)

We have custom outfitted a 381L capacity refrigerated incubator system with full LED spectrum lighting and a filtered air source (**Figure 2**). This system supports 80 x 13.5 ounce container replicates for growth trials and 32 x 1 L flasks for long-term cultures. Individual bulk cultures of gametophytes released from 7-10 kelp plants from each population have been maintained in Petri dishes for future bulking of biomass, as well as for isolation for germplasm conditions.

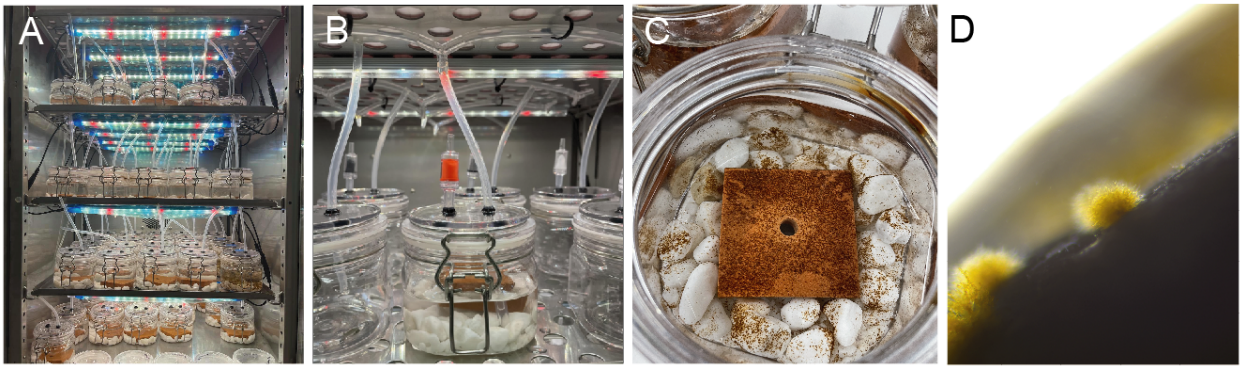

Figure 2 (**A**) Incubator shelves containing experimental replicate containers from San Diego (n = 36) and Santa Cruz (n = 36). (**B**) Close-up of container with 3.5 oz of aquarium-grade gravel and one small natural stone tile submerged in 200 mL of enriched seawater media, connected to a filtered air source. (**C**) Seeded substrates reared for 8-weeks. (**D**) Magnified gametophytes growing on gravel surfaces.

Experimental Field Deployment (Goal 2 and Goal 4)

Shelved experimental mesocosms

Gravel seeded with zoospores from Santa Cruz (K1 population) and San Diego (K4 population) was deployed on 4 September 2022. For the deployment of these gravel substrates, we deployed experimental mesocosms (20" x 20" x 6" shelves), within which we simulated a natural reef with small cobbles and boulders (**Figure 3**). Seeded gravel will be placed in these enclosures (n = 5), so we can monitor differential growth between K1 and K4 populations through time (Project Aim 2, Goal 4). Each cage will contain approximately 4 pounds of seeded gravel, pooled from 10-12 containers from the respective source population. Kelp survival will be monitored weekly for the first month. Alongside the deployment of cages with seeded gravel, we also deployed unseeded gravel within natural crevices in the rocky reef at our site and assess retention within our plots, informing the success of future deployment of un-caged, seeded gravel.

Deployment of fixed tiles

Although seeded gravel is the easiest and most scalable method for deployment of restoration outplants, loose/un-fixed gravel may be lost before establishment and is difficult to monitor. Thus, we constructed temporarily fixed small natural terracotta tiles (approximately 4 square inches in size) as a tandem experimental approach alongside seeded gravel. These tiles are unlikely to be lost and will allow for easy monitoring, as confirmed by Dr. Cayne Layton from the University of Tasmania. When seeded tiles have sporophytes that are approximately 1-3mm (approximately 8 weeks post-inoculation), they will be bolted 3 inches above concrete stations (n = 10) and deployed alongside experimental mesocosms. This elevation above the station will increase outplant visibility when monitoring and ensure that the kelps are not smothered in sand. Each concrete station is approximately 12 x 12 in and has five holes drilled into it which we set into place 4 galvanized bolts and one hole used for a unique tag.

Monitoring

Experimental units were scheduled to be monitored 1 week and 2 weeks post-deployment, followed by monthly checkpoints for 10 months before retrieval. However, all deployments were unable to be located after storm conditions at our deployment site, with several cages found onshore nearby. In tandem trials applying identical protocols conducted by our collaborator Andrea Paz-Lacavex in Baja California, Mexico, we have observed overgrowth success onto nearby substrates (**Figure 3**).

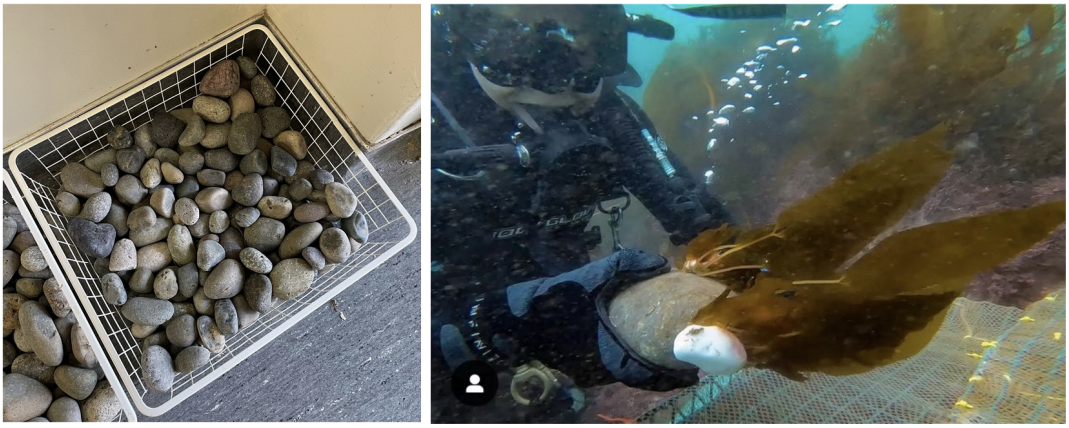

Figure 3 Experimental mesocosms deployed 4 September 2022 to simulate natural reef substrate using small cobbles and boulders (left). Overgrowth success observed in tandem trials in Baja California, Mexico, conducted by PhD student collaborator Andrea Paz-Lacavex (right).

DEVELOPMENT OF A LABORATORY REARING PROTOCOL

In order to implement *Green Gravel* in California, we amended existing protocols to incorporate rearing conditions that are appropriate for the Southern Californian canopy-forming giant kelp, *Macrocystis pyrifera* (**Figure 4**).

Figure 4 General diagram illustrating the phases of the Green Gravel method included in protocol.

1. Facilities and Material Preparations

1.1. Incubator systems with a built-in electric outlet or an access port for wires and tubing can be fitted with lights and a filtered air source for kelp culture.

1.2. Rearing temperatures should be site- and season- specific, ranging between 10 - 15°C. Temperature of your growth media can be measured using thermometers or temperature guns.

1.3. Full-spectrum lights are set to a 12 hour light : 12 hour dark photoperiod, using the timing settings on the light source or by plugging the light source into a timer with a programmable cycle. Light intensities range between 0 - 180 uE, and are set based on the development of your kelp cultures. Light intensity can be measured with a PAR quantum meter and adjusted using a dimmable light source or by layering cellophane or mesh over the light source.

1.4. Aeration is important for haptera development and holdfast strength for Macrocystis cultures, and can be added to cultures with the use of air pumps. Filters (.2 µm) are optional and recommended, as they help to reduce air-borne bacterial contamination. Aeration pressure must be sufficient to circulate water in all culture containers.

1.5. All materials (i.e. tweezers, scissors, and glassware) must be cleaned with soap Alconox (5%), rinsed with distilled water, and if applicable, autoclaved at 160 PSI for 15 minutes, and stored away from airflow.

2. Seawater Filtration

2.1. Large volumes of filtered seawater will be needed for your growth media. Calculate how much seawater you will need to replenish your culture containers each week and schedule this filtration task accordingly. Large batches of filtered seawater can be prepared ahead of time and stored for up to 6 months.

2.2. Using a clean flask, pour your unfiltered seawater into a filter with a pore size of .1 - .55 µm. Using a vacuum filtration system, pull the unfiltered seawater into a sterile bottle. Turn your vacuum source off before all the water is pulled through to avoid damaging the filter, and pour the filtered water into a dedicated sterile container. Keep count of the volume of seawater filtered; this volume will be used to calculate the necessary amount

of nutrients and media to be added. Store filtered seawater away from sunlight in a refrigerated facility at 8 to 10°C if possible.

2.3. If access to natural seawater is limited, artificial seawater can be used. Using a refractometer or salinometer, *Instant Ocean* should be diluted using distilled water (approximately 76 g Instant Ocean for 2 L of distilled water) to a salinity of 32 - 34 ppm. Alternatively, natural seawater that has already been filtered, sanitized, and pH balanced can be purchased from aquariums and pet stores in bulk and does not require mixing, measuring, or adjusting. Media enrichment for these options is still necessary.

3. Seawater Enrichment

3.1. Enrichment of seawater with nutrients and vitamins will help to support *Macrocystis* growth. Provasoli Enriched Seawater media, also known as P.E.S., is a common and widely used medium designed for algal growth. P.E.S. can be purchased from algal culture centers, but can be costly at scale. Preparations of P.E.S., as well as additional vitamins are described in the New England Seaweed Culture Handbook (Redmond et al. 2014).

3.2. Provided here is (1) a tool to calculate the proportion of solutions needed for the change of culture media based on the amount and volume of culture containers, and (2) a guideline of expected laboratory operational expenses.

4. Sori Collection

4.1. *Macrocystis* individuals begin fruiting once they attain a size of four to eight stipes in their first year of growth. For reproductive tissue collection, 3 - 5 sporophylls from 10 - 15 fertile individuals of *Macrocystis* with visible sori in healthy condition are selected from each source population. Reproductive tissue is stored according to source individuals for later isolated cultures.

4.2. Fresh tissue is kept in bags of ambient seawater and stored in coolers at 12°C for transportation before sporulation in the laboratory.

4.3. In the event that sporophylls are collected from other locations and need to be shipped to our lab, they are wrapped in moist paper towels soaked in filtered-sterilized seawater, and wrapped again in aluminum foil to avoid light penetration. These aluminum packages containing sporophyll samples are wrapped in bubble wrap or cardboard and then placed in a cooler containing ice and prepared for FedEx overnight shipping.

5. Sporulation and Inoculation

5.1. Upon arrival at the lab, sporophylls are cleaned of epiphytic organisms gently with gauze cotton pads and a freshwater rinse.

5.2. Then, sori from the individuals are cut out and placed in a moist paper towel in the fridge for 24 hours to desiccate the tissue.

5.3. Finally, complete a post-desiccation immersion of the reproductive material in a filtered‐sterilized seawater medium for zoospore release for a maximum of 4 hours in 4℃.

5.4. A spore solution of 500 kelp spores ml^{-1} is determined using a hemocytometer and added to modified containers containing glass slides for monitoring kelp development and aquarium-grade gravel submerged in enriched seawater media for algal growth (27 mL Provasoli media / 973 mL seawater batch).

5.5. Provided here is a spreadsheet of tools that support growers to: (1) calculate spore density for gravel inoculation, (2) calculate the proportion of solutions needed for the change of culture media based on amount of containers and their volume, (3) a calendar as a guideline for kelp development, juvenile development expectations and weekly hours investment by a laboratory technician, (4) track weekly requirements, (5) to provide guidelines of expected laboratory operational expenses and (6) share a template to log laboratory activities.

6. Maintenance and Monitoring

6.1. Glass slides within containers are monitored every day for the first two weeks to assess germination success and gametophyte development. Life history traits including survivorship, germination rate, vegetative development, reproductive maturity and fecundity are noted (**Figure 5**).

6.2. Light conditions are adjusted according to observed developmental stages (see **Giant Kelp Rearing**, below). Enriched seawater media is drained from each container and re-filled weekly to replenish necessary nutrients and minerals for *M. pyrifera* growth.

6.3. Provided here is (1) a calendar as a guideline for kelp development, juvenile development expectations and weekly hours investment by a laboratory technician, (2) an outline of weekly requirements, and (3) a template to log laboratory activities and observations.

7. Giant Kelp Rearing

7.1. Temperature is set to 12℃ in our experimental laboratory incubation system. Full spectrum LED lights for aquatic plants outfitted above each shelf are set to a 12 light: 12 dark cycle, with a light intensity of 0 - 10 through spore settlement (1 d), and 10 - 20 µE through gametogenesis (approximately 2 wk). Aeration with a filtered air source is provided.

7.2. When embryonic sporophytes are observed (approximately 3 wk), light intensity is increased every week by approximately 20 µE.

7.3. When initial kelp blades are visible, aeration is increased until soft bubbles at the water surface indicate sufficient flow within culture containers — this is important for haptera development and holdfast strength.

7.4. When sporophytes reach 1 - 2 cm in length (approximately 8 wk), light intensity reaches approximately 180 µE. Kelps grown for longer periods in the lab may become adapted to lab conditions and perform poorly after deployment.

8. Giant Kelp Vegetative Culturing

8.1. Population genetics plays a critical role in the success of *ex situ* conservation efforts. Long-term storage of many individual gametophytes from distinct populations will act as biological insurance of genetic diversity in a changing environment. In order to isolate individual females and males from our bulk cultures in low volume dishes, spore solution of 500 kelp spores ml^{-1} is added to Petri dishes containing 25 mL of artificial seawater media enriched with Provasoli (38 g Instant Ocean / 1 L seawater batch).

8.2. Once gametogenesis has occurred and females and males are distinguishable, individuals are isolated first in 24-well plates and then in 5-mL tubes for long term germplasm preservation.

8.3. For these germplasm conditions, gametophytes are stored at 4 - 8℃ in red light to disrupt gamete production at an intensity of 1 - 5 µmol $m^{-2}s^{-1}$ in a 4 light : 20 dark cycle.

9. Giant Kelp Vegetative Culturing Seed Bank

9.1. To reduce lab culturing dependency on reproductive tissue collection from the natural reef, axenic, high-quality gametophyte stocks can be vegetatively propagated indefinitely and readily available for reproduction, growth, and deployment. For vegetative conditions, gametophytes are stored in 1-L round, flat-bottomed flasks at 12℃ in red light at an intensity of 5-10 µmol $m^{-2}s^{-1}$ in a 12 light : 12 dark cycle. In these conditions, gametophytes produce biomass asexually without gamete production.

9.2. To induce reproduction for experimental growth trials, bushy gametophytes can be pipetted into 1.5-mL epitubes and ground with a pestle and full spectrum LED light is gradually increased from 5 - 10 µmol $m^{-2}s^{-1}$ to 45 - 60 µmol $m^{-2}s^{-1}$ by 10 µmol $m^{-2}s^{-1}$ daily for photo-acclimation.

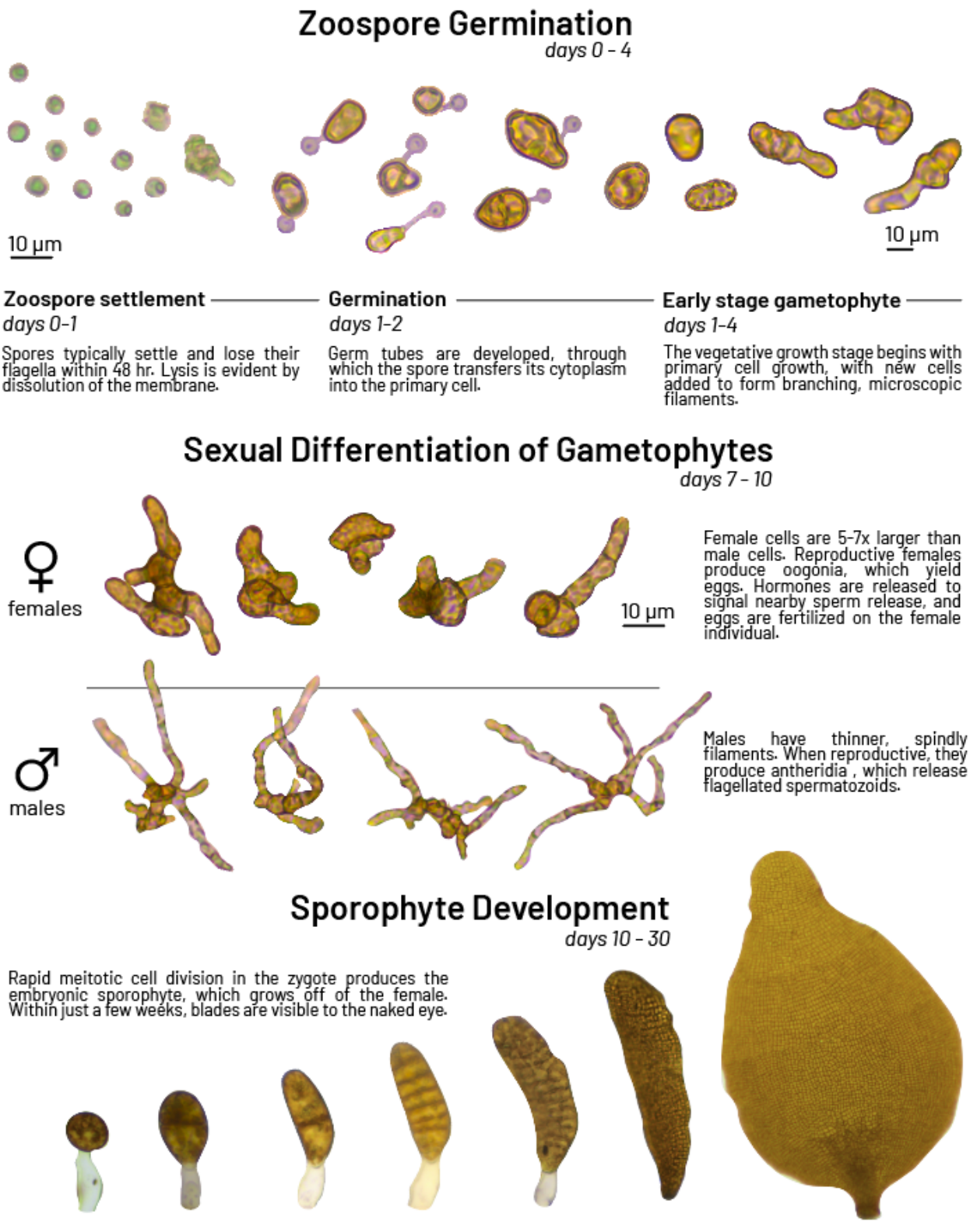

Figure 5 Developmental life history stages of giant kelp from laboratory growth trials in the Lamb Lab. Photos by Dawkins and Paz-Lacavex.

AIM 2 – METHODS, RESULTS & OUTCOMES

Thermal acclimatization to future-proof giant kelp restoration

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Manuscript in early stages of preparation
Additional Materials Data Collection Protocol

BACKGROUND

Climate change and anthropogenic disturbances are currently outpacing the adaptive capacity of natural populations, challenging traditional conservation aims of restoration to historic states (Hobbs et al. 2009; Perring et al. 2015; Oppen et al. 2015; Breed et al. 2018, 2019; Gurgel et al. 2020). Consequently, conservation frameworks have necessarily expanded to include anticipatory management that proactively consider resilience and adaptive capacity (Coleman et al. 2020). This includes a tool-box of assisted evolution methods that range from more revolutionary approaches such as genetic modification to more conservative approaches such as translocation of resilient genotypes to areas within their distribution and non-genetic manipulation such as acclimatizing individuals to environmental stress (Oppen et al. 2015).

Long-term persistent ocean warming compounded by the increasing frequency of extreme temperature anomalies have critical implications for canopy-forming kelps (Dayton et al. 1999; Jueterbock et al. 2013; Voerman et al. 2013; Bennett et al. 2015; Wernberg et al. 2016; Smale et al. 2019; Rogers-Bennett & Catton 2019). Increased ocean temperatures are associated with reduced upwelling and nutrient limited conditions (Zimmerman & Kremer 1984), while physiological thresholds to heat stress can result in mortality (Rothäusler et al. 2011). For instance, severe temperature anomalies during the 2014-2016 El Niño event pushed large-scale Southern California *Macrocystis* populations beyond their temperature thresholds that resulted in massive declines, emphasizing the significance of temperature as an environmental stressor (Cavanaugh et al. 2019). Projected intensification of climate-driven environmental stressors calls for anticipatory management that will **future-proof** performance of restoration efforts. In line with the California Ocean Protection Council's (OPC) management goal of safeguarding coastal marine ecosystems and communities in the face of climate change (Strategic Plan to Protect California's Coast and Ocean 2020–2025), we propose to incorporate modern restoration initiatives that enhance the inherent ability of kelp to adapt or acclimatize to improve transplantation efforts.

Adaptation and acclimatization processes influence an organism's ability to respond to environmental change. While adaptation involves genetic changes from one population to the next, acclimatization is a non-genetic phenotypic response that occurs through epigenetic processes (van Oppen et al., 2015). Both processes may alter performance and impact an organism's susceptibility to warming (Stillman 2003; Sanford & Kelly 2011; Howells et al. 2013). Selection pressures along thermal gradients can lead to local adaptation that can cause differential thermal-tolerance limits among populations (Stillman 2003; Sanford & Kelly 2011; Howells et al. 2013). For instance, a recent study suggested that high-latitude *Macrocystis* populations in California are more vulnerable to climate- or ENSO-driven

warming events (Hollarsmith et al. 2020). Conversely, organisms can acclimatize to stress, with thermotolerance induced by exposure to short-term, mild temperature increases (de Klerk & Pumisutapon 2008; Pumisutapon et al. 2012) or through gradually increasing to lethal temperatures (Larkindale & Vierling 2008).

Acclimatization and genetic adaptation mechanisms can be accelerated conservatively through the use of widely accepted techniques of adapted evolution. The application of assisted evolution practices are already being implemented for tree species to address climate change in forest ecosystems (e.g., (O'Neill 2008) and has been suggested for other large-scale restoration efforts to maximize evolutionary potential of outplants (e.g., Broadhurst et al., 2008; Vitt et al., 2010). While these strategies are inherently easier to manipulate in terrestrial systems, marine ecologists are beginning to explore their potential and feasibility as well (Buerger et al., 2020; Chakravarti & van Oppen, 2018; van Oppen et al., 2015, 2017). For instance, coral reefs, important foundational habitats, are threatened by a myriad of anthropogenic stresses that have led to unprecedented loss (Hughes et al. 2003; Harborne et al. 2017). In response to the deterioration of reefs globally, intervention strategies, such as active restoration and assisted evolution techniques have been increasingly advocated to protect remaining reefs and associated functions (Anthony et al., 2017; Darling & Côté, 2018; van Oppen et al., 2015). One such technique involves translocation of individuals between conspecific populations within the current distribution range of the species to increase tolerances to heat stress (van Oppen et al., 2014). Another method involves acclimatization of bacterial associates (Ziegler et al. 2017) or the algal symbionts (Buerger et al. 2020) to temperature extremes to yield heat-tolerant hosts. Thus, coral reef conservation has offered a solid framework for the use of assisted evolution in other threatened natural systems, including kelp forests.

DEVELOPMENT OF LABORATORY EXPERIMENTAL SYSTEMS

Experimental Laboratory Acclimatization System (Goal 3 and Goal 4)

In order to overcome obstacles associated with culturing in a flow-through seawater system, we designed and acquired an aquarium system to overcome diatom contamination, increase experimental replication, and enable treatment of individual tanks (**Figure 6**). This system contains 12 x 25 L tanks, each supplied with its own water source, filtration system, and water chemistry and temperature regulation. Thermal acclimatization experiments were conducted in this system.

Rationale for Thermal Experiments

Kelp forests along the California coast are facing unprecedented loss due to climate-driven environmental stressors, necessitating innovative restoration strategies. Projected intensification of environmental stressors calls for anticipatory management that will future-proof performance of restoration efforts. Experimental manipulation of source populations and experimental hardening of Green Gravel will inform management strategies that will maximize survival and retention of transplanted kelps under projections of future climate stressors. In these trials, we assess if thermal acclimatization of two distinct populations at microscopic, pre-reproductive life stages influence the eventual population's ability to respond to environmental change.

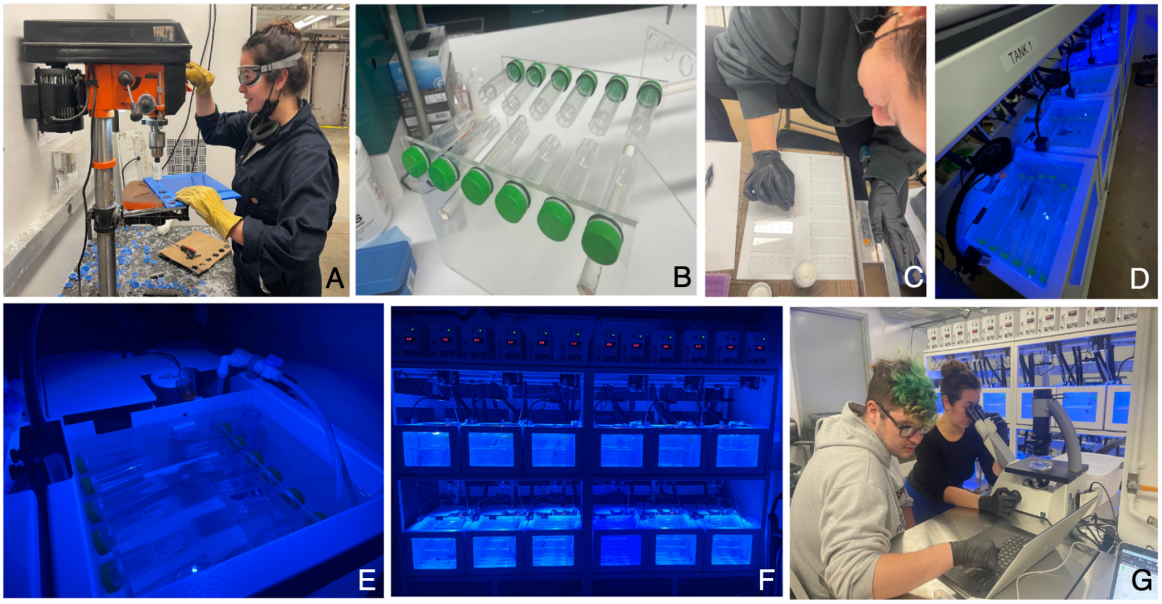

Figure 6 Photo panel of: **(A-B)** the construction of a multi-level rack system to fit 50mL Falcon tube replicates, **(C)** preparation of gridded glass sides for monitoring microstage kelp development, **(D-F)** acclimatization system in the Lamb Lab, and **(G)** team members Phoebe Dawkins and Evan Fiorenza imaging glass sides using an inverted microscope.

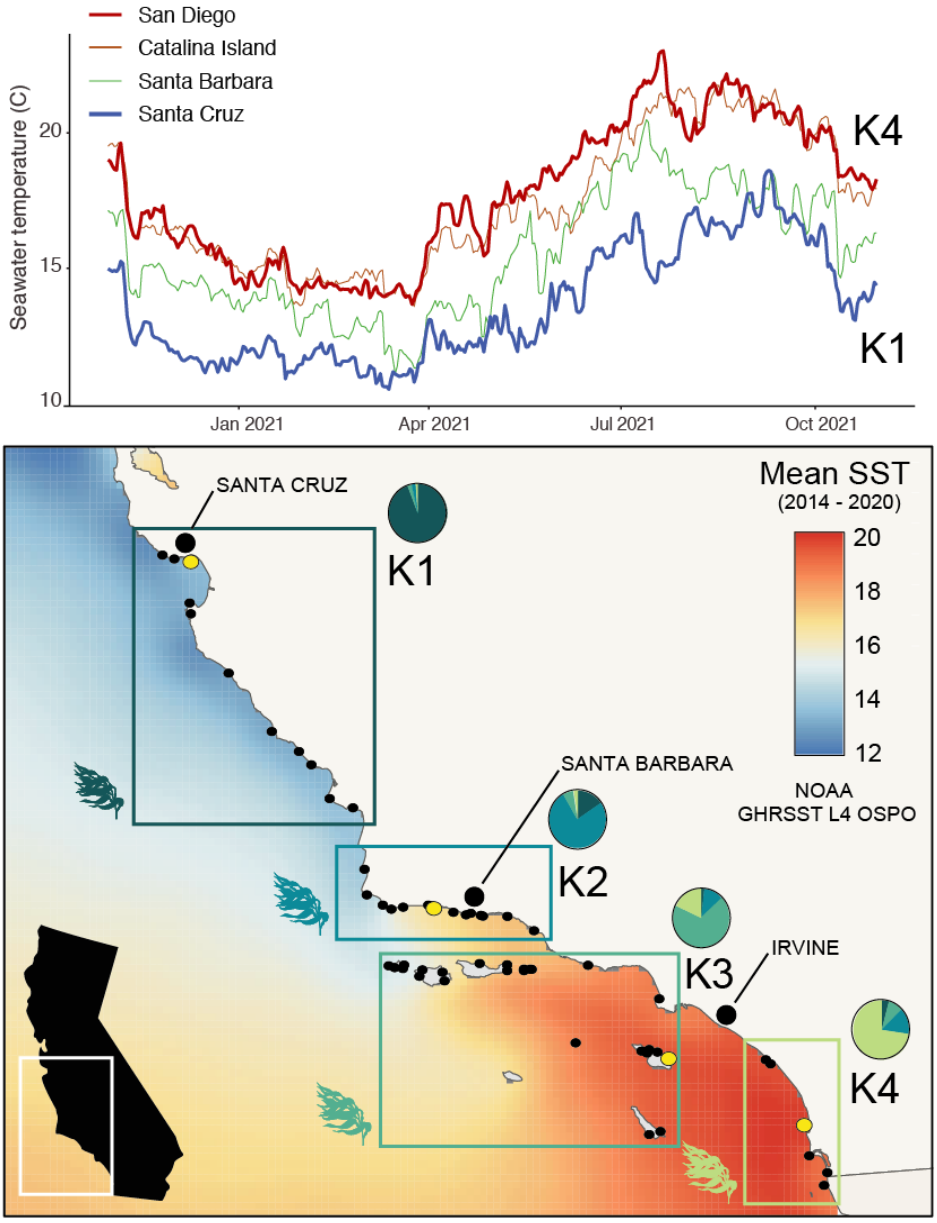

Figure 7 Seawater temperature monitoring from November 2020 to November 2021 (Year 1, Top panel) and genetic continuity and geographic clusters of *Macrocystis* in California overlaid on average sea surface temperature (SST) data from June 2014 to May 2020 (Bottom panel). Black filled circles represent sampling locations obtained from Johansson et al. (2015) and are enclosed by colored boxes that represent the predominant genetic cluster (K1-K4 regions). Pie charts represent the average *Macrocystis* genotype for each region. Mean SST was generated using datasets from the National Oceanic and Atmospheric Administration's Office of Satellite and Product Operations (OSPO). Map generated using R v4.1.2.

Experimental Design

We conducted a long-term trial beginning on 6 March 2022 to elucidate temperature dependent effects on the development of microstages and juvenile stages of *M. pyrifera* collected from the K1 (Santa Cruz, 36.60167° N, 121.88508° W) and K4 (Santa Diego, 32°51'01.3" N 117°16'33.6" W) populations (**Project Aim 2, Goal 3, Figure 7**). In preparation for these experiments in our newly constructed aquarium system (see Year 1 Annual Report), we developed and constructed a multi-level racks system to fit 50mL Falcon tube replicates, each containing a glass slide with standardized grid cells (fixed fields) in order to quantify early life history stages and traits using high resolution images from our Leica DMi1 inverted microscope and Leica C1 FLEXACAM.

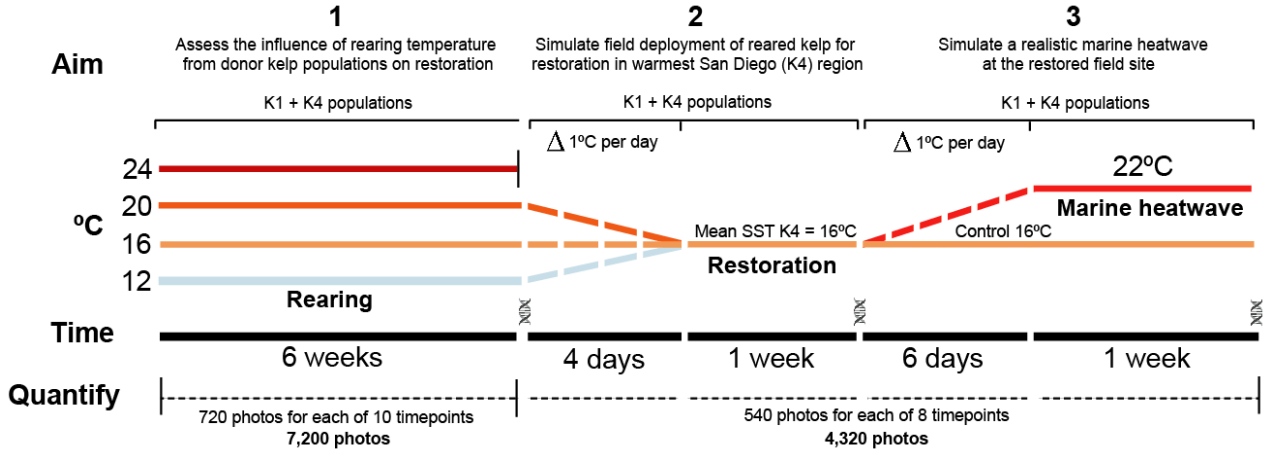

Figure 8 Illustration depicting the Aims 1-3 and respective rationales of our thermal experiments, with shifting temperature regimes and image quantification.

Experiment A

For the first 40 days of our experimental trial (Experiment A, 6 March 2022 - 16 April 2022), we assessed the influence of rearing temperature from two distinct donor kelp populations (**Aim 1, Figure 8-9**). We reared K1 and K4 kelps in 4 distinct thermal conditions (12°C, 16°C, 20°C, and 24°C; see rationale **Table 2**), with nutritional and light conditions held constant for each treatment. Approximately every 4 days, we imaged fixed fields of our glass slide replicates to track the development of individuals throughout time (4 thermal conditions x 3 replicate tanks x 2 populations (K1 and K4) x 6 slide replicates x 5 fixed fields = 720 images per time point x 11 timepoints = 7,920 photos). By the end of Experiment A, there was no kelp survival in the 24°C thermal condition, and thus this treatment was terminated and excluded from Experiment B.

Experiment B

In days 41-68 of growth (Experiment B, 17 April 2022 - 14 May 2022), we simulated a field deployment of reared kelp for restoration in the warmer K4 region (**Aim 2**) followed by a marine heatwave (**Aim 3**). After 40 days of growth, the temperature of each aquarium was brought to 16°C (average SST in our most southern population, and the average of our remaining thermal conditions) at a rate of 1°C per day to assess thermal acclimatization. Once brought to this control temperature, all tanks experienced an acute marine heatwave (MHW) condition of 22°C over a 4 day period, to simulate a realistic MHW condition that occurred in southern limit of *M. pyrifera* ranges in the northern hemisphere

(http://www.marineheatwaves.org/tracker.html). Differences in K1 and K4 populations to this thermal stress will be quantified at both pre-MHW and post-MHW conditions via sporophyte characterizations of biomass and morphology. For Experiment B, we imaged fixed fields of our glass slide replicates at 5 timepoints (3 thermal conditions x 3 replicate tanks x 2 populations (K1 and K4) x 6 slide replicates x 5 fixed fields = 540 images per time point x 5 timepoints = 2,700 photos). We have also collected DNA samples to assess the genetic composition of individual sporophytes pre-MHW and post-MHW.

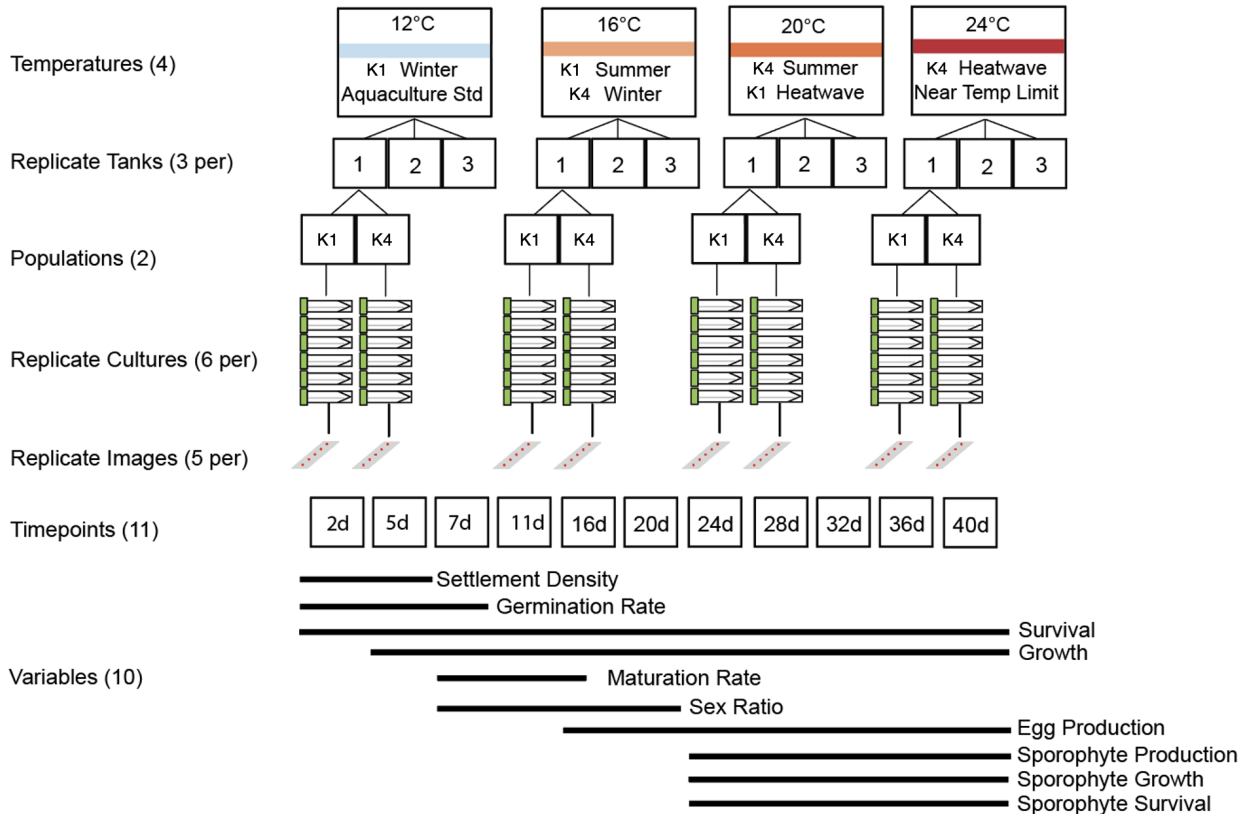

Figure 9 Illustration depicting experimental design for Experiment A (4 thermal conditions x 3 replicate tanks x 2 populations (K1 and K4) x 6 slide replicates x 5 fixed fields = 720 images x 11 time points), resulting in a total of 7,920 images.

Table 2. Rationale for experimental temperature treatments.

Temperature	Rationale
12°C	standard for aquaculture, winter SST of K1
16°C	summer SST of K1, winter SST of K4
20°C	summer SST of K4, 4C heatwave of K1
24°C	Upper limit of gametophyte survival (Fain and Murray 1982), Heatwave of K4/near max SST of K4

Characteristics of Life Cycle and Criteria for Life History Stage Determination

All *Laminarian* kelps, including *M. pyrifera*, demonstrate a heteromorphic haploid-diploid life cycle with an alternation of haploid dioecious gametophytes and macroscopic diploid sporophytes. Male and female gametophytes exhibit unambiguous sexual dimorphism; post-gametogensis, female cells are 5-7 times larger than male gametophytes, and males display highly branched filaments. Furthermore, females produce eggs that — once fertilized — produce diploid sporophytes, from which adult giant kelps grow.

Eight microscopic life-history stages were defined, based on developmental characteristics and reproductive structures: (1) settled meiospores, (2) germinated spores, identified by a germination tube, (3) 1-2 celled gametophytes, (4) gametophytes of >2 cells, (5) reproductive female gametophytes (i.e. bearing oogonia), (6) fertilized female gametophytes, (i.e. bearing microscopic sporophytes), (7) male gametophytes, and (8) multicellular (>4 cell) sporophytes. All microscopy photos were taken with the Leica Inverted Microscope DMi1 and FLEXACAM C1 Camera, and analyzed with ImageJ software.

Life History Traits Estimations and Data Analyses

Observations were made over two months on days 2, 5, 7, 11, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 60, and 68. Life history stages were counted on five evenly spaced fixed visual fields per slide. A small dot was etched using chemical media (Armour Etch Glass Etching Cream), to mark the top-right point of each of the five microscopy fields and ensure fixed fields through time.

All data will be collected using ImageJ software (**Figure 10**, Shneider et al. 2012). According to methods outlined by (Oppliger et al. 2012), for each observation date, survivorship will be estimated on day 2 of culture by counting the number of well-colored gametophytes over the initial count on the same area. The germination rate will be estimated on days 2 and 5 of culture by counting the number of germinated spores of the total number of surviving spores. On days 11, 16, and 24 of culture, the frequencies of female gametophyte of 1-2 cells and >2 cells will be quantified to characterize vegetative development. Sex ratio will be estimated after 15 days, expressed as the frequency of males/ (males+females). Reproduction will be estimated at days 11, 16, and 24 of culture; (1) the frequency of mature females/ 30 females in each visual field, and (2) female fecundity will be estimated using frequency of females bearing juvenile sporophytes in each visual field.

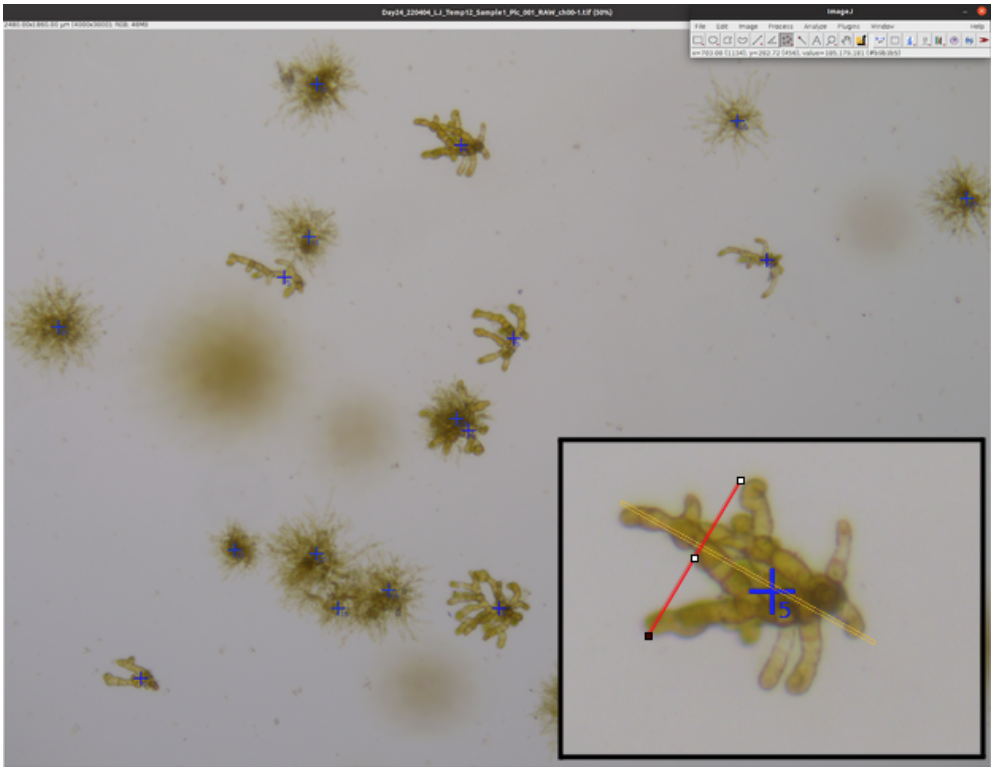

Figure 10 Example of a single visual field viewed and analyzed in ImageJ software. Counted gametophytes are marked by blue (+). Bottom right image illustrates measurements of minor and major axes.

PRELIMINARY RESULTS & DISCUSSION

In this preliminary stage of data collection, gametophyte density and sex ratio has been estimated at 24 days for K1 and K4 treatments at 12℃ (standard for aquaculture, winter SST of K1) and 20℃ (summer SST of K4, 4C heatwave of K1). Current image analysis of survivorship, germination rate, reproduction, and female fecundity of individuals through time (**Figure 11**) and in response to a simulated marine heatwave will further investigate the effects of local thermal adaptation and inform rearing practices that support effective kelp restoration. To test for differences in density and sex ratios, we employed generalized linear mixed effects models with Poisson distribution using the function *glmmTMB()* in package *glmmTMB* and pairwise comparisons were conducted with *emtrends()* from package *emmeans*.

Response to thermal variability was shown to be different among K1 and K4 differentiated populations ($t = 2.7$, $p = 0.007$) , where temperature did not have an effect for the warmer K4 population (estimate = -0.01, standard error = 0.01, CI = [-0.03, 0.01]) , and did have an effect for the cooler K1 population (estimate = -0.06, standard error = 0.02, CI = [-0.10, -0.03]) (**Figure 12**) , suggesting a possible adaptive divergence in thermal tolerance traits. Kelp gametophytes are often depicted as a resistance stage (e.g. Ladah & Zertuche-González 2007), suggesting they produce an all-purpose phenotype relatively insensitive to environmental variability. However, these results suggest that thermal variability imposes a significant pressure at this early stage.

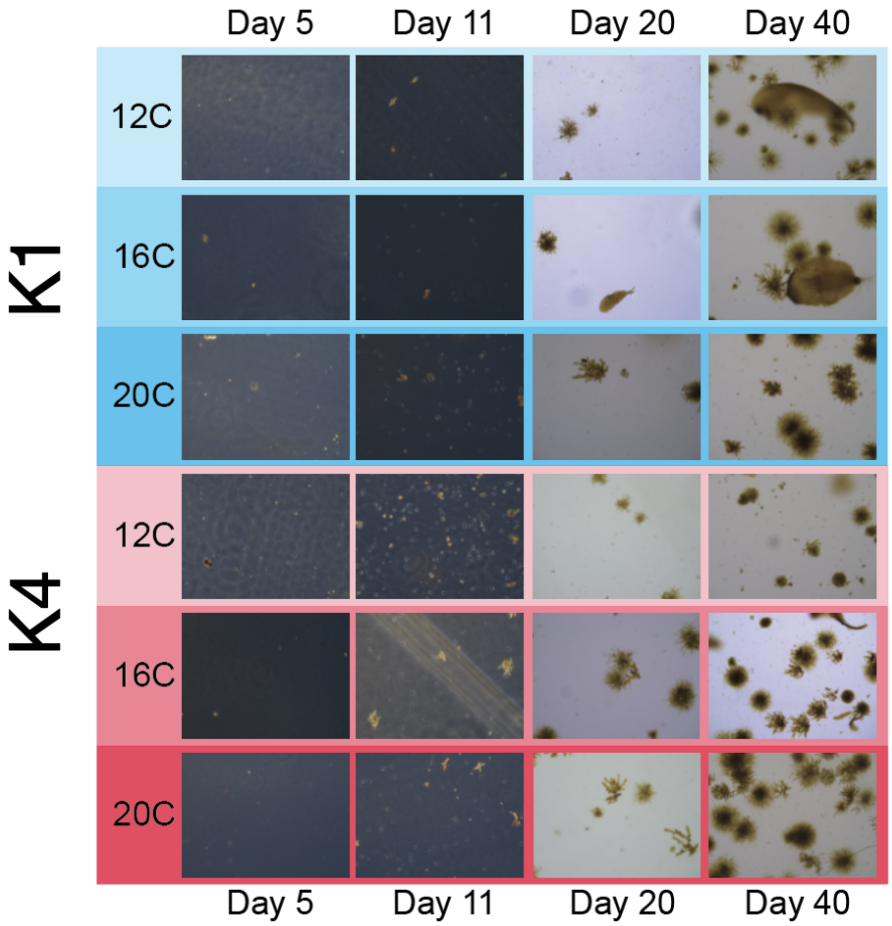

Figure 11 Example image time series following the growth and development of gametophytes and sporophytes originating from two populations cultured at three different temperatures. K1 = Santa Cruz, K4 = San Diego.

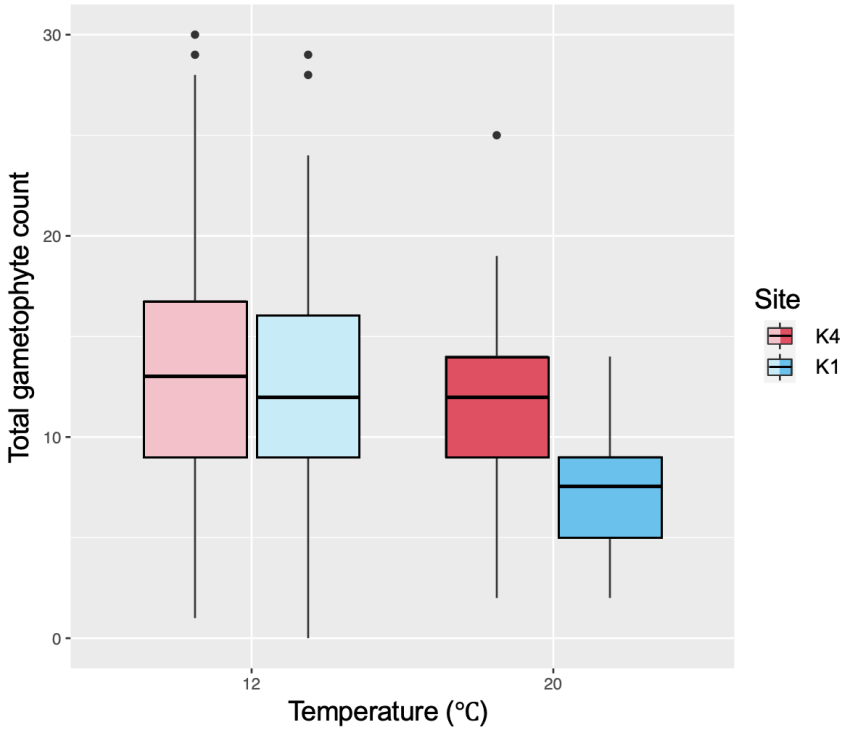

Figure 12 Boxplot illustrating total gametophyte count of two populations cultured at two different temperatures (N = 300 images from 60 samples). K1 = Santa Cruz, K4 = San Diego.

GAMETO-FINDER – Development of Artificial Intelligence Tool

To assist in the quantification of 10,000+ photos collecting during our laboratory-based temperature tolerance and acclimatization trials, we have started a collaboration with computing and information science doctoral researcher Brendan Rappazzo at the Institute of Computation Sustainability at Cornell University to develop AI software, which we named GAMETO-FINDER. This system will use deep learning methods to automatically annotate and differentiate kelp life stages (spores, germinated spores, gametophytes, sporophytes) and quantify features of interest (length of germination tube, gametophyte sex, size, and growth morphology, number of eggs, sporophyte cell division and size). In order to train the required large segmentation convolutional networks (CNN) architectures to automatically create these annotations, we will manually label approximately 10% of our photos in the software, simultaneously generating the training data needed to develop GAMETO-FINDER while quantifying data from our aquarium experimental trials. Ultimately, this trained CNN and software program will suggest annotations that will be integrated into our established workflow for data analysis, with the option to verify and edit AI outputs. In similar ecological applications (co-authored by CA Sea Grant supported doctoral student Phoebe Dawkins), deep learning modules used for image classification resulted in data collection at a rate that was 5,000 times faster than manual data collection.

MENTORSHIP AND TRAINING

We received funding from California Sea Grant through the CA-SURE mentorship and training program to expose undergraduate students to experimental field and laboratory research methods, while developing transferable skills to meet their individually developed academic and professional goals. Distribution of mentoring through peers can greatly increase the opportunities for individualized support, and significantly increase sustained engagement. We have leveraged our funding from the CA-SURE program at UCI and have seeked additional support in order to engage three undergraduate students (Min Hanh, Makena Rush, and Caitlin Yee; see **Figure 13**) in Year 2. Our goal is to foster collaboration and community through this research training program using a peer-mentoring structure and integration with the research community. The CA-SURE supported undergraduate student has participated in experimental acclimatization research exploring anticipatory management strategies to future-proof kelp restoration efforts by proactively anticipating climate conditions. During the first month of the project, they participated in a workshop that focused on laboratory-based rearing techniques and boat-based field collection of reproductive tissue using detailed protocols developed over the past year. Undergraduates will form an important piece of our larger research objective, participating in acclimatization experiments in order to learn and apply techniques to quantify different metrics of success for kelp restoration. By the end of this program, undergraduate students gained skills that are transferable to a number scientific research careers, including experimental design and hypothesis testing, laboratory and field research experiences, microscopy techniques and imaging software, pipetting, sterile techniques, chemical handling, statistical analyses and R programming, data visualization, licensing and certifications, interpreting, writing, and presenting scientific research, publishing scientific research in peer-reviewed journals, time management and leadership, and strategies for working in teams and problem solving.

SCIENCE COMMUNICATION AND OUTREACH

California had one of the largest oil spills in recent history, with over 150,000 gallons of oil impacting our coastal ecosystems. Given the potential impact the oil spill could have on the restoration of giant kelp, we used this opportunity to communicate the current restoration efforts in response to local declines associated with a multitude of stressors. This story was featured on the cover of the LA Times (view here, https://tinyurl.com/fwc6vz8b). We filmed a short documentary series about the decline of kelp habitats along the California coast and our current field and laboratory research funded by California Sea Grant to future-proof our restoration efforts for Channel News Asia, which broadcasts to a network to 29 territories across Asia and Australia (view here, https://tinyurl.com/2skseshj). See **Figure 14.**

As part of an UCI Alumni Homecoming event titled ‘Even COVID can’t stop us from saving the environment,’ PhD student Phoebe Dawkins served as a panelist, presenting the issues around local conditions of kelp loss and proposed Green Gravel methods for restoration. This event was hosted by the NSF funded Ridge to Reef program, which trains students to work interdisciplinarily across research and practitioning to manage natural resources in and around urban complexes. Andrea Paz-Lacavex promoted our restoration research and discussed the importance of kelp at the Baja Splash Festival hosted by the Aquarium of the Pacific from 2-3 October 2021 (view here, https://tinyurl.com/k6jahmaa). This festival is a cultural and educational event targeted for Hispanic populations in the Los Angeles area.

Figure 13 Mentees Phoebe Dawkins, Evan Fiorenza, and Andrea Paz Lacavex (PhD students), and Makena Rush, Caitlin Yee, and Minh Han (undergraduate students).

Figure 14 Media communication of field and laboratory research to future-proof kelp restoration efforts.

Doctoral student Phoebe Dawkins presented at the Art and Ecology: Stories that Build Connection (NSF Ridge 2 Reef, 3 June 2022). This event explored different tools, techniques, media, and outcomes that can be used to compose and share stories about ecology and the environment. The art piece, titled 'Starry Night,' comprised of three high resolution images from our Leica DMi1 inverted microscope and Leica C1 FLEXACAM taken during our thermal experiments, depicting both haploid gametophyte and diploid sporophyte phases (see **Figure 15**). Phoebe used this opportunity to share knowledge with the invited community and her peers about complex kelp life histories, diversity of local kelp beds, and factors affecting their global decline.

Phoebe Dawkins also gave an oral presentation to the University of California - Irvine's Masters in Conservation and Restoration Science program, a professional degree program designed to provide the graduate with the skills and knowledge base necessary to hold leadership and management positions in conservation, restoration, and sustainability fields. She discussed kelp ecology, global declines, and Sea Grant-funded efforts to develop urgently needed restoration solutions.

PIs Joleah Lamb and Matthew Bracken are key contributors to the Orange County Museum of Art's (OCMA) upcoming exhibition, 'Sea Change: Art Effecting Change in the Pacific Ocean.' As the only contemporary art museum with a focus on the Pacific Rim, OCMA's mission offers a unique lens with which to assess the topic of environmental degradation at a dire moment in history. PI Joleah Lamb has been engaging in conversations with Professors Marek Ranis and Maja Godlewska of the University of North Carolina, Charlotte to increase local awareness and appreciation of coastal kelp ecosystems. The mixed media art piece, titled 'Pom Poms,' depicts early stage gametophytes (see **Figure 15**).

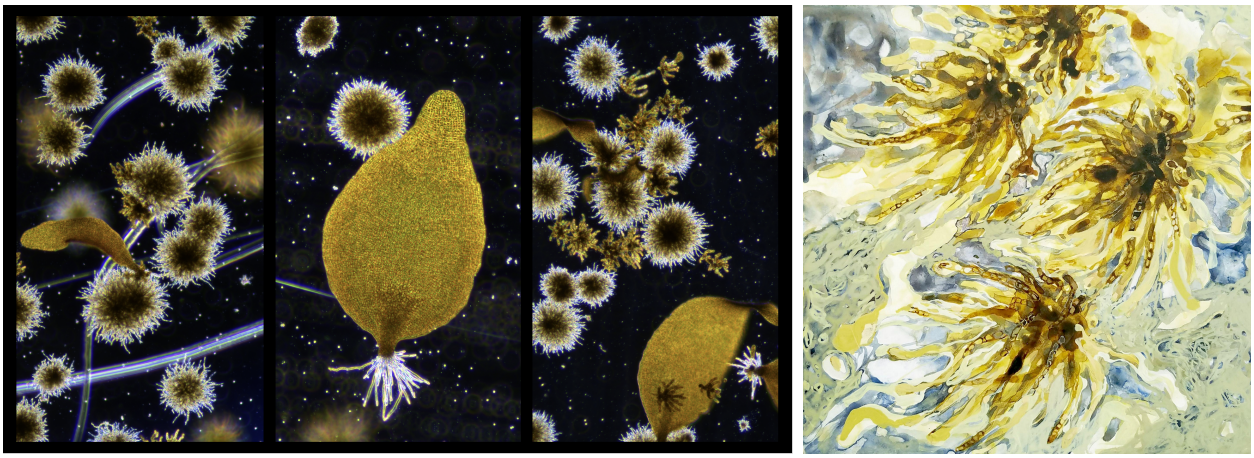

Figure 15 Art pieces and presentations resulting from this Sea Grant-funded project: left, titled ‘Starry Night,’ (Phoebe Dawkins) and right, titled ‘Pom Poms’ (Marek Ranis and Maja Godlewska).

CONFERENCE AND WORKSHOP ATTENDANCE

PI Joleah Lamb spoke at the PSA Presidential Symposium at the Joint Aquatic Sciences Meeting (**Figure 16** JASM, 14-20 May 2022, view here, tinyurl.com/m9w4kxzd) about our research on future-proofing kelp restoration. The conference was attended by co-PI Matthew Bracken and doctoral student Phoebe Dawkins. PI Joleah Lamb gave a presentation at the Eastern Pacific Kelp Forest Restoration Workshop hosted by the Nature Conservancy (EPKFRW, 3-4 October 2022, view here, tinyurl.com/3hmpd9vu). This workshop was attended by doctoral students Phoebe Dawkins and Evan Fiorenza.

Figure 16 Presentation cover slide for JASM PSA Presidential Symposium.

Discussions with Dr. Filipe Alberto and his lab (kelpbreeding.com) at the University of Wisconsin, Milwaukee and participation in their 3-day kelp culturing workshop have provided insight for our experimental growth trials, vegetative culturing, and seed banking (**Figure 17**). During this workshop, we developed skills for growing, crossing, and maintaining gametophyte lines to meet Aim 1, and we have modified rearing conditions and optimized nutritional media for *Macrocystis pyrifera* reproduction.

Figure 17 California Sea Grant funded workshop attended by project graduate research students Phoebe Dawkins and Andrea Paz-Lacavex at the University of Wisconsin, Milwaukee hosted by the Alberto Lab.

References

Anthony K et al. 2017. New interventions are needed to save coral reefs. Nature Ecology & Evolution **1**:1420–1422. Nature Publishing Group.

Bennett S, Wernberg T, Arackal Joy B, de Bettignies T, Campbell AH. 2015. Central and rear-edge populations can be equally vulnerable to warming. Nature Communications **6**:10280. Nature Publishing Group.

Boyko A, Blevins T, Yao Y, Golubov A, Bilichak A, Ilnytskyy Y, Hollander J, Meins F, Kovalchuk I. 2010. Transgenerational Adaptation of Arabidopsis to Stress Requires DNA Methylation and the Function of Dicer-Like Proteins. PLoS ONE **5**. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2831073/ (accessed May 15, 2020).

Boyko A, Kovalchuk I. 2011. Genome instability and epigenetic modification—heritable responses to environmental stress? Current Opinion in Plant Biology **14**:260–266.

Breed MF et al. 2018. Priority Actions to Improve Provenance Decision-Making. BioScience **68**:510–516.

Breed MF et al. 2019. The potential of genomics for restoring ecosystems and biodiversity. Nature Reviews Genetics **20**:615–628.

Broadhurst LM, Lowe A, Coates DJ, Cunningham SA, McDonald M, Vesk PA, Yates C. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications **1**:587–597.

Buerger P, Alvarez-Roa C, Coppin CW, Pearce SL, Chakravarti LJ, Oakeshott JG, Edwards OR, Oppen MJH van. 2020. Heat-evolved microalgal symbionts increase coral bleaching tolerance. Science Advances **6**:eaba2498. American Association for the Advancement of Science.

Carney LT, Waaland JR, Klinger T, Ewing K. 2005. Restoration of the bull kelp Nereocystis luetkeana in nearshore rocky habitats. Marine Ecology Progress Series **302**:49–61.

Cavanaugh KC, Reed DC, Bell TW, Castorani MCN, Beas-Luna R. 2019. Spatial Variability in the Resistance and Resilience of Giant Kelp in Southern and Baja California to a Multiyear Heatwave. Frontiers in Marine Science **6**. Frontiers. Available from https://www.frontiersin.org/articles/10.3389/fmars.2019.00413/full (accessed April 7, 2020).

Chakravarti LJ, van Oppen MJH. 2018. Experimental Evolution in Coral Photosymbionts as a Tool to Increase Thermal Tolerance. Frontiers in Marine Science **5**. Frontiers. Available from https://www.frontiersin.org/articles/10.3389/fmars.2018.00227/full (accessed May 14, 2020).

Coleman MA, Wood G, Filbee-Dexter K, Minne AJP, Goold HD, Vergés A, Marzinelli EM, Steinberg PD, Wernberg T. 2020. Restore or Redefine: Future Trajectories for Restoration. Frontiers in Marine Science **7**:237.

Coleman-Derr D, Tringe SG. 2014. Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. Frontiers in Microbiology **5**. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4047557/ (accessed May 15, 2020).

Darling ES, Côté IM. 2018. Seeking resilience in marine ecosystems. Science **359**:986–987. American Association for the Advancement of Science.

Dayton PK, Tegner MJ, Edwards PB, Riser KL. 1999. Temporal and Spatial Scales of Kelp Demography: The Role of Oceanographic Climate. Ecological Monographs **69**:219–250.

de Klerk G-J, Pumisutapon P. 2008. Protection of in-vitro grown Arabidopsis seedlings against abiotic stresses. Plant Cell, Tissue and Organ Culture **95**:149–154.

Denny MW, Gaylord BP, Cowen EA. 1997. 1Hopkins Marine Station of Stanford University, Department of Biological Sciences, Pacific Grove, CA 93950, USA and 2Civil Engineering Department, Stanford University, Pacific Grove, CA 93950, USA:19.

Edwards M, Watson L. 2015. Aquaculture Explained: Cultivating Laminaria digitata.

Fredriksen S, Filbee-Dexter K, Norderhaug KM, Steen H, Bodvin T, Coleman MA, Moy F, Wernberg T. 2020. Green gravel: a novel restoration tool to combat kelp forest decline. Scientific Reports **10**:1–7.

Gurgel CFD, Camacho O, Minne AJP, Wernberg T, Coleman MA. 2020. Marine Heatwave Drives Cryptic Loss of Genetic Diversity in Underwater Forests. Current Biology **30**:1199-1206.e2.

Elsevier.
Harborne AR, Rogers A, Bozec Y-M, Mumby PJ. 2017. Multiple Stressors and the Functioning of Coral Reefs. Annual Review of Marine Science **9**:445–468.
Hobbs RJ, Higgs E, Harris JA. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599–605.
Hollarsmith JA, Buschmann AH, Camus C, Grosholz ED. 2020. Varying reproductive success under ocean warming and acidification across giant kelp (Macrocystis pyrifera) populations. Journal of Experimental Marine Biology and Ecology **522**:151247.
Howells EJ, Berkelmans R, Oppen MJH van, Willis BL, Bay LK. 2013. Historical thermal regimes define limits to coral acclimatization. Ecology **94**:1078–1088.
Hughes TP et al. 2003. Climate Change, Human Impacts, and the Resilience of Coral Reefs. Science **301**:929–933. American Association for the Advancement of Science.
Jueterbock A, Tyberghein L, Verbruggen H, Coyer JA, Olsen JL, Hoarau G. 2013. Climate change impact on seaweed meadow distribution in the North Atlantic rocky intertidal. Ecology and Evolution **3**:1356–1373.
Ladah LB, Zertuche-González JA. 2007. Survival of microscopic stages of a perennial kelp (Macrocystis pyrifera) from the center and the southern extreme of its range in the Northern Hemisphere after exposure to simulated El Niño stress. Marine Biology **152**:677–686.
Larkindale J, Vierling E. 2008. Core Genome Responses Involved in Acclimation to High Temperature. Plant Physiology **146**:748–761.
Layton C, Coleman MA, Marzinelli EM, Steinberg PD, Swearer SE, Vergés A, Wernberg T, Johnson CR. 2020. Kelp Forest Restoration in Australia. Frontiers in Marine Science **7**. Frontiers. Available from https://www.frontiersin.org/articles/10.3389/fmars.2020.00074/full#B71 (accessed May 2, 2020).
North WJ, Jackson GA, Manley SL. 1986. Macrocystis and its environment, knowns and unknowns. Aquatic Botany **26**:9–26.
O'Neill GA. 2008. Assisted migration to address climate change in British Columbia: recommendations for interim seed transfer standards. Ministry of Forests and Range, Forest Science Program, Victoria, B.C.
Oppen MJH van et al. 2017. Shifting paradigms in restoration of the world's coral reefs. Global Change Biology **23**:3437–3448.
Oppen MJH van, Oliver JK, Putnam HM, Gates RD. 2015. Building coral reef resilience through assisted evolution. Proceedings of the National Academy of Sciences **112**:2307–2313. National Academy of Sciences.
Oppliger LV, Correa JA, Engelen AH, Tellier F, Vieira V, Faugeron S, Valero M, Gomez G, Destombe C. 2012. Temperature Effects on Gametophyte Life-History Traits and Geographic Distribution of Two Cryptic Kelp Species. PLOS ONE **7**:e39289. Public Library of Science.
Perring MP, Standish RJ, Price JN, Craig MD, Erickson TE, Ruthrof KX, Whiteley AS, Valentine LE, Hobbs RJ. 2015. Advances in restoration ecology: rising to the challenges of the coming decades. Ecosphere **6**:art131.
Pumisutapon P, Visser RGF, de Klerk G-J. 2012. Moderate abiotic stresses increase rhizome growth and outgrowth of axillary buds in Alstroemeria cultured in vitro. Plant Cell, Tissue and Organ Culture (PCTOC) **110**:395–400.
Redmond S. 2013. Effects of Increasing Temperature and Ocean Acidification on the Microstages of two Populations of Saccharina latissima in the Northwest Atlantic:58.
Rogers-Bennett L, Catton CA. 2019. Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. Scientific Reports **9**:15050. Nature Publishing Group.
Rothäusler E, Gómez I, Hinojosa IA, Karsten U, Tala F, Thiel M. 2011. Physiological Performance of Floating Giant Kelp Macrocystis Pyrifera (phaeophyceae): Latitudinal Variability in the Effects of Temperature and Grazing1. Journal of Phycology **47**:269–281.
Sanford E, Kelly MW. 2011. Local Adaptation in Marine Invertebrates. Annual Review of Marine Science **3**:509–535.
Shukla P, Edwards MS. 2017. Elevated pCO2 is less detrimental than increased temperature to early

development of the giant kelp, Macrocystis pyrifera (Phaeophyceae, Laminariales). Phycologia **56**:638–648. Taylor & Francis.
Smale DA et al. 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nature Climate Change **9**:306–312. Nature Publishing Group.
Stillman JH. 2003. Acclimation Capacity Underlies Susceptibility to Climate Change. Science **301**:65–65. American Association for the Advancement of Science.
Tracey SR, Baulch T, Hartmann K, Ling SD, Lucieer V, Marzloff MP, Mundy C. 2015. Systematic culling controls a climate driven, habitat modifying invader. Biological Invasions **17**:1885–1896.
Vadas RL. 1972. Ecological Implications of Culture Studies on Nereocystis Luetkeana1. Journal of Phycology **8**:196–203.
van Oppen MJH, Puill-Stephan E, Lundgren P, De'ath G, Bay LK. 2014. First-generation fitness consequences of interpopulational hybridisation in a Great Barrier Reef coral and its implications for assisted migration management. Coral Reefs **33**:607–611.
Vitt P, Havens K, Kramer AT, Sollenberger D, Yates E. 2010. Assisted migration of plants: Changes in latitudes, changes in attitudes. Biological Conservation **143**:18–27.
Voerman SE, Llera E, Rico JM. 2013. Climate driven changes in subtidal kelp forest communities in NW Spain. Marine Environmental Research **90**:119–127.
Wernberg T et al. 2016. Climate-driven regime shift of a temperate marine ecosystem. Science **353**:169–172. American Association for the Advancement of Science.
White N, Marshall C. 2007. Sugar kelp (Saccharina latissima):21.
Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR. 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. Nature Communications **8**:14213. Nature Publishing Group.
Zimmerman RC, Kremer JN. 1984, August. Episodic nutrient supply to a kelp forest ecosystem in Southern California. Sears Foundation for Marine Research. Available from https://www.ingentaconnect.com/content/jmr/jmr/1984/00000042/00000003/art00008 (accessed May 14, 2020).