A MULTI-PRONGED APPROACH TO KELP RECOVERY ALONG CALIFORNIA'S NORTH COAST

Executive Summary

Brian Gaylord, Marissa Baskett, Matthew Edwards, Jason Hodin, Brent Hughes, Sean Place, Aurora Ricart, and Mackenzie Zippay

The problem

Since 2014, the abundance of the bull kelp, *Nereocystis luetkeana*, a key canopy-forming seaweed that provides habitat and resources for numerous other species along California's north coast, has declined precipitously. This alarming trend has garnered intense interest among managers, policymakers, and conservation and restoration practitioners, all of whom are striving to identify and potentially implement approaches to reverse it.

Assumed causes

The marked decrease in bull kelp cover appears to derive from three intersecting factors (Fig. i, top panel). First, strong shifts in oceanographic conditions led to the "blob," a major warming event that raised temperatures regionally and imposed physiological stress on bull kelp. Second, an outbreak of purple urchins (*Strongylocentrotus purpuratus*) elevated grazing pressure on the kelp, with the result that many forests transitioned to a barren state, characterized by open reef with no surface canopy and little to no fleshy algae even in the benthos. A third contributor has been the absence of strong counteracting predation on urchins, due to a disease epidemic that locally extirpated sunflower sea stars (*Pycnopodia helianthoides*), exacerbating the historical loss of sea otters which also used to place a check on urchin numbers.

Goals for our project

Recognizing the above perturbations and their likely origins, we assembled a multi-faceted team to address questions centered on each of the three nodes of the problem: vulnerability of kelp, overabundance of urchins, and loss of sea star predators. We also included a modeling component to isolate the most effective policy "levers" that could be used to combat the disappearance of bull kelp.

Research approach

We merged the varied expertise of eight principal investigators and their research groups, from four universities across California and the west coast more generally, to help address the loss of *Nereocystis* (Fig. i, bottom panel).

With regards to <u>experiments targeting kelp</u> directly, we examined three issues.

1.A. Temperature effects

–We evaluated whether certain populations of bull kelp, originating from different locations within the range of *Nereocystis* along California's shores, might exhibit greater tolerance to warm water. In these efforts, we collected reproductive blades, induced spore release from their sori, then cultured ensuing gametophytes and young sporophytes under ambient and elevated temperatures, keeping track of the source populations, and recording algal densities and rates of growth in each treatment.

–We explored whether laboratory-reared, early life stages of bull kelp could be successfully outplanted to the field. We settled *Nereocystis* spores on hard substrates ("green gravel") or larger limestone pavers, and then deployed those substrates into the field, monitoring the appearance of young sporophytes and their success over subsequent weeks and months.

–We tested whether laboratory acclimation of spores, gametophytes, and young sporophytes to higher seawater temperatures encouraged physiological acclimation, in the form of elevated levels of heat shock proteins (Hsp70). We also explored whether heat-acclimated cultures of early life stages of bull kelp exhibited altered photosynthetic capacity.

1.B. Spore dispersal

–Recognizing that some level of recovery could proceed via natural spore production by remnant kelp patches, we refined an existing model of spore dispersal, originally developed for giant kelp, to predict distances over which spores of bull kelp can be transported in ocean currents. Resulting information is useful for assessing how best to space restoration efforts along the coast.

1.C. Banks of microscopic stages

–We tested whether extant reservoirs of microscopic stages of bull kelp might be present in the field, which could allow for potentially broad recruitment should future conditions improve. In this study component, we collected small boulders from multiple sites along the north coast, placed them in laboratory culture, and monitored for the appearance of young sporophytes even in the absence of new inoculation of spores. We also examined a possible role of urchin feces in provisioning nutrients to bull kelp gametophytes and young sporophytes.

With regards to levers that might be activated to offset overabundance of purple sea urchins, we explored three issues.

–We tested whether gametes from purple sea urchins collected at barren sites lead to inferior fertilization or aberrant development of larvae. This question has been of interest due to concerns that certain methods of urchin culling (in particular, smashing them *in situ*) might result in unintended gamete release and increased production of offspring.

–Based on anecdotal observations of improved *Nereocystis* survival in locations with modest freshwater influx, we conducted experiments to see whether such patterns might derive from disproportionate susceptibility of larval sea urchins to reduced seawater salinity, which could presumably reduce numbers recruiting into forests and subsequent grazing impacts.

–In related trials, we explored whether adult purple sea urchins exhibit reduced feeding rates in lower salinity seawater.

Our third arm of empirical research focused on missing sea star predators of urchins.

–Here we linked to an ongoing project aimed at developing best practices for rearing *Pycnopodia* in culture, and used it as a platform for quantifying rates of predation by young sunflower stars on juvenile purple sea urchins.

The fourth and final element of our research employed mathematical theory to identify the most impactful management or restoration interventions. Here we analyzed two model constructs.

–The first model examined the relative benefits of removing urchins versus reintroducing kelp.

–The second model identified the relative importance of factors governing kelp resilience.

Key findings

Our experiments verified strong responses of bull kelp to elevated seawater temperatures. We found less evidence of population-level variation in heat tolerance, with the exception of growth rates of young sporophytes, which appeared to be higher in kelp sourced from central California. Due to limitations of experimental protocol, we were unable to detect patterns in expression of heat shock proteins across temperature and source population. Photosynthetic performance depended on acclimation temperature, but not source population, in kelp samples composed of combinations of gametophytes and young sporophytes.

Physically based modeling indicates that *Nereocystis* likely exhibits a bimodal pattern of spore dispersal. An appreciable subset of spores disperse short distances (< 1 to several meters), facilitating self-recruitment to the originating forest. This trend likely aids local persistence in this annual species where "holdover" adults may not be present to help foster the next generation. However, many spores will also travel multiple kilometers (> 1 to 10 km), and may thus contribute to persistence or recolonization of other forests.

Microscopic stages of kelp can be found on hard substrates in the field. Such stages may help bolster bull kelp recruitment, especially near remnant kelp patches or restored forests where the abundance of these microscopic stages appears to be greater. Such microscopic stages may also benefit from the presence of urchins through the latter's provision of nutrients for growth, though the overall impact of urchins is clearly negative.

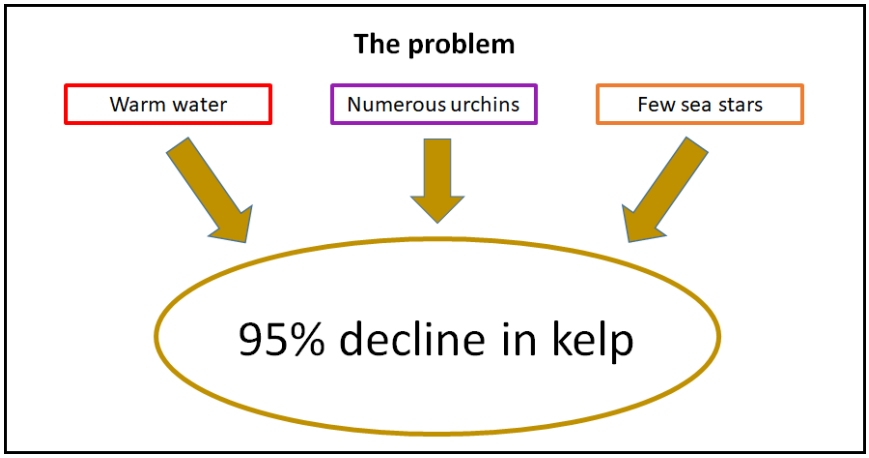
Observations have suggested a possible link between modest freshwater influx and improved survival of kelp, and a possible mechanism is impaired health or performance of urchins under reduced salinity. Our work suggests the possibility that settlement of purple urchin larvae could decrease in reduced-salinity seawater, if salinities are low enough. Adult urchins may also exhibit slower grazing rates under decreased salinity. We additionally found modestly lower rates of grazing by adult urchins on gametophyte stages of bull kelp under elevated seawater temperatures compared to ambient temperatures.

The loss of sunflower stars from bull kelp ecosystems in California raises important questions about how much control this species exerted previously on urchin abundance. Although most attention has focused on consumption of adult urchins by *Pycnopodia*, results of our work suggest that predation by sunflower stars on juvenile urchins may be a strong axis by which population limitation could occur.

Science synthesis and training

In the final months of the project, we additionally hosted a two-day working group, composed of participants from our team plus each of the other projects funded through CA Sea Grant's kelp recovery research program, as well as personnel from key agencies, including the California Ocean Protection Council and California Department of Fish and Wildlife. The intent was to provide a venue by which results could be compared and dissected among the various research teams, thereby supporting efforts to develop comprehensive strategies for combating kelp declines. Beyond such research integration, our team mentored four undergraduates

through CASG's CA-SURE program, provided internship and training opportunities for an additional 12 students or early-career scientists, and supported thesis and dissertation chapters of five graduate students.



Unknowns and potential interventions explored in this study

Kelp	Urchins	Sea stars
• Do heat tolerant strains of bull kelp exist? • Can heat tolerant strains of kelp be cultured and outplanted? • Which stages of kelp are most heat sensitive? • How does temperature affect key physiological processes of kelp? • Over what spatial scales might kelp patches by connected? • To what extent are microscopic kelp stages extant in the field?	• How does temperature affect rates of purple urchin grazing on kelp? • Do urchins from barrens produce disfunctional gametes? • Are urchin larvae vulnerable to low salinity? • How does low salinity influence adult urchins?	• It is feasible to rear sunflower stars in the laboratory? • Can sunflower stars be cultured through their full life cycle? • To what extent do sunflower stars prey on juvenile sea urchins?
	• When considering different classes of restoration approaches, which are likely to be most impactful?	

Figure i. Multiple drivers underlie dramatic declines in the abundance of bull kelp (*Nereocystis luetkeana*) along the north coast of California. Note that effects of sea stars on kelp are indirect (arising because the absence of seastars decreases their predation on urchins, which in turn elevates urchin consumption of kelp) and that additional interactions (e.g., temperature effects on urchins and seastars) may also operate but are not shown for visual simplicity. Efforts to combat the kelp decline involve a number of possible intervention activities; each is accompanied by questions and unknowns including several explored in this project.

California Sea Grant Kelp Recovery Research Program (2020-2023)

FINAL PROJECT REPORT

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Brian Gaylord[1], Marissa Baskett[1], Matthew Edwards[2], Jason Hodin[3], Brent Hughes[4], Sean Place[4], Aurora Ricart[1], and Mackenzie Zippay[4]

[1]University of California, Davis
[2]San Diego State University
[3]University of Washington
[4]Sonoma State University

INTRODUCTION AND BACKGROUND

The bull kelp, *Nereocystis luetkeana*, has historically been the dominant forest-forming macroalga along most of California's north coast, providing habitat for numerous species, including taxa of commercial and recreational interest (e.g., red urchins, abalone and rockfish; Springer et al. 2010). However, populations of this species underwent a more than 90% decrease in abundance since 2014.

Causes of the decline are not fully understood, but three putative drivers have received the bulk of attention (Rogers-Bennett & Catton 2019). First, anomalous oceanographic conditions – a marine heatwave called the "warm blob" and a strong El Nino-Southern Oscillation event – resulted in a prolonged period of elevated seawater temperatures in the region (Bond et al. 2015; Fewings & Brown 2019). Such conditions may have induced physiological stress in bull kelp with potential population consequences. Second, massive increases in the numbers of purple sea urchins (*Strongylocentrotus purpuratus*) caused overgrazing of bull kelp and left "urchin barrens" devoid of fleshy algae (Rogers-Bennett & Catton 2019). Third, a possible control on urchins was eliminated when an outbreak of sea star wasting disease decimated a

key invertebrate predator, the sunflower sea star, *Pycnopodia helianthoides* (Burt et al. 2018; Harvell et al. 2019). Warmer seawater conditions may have exacerbated disease symptoms, and the *Pycnopodia* declines also amplified an existing dearth of urchin predators initiated by the loss of sea otters, which were extirpated from the region a century ago. The more recent changes, moreover, followed a 2011 harmful algal bloom that killed large numbers of invertebrates, including sea stars, along 100 km of north-coast shoreline (Jurgens et al. 2015).

Figure 1.0. Kelp presence and abundance have plummeted along the northern coast of California in recent years, spurring intense interest in developing methods for managing and restoring this crucial constituent of the nearshore marine ecosystem.

While losses of bull kelp have been profound (Fig. 1.0), residual forests do remain in some areas. Whether these remnant patches represent refugia that will continue to persist, or whether such patches will disappear or reconfigure through time has remained unclear. Early observations suggested that kelp stands in localities with modest (but not extreme) freshwater inflow have exhibited improved survival, creating impetus for examining this potential mechanism of resilience. Additionally, while recovery of these forests may be aided by dispersal of spores from nearby residual forests, or whether a bank of microscopic life stages (gametophytes and/or embryonic sporophytes) persists in these barren grounds and can facilitate rapid recovery of the forests, also remains unknown.

Based on uncertainties such as those above, we conducted the following research activities to guide ongoing and forthcoming efforts in kelp management and restoration. Our efforts targeted the following aims.

OBJECTIVES

1. Foster kelp resilience and reseeding potential. This objective had three components.

A) *Identify heat-tolerant strains of bull kelp*.

Here, we conducted laboratory experiments to assess effects of anomalous warming on growth and survival of early life stages of multiple population-level strains of bull kelp, accompanied by cellular analysis of stress markers and eventual targeted sequencing of genotypes. In 2020-2021, we ran a 5-month warming experiment testing for temperature effects on micro-to-macroscopic development of bull kelp, and explored whether individuals sourced from four northern California and two central CA sites differed in their responses. We then ran a series of two outplanting "green gravel" field experiments that tested for the effects of 1) population source (from central to northern California), and 2) the influence of habitat type on bull kelp restoration success. Our team additionally tested for physiological differences in early life stages of bull kelp acclimated to ambient versus elevated seawater temperatures, focusing on markers of heat stress and physiological capacity.

B) *Quantify spatial scales over which remnant kelp patches can reseed*.

In these efforts we merged physically based models with laboratory experiments that assayed spore sinking speeds and hydrodynamic measurements of wave and current conditions in the field. Resulting information was used to estimate transport distances of kelp spores, important for ascertaining the extent to which *Nereocystis* might self-recruit to its source forest, or exchange spores with other forests.

C) Determine the extent to which a bank of microscopic stages is available to aid forest recovery.

In this study component we assessed whether microscopic life stages (gametophytes and/or embryonic sporophytes) occur on natural substrates in areas where kelp forests have been lost. We explored sporophyte recruitment on small boulders collected from these sites and determined whether sporophytes appeared on the boulders after their transport to the laboratory, even in the absence of additional spore settlement. A second aim examined whether urchin feces might serve as a nutrient source for kelp microscopic stages, thereby enhancing gametophyte and early sporophyte growth.

2. Explore easier methods for urchin removal, and determine urchin vulnerabilities.

Here we tested whether reproduction is poor in malnourished urchins from kelp-depleted areas. These efforts addressed the question of whether simpler methods of urchin culling (i.e., crushing) would be expected to increase gamete release and unintended production of young. We additionally quantified how reductions in seawater salinity might influence larval and adult purple urchins. Our results here are relevant for ascertaining whether fewer urchins and improved kelp survival might be expected in locales with modest freshwater influx.

3. Build knowledge and capacity for culturing invertebrate predators of urchins.

In this project component, we leveraged ongoing efforts to rear sunflower sea stars (*Pycnopodia helianthoides*) in the laboratory. Specifically, we quantified predation rates of young sunflower stars on purple sea urchin recruits. Given concerns about the long-term persistence and probable consequences of the near-absence of *Pycnopodia* from California's shores, this phase of our work represents a key precursor to determining if restocking of sunflower stars could have utility in kelp restoration.

4. Isolate the best levers for management action.

In accompaniment to the three experimental components of the project, we developed and analyzed multiple models of the kelp-urchin-sea star system. Our aims were to project long-term outcomes from the empirical interventions explored in our work, while also providing decision support for adaptive management.

ACTIVITIES AND FINDINGS

Objective 1: Facilitate kelp resilience and reseeding capacity

1.A. Temperature experiments

As noted above, initial declines of north-coast kelp forests were associated with anomalously high seawater temperatures. We therefore conducted laboratory culturing of early life stages of bull kelp (*Nereocystis luetkeana*) to investigate the role of ocean warming and population source on abundance and growth (Table 1.1). We also explored the feasibility of outplanting laboratory-acclimated kelp to the field. In additional experiments, we examined physiological metrics of heat stress and photosynthetic capacity in early life stages of bull kelp exposed to ambient or elevated seawater conditions.

1.A.i. Effects of temperature on early life stages of bull kelp

In October of 2020 we initiated laboratory culturing of bull kelp, led by co-PI Hughes and his team, to test for population-level variation in heat tolerance in early life stages (gametophytes and young sporophytes). We began by collecting ~30 *Nereocystis luetkeana* blades with reproductive sori from different individuals sourced from six different persistent kelp sites throughout central and northern California. These sites, moving from south to north, included two central California sites (Point Sur and Point Pinos), and four northern California sites: Russian Gulch, Shell Beach, Navarro River, and Big River (Table 1.1). Collaborators at the University of California Santa Cruz and Moss Landing Marine Laboratories assisted with the central California kelp collections. Kelp persistence, kelp reproductive health, and shore access to these sites were considered when determining the populations for collection. To collect the sori, we clipped the reproductive blades at their intersection with the pneumatocyst, and collected only one blade from each individual. Additionally, to maximize the area of the kelp forest sampled to account for individual variation, we ensured each individual was > 2 m apart from another individual kelp. After collection, we immediately wrapped the blades in seawater-soaked paper towels and placed them in a chilled cooler for transportation back to the University of California Davis' Bodega Marine Laboratory (BML).

Following return to BML, we stimulated spore release from the reproductive sori, with an aim of establishing kelp cultures in long-term temperature acclimation tables of seawater where we would characterize growth of ensuring life stages. We processed the kelp for this purpose by cutting off unnecessary vegetative sections and leaving only the soral material. We rinsed the sori in 10% iodine ratio, to remove any fouling organisms living on the kelp, and then rinsed them in filtered seawater. The seawater used for culturing was UV sterilized (Jebao, JUVC-55), passed through a 20 micron filter, and finally filtered at 1 micron to remove larger

contaminants. We laid out the sori for ~1 hour to dry on paper towels (Hernández-Carmona et al. 2006; Muth et al. 2019), then cut them into 54 cm^2 pieces to standardize the amount of reproductive material. Next, we placed the standardized soral pieces into pyrex dishes (2.6L glass rectangles, ~24 X 20 X 7.5 cm) with filtered seawater, at ambient temperature, to osmotically induce spore release (Hernández-Carmona et al. 2006; Muth et al. 2019), inserting three 54 cm^2 pieces of sori, separated by site, into each pyrex dish. After allowing the sori to release their spores in the pyrex dishes for 1 hour, we removed and discarded the adult kelp tissue. The expelled spores sank to the bottom of the dishes and settled within the first 24 hours onto microscope slides also added to the dishes (6 labeled slides per dish). We consolidated the culture design within each site to get a broad picture of the entire kelp bed sampled. For every site sampled, we used six pyrex dishes for each of the two temperature treatments: ambient and warm temperatures. The settlement surfaces of the microscope slides allowed us to track the kelp growth and percent cover throughout the entirety of the experiment.

The pyrex dishes with settled spores were placed into a temperature controlled environmental room at BML. This room contained a total of four seawater tables (251 X 83 X 23 cm) divided equally across two temperature treatments. The ambient air temperature for the environmental room was 13° C which facilitated each set of two ambient sweater tables remaining stable at ~ 13° C. This temperature represented pre-warming conditions in northern California (Rogers-Bennett and Catton 2019). We added two heaters to each of the warm seawater tables making the water temperature about ~17° C. This warmer temperature represented the average sea surface temperature in northern California during the marine heatwave known as “the blob” (Bond et al. 2015). To ensure the warm seawater tables did not have localized warm or cool areas, a seawater pump was added to improve circulation. All seawater tables contained two HOBO Onset data loggers to record the temperature of the seawater every 15 minutes for the duration of the experiment. The average temperature recorded by the HOBO loggers for the duration of the experiment for the two warm seawater tables was 16.7° C ±1° C and 11.5 ± 0.4° C for the ambient seawater tables.

Above each table were two wide-spectrum LED grow lights covered with a garden screen. We measured light intensity using photosynthetically active radiation (PAR) set at ~20 µmol, and implemented a 12-hour photoperiod, mimicking intensity and duration of light in the field during spore settlement and early growth (spring).

We ran the culturing and acclimation experiment for five months, during which the settled spores quickly germinated into gametophytes, which in turn released gametes that fertilized and produced young sporophytes that grew over time. Each of these stages of kelp remained in

their respective temperature treatments, and we monitored their growth. We conducted water changes weekly to replenish naturally occurring nutrients. To do this, we removed the microscope slides and placed them into clean pyrex dishes and wiped down the sides and bottom of the original pyrex dishes with a paper towel to remove any residue growing on the sides or bottom. Lastly, we added filtered seawater to the original pyrex dish and transferred the slides back into the cleaned pyrex dish, where we added a 20 µl/L concentration of germanium dioxide to each dish to control for diatom growth.

During the culturing, we modulated the intensity of the grow lights to simulate seasonal variation in solar exposure. After two months (October to mid-December) of kelp growth, we lowered the light intensity by adding an additional screen cover over the grow lights, bringing the light intensity (photosynthetically active radiation; PAR) to ~5 µmol, to induce a state of dormancy to simulate winter conditions (Kinlan et al. 2003). Responding to the reduced growth of the kelp, after about 1.5 months (mid-December to late-January), we removed the second screen to bring the light intensity back to 20 µmol to continue growing the culture (late January-March) for the remaining months (5 months total).

Throughout the duration of the 5-month culturing, we took photographs to monitor bull kelp growth and percent cover of each of the successive life stages (starting with settled spores, then gametophytes, microscopic sporophytes, and eventually macroscopic sporophytes). During the microscopic life history stages, periodic images of the slides started on day 1 after spore release and continued through day 45. To do this, we chose one representative field of view for every slide that was analyzed. We imaged one representative field of view under the microscope from three of the six pyrex dishes per site each week to have enough microscope slides to monitor growth for the full duration of the experiment. After viewing the kelp slides under the compound microscope (Nikon), the slides were discarded due to their exposure to bright light and heat. Once there were visible macroscopic sporophytes, starting on day 57, we began taking pictures of the entire microscope slide with a camera (Nikon Coolpix) every two weeks. We used these images to quantify size and development through the various life history stages over time in the different temperature treatments from the collection sites.

To extract data from the images, we used ImageJ (version 1.53s) to measure size and percent cover from the slides. The area of both the gametophytes and sporophytes determined the size of the kelp. We randomly chose up to ten gametophytes or sporophytes (as long as the entirety was visible in the field of view) and measured them by outlining the shape and size to calculate the area. In addition, we recorded the percent cover by using a grid macro (uniform point contact) and noted the life history stage of bull kelp on each intersection. There were approximately 20-point contacts to assure there were enough data points to accurately

describe the percent cover of the microscope slides. We divided the number of intersections under each category by the total number of point contacts to yield the percent cover of bull kelp in each stage on the microscope slide. Using both bull size and kelp cover are important metrics because they each capture unique aspects of development. The area of the bull kelp provides insight into the health and the size of development, and percent cover shows the abundance (Hughes, et al. 2017) of bull kelp on the slides between region and temperature treatment. Being able to compare size and abundance of bull kelp in the early laboratory life history stages to what is seen in ideal conditions is extremely valuable in understanding how temperature affects each of these factors.

Growth and development over time in the laboratory resulted in a complex picture. We had hypothesized that the kelp from central California would perform better in warmer conditions, due to its historical exposure to higher temperatures characteristic of lower latitudes. Overall, we found that the metric of size and the later sporophyte life history stage of bull kelp were more susceptible to region and temperature differences than the metric of percent cover and the earlier gametophyte stage. Bull kelp sporophytes successfully entered a dormancy phase, which stunted growth, and were highly susceptible to regional and temperature differences during this phase. Increased temperature typically had a negative effect on early stages of bull kelp regardless of its source, and the kelp from central California were larger and more abundant regardless of the temperature treatment. Investigations of the peak growth at each life-history stage showed that macroscopic sporophytes of central California kelp had a greater abundance and trended towards larger sizes in the warm temperature treatment than the kelp from northern California in the warm temperature treatment (Fig. 1.1). These results suggest that population-level thermal resistance varies by bull kelp life history stage. This resistance to temperature stress at this species' southern range limit informs conservation and management, and suggests that source population may matter, or that selective breeding could be profitable for future restoration.

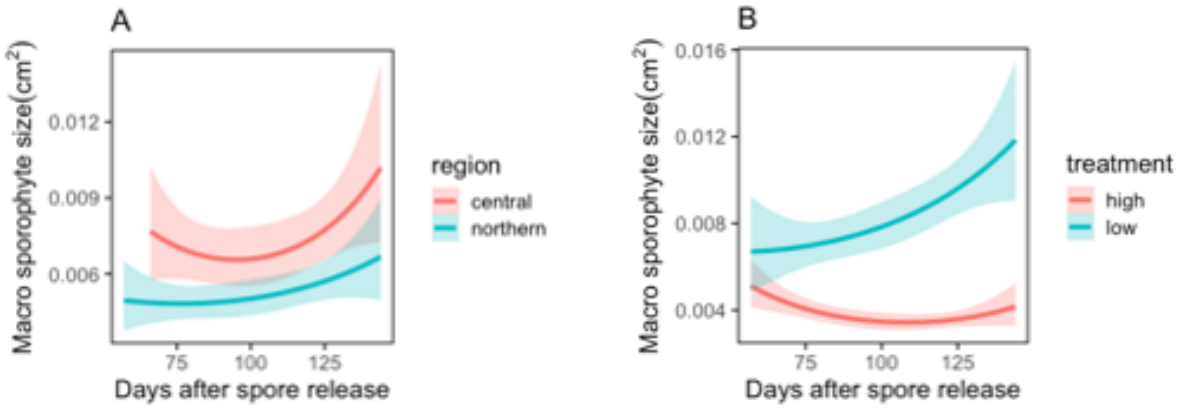

Figure 1.1. Time series analysis of macroscopic sporophyte size. A) Macroscopic sporophyte size by region (Table 1.1) and, B) macroscopic sporophyte size by temperature treatment (low -12°C vs. high 17°C). There was a significant regional effect with central California having bigger macroscopic sporophytes. The high temperature treatment significantly reduced the size of macroscopic sporophytes. Kelp abundance decreased over time, most likely due to density dependence competition. Reproduced from Karm et al. in prep.

1.A.ii. Kelp outplants

In 2021-2022, we additionally conducted outplants of laboratory-reared kelp, for the purposes of informing restoration (Table 1.1). Using methods similar to those described above for acquiring spores of bull kelp, co-PI Hughes and his group seeded batches of gravel with spores ("green gravel") for subsequent placement into the field. These sets of green gravel were generated using source kelp collected across multiple sites, spanning central to northern California.

The outplants were modestly successful in the first year of the project, in that we were able to keep outplant units stable through the winter storm period (2021-2022). However, we detected no kelp on the transplant units in the spring of 2022 after 6 months post-restoration. Therefore, in a subsequent outplanting experiment in summer to fall 2022, we modified our outplanting technique by scaling up transplant units from "green gravel" (Fig. 1.2) to larger pieces of limestone rock; this alternative approach resulted in improved restoration success and detectable growth of kelp into early fall 2022. For our 2022 kelp restoration experiments, we also took advantage of various reef habitat and community types (fringing kelp, subsurface kelp and other large brown algae, and urchin barrens (N = 10-12 outplant units per habitat type), in work conducted as part of Masters research in co-PI Hughes' lab by Sonoma State University students A. Dias and M. Velazquez. These outplant units were deployed in June 2022 and were checked every 2-4 weeks until October 2022. We only detected successful outplant growth in plots dominated by the subsurface kelp *Pterygopohora californica* (20% of outplants had detectable kelp growth), but it was short-lived as all outplants had no detectable kelp growth

after two months post-outplant; we attribute this outcome to an urchin front that moved through the system, as documented in a forthcoming M.S. thesis by A. Dias.

Table 1.1. Sites where kelp was collected and cultured in 2020-2021, and outplant sites in 2021 and 2022. Outplanting sites for 2021 received green gravel bags (~10 cm x 15 cm mesh deployed on a bolted line on the benthos, Fig. 1.2) of bull kelp cultured under warm (17°C) and ambient conditions (12°C) from two central California and two northern California sites, each site received 5 replicates for each treatment for a total of 40 green gravel bags per site (Fig. 1.2). Outplanting in 2022 was done with kelp on 3 x 5 cm limestone pavement stones, and kelp was grown at ambient temperatures from one northern California population (Big River).

Site	**Latitude**	**Longitude**	**Research activities**
Big River (Portuguese Beach)	39.30422	-123.804183	Culture (2020,2021), Outplant (2021 and 2022)
Navarro River	39.188281	-123.758651	Culture (2020)
Sonoma Sea Ranch Shell Beach	38.724602	-123.479768	Culture (2020, 2021)
Timber Cove	38.531041	-123.273929	Culture (2021), Outplant (2021)
Russian Gulch	38.465866	-123.157029	Culture (2020)
Point Piños	36.642011	-121.938221	Culture (2020, 2021)
Point Sur	36.586851	-121.984712	Culture (2020, 2021)
Soberanes Point	36.454552	-121.925698	Culture (2021)

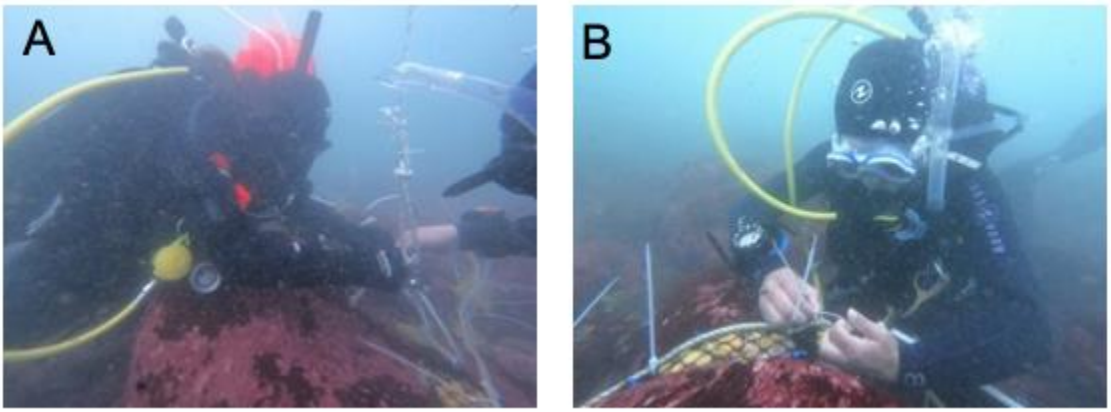

Figure 1.2. Experimental work at various locations along the Sonoma and Mendocino County Coast: A) Divers deploying a sensor mooring and green gravel bags, B) divers deploying green gravel bags. Photo credits: B. Hughes, R. Karm.

1.A.iii. Physiological responses of kelp to elevated temperatures

In addition to effects of temperature and source population on growth of early life stages of bull kelp, we also examined markers of stress responses. Led by co-PI Zippay and her research group, we focused on the expression of heat shock protein Hsp70. Our efforts involved three temperature acclimation experiments, two which occurred during COVID-19. As a result of the pandemic conditions, we were unable to acquire complete results from the first two trials. In particular, they did not provide us with the appropriate individual sampling nor enough replication to run our molecular analysis. However, we used the earlier runs to troubleshoot the appropriate amount of tissue that is needed from different life history stages to run our assays, and we optimized how to minimize diatom growth, which in many experiments can outcompete and decrease survival of kelp in culture. These preliminary efforts allowed our third temperature acclimation experiment with *Nereocystis* to proceed successfully.

In these latter efforts associated with the third trial, we collected reproductive sori and vegetative sporophyte blades (non-reproductive tissue) from two different locations, including one northern site at Big River in Mendocino County, and one site further south at Russian Gulch. These samples were transported to BML, where we wiped the sori and sporophyte blades with a damp freshwater paper towel to remove any diatoms and small microbes, then cut open the blades and washed them with iodine-seawater solution (1:9 iodine in seawater filtered at 1um). We subsequently left the sori on paper towels to air dry for one hour before dividing blades in half and placing them in pyrex dishes filled with filtered seawater for four hours to allow their spores to be released. After spore release, the sori blades were removed and the resulting solution of swimming spores was divided equally into 1 L flasks with filtered seawater, which were then placed into two acclimation tanks: control (ambient) at ~12 °C ±1°C and elevated (warm) at ~17 °C ±1°C. The spores were allowed to settle overnight, and the resulting flasks with settled spores, which had now germinated into male and female gametophytes, were used to grow the kelp to maximize sample collection for later analysis of gene expression and protein abundance, while also conserving space in our acclimation sea tables. The vegetative (non-reproductive) sporophyte blades were treated similarly, though no spores were released and the blades were placed in a 12 x 8-inch glass pyrex dish filled with filtered seawater. The pyrex dishes were transferred to the same two acclimation tanks (control- ambient- ~12°C ±1°C and elevated - warmed- ~17°C ±1 °C). We changed the water 3 times weekly in both the flasks (containing settled gametophytes) and pyrex dishes (containing non-reproductive sporophytes) and added germanium dioxide to the filtered seawater to deter diatom growth.

After 7 days of acclimation, the non-vegetative sporophyte blades from all sites received a one-hour heat shock to explore responses to acute temperature stress. The blades were cut into

one-inch portions and placed in a 5 mL microcentrifuge tube filled with filtered seawater. Blades were heat shocked in an aluminum gradient heat block at 12, 17, 20 and 23 °C. However, if blade quality deteriorated or did not have enough quantity to heat shock, the 23 °C heat shock temperature was eliminated. After the one-hour heat shock, bull kelp samples were flash frozen on dry ice and stored in -80 °C freezer until further use.

We also examined the earlier life stages of bull kelp that we were culturing in flasks. After a 40-day acclimation, the flask walls and bottoms had developed a golden-brown "fuzz," which was likely a mixture of microscopic gametophytes and embryonic sporophytes. This combination was scraped from the flask bottoms with a modified spatula. A sample of this fuzz in seawater was transferred to a 50 mL falcon tube and centrifuged at 1200 rpm to aggregate the kelp material. The water solution was decanted and the process was repeated until the whole flask volume was centrifuged. Once the volume of bull kelp material was condensed to a 2 mL centrifuge tube, it was then exposed to the same one-hour heat shock protocol as described for the adult, vegetative *Nereocystis* samples.

Ultimately, we aimed to quantify gene expression in the experimental samples described above. However, this work required considerable troubleshooting to extract total RNA. Eventually we were able to modify a protein extraction protocol for bull kelp that uses Trizol, and found a more recent study that has had some success with a different Hsp70 antibody. After identifying the best Hsp70 antibody we began trying it on the different extraction methods (Fig. 1.3). Lane #3 and #4 yielded the least non-specific binding thus we moved forward in extracting and processing our experimental heat shocked tissues starting with the adult, non-reproductive sporophyte blade samples. Although we are not yet done with this work, and although the current project is formally concluded, we hope to use other sources of support in coming months to answer the following hypotheses that originally motivated this study:

H1: Microscopic gametophytes and embryonic sporophytes acclimated to warmer temperatures will exhibit increased Hsp70 gene expression compared to those acclimated at ambient (13°C) seawater temperatures.

H2: Juvenile sporophyte Hsp70 gene expression will show no significant differences across collection sites.

H3: Adult sporophytes acclimated to warmer temperatures will have increased Hsp70 gene expression compared to sporophytes acclimated at ambient (13°C) seawater temperatures.

H4: Adult sporophytes will show no significant differences in Hsp70 gene expression across collection sites.

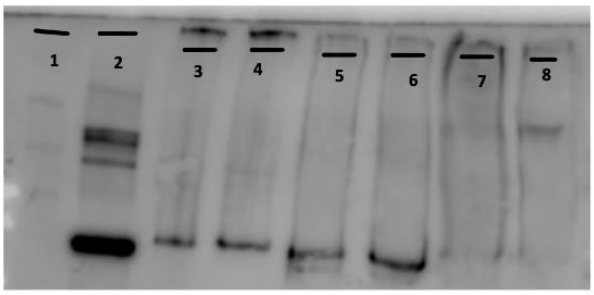

Figure 1.3. Example western blot from bull kelp protein extraction with Hsp70 antibody. Wells labels: 1=size marker, 2=Hsp70 positive control, 3=protein extract from Trizol method #1, 4=protein extract from Trizol method #2, 5=protein extract using Hammann method #1, 6=protein extract using Hammann method #2, 7=protein extract using Wang method #1, 8=protein extract using Wang method #2.

In collaboration with co-PI Edwards at San Diego State University (SDSU), we also measured chlorophyll fluorescence parameters associated with light harvesting during photosynthesis of the microscopic life stages in the flasks. This work was conducted using a Diving PAM fluorometer (Walz GmbH, Effeltrich, Germany). Specifically, we determined the effective (F_{PSII}) and maximum (F_v/F_m) quantum yields of photosystem II after exposure to actinic light and quasi-dark condition for 30 sec, respectively. This approach allowed us to assess any stress to photosystem II. Rapid Light Curves (RLCs) were then constructed under various light conditions, and the electron transport rate (hereafter ETR) was determined for each light intensity. From this information, the photosynthetic parameters of maximum ETR capacity (ETR_{max}), light harvesting efficiency under non saturating irradiances (a), light saturation intensity (E_k), and photoinhibition light intensity (I_b) were calculated.

In undertaking these assays, one flask from both temperature acclimation treatments (12 and 17°C) for each individual (5 individuals per site; 20 flasks total) were transported from Bodega Marine Laboratory (BML), where the culturing was being done, to SDSU in two temperature controlled (12 and 17°C) coolers (10 flasks per cooler). Samples were tested within 12 hours of their removal from the BML water tables. Flasks were placed over a white copy paper to help mitigate misreading of the PAM fluorometer. The probe of the PAM fluorometer was placed in high concentration clumps of gametophytes/embryonic sporophytes in order to get proper readings for the vial. The probe used the same population of microscopic life stages for all of its readings per vial.

Temperature explained about 60% of the variation for both saturation and photoinhibition (Fig. 1.4). These patterns mirror each other in magnitude for the two parameters. Specifically, warmer temperatures resulted in the microscopic thalli becoming both light saturated and light inhibited at higher light levels. Thus, warmer temperatures led to the thalli being able to tolerate higher light levels. In contrast, temperature did not affect light harvesting at lower light

levels or alter the maximum electron transport rates. Together, these results suggest that low temperatures make young bull kelp more low-light adapted while warmer temperatures make them high-light adapted, which follows basic metabolic theory. In all, the microscopic thalli handled higher light levels, which is similar to broader patterns known for kelp, where warmer temperatures tend to elevate a variety of physiological processes and metabolism.

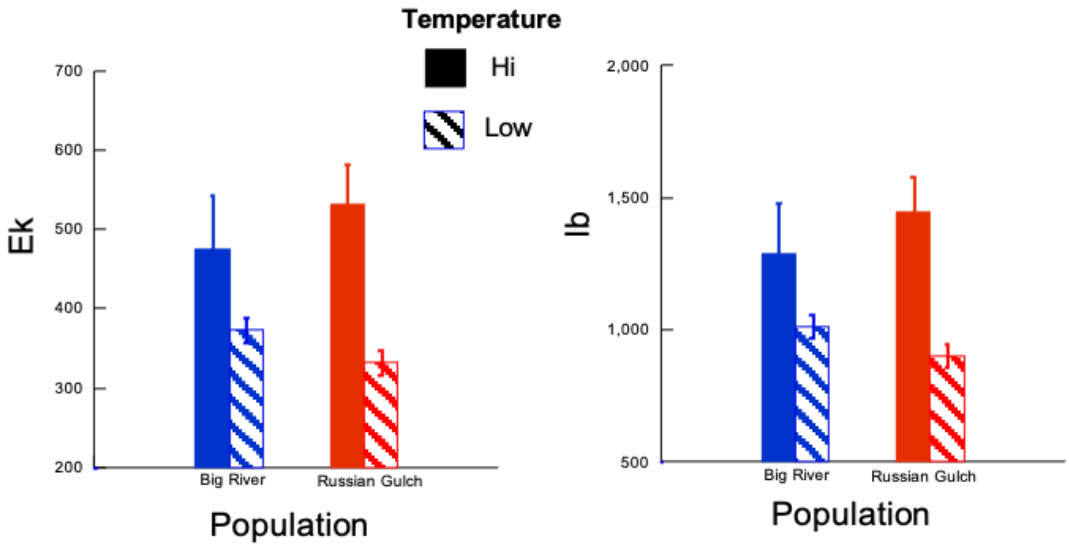

Figure 1.4. Ek (saturation irradiance) and Ib (photoinhibition) were not significant for population but both varied significantly between the two temperatures.

Indeed, although not significant, higher temperatures appear to have a slightly higher electron transport rate (ETR; Fig. 1.5). This trend suggests that elevated temperatures raise metabolism and allow juvenile sporophytes to handle higher light levels as noted.

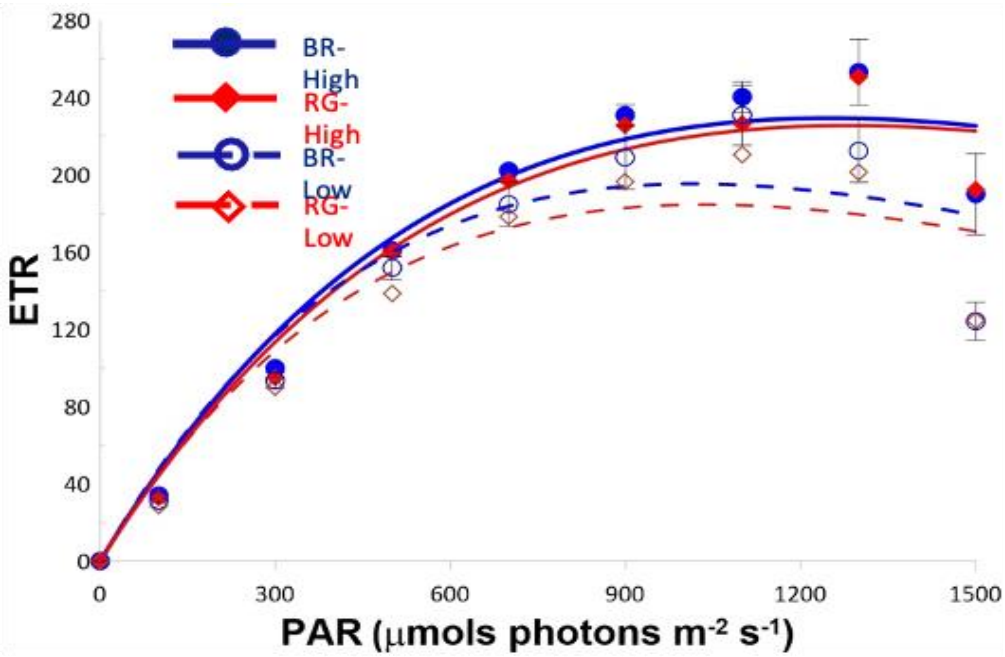

Figure 1.5. Electron transport rate (ETR) for gametophytes acclimated at low (control temperature of 12 C) and high (warm temperature of 17 C) from different sites – Big River (BR) and Russian Gulch (RG).

In our analysis of photosynthetic responses, normality was verified using Q-Q (probability) plots. Equality of variances was checked using Bartlett tests, and ETRmax passed but Ek and Ib did not. Ek and Ib were log transformed and retested, which then passed assumptions of parametric statistics. A two-way Model I (fixed effects) ANOVA was run for each parameter. For ETR neither site nor temperature were significant, nor did they interact. Indeed, almost all variation (98-99%) was due to natural variation (see Table 1.2). Thus, neither temperature nor population affected either light harvesting efficiency under non saturating irradiances (Ek) nor maximum electron transport rates (ETR). But there was a tendency for the low temperatures to be more efficient at lower light levels (greater alpha) and have a lower saturation irradiance (similar to a low light adapted plant).

Table 1.2. Statistics for electron transport rate (ETRmax), saturation irradiance (Ek), and photoinhibition (Ib).

ETRmax

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
POP$	0.005	1	0.005	1.99	0.184
TEMP$	0.003	1	0.003	1.337	0.27
POP$*TEMP$	0	1	0	0.057	0.815
Error	0.028	12	0.002		

Ek

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
POP$	0	1	0	0.003	0.955
TEMP$	0.47	1	0.47	21.05	0.001
POP$*TEMP$	0.058	1	0.058	2.601	0.133
Error	0.268	12	0.022		

Ib

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
POP$	0	1	0	0.003	0.955
TEMP$	0.471	1	0.471	21.055	0.001
POP$*TEMP$	0.058	1	0.058	2.596	0.133
Error	0.268	12	0.022		

Our team had originally hoped to link physiological differences with genotypic differences. To this end, we undertook a small pilot RADseq sequencing project to look for polymorphisms in bull kelp populations that displayed differences in thermal tolerance or photosynthetic capacity. Ultimately we were unable to generate sequencing data from enough individuals to tease out any potential polymorphisms that were enriched in the populations displaying the greatest variance in physiological performance. After conferring with colleagues familiar with these approaches, it was concluded that the approach with the greatest chance of identifying such genetic linkages would be a significantly larger scale sequencing project that employed whole genome sequencing as opposed to the reduced representation approach employed in the RADseq approach.

1.B. Spore dispersal and population connectivity in bull kelp

An important question in management and restoration efforts targeting *Nereocystis luetkeana* is the extent to which remnant kelp patches might be expected to self-replenish themselves. Likewise, it has remained unclear how much population connectivity might exist among multiple forest patches that may be separated by differing distances along the shoreline. Understanding scales of dispersal of kelp spores has therefore been a limiting unknown.

Our project team refined and applied a mathematical model of *Nereocystis* spore dispersal to better understand scales over which adjacent kelp forests might be linked demographically (Fig. 1.6). This work, led by postdoc Nick Burnett and PI Gaylord, may be especially relevant to ascertaining whether remnant kelp patches could operate as spore sources for nearby depleted reefs. To the extent that spores might be delivered from extant forests to depauperate sites by means of oceanographic currents, such processes could foster recovery once environmental conditions (e.g., temperatures) and biotic factors (density of sea urchins) return to more suitable states (Pearse and Hines 1979, 1987; Ebeling et al. 1985). Transport of spores from existing to degraded forests has even greater value given that this mechanism for recovery is natural and does not require additional human intervention.

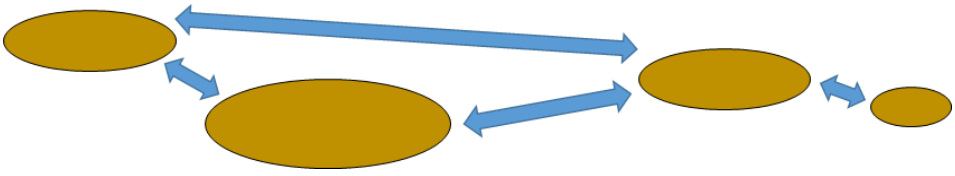

Figure 1.6. Schematic highlighting how spore dispersal among disjunct forests (brown ovals) connected via oceanographic currents (blue arrows) can foster persistence and recovery within a broader metapopulation framework. In particular, self-recruitment back to an originating source forest (not depicted) can mitigate against local extinction of a forest, while transport of spores to another location can bolster recruitment there, improving restoration efforts and increasing colonization rates of new forests in places where they have disappeared.

A crucial parameter in our dispersal model is the sinking speed of spores (Gaylord et al. 2002, 2006). If spores are substantially denser than seawater, and sink quickly, then they will tend to disperse shorter distances compared to if they sink more slowly. Shorter dispersal distances could increase self-recruitment and thereby foster persistence of a given forest, but would not facilitate recolonization of nearby forests that have disappeared. Our group therefore conducted experiments to quantify spore sinking rates in bull kelp, led by co-PI Ricart. Findings documented that bull kelp spores sink in still seawater at a rate of approximately 0.002 mm/s, twice as fast as spores of giant kelp (*Macrocystis pyrifera*) (Fig. 1.7; see also Gaylord et al. 2002).

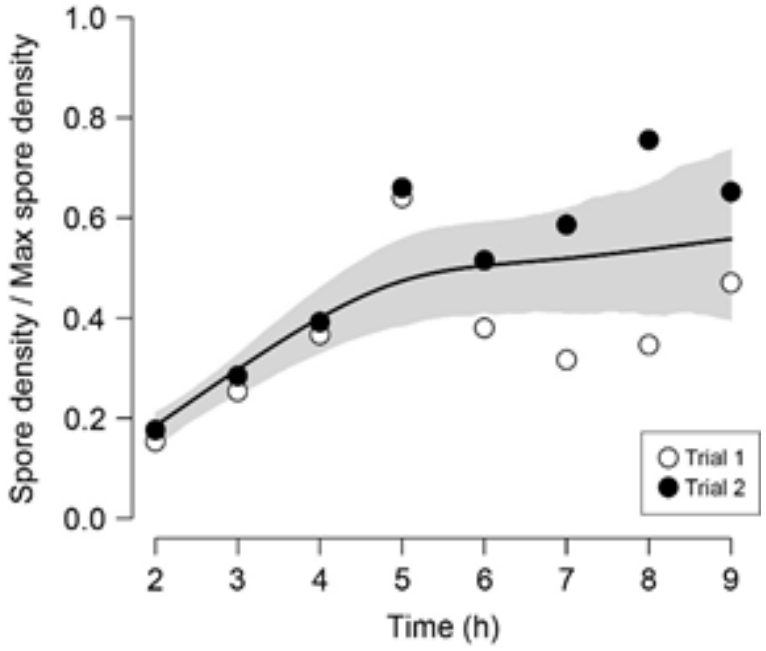

Figure 1.7. Spore settlement densities over time from an experimental assay to determine sinking speeds of *Nereocystis* spores. Data are normalized to the maximum settlement density observed in each trial (open circles = trial 1, closed circles = trial 2). The black line shows a cubic splining curve calculated via the 'smooth.spline' function in R Statistical Software (https://www.r-project.org), and the shaded area represents 95% confidence intervals.

In addition to spore properties like sinking speed, model predictions are sensitive to prevailing oceanographic conditions (wave heights and current speeds; Gaylord et al. 2007, 2012). PI Gaylord's research group, in particular then-PhD student Kristen Elsmore, made a series of field measurements using acoustic Doppler current meters and wave sensors to characterize common conditions at sites along the northern California coast (see also Lyman et al. 2020; Elsmore et al. 2022, 2023). These two lines of information were then incorporated into our

numerical model of spore dispersal, to enable a series of model runs that predict how far spores travel under everyday current and wave conditions.

Importantly, bull kelp can release spores from both sori on reproductive blades near the water's surface, as well as from sori that abscise from the blades and fall to the seafloor. Our model results suggest that an almost bimodal pattern of spore dispersal may arise from the dual option of near-surface and near-seabed release of spores (Fig. 1.8). This tendency differs somewhat from that predicted for the giant kelp (*Macrocystis pyrifera*) in southern California (Gaylord et al. 2002). One consequence of bull kelp exhibiting a bimodal dispersal pattern, unrecognized until now, is that established *Nereocystis luetkeana* forests may exhibit high levels of self-recruitment (but see Raimondi et al. 2004 for additional possible consequences), while simultaneously provisioning more distant locations with spores to facilitate the recovery or persistence of other forests within a broader metapopulations framework (Reed et al. 2006). The former life-history feature may be especially advantageous to an annual species like bull kelp, where population replenishment must occur every year, without the benefit of "holdover" adults from prior cohorts. The latter attribute may similarly have benefits given the capacity for large wave events (or other factors) to drive local extinctions of individual forests, which then rely on delivery of spores from elsewhere for recovery (Reed et al. 1988).

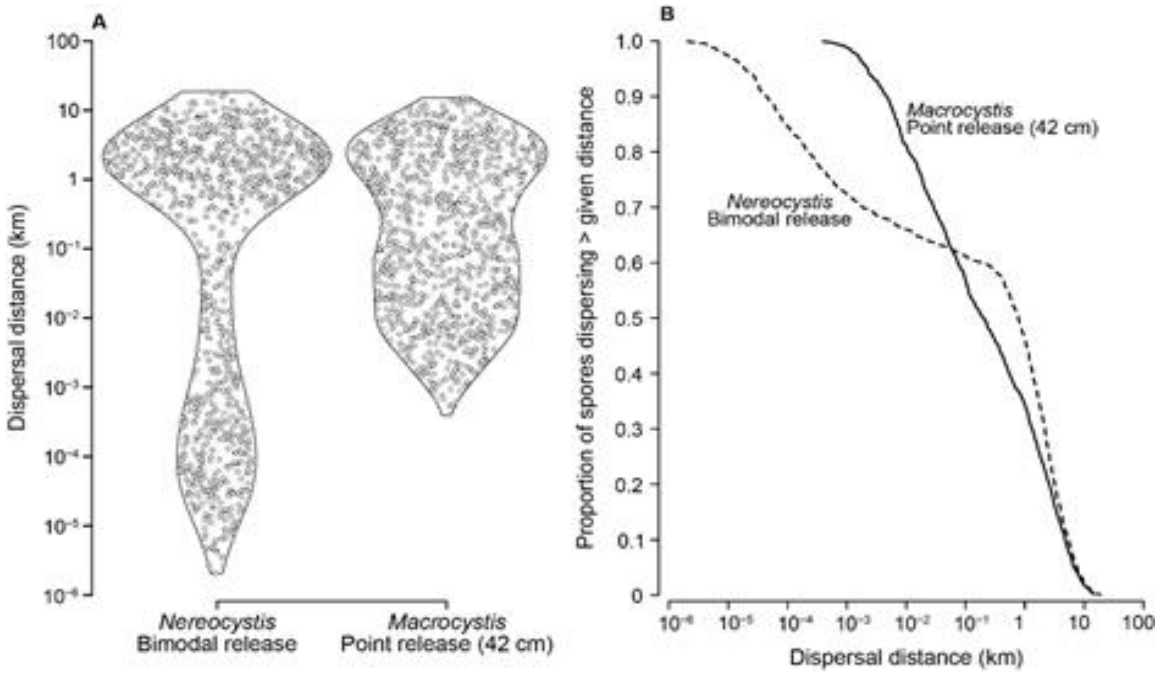

Figure 1.8. Comparisons of spore dispersal for *Nereocystis* and *Macrocystis pyrifera* (giant kelp, the dominant canopy-forming kelp in southern California) under the same flow conditions. A) Violin plots showing distributions of dispersal distances, with points representing individual spores (n = 1000 spores per simulation). B) Proportion of spores dispersing beyond a given distance for *Nereocystis* (dashed line) and *Macrocystis* (solid line). Reproduced from Burnett et al. (in prep).

Operationally, the spore dispersal model estimates the time and horizontal distances potentially traveled by spores, from the time they are released at prescribed heights in the water column to the moment they settle on the seafloor (Gaylord et al. 2002, 2006). Horizontal movements of spores are driven at large scales by the velocities of prevailing currents, and by localized transport due to waves and mixing from turbulence (Gaylord et al. 2012). At the tiniest of scales, spore motion is driven by molecular diffusivity (negligible beyond distances of millimeters), and the natural downward swimming speeds or passive sinking rates of the spores. Water velocity profiles, spanning these scales of motion, are modeled across the water column and through time as functions of the water depth, bottom shear stress, current velocity, seafloor roughness, wave period and height, and the kinematic viscosity of seawater. For each model simulation, spores are released at a specified height in the water column. At each time point, spores move horizontally following the horizontal water velocity at their respective depth, and they move vertically according to their sinking speed and the local molecular-turbulent diffusivity (mixing) at their respective depths (see also Cie and Edwards 2011). The degree to which spores move up or down in the water column is determined by chance through vertical mixing processes (e.g., turbulent eddies move some water masses higher in the water column and some masses lower), and is incorporated into the model as a depth-dependent random-walk. At the end of each time step, the new horizontal and vertical positions of every spore are updated, thereby informing the water velocity and mixing conditions of the following time step. Once the spores contact the seafloor, they are assumed to settle at that point. The model is allowed to run for a simulated duration that corresponds to the viability of spores in nature. Despite spores being released at the same starting point, the vertical velocity gradients and the stochasticity associated with vertical mixing result in a distribution of settling times and distances.

1.C. Persistence and success of microscopic kelp stages in the field

Our research consortium, led by co-PI Edwards and his lab group, also aimed to explore the extent to which banks of persistent but cryptic microscopic stages of bull kelp might remain extant along the north coast of California (Edwards 1999, 2000; Carney et al. 2005, 2013). If present and widespread, such “seed banks” could foster relatively rapid and potentially extensive recovery of kelp forests, if and when environmental conditions improve and the intensity of urchin grazing subsides (Edwards and Hernandez-Carmona 2005; Ladah and Zertuche-González 2007; Dobkowski et al. 2019). Our team also examined interactions between nutrient subsidies that might be emitted by sea urchins, and growth of microscopic kelp stages.

1.C.i. Evaluation of presence of cryptic kelp stages on small boulders

In this component of the project we collected small boulders from forested and non-forested sites and brought them to the laboratory where we cultured them in flowing seawater to determine if they produced visible sporophytes. Here, SDSU/UCD Joint Doctoral student T. Winquist led field activities where she collected ~10 small boulders from each of nine sites in northern California (Fig. 1.9) on SCUBA (Fig. 1.10) in September and October 2022 and brought them to BML. Two of these sites were still forested, five were deforested (urchin barrens) and two were restoration sites where urchins had been actively removed. Specifically, the two forested sites were Noyo Harbor and Portuguese Beach. The five urchin barren sites were Ocean Cove, Timber Cove, Russian Gulch, Stillwater Cove, and South Van Damme State Park. The two restoration sites were Albion Cove and Caspar Cove. Additionally, urchin densities were also recorded at each site. At BML, we cultured the boulders in flowing seawater tanks under full-spectrum LED lights for 4-8 months and observed them for sporophyte recruitment (Fig. 1.11). Additionally, we seeded a set of boulders from each site type (forested, deforested, restored) with bull kelp spores to serve as a procedural control for the culturing methods. We monitored these boulders weekly for the appearance of bull kelp sporophytes to ensure that conditions were in fact suitable for sporophyte emergence, given spore presence.

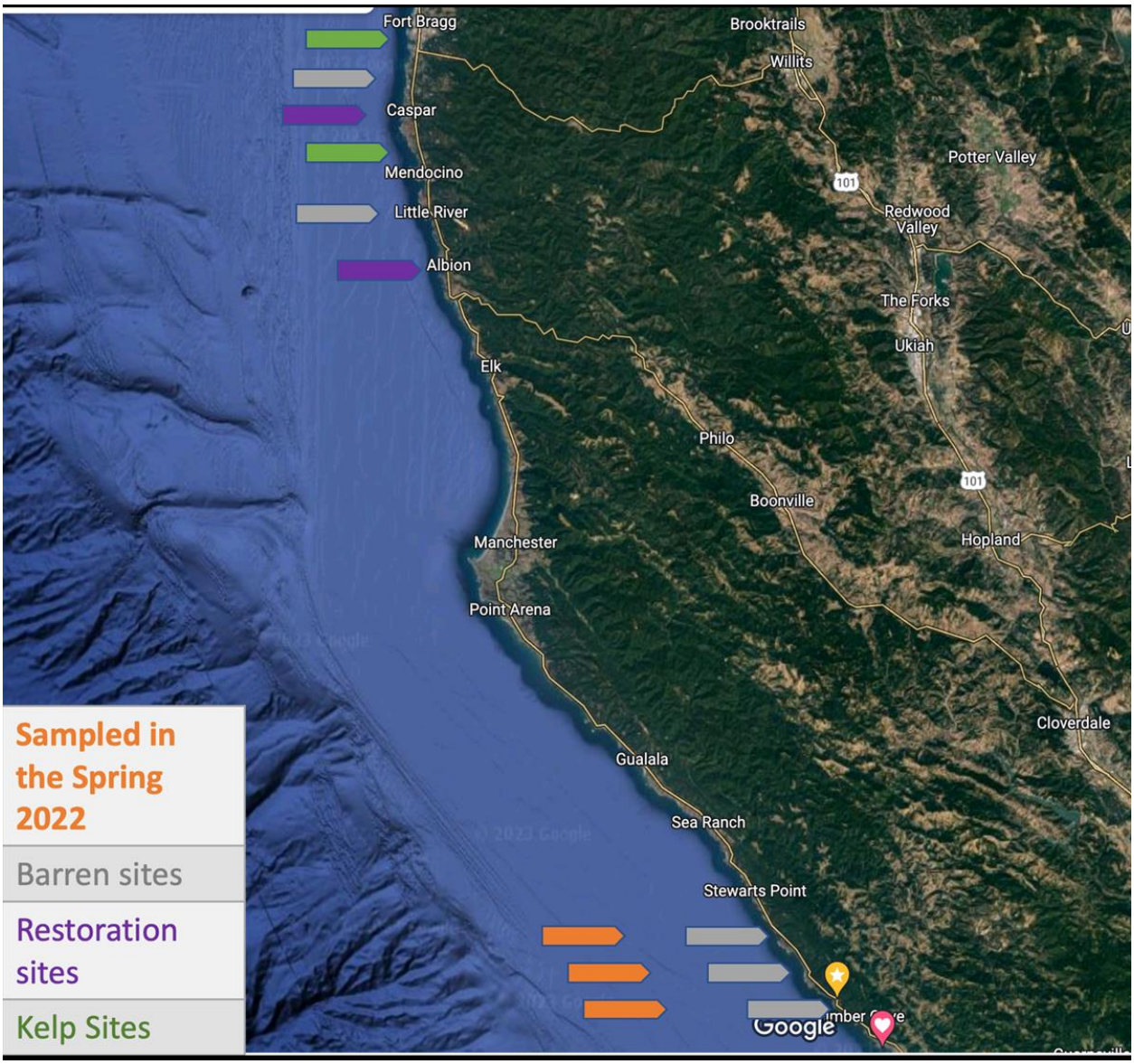

Figure 1.9. Map showing locations of sites from which boulders were collected and brought to Bodega Marine Laboratory (BML) for evaluation of whether microscopic kelp stages were present on benthic substrate.

Figure 1.10. Graduate student T. Winquist (left) and field assistant heading to a dive site at Stillwater Cove to collect boulders.

Figure 1.11. Photographs of boulders collected from forested, deforested and restoration sites in northern California (left panel) and flow-through seawater tanks at Bodega Marine Laboratory where the boulders were placed in culture under full-spectrum lights (middle panel) and observed for sporophyte production (right panel). The sporophytes pictured in the right panel were later collected from the boulders.

Results of the boulder experiment revealed that there were indeed microscopic life stages on the boulders, as we observed kelp recruitment on boulders collected from each site type (Fig. 1.12). Although more sporophytes were observed on boulders collected from kelp and restoration sites, sporophytes were observed on boulders collected from barren sites where no adult bull kelp occurred. This result supports the hypothesis that a bank of microscopic stages does occur naturally. However, this outcome was not consistent across all sites, as no sporophytes were observed on boulders collected from three of the barren sites but were observed on all boulders collected from kelp and restoration sites (Fig 1.13). Further, the greatest number of sporophytes were observed on control boulders that were seeded with spores in the laboratory, which suggests that our culture conditions were appropriate for producing sporophytes if they occurred on the boulders (Fig. 1.14). Altogether, these findings suggest that there is indeed a bank of microscopic stages occurring on natural substrates in areas that have been deforested by urchin grazing, although their densities are lower than in forested areas. Thus, there are hints that extant microscopic stages may serve as a natural pathway for forest recovery should the effects of urchin grazing be removed.

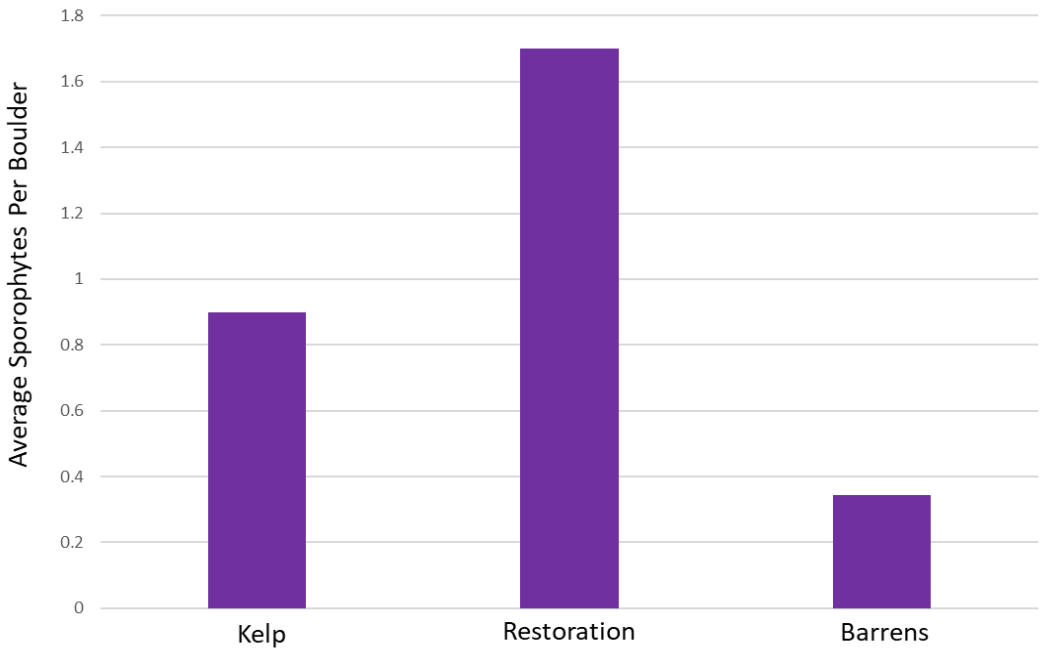

Figure 1.12. Average number of bull kelp sporophytes observed on boulders collected from sites in northern California.

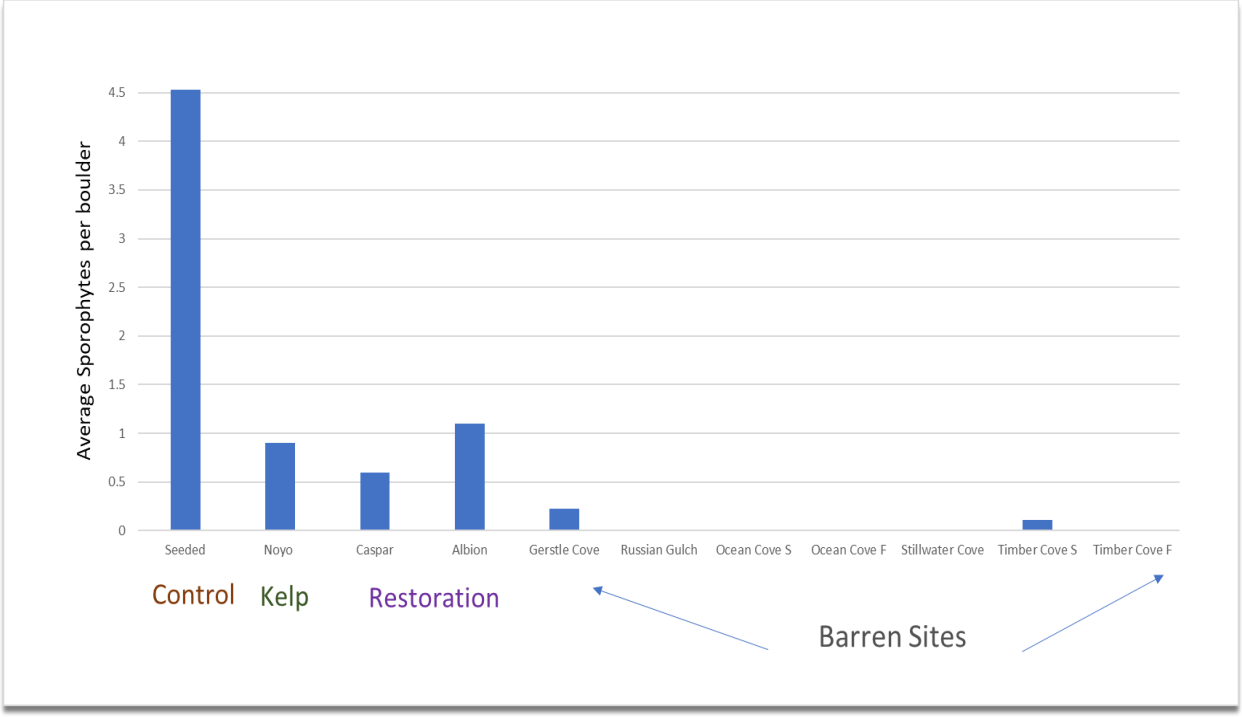

Figure 1.13. Average number of bull kelp sporophytes recruiting on boulders collected from each of the collection sites (by type) and on control boulders that were experimentally seeded with bull kelp spores.

1.C.ii. Role of urchin feces as a nutrient source for microscopic kelp stages

An additional experiment was conducted at SDSU where we examined the potential of urchin feces to serve as a nutrient source for kelp microscopic stages. Working with CA-SURE undergraduate student I. Elizarraras who was mentored by co-PI Edwards, we first collected giant kelp (*Macrocystis pyrifera*) sporophylls and induced them to release their zoospores into 30 petri dishes filled with raw seawater. We then added commercial nutrient media (AlgaGrow) to ten of these dishes. We also collected feces from urchins and mixed these with raw seawater for 24 hrs. The resulting seawater was added to ten of the dishes. We cultured all 30 dishes containing giant kelp gametophytes for six weeks in Percival growth chambers under full-spectrum lights and monitored them weekly for gametophyte growth and sporophyte production. Our results clearly showed that the gametophytes in dishes with both commercial fertilizer and urchin feces grew faster and larger than those under raw seawater (Fig. 1.14). Additionally, the gametophytes in both the urchin feces and commercial fertilizer treatments produced embryonic sporophytes after three weeks, while the gametophytes in the raw seawater never produced them in over six weeks (Figs. 1.15, 1.16). Interestingly, although there were no differences in the number of sporophytes produced between the AlgaGrow and urchin

feces treatments, the sporophytes in the urchin feces treatment grew larger than those in the ALgaGrow treatment (Fig. 1.17).

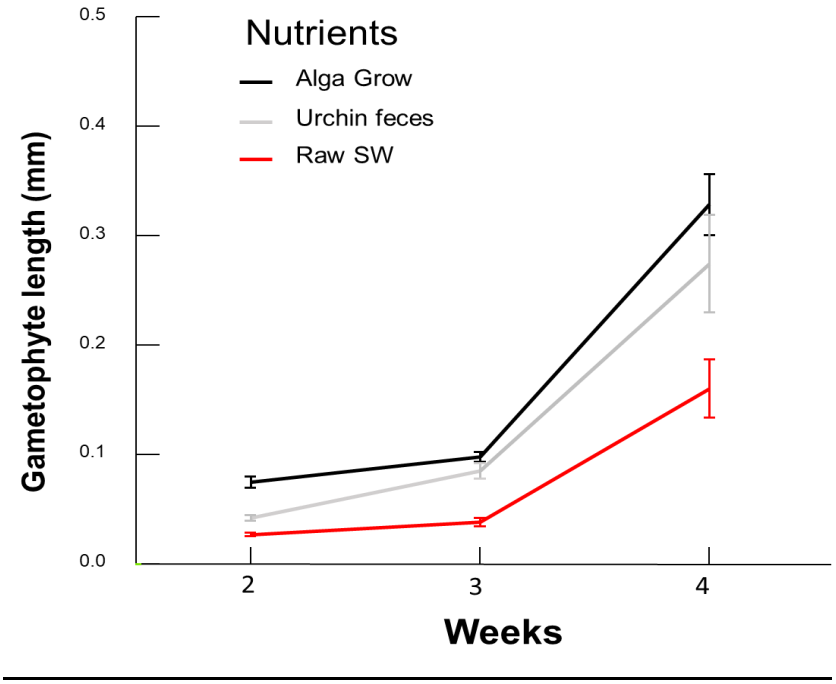

Figure 1.14. Average size (longest axis length) of *Macrocystis pyrifera* gametophytes cultured in raw seawater and seawater with both Alga Grow (commercial fertilizer) and urchin feces over the first four weeks of the experiment.

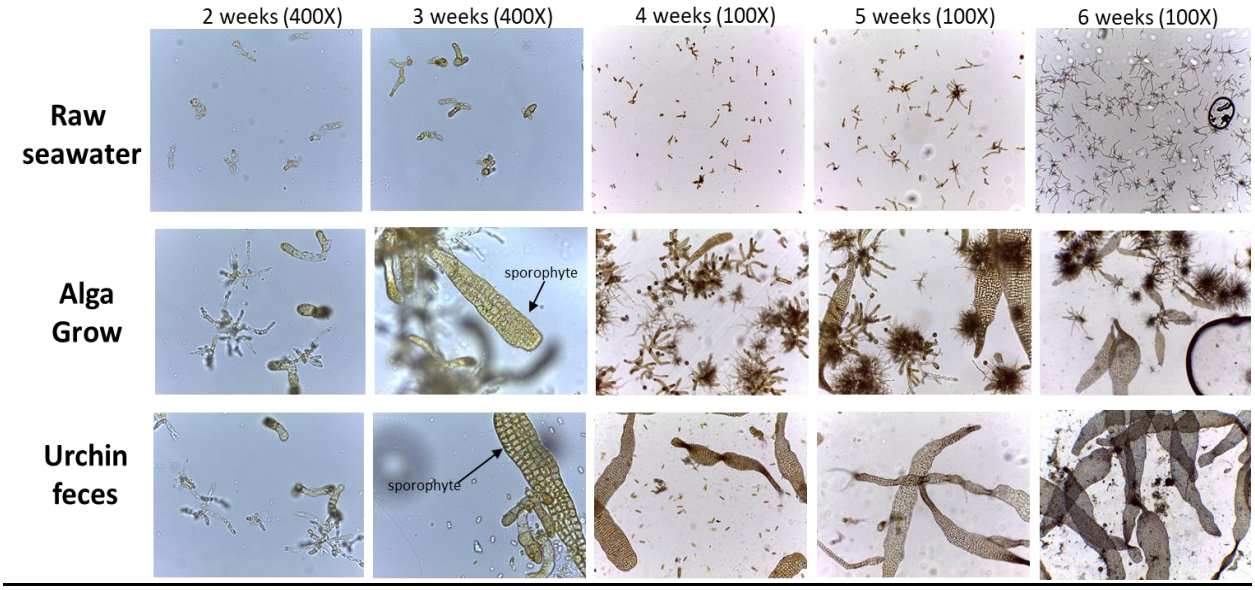

Figure 1.15.Photographs of *Macrocystis pyrifera* gametophytes and embryonic sporophytes in petri dishes cultured in raw seawater and seawater with AlgaGrow and urchin feces added.

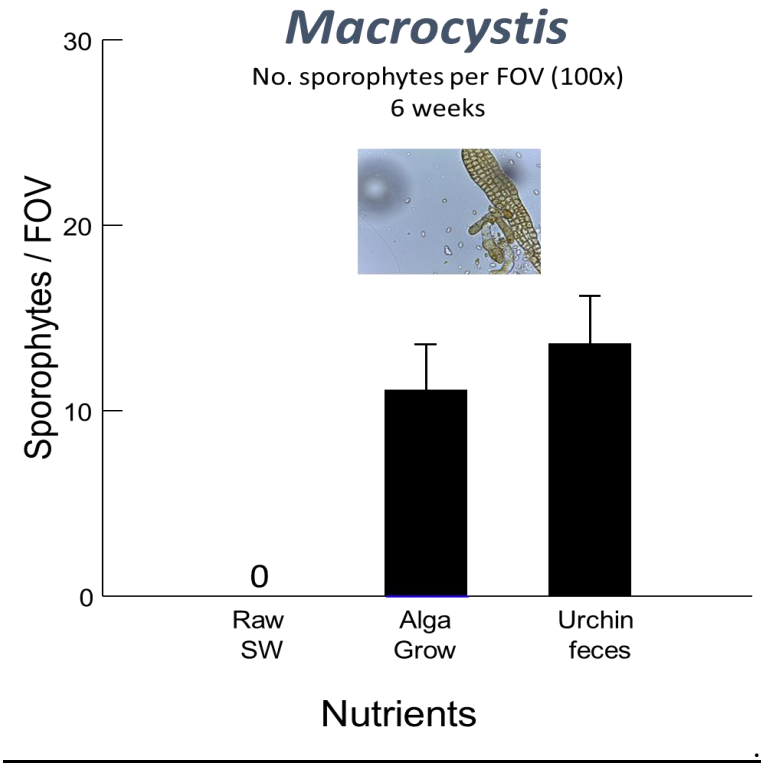

Figure 1.16. Average number of *Macrocystis pyrifera* sporophytes (+SE) produced after six weeks in Petri dishes with raw seawater (SW) and with AlgaGrow and urchin feces added.

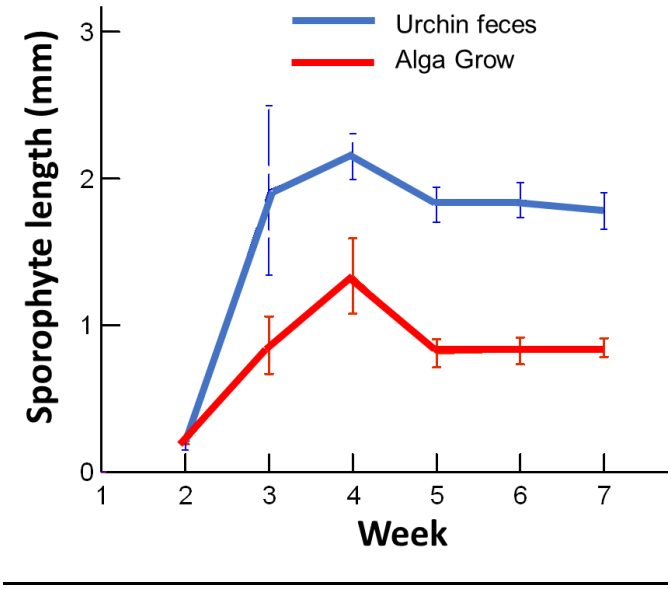

Figure 1.17. Average length of *Macrocystis pyrifera* sporophytes grown in the AlgaGrow and urchin feces treatments over seven weeks in culture.

Following the urchin feces experiments with giant kelp, we obtained bull kelp sporophylls from colleagues at Moss Landing Marine Laboratories, brought them to SDSU and induced spore release. We followed the same protocols described for giant kelp and cultured the resulting bull kelp gametophytes for five weeks under the same nutrient conditions. Similar to giant kelp, the *Nereocystis* gametophytes grew larger in the AlgaGrow and urchin feces treatments than in raw seawater (Fig. 1.18). However, while we did observe some sporophyte production in both the AlgaGrow and urchin feces treatments (Fig. 1.19), but not the raw seawater treatment, we did not observe sufficient sporophyte production to determine the effects of nutrients on development for bull kelp. We believe our light levels were too low for adequate sporophyte production.

Findings from this project component clearly show that urchin feces can serve as a nutrient source for kelp gametophytes and aid in their growth and development. Moreover, results also suggest that urchin feces promote emergence and growth of kelp sporophytes. These data may be important given the large number of urchins occurring in the barren areas, especially if ocean warming leads to reduced nutrient supply. We have also collected the seawater from each treatment on a weekly basis and all the microscopic life stages from both experiments and have analyzed them for their nitrogen and carbon stable isotopes using mass spectrometry. Resulting data will inform us of the form of nitrogen (nitrate vs. ammonium) that was assimilated into the thalli of the microscopic stages and further confirm if they indeed take up

the nitrogen from the urchin feces. While these samples have now been fully analyzed, at the time of the writing of this report, the data have not yet been interpreted.

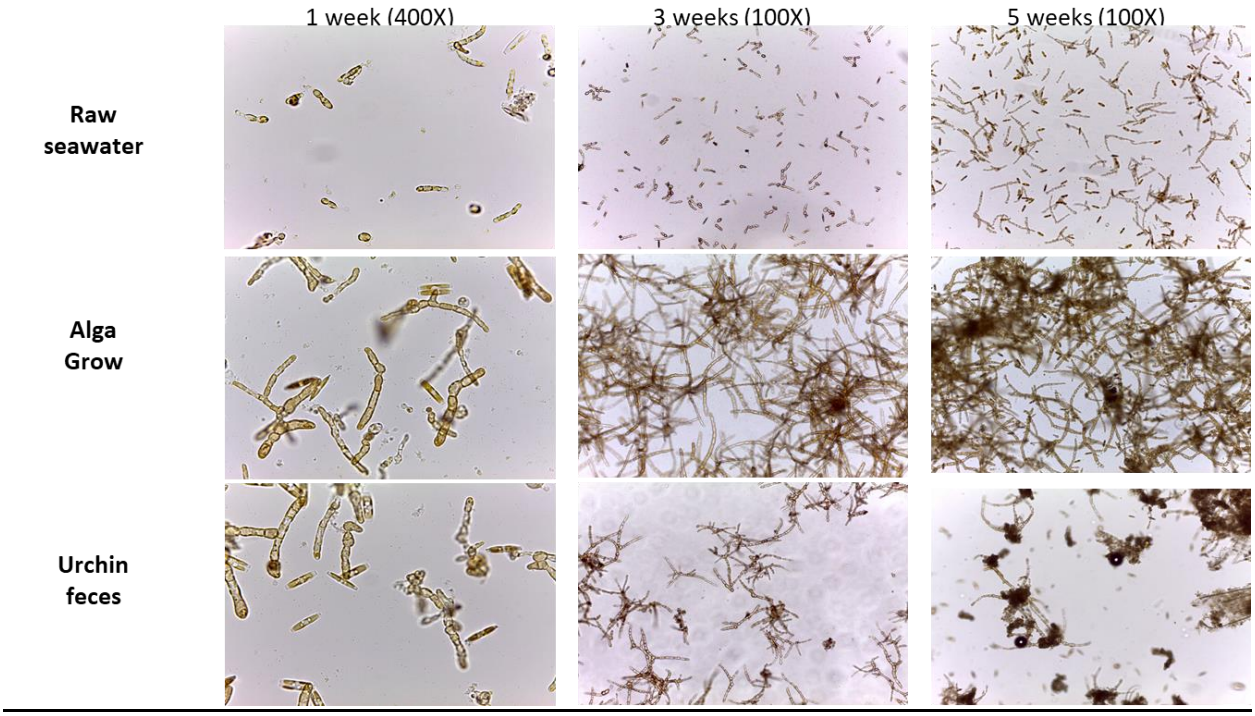

Figure 1.18.Photographs of bull kelp gametophytes cultured in raw seawater, and seawater with both AlgaGrow and urchin feces added.

Figure 1.19. Photograph of bull kelp sporophyte produced in a petri dish with raw seawater and urchin feces added after five weeks in culture.

Objective 2: Mitigate against overabundant urchins

2. Experiments on sea urchins

Our team explored several questions relevant to understanding how the overabundance of purple sea urchins might be addressed to assist the recovery of bull kelp. Efforts at culling sea urchins have been complicated by the perception that smashing urchins in place might result in the unintentional release of gametes and subsequent increases in fertilization of this species. However, one anecdotal observation by researchers at Bodega Marine Laboratory suggested that sea urchins in barrens, which can exist for extended periods of time in states of low nutrition, may produce low-quality gametes that fertilize poorly and lead to larvae that exhibit aberrant early development. If so, then smashing urchins *in situ* would incur minimal elevated risk of increased fertilization. We tested this possibility in purple sea urchins by comparing 'barren' urchins compared to those collected from a kelp-rich area in Jan-Feb 2022.

Other anecdotal observations have suggested improved kelp survivorship in regions that experience modest, chronic exposure to freshwater inputs. The idea here has been that sea urchins might be more sensitive to reduced seawater salinity than kelp. We investigated this latter concept for purple sea urchin larvae. A related possibility is that adult sea urchins might feed at altered rates in waters of decreased salinity, and we tested this hypothesis in laboratory trials as well.

2.A. Gamete viability of starved purple sea urchins

Co-PI Hodin, working collaboratively with students from our California institutions, compared fertilization and early development in urchins collected from a barren in comparison with non-barren California urchins. The barren site was Ocean Cove (Jenner, CA), a site that both historically and at the time of urchin collection (8 Feb 2022) was devoid of kelp. The kelp-rich area from which we collected the control urchins (19 Jan 2022) was Big River (Mendocino, CA). We transported all urchins immediately following collection to holding tanks at Bodega Marine Lab (BML). The Big River urchin were fed bull kelp fronds at BML for the three weeks until fertilization; barren urchins were starved for the 48 hrs of pre-fertilization holding at BML. We used standard methods for spawning (0.55 M KCl), fertilization, and early embryonic culturing (Strathmann 1987; Hodin et al. 2019), in single male x female crosses.

We injected a total of seven barren urchins and 14 control urchins. All seven barren urchins spawned (5 males and 2 females), whereas 12 of the 14 control urchins spawned (3 males and 9 females). For the control fertilizations, we used five females and three males in 10 total crosses. For the barren urchins we used two females and four males in eight total crosses. We maintained urchins and embryos at 14°C on an approximately 12:12 photoperiod throughout the study. We did not quantify sperm suspension concentrations, but we assessed each sperm suspension for approximate equivalent opacity before adding the same volume of each sperm suspension to a beaker with a monolayer of eggs at the bottom.

At 1 hr post fertilization (1 hpf), all crosses were scored for the presence of fertilization envelopes, thus denoting successful fertilization, in 20 haphazardly sampled embryos per cross at 100x magnification. Then, cultures were allowed to develop for two days to assess proportion hatching (24 hpf) and proportion normal gastrulation (48 hpf).

Results of these trials do not support the hypothesis that embryos deriving from urchins collected in barrens are compromised relative to embryos deriving from adults collected in kelp-rich areas (Fig. 2.1). All eight individual barren crosses had 10% or fewer unfertilized, unhatched or abnormally gastrulating embryos; results from the 10 control crosses were similar.

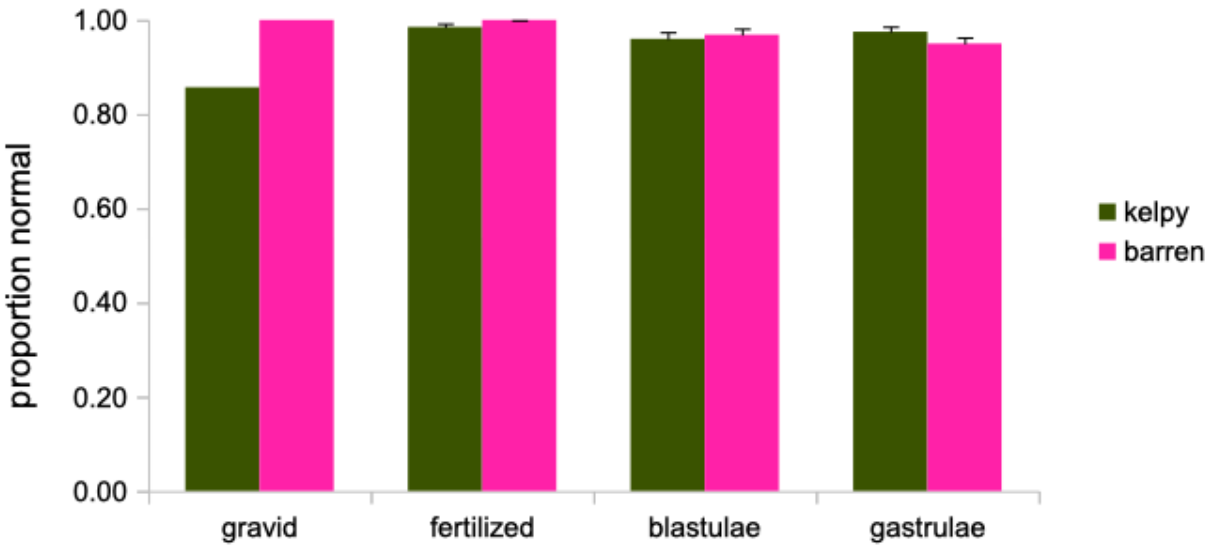

Figure 2.1. Proportion of purple sea urchin embryos that developed normally, when sourced from a deforested site ("barren"), versus a kelp-replete site ("kelpy").

Although we saw no evidence for compromised early development in barren urchins, it is possible that defects would manifest during later larval stages. In our experiment, we did not have the opportunity to continue raising the embryos through later stages, so we were unable to directly assess issues of relative quality and performance in barren-derived urchin larvae. However, egg size has been used in planktotrophic echinoids and other invertebrates as an indirect (albeit imperfect) measure of larval performance (reviewed in Marshall and Keough 2007). Therefore, we measured egg sizes in ten haphazardly-selected unfertilized eggs from the two barren females and three of the control females. We again detected no statistical difference (at $p<0.05$) in egg sizes between barren (80.1 $\pm$ 1.5 [s.d.] µm) and control (79.2 $\pm$ 1.5 µm) females.

Therefore, while barren urchins in other studies have been observed to have a reduced reproductive capacity (small gonads), this is not universally true, and our results suggest that those that are gravid in barrens could reproduce successfully. Furthermore, since gravid male and female echinoids store fully mature (ready to fertilize) gametes, crushing a gravid urchin *in situ* does have the potential to result in successful fertilizations. Whether the impacts of such anthropogenically-aided fertilizations would outweigh the potential population control benefits of crushing urchins *in situ* requires further study. Of course, if such urchin crushing efforts were conducted out of the urchin's reproductive season, then anthropogenically-aided fertilizations would be of minimal concern.

2.B. Effects of reduced salinity on larval sea urchins

As noted previously, some persistent bull kelp patches in Northern California are situated near river mouths, and hence subject to fluctuating salinities. This pattern raises the question of whether such locations could serve as refugia for kelp from intense urchin grazing. Surprisingly, little work has been published on purple urchin sensitivity to salinity across their life history, but a few papers point to the sensitivity of adult purple urchins to low salinity events (<21 psu; see Hendler 2013), and one study on embryos and larvae points to a greater sensitivity of *S. purpuratus* at these early stages as compared to embryos and larvae of their euryhaline congener, *S. droebachiensis* (Roller and Stickle 1985). Finally, field data seems to indicate better recruitment success in higher salinity conditions (reviewed in Rogers-Bennett and Okamoto 2020), but the responses of settlement-stage purple urchin larvae to low salinity has not been previously reported.

Co-PI Hodin had conducted prior, unpublished experiments involving direct observations of metamorphic stage purple urchin larvae from the salish sea in a chilled, stable water column containing a halocline between 30 psu and 20 psu (layered on top). This situation – a lens of low salinity water over top of higher salinity ocean water – mirrors what one sees during seasonal river discharge events in the Salish Sea and elsewhere. In this work Hodin and his research group video-recorded 10 metamorphic stage purple urchin larvae interacting with the halocline. Each larva followed an identical pattern: spiral upward swimming through the water column until the larva encountered the halocline, and then precipitous sinking back down into the 30 psu water. This avoidance behavior in response to low salinity water was not observed in a co-ocurring, euryhaline asteroid species, the ochre star, *Pisaster ochraceous*. Larvae of this latter species swam up to and then concentrated at the halocline, and sometimes even passed through it into the 20 psu water (S. George, pers. comm.). If sea urchin larvae in the field avoid regions of low salinity water as they did in these earlier laboratory trials, such behaviors could impact their likelihood of moving into such regions.

In addition to avoidance behaviors while in the water column, it is also possible that purple urchin larvae might be less likely to settle and complete their transformations into benthic juveniles, in locations with lower salinity seawater. We undertook a series of laboratory experiments to address this question during our project, both in the Salish Sea (where mean salinities are lower than in open ocean California waters) and in Bodega Bay, California. In each experiment we raised mixed parentage batches of larvae through their one month larval period, and when they reached competence, we exposed them to reduced salinity seawater for 24 hours and then provided them, at that salinity, with a natural settlement cue (Gaylord et al. 2013): fronds of the coralline alga *Calliarthron tuberculosum*.

We conducted our first experiment with Salish Sea larvae, comparing settlement responses in 30 psu (ambient) versus 20 psu (reduced) seawater. The basic results were clear: whereas 38 ± 15% (s.e.m.) of larvae in the ambient salinity treatment settled in this experiment, no larvae (0%) settled in the 20 psu treatment after 24 hrs of exposure to the cue. Interestingly, providing these same larvae with kelp detritus for 24 hours or a 3 min exposure to intense fluid turbulence increased the proportion of settling larvae at 20 psu to 10 ± 7 % and 6 ± 3%, respectively. Combining turbulence, kelp detritus and low salinity further increased the proportion settled to 22 ± 14%. Still, this proportion undergoing settlement is approximately half of what we saw with larvae exposed to turbulence, kelp detritus and high salinity in combination (47 ± 13% settled at 24 hrs). In summary, although purple urchin larvae are capable of settling in low salinity conditions, they appear to avoid doing so unless the low salinity is accompanied by other conditions characteristic of nearshore habitats where adults often live. As such, there is some evidence to suggest that purple urchin larvae may tend to settle in reduced numbers in kelp patches with lower salinity, relative to higher salinity regions with otherwise similar conditions.

In our second experiment of this type, we used California purple urchin larvae raised in California seawater conditions (35 psu). The experimental design was similar to the first experiment, except that we only manipulated salinity, and we used four salinity levels (35, 31.5, 28 and 25 psu). The results here were variable and less clear than in the first experiment (Fig. 2.2). Larvae settled in similar levels across treatments, with, if anything, a non-significant trend towards peak settlement responses at 28 psu. These results indicate that a 10 psu drop in salinity in California larvae does not in itself result in a substantial block in settlement as it did in the first experiment. One possibility could be that absolute levels of salinity, as opposed to the scope of the shift, is what impacts settlement behavior.

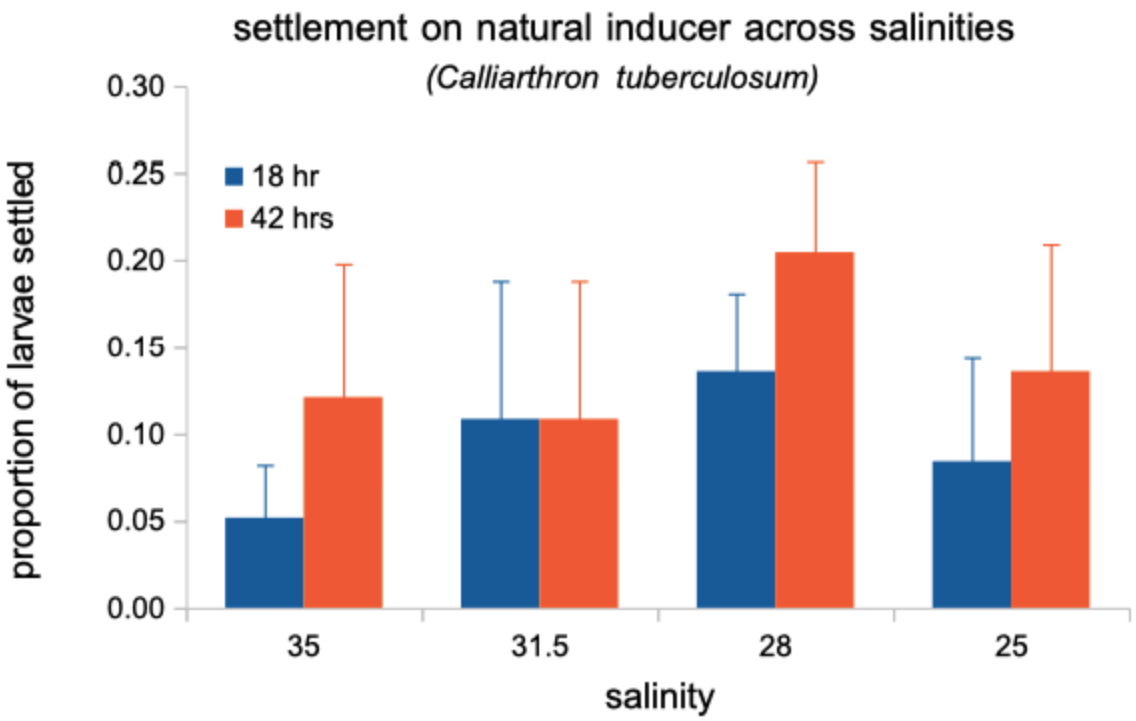

Figure 2.2. Settlement responses under different salinities, of purple urchin larvae of California origin, 18 and 42 hours after they were provided with a natural settlement inducer.

It should be noted that in the first two experiments we did not distinguish between direct effects of salinity conditions on the larvae, on the coralline algae, or both. Furthermore, our manipulations of salinity in these experiments involved the standard method of diluting natural seawater with distilled (or in our case reverse osmosis-filtered) water. However, this is a poor proxy for river water mixed with ocean water, as the chemistry of river water can be quite variable, and the alkalinity is often >>0.

Therefore, in a third experiment, again in the Salish Sea, we manipulated alkalinity independent of salinity, using a combination of carbonate and bicarbonate to mimic the alkalinity of our ambient seawater. Furthermore, we raised some of our larvae for the latter half of their larval period in elevated salinity conditions to mimic open-ocean seawater conditions in California. We accomplished this elevated salinity by adding a small quantity of instant ocean to ambient (31 psu) seawater to raise the salinity to 35 psu. We also raised a cohort of larvae alongside these in ambient conditions (31 psu). Then, we exposed larvae from one of these two cohorts to the following pre-settlement conditions for 24 hrs resulting in eight treatments:

	larval salinity		pre-settlement salinity	diluted with...
(1)	35 psu	--->	35 psu	n/a
(2)	35 psu	--->	25 psu	elevated alkalinity RO
(3)	35 psu	--->	25 psu	RO
(4)	31 psu	--->	31 psu	n/a
(5)	31 psu	--->	26 psu	elevated alkalinity RO
(6)	31 psu	--->	26 psu	RO
(7)	31 psu	--->	21 psu	elevated alkalinity RO
(8)	31 psu	--->	21 psu	RO

We performed settlement tests as described for the first two experiments, and scored the proportion of larvae that had settled at 24 hrs (Fig. 2.3; error bars are s.e.m.). We found that the alkalinity of the seawater does not appear to impact the results in any noticeable fashion [see treatments (2) vs (3), (5) vs (6), (7) vs (8)], thus validating the methods used in our initial two experiments. We also saw that the elevated salinity-reared larvae settled in higher numbers than the ambient reared larvae [see, in particular, treatments (2,3) vs (5,6)], indicating more rapid development at higher salinity (earlier competence). And, finally, our results indicated that reduced salinity only resulted in decreased settlement in larvae transitioned from 31 --> 21 psu [i.e., treatment (4) vs (7,8)]

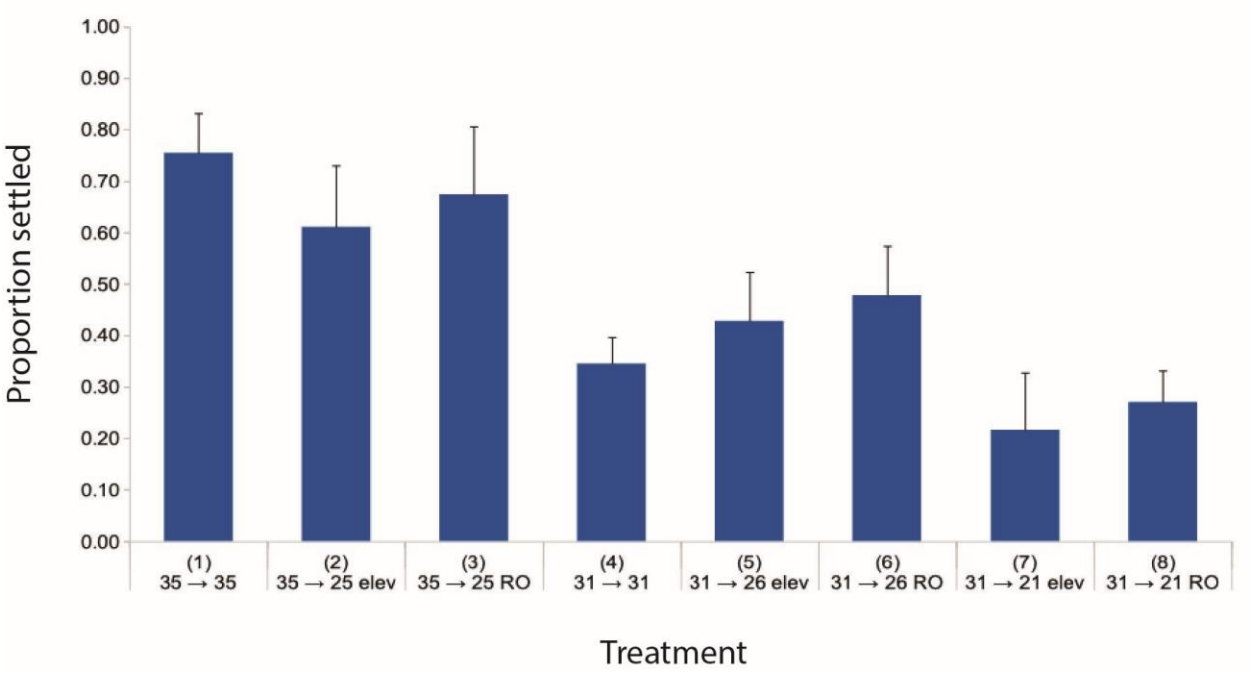

Figure 2.3. Settlement responses of purple urchin larvae exposed to a wider range of low-salinity conditions and transitions between salinities. "Elev" indicates seawater diluted using fresh water characterized by elevated alkalinity, in comparison to treatments using fresh water acquired via reverse-osmosis (RO) filtration.

All told, our three experiments indicate that strong salinity reductions could impact larval settlement but only if the salinities drop to nearly 20 psu. Such salinities are observed in nearshore regions of the California coast, but are typically seen only at specific times of year, in close proximity to larger rivers or streams, or episodically in association with strong rainfall events. As a result, although it appears possible that settlement-stage larvae in California might be discouraged from settling in kelp forests located near freshwater inputs, it would likely take relatively large departures from full-strength seawater. Our results do not address the additional question of whether newly settled juveniles might be more sensitive than larval stages to freshening events.

2.C. Effects of temperature and salinity on grazing activities of adult urchins

In experiments led by co-PI Hughes and his research group at Sonoma State University, we examined whether adult purple sea urchins consume differing amounts of kelp in ambient or warmer seawater, and in seawater of lower or higher salinity. The first experiment tested for the effects of warming on urchin herbivory. We cultured bull kelp in a temperature-controlled wet lab at BML using methods adapted from Muth et al. (2019) and Hernandez-Carmona et al. (2006). We collected about thirty reproductive blades from different individuals about 1 m apart from each other from Russian Gulch in Sonoma County, CA (Fig. 1.2). We placed the blades in a mesh bag for transportation to the vehicle. To ensure the bull kelp blades remained

fresh for the 2-hour drive back to the lab, We created a “kelp lasagna”. We individually wrapped sori in paper towels moistened with seawater. Every ten blades, we placed an ice pack on top before adding another “kelp lasagna” layer to the cooler. The “kelp lasagna” ensured the sori remained cool and did not release their spores during transportation.

Once in the lab, we unwrapped the bull kelp sori from their “kelp lasagna” and quickly dipped them in a 10% iodine solution (10mL iodine with 90mL filtered seawater – 20µm filter, 1µm filter, and an ultraviolet light filter) to rinse off any epiphytes, and then rinsed with filtered seawater to remove the iodine. We then let the sori air dry on paper towels for 1 hour. We filled thirty-two pyrex dishes (1.1 L) with filtered seawater (20µm filter, 1µm filter, and an ultraviolet light filter) to reduce diatom fouling over time. After 1 hour, we cut the sori into 54 cm^2 pieces so that each pyrex dish had a standardized amount of material. We placed three random sori pieces into each pyrex dish and soaked them in an ambient seawater bath in the temperature-controlled lab (the temperature of the room was kept at 14 °C) to osmotically stress sori to induce spore release onto microscope slides. After 1 hour, we removed the sori. We also placed ten microscope slides in each pyrex dish for released spores to settle upon. For the feeding trials, two microscope slides were used as one replicate and were randomly selected from one pyrex dish. We covered each pyrex dish with saran wrap and poked seven holes in it to avoid contamination from fouling species (e.g., diatoms) in the water table. We placed a rubber band around the pyrex dish to hold the saran wrap in place.

Following spore release and subsequent settlement on the microscope slides, we grew early life stages of bull kelp in two temperature treatments (ambient – 13 °C and high – 17 °C) for a month to use in four feeding trials. In these trials we explored rates at which adult purple sea urchins fed upon spores and gametophytes of three ages, which we standardized by days after spore release: 1) post-settlement spore stage (1 day after spore release), 2) early gametophyte stage (8 days after spore release), 3) mid gametophyte stage (15 days after spore release), and 4) late gametophyte stage (22 days after spore release). The elevated temperature treatment simulated the thermal threshold for bull kelp and temperature conditions during “the blob” (Muth et al. 2019; Rogers-Bennet and Catton 2019). Each trial had 80 microscope slides (40 slides per temperature treatment).

During culturing of gametophytes on the microscope slides, we placed pyrex dishes in seawater tables, allowing them to be partially submerged in still seawater in a temperature-controlled room set to 14 °C (typical summer-time temperature conditions for northern California). The high temperature treatment tables contained two titanium heaters with temperature probes and a digital controller (True Temp T3-150, JBJ Inc.) set to 17 °C and two small aquarium pumps to ensure that the entire table had a consistent temperature. We placed a tarp over the table to

help maintain the desired elevated temperature for culturing and minimize evaporation. We placed a temperature and light intensity sensor (HOBO Onset Data Logger) inside a control pyrex dish and submerged another in the water table for each temperature treatment; the HOBO loggers collected data every 15 minutes. Each table had a multi-spectrum LED grow light covered with thin black mesh to not photoshock the growing bull kelp gametophytes (~20 µmol of photons per msq per s) hanging above the pyrex dishes.

We conducted weekly water changes for the bull kelp cultures to reduce contamination from green algae and diatoms. We changed the water one pyrex dish at a time by temporarily placing the microscope slides in a different dish while we emptied the water from the pyrex dish and scrubbed the bottom of the dish clean with a paper towel to remove any fouling organisms. We placed filtered seawater in the pyrex up to about an inch from the top of the dish, placed the microscope slides back into the dish, added 60 µL of germanium dioxide, and wrapped a new piece of saran wrap over the dish. We refilled the water in the high temperature treatment table about twice a week due to evaporation caused by the heaters, but only refilled the water in the ambient temperature treatment table about every two weeks since evaporation was not an issue. As noted above, in total, we cultured bull kelp gametophytes for 22 days.

The purple sea urchins we used in the feeding trials were adults (n=40) of roughly the same test size (50–70 mm), gathered from Timber Cove and transported to BML in a cooler filled with seawater. In the lab, we split the urchins evenly between the two temperature treatments (n=20 per treatment) and placed them in a 5-gallon tank with two air stones and flowing seawater with a mesh on top of the tank to deter them from escaping. We acclimated the urchins in their respective temperatures for one week. During this time, the purple sea urchins were also starved to prepare them for the feeding trials. During their starvation period, we siphoned their tank every day to remove fecal matter.

To create the feeding arenas, we drilled two holes in the lids of forty pyrex dishes to allow a tube to fit into one of the holes for flowing seawater and for seawater to flow out of the other hole. We attached the tubes to pipes that provided flowing seawater directly from the ocean for the ambient temperature treatment and to a sump inside the room for the elevated temperature treatment. The sump and elevated temperature water table for bull kelp culturing each contained two titanium heaters with temperature probes and a digital controller (True Temp T3-150, JBJ Inc.) set to 17 °C. Like the elevated temperature culturing table, we also covered the elevated temperature feeding trial table with a tarp. The tarp allowed the elevated temperature tables to maintain a constant elevated temperature above the ambient temperature of the room (14 °C). Each temperature treatment had twenty pyrex dishes. After

the 1-week starvation period, we placed one urchin per labeled pyrex for the duration of the experiment.

Each feeding trial lasted 19 hours; we chose this time based on a preliminary trial during which we monitored urchins every two hours to determine the adequate period for the feeding trial. During the feeding trial, we left the purple sea urchins undisturbed with two microscope slides from their respective temperature treatment. Between feeding trials, we left purple sea urchins in their labeled pyrex dish in their respective temperature treatments with flowing sea water; we did not feed them in between trials. The control pyrex dish contained no urchins, only two microscope slides with bull kelp from its respective temperature treatment. This approach allowed us to see if any changes occurred within the alga itself. In total, each trial had twenty-one pyrex dishes (20 experimental + 1 control).

Using a digital microscope with a camera attachment (Amscope Compound Microscope with 5MP Digital Camera), we took pictures at 40x at six 1 cm increments along each microscope slide before the feeding trial. We measured each urchin's wet weight (g) and test diameter (mm) before the trial. After each feeding trial, we photographed the microscope slides as described previously. We used these pictures to determine the life stage of the bull kelp at the start of the trial and to quantify the change in kelp percent cover during feeding trials. We analyzed all images on ImageJ.

The laboratory experiment showed no preference of purple sea urchins on bull kelp life history stage, i.e., spores versus gametophytes of various ages (Fig. 2.4). However, it did show that purple sea urchins consumed more gametophytes in the ambient temperature treatment than in the elevated temperature treatment. These results verify that purple sea urchins do consume microscopic bull kelp and suggest the possibility that purple sea urchin herbivory on microscopic bull kelp stages could decrease as the ocean warms. This study helps fill the data gap on purple sea urchin herbivory on microscopic bull kelp life history stages.

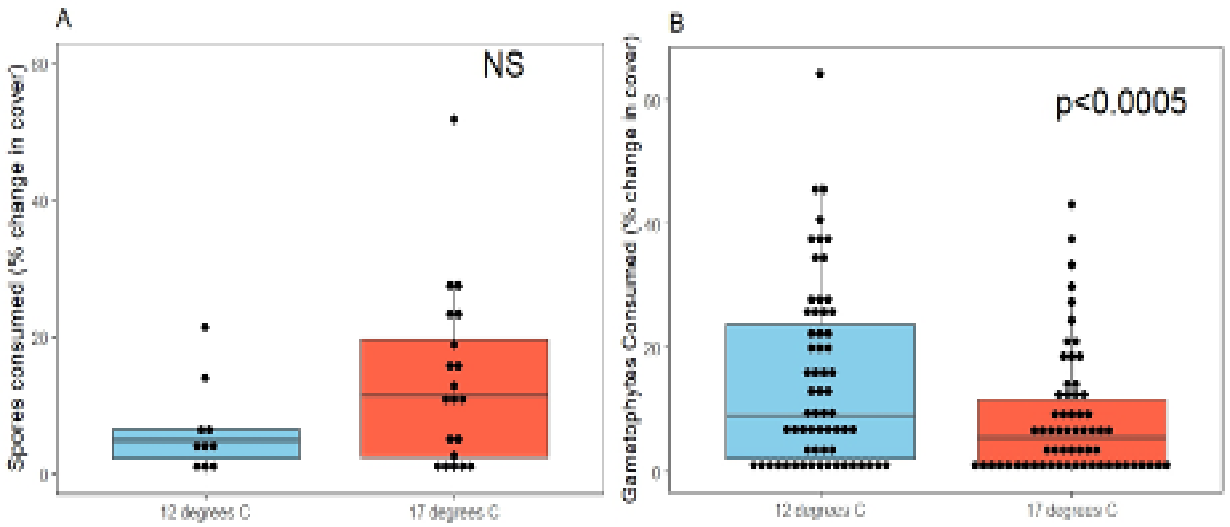

Figure 2.4. Bull kelp spores and gametophytes consumed as a function of temperature. A) There was no significant difference in spore consumption between temperature treatments (Trial 1). B) There was a significant difference in gametophyte consumption between temperature treatments (Trials 2–4). From Gomez et al. 2023 in prep.

Given anecdotal observations that remnant kelp patches appear to persist preferentially in locations with modest freshwater input, we also investigated the effect of salinity on rates of herbivory by adult purple urchins on bull kelp. In these efforts, co-PI Hughes led a feeding assay experiment in static (no-flow) aquaria at a temperature controlled laboratory (12 °C) at Sonoma State University. For this purpose, we collected 60 similarly sized urchins (50-70 mm) from Arena Cove. To acclimate the urchins, we placed urchins into two 20 L holding tanks (~30 urchins per tank) with flowing seawater for four days without food. On the fourth day of laboratory acclimation, we collected four different fresh bull kelp stipes that had been deposited by waves on Doran Beach, California. We transported the kelp to the laboratory, kept it in a 4 °C refrigerator overnight, and the day after cut the kelp stipes into five-gram rings to feed urchins in the assay.

The urchin feeding experiment ran for 72 hours and included four salinity treatments (n = 10 replicate aquaria per treatment: 20, 25, 30, and 35 psu. For each salinity treatment except the 35 psu treatment, we diluted seawater with deionized water until the appropriate salinity was reached, as quantified using a multiparameter seawater sensor with salinity probe (YSI; Yellow Springs Instruments, Ohio). Seawater was collected and sand filtered at BML and transported to a climate controlled room at Sonoma State University. The water was collected shortly after a heavy rain and the salinity was lower than the expected average of 35 psu. To create the 35 psu treatment concentration we used Instant Ocean Sea Salt (Instant Ocean, Blacksburg, Virginia) to raise salinities to 35 psu. After a treatment batch of seawater was diluted, we filled the corresponding aquaria, placed an urchin in each of them, placed two five-gram pieces of bull

kelp stipe and placed the lids back on with the kelp, urchin, and seawater treatment inside. The aquaria (1.1 L, N = 40) had a lid (a small hole was cut out of each of the lids to allow a tube for airflow to enter) with tubing to run from air supplied valves to supply sea urchins with adequate oxygen. We inspected urchins at 0, 24, 48, and 72 hours after initiation of the experiment, and noted health indicators (observed grazing, upright spines, waving pedicellariae) to ensure each urchin remained healthy in each aquaria. At completion of the trials, we gathered all remaining kelp and measured fresh weights. For this component of the study we let the samples sit in paper towels at room temperature in air for one hour to rid them of any excess water that would affect the true mass of the kelp. We recorded the final mass of all kelp and urchins, and determined kelp consumption by subtracting the final kelp mass from the initial mass.

We found that low salinities are an important environmental factor limiting runaway urchin herbivory in northern California bull kelp forests (Fig. 2.5).

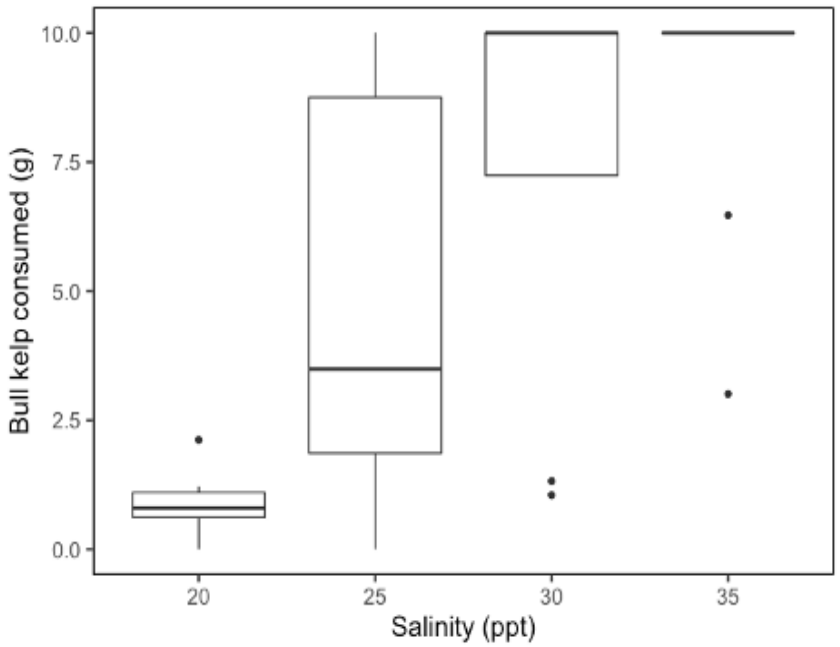

Figure 2.5. Box plots showing the differences in purple urchin herbivory across four salinity treatments (N = 10 aquaria replicates per treatment). The effect of salinity was significant ($P < 0.0005$) using a generalized linear model with a tweedie distribution. Darkened bars represent the median; upper and lower ends of boxes represent the 75th and 25th percentiles, respectively; whiskers represent the 95% confidence intervals; and the points represent data 2 SD outside the mean of each treatment. From Ricart et al. 2023 in prep.; salinity experiment run by CA-SURE Intern Taylor Nelson.

Objective 3: Bolster a predator of sea urchins

3. Experiments with sunflower stars

A third component of our project explored ways to assist recovery of sunflower sea stars, which historically have been a key urchin predator. This species has declined precipitously due to sea star wasting disease, and we conducted work, led by co-PI Hodin, to develop ways to culture these animals in the laboratory as a hedge against further losses in the wild (Fig. 3.1). In particular, we refined sunflower star husbandry and juvenile rearing techniques, such that juvenile stars grow quickly and have high rates of survival.

Figure 3.1. Two-year old adult *Pycnopodia helianthoides* individuals reared in the laboratory, demonstrating that with careful husbandry, these animals can be successfully cultured to reproductive age and beyond. Research assistant Augie Kalytiak-Davis, now in a PhD program, is visible in background. Photo credit: Joey Ullmann.

Intriguingly, we also learned that juvenile sunflower sea stars (*Pycnopodia*) will feed on both red urchin juveniles and purple urchin juveniles, but prefer purple urchins. Moreover, the maximal feeding rates of juvenile *Pycnopodia* on juvenile purple urchins are notable, reaching 5-10 times higher than reported predation rates of adult sunflower stars on adult urchins (Fig. 3.2).

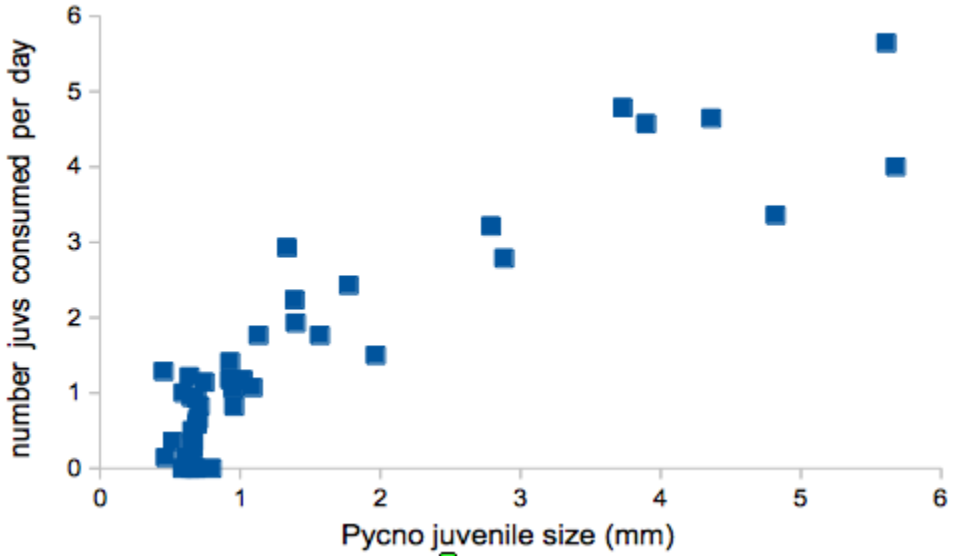

Figure 3.2. High rates of consumption of juvenile purple sea urchins by juvenile sunflower stars.

By means of joint funding from this project and other sources, co-PI Hodin and his group additionally refined techniques to complete the life cycle in sunflower stars, successfully settling offspring from the adults his research team had been culturing for multiple years. Co-PI Hodin determined the best settlement substrates and biofilm attributes to maximize successful transition of larvae from their pelagic phase into juvenile recruits (Fig. 3.3). Furthermore, the lab-reared sunflower stars the team has been rearing have now obtained reproductive maturity in captivity - at 3 years of age, an important landmark for any captive breeding program. Hodin's team also determined that sunflower star larvae and juveniles are quite robust to high temperatures (several degrees C above the seasonal temperatures they experience in the region from which they were collected - the Salish Sea).

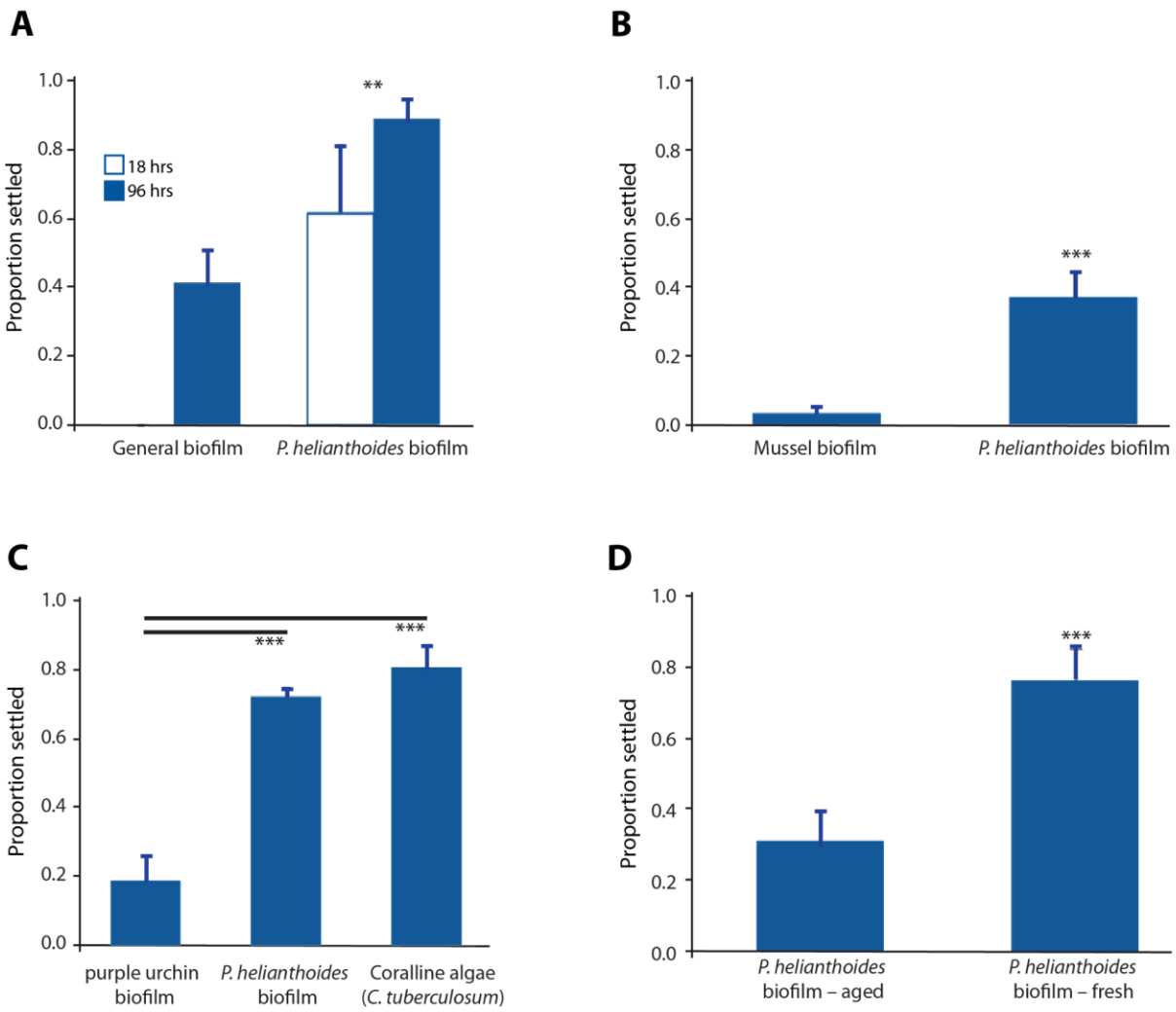

Figure 3.3. Settlement of sunflower start larvae on various substrata. (A) Larvae settled both more quickly (open bars; $F_{1,8}=9.263$, $p=0.016$) and in higher numbers (filled bars; $F_{1,8}=23.459$; $p=0.0013$) when exposed to bioflm grown in the presence of conspecifc adults, as compared to "general" bioflm (i.e., no macroinvertebrates present). (B) The response to conspecifc bioflm was not due to the confounding effect of the presence of their prey (Mytilus *edulis* mussels; $Z=5.357$, $p<0.001$). (C) Larvae also responded more readily to fronds of an articulated coralline alga (*Calliarthron tuberculosum*) or to conspecifc bioflm ($Z=5.250$; $p<0.001$) when compared to bioflm grown in the presence of another known adult prey species: the purple urchin, *Strongylocentrotus purpuratus*. (D) Conspecifc bioflm is much more active when freshly collected (tested 9 d after exposure to adults), compared to similar bioflm aged for 25 d after exposure to adults. Error bars: s.e.m. **p<0.01; ***p<0.001. Figure reproduced from Hodin et al. (2021).

Objective 4: Link experimental results with ecological theory

4. Identification of best management and restoration practices

Under the leadership of co-PI Baskett, our research group developed two models to inform kelp restoration management decisions: (1) a spatial integral difference equation model to quantify the interaction between urchin removal and kelp reintroduction in their effect on kelp recovery and spread rate, and (2) an ordinary difference equation model to quantify how sea star recovery or reintroduction might affect multiple aspects of kelp system resilience (recovery rate as measured from stability analysis, recovery likelihood as measured from the basin of attraction to a kelp-dominated state, and resistance as measured by quasi-potential) to future climate change. In both cases, we used global sensitivity analysis to quantify the relative effect of different ecological processes on kelp system outcomes and identify which processes are most important to resolve empirically for more precise future predictions.

4.A. Model examining urchin removal and kelp reintroduction

For the first modeling framework, we found that (a) as kelp outplanting increased, the amount of urchin removal necessary for kelp recovery decreased (Fig. 4.1, where the threshold values to achieve recovery depended most on urchin grazing rate on kelp and the kelp density that led to a shift in urchin grazing behavior from active grazing to passive grazing on drift kelp), and (b) after initial urchin removal or kelp outplanting to cross the threshold necessary for kelp recovery, continued kelp reseeding had the greatest effect on kelp spread rate (as compared to continued kelp outplanting or continued urchin removal; Fig. 4.2). Results of this first model are now published (Arroyo-Esquivel et al. 2023).

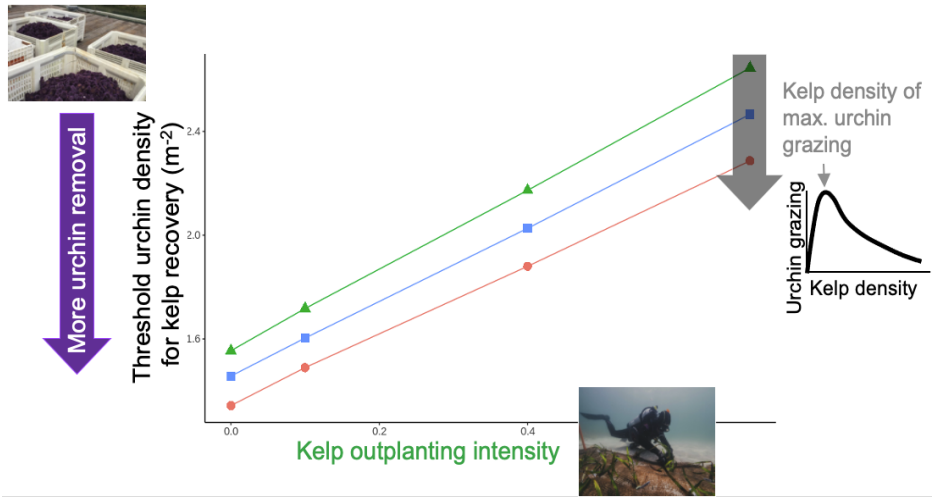

Figure. 4.1. Threshold urchin density necessary for kelp recovery as a function of kelp outplanting intensity. Different lines represent different values of the kelp density of maximum urchin grazing changed by +/-10% from its baseline value. Reprinted from https://doi.org/10.1002/eap.2850.

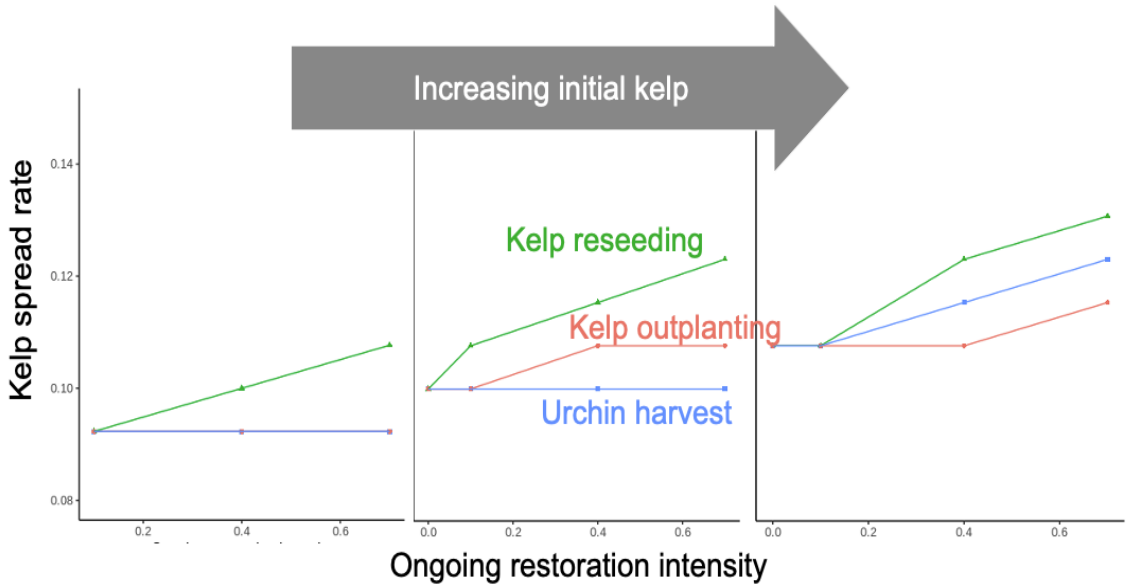

Figure. 4.2. Kelp spread rate under different restoration strategies with increasing intensity following urchin removal below threshold value necessary for kelp recovery with varying initial kelp density. Each line represents a different strategy: kelp outplanting in red circles, kelp seeding in green triangles, and sustained urchin harvest in blue squares. Each panel shows ongoing restoration efforts with the baseline, double, or triple (left to right) the initial kelp density in the oasis. Reprinted from https://doi.org/10.1002/eap.2850.

4.B. Kelp resilience model

For the second modeling framework, results indicate that the most influential ecological processes on resilience depends on the resilience metric: kelp production rates (direct and of drift kelp) have the greatest effect on kelp recovery rate, urchin production has the greatest effect on recovery likelihood, and seastar production has the greatest effect on resistance to disturbance (Fig. 4.3). These findings suggest different interventions can influence different aspects of the recovery process: kelp enhancement can speed up kelp recovery following disturbance, urchin removal following disturbance can decrease the likelihood of a shift from a kelp-dominated to an urchin-dominated state, and sea star recovery or reintroduction can decrease the impact of future disturbances. Therefore, consideration of multiple resilience metrics is necessary to understand the potential role of sea star reintroduction or recovery in future kelp resilience, and its potential role is most likely to be realized as mitigating the impact of future marine heat waves. We have recently finalized results of this second model and are developing a manuscript, again with continued discussions with OPC and CDFW (Arroyo-Esquivel et al., in prep.).

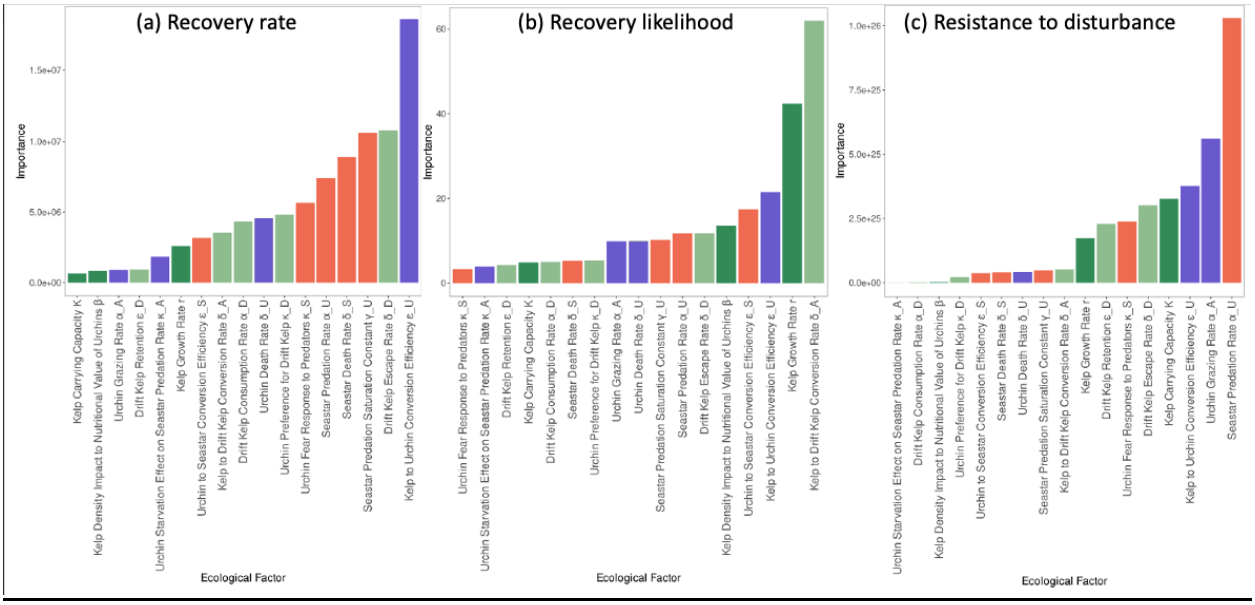

Fig. 4.3. Preliminary results for importance ranking of all parameters from the global sensitivity analysis in the kelp-urchin-sea star model of (a) recovery rate, (b) recovery likelihood, and (c) resistance to disturbance. Green bars represent parameters related to kelp dynamics, purple bars represent parameters related to urchin dynamics, and orange bars represent parameters related to predator dynamics, with lighter bars for indirect effects and darker bars for direct effects.

Science Synthesis

Our group organized and led a two-day workshop in January of 2023, near the end of the project period, to facilitate information exchange and foster creative evaluation of findings across the multiple groups funded by California Sea Grant and the California Ocean Protection Council (OPC). This workshop was held at Bodega Marine Laboratory, and participants from each of the project teams attended, together with personnel from California Sea Grant, California Department of Fish and Wildlife, and OPC (Fig. 5). Each team presented results, and vigorous discussion of commonalities, differences in outcomes, and points of complementary information occurred at length in multiple plenary sessions.

Figure 5. Attendees at the workshop to discuss research insights held at Bodega Marine Laboratory in January, 2023.

Training of next-generation researchers

Our project supported mentoring and internship opportunities for multiple undergraduates, early-career professionals, and graduate students. Five graduate students (2 PhD and 3 MS) conducted work that contributed to thesis or dissertation chapters, and 14 undergraduates received exposure and training as they undertook guided research. Four of the undergraduates participated by means of California Sea Grant's CA-SURE program, which linked to our scientific efforts and forefronted involvement of individuals from underrepresented groups. The project also provided a framework for a postdoc, Dr. Nick Burnett, to contribute to theoretical components of the research, enabling expansion of his professional toolkit. Two early-career professionals received training in animal rearing while also learning techniques in larval development, through their work with co-PI Hodin at the University of Washington.

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