

PROCEEDINGS REPORT 2022

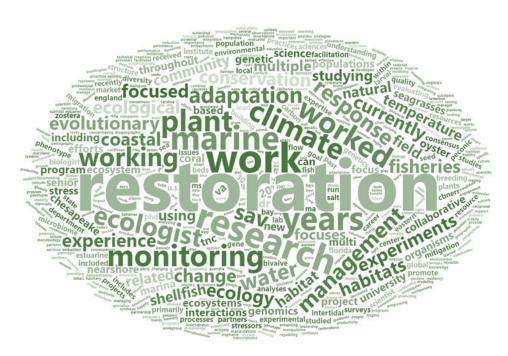


A PATH FORWARD: Building Eelgrass Resilience

ALONG THE MID-ATLANTIC AND NEW ENGLAND COAST

Acknowledgements

The Steering Committee would like to thank all the participants of the Building Eelgrass Resiliency Workshop for their expertise, enthusiastic participation, and thoughtful contributions that made this workshop a success. In particular, we would like to thank our panelists Drs. Collin Timm, Dina Proestou, Hollie Putnam, Katie E. Lotterhos, Kelly Racette, and Thomas G. Whitham for sharing their experiences and leading great discussions that made the short time together so valuable. This workshop would not have been possible without the expert facilitation team at the Consensus Building Institute, led by Bennett Brooks and Stephanie Horii, and we thank them as well.



This word cloud built around participant responses demonstrates the unity around the concept of restoration, despite the diverse backgrounds of participants.

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PART 1 Workshop Overview



Seagrasses provide some of the world's most unique and important habitats, and they are increasingly under threat. These forests of the sea provide a foundation for entire food chains in coastal waters. Across the Mid-Atlantic and New England coast of the United States, this critical habitat is provided by one particular species, eelgrass (*Zostera marina*), which has already experienced enormous losses in the past century due to nutrient pollution, dredging, and disease.

Adding to this challenge, climate change has been altering the distribution of marine species around the globe and is expected to have particularly heavy impacts in the Mid-Atlantic and New England coast. Sea surface temperatures from North Carolina to Maine have risen at a rate nearly twice the global average. This temperature stress has already altered eelgrass growth rates, reduced its extent, shifted its distributions, and changed its patterns of sexual reproduction. It will continue to exacerbate local extinctions of eelgrass as impacts from these stressors compound continued warming. The innate ability of plant populations to adjust and adapt to these temperature changes is likely to be outpaced by the rapid temperature increase. In the face of this threat, we posed the question:

What interventions can increase the pace and effectiveness of climate adaptation in eelgrass to maintain its presence and associated services along the Mid-Atlantic/New England coast?

To help answer this question, we convened an interdisciplinary team of experts working in terrestrial and marine restoration and the agricultural sciences, all of whom faced similar climate challenges in their work. Together they explored the questions "what can be done?" "what should be done?" and "how do we do it?" The workshop participants arrived at several conclusions on the most tenable and likely avenues of success. Many of the options they discussed rely upon developing a foundational understanding of which populations and genotypes are more resilient and/or resistant to thermal stress, and why. Fortunately, in light of the urgency of this effort, there is a well-established tool for exploring these questions, known as common garden experiments, which was highly recommended by those that have used it and was well received by the workshop attendees.

In common garden experiments, plants from different environments are relocated to a single location to observe individual responses to those conditions. Which can be a fairly low-investment intervention with potentially high yields in identifying and understanding population resilience. For eelgrass, we could collect seeds from southerly populations or warmer areas, plant them in northern or cooler areas alongside local seeds, and gauge which varieties best persist and confer climate resistance.



At the conclusion of the workshop, the participants created a list of the next steps for implementing the approaches discussed. These steps require coordination and communication.

The **coordination** of this effort, potentially across multiple jurisdictions and environmental gradients, is an essential first logical step to help the restoration community at large. To help with coordination, we propose the following steps.

- Create a steering committee to oversee this process, which would include developing common work plans and standardized methodologies, and forming a regional common garden network.
- Create an organizational entity and identify fiscal sponsors.
- Hire a full-time coordinator to manage the logistical and communication framework.
- Acknowledge and engage existing restoration and conservation efforts for eelgrass within the region to leverage knowledge and resources.

Alongside coordination of efforts, **communication** of results and lessons learned will be key to success.

- Create a platform for open and transparent discussions of outcomes from within and across programs.
- Compile existing genetic data, physical parameters and biological parameters across the region.
- Identify and share where resilient populations may be found that may be included in common garden experiments.
- Create a standardized approach and set of best practices for establishing common garden experiments, including seed collection, handling, and moving.
- Begin selective breeding demonstration experiments to confirm that this approach will work for *Zostera*.

The proceedings that follow attempt to capture an in-depth view of the lively discussion that occurred over three days, while also providing a higher-level view of the processes that led the workshop participants to engage so actively in trying to solve this climate challenge.

Background

Seagrasses provide some of the world's most unique and valuable habitats. They are a foundation species that provides structural habitat, primary production, oxygenation and sediment stabilization for shallow marine ecosystems. As such, they represent some of the most valuable, and at the same time, vulnerable ecosystems on earth. Seagrasses are particularly sensitive to local environmental degradation such as changes in water clarity, salinity and temperature, all of which are further affected by climate change. These disturbances collectively affect ecosystem stability and resilience. When the grasses are lost, so is the habitat on which important commercial fish for nursery or feeding grounds. Carbon that would be sequestered in their root structure and sediments is now free to return to the atmosphere, increasing global warming. Coastal food webs and the economy that is built around them begin to fail.

Seagrasses have been disappearing for centuries, primarily driven by poor management of waste streams that flow from our coastlines, cloud the water with sediments, and fuel algal blooms that shade out these plants. Estuaries along the Mid-Atlantic and New England coast are particularly threatened by thesehuman disturbances, as one-third of the coastal population of the entire United States lives in this region. Since 1995, rates of loss in some coastal embayments in the Mid-Atlantic/New England region have been as high as 3 to 5% each year.

A new threat arose within the last few decades, another mismanagement of our waste, this time through the air. Greenhouse gas emissions have fueled climate change and led to an overall warming trend punctuated by extreme events that have driven seagrasses to the brink of existence. The new warmer, murkier conditions

that humans have created are changing more rapidly than seagrasses can adapt. Once-great meadows that covered many of our bays are now contracting into patchy discontinuous beds that only occur as fringing areas near cooler, clear water.

In the Mid-Atlantic/New England region, temperature change poses a primary threat to meadows of eelgrass (*Zostera marina*). Increases of 1–5°C above normal

summertime temperatures can trigger large-scale die-offs of eelgrass. In the absence of intervention, the outlook is poor for eelgrass, a predominantly cool-water species, in a steadily warming western Atlantic. The consequences of the loss of eelgrass will be dramatic, as it is the main species of seagrass along much of this range, and the ecosystem functions that it provides will be completely lost in these coastal systems.

Some eelgrass meadows in the western Atlantic have declined by **more than 90% in the past century**. Without human intervention within the next 10–15 years, we could see a catastrophic loss of eelgrass in the southern half of its current distribution and irreversible degradation of our mid-western Atlantic coastal communities.

Restoration Efforts

Although recent years have seen creative efforts to restore eelgrass across many regions of the Mid-Atlantic and North Atlantic, many of these efforts have failed to meet their restoration goals and the gains have not kept pace with the losses. Even those initiatives that achieved their restoration goals struggle to maintain them and those gains may soon be lost. Restorations with larger and denser plantings tend to fare better, but as water temperatures continue to rise, even larger-scale restorations may not be enough to save eelgrass.

How to help plant populations adapt to a rapidly changing environment has been an increasing focus of research and restoration methods for other marine and terrestrial ecosystems for decades. Arguably humans have been adapting plants to new climates since before Mesopotamian civilization, and the lessons learned along the way will be valuable for conservation and restoration.

As the urgency to preserve these habitats from extinction grows, the resource management and restoration community needs to prioritize collaborative and complementary efforts that leverage collective learning across geographies. It is key to have a well-designed plan in place that is driven by the best available science and is implemented following a set of guiding principles to ensure equitable engagement across all stakeholders. A lesson shared from efforts to save coral reefs: the lack of a coordinated plan led to duplicative or wasted efforts, as many were desperate to try anything, including unintentionally repeating actions that had already failed. We fear a similar scenario playing out for eelgrass along the Mid-Atlantic/New England coast.

Workshop Charge

In June 2022, a diverse set of scientists, practitioners, managers, and stakeholders came together to tackle these eelgrass challenges and brainstorm possible options to assist eelgrass survival along the East Coast of the United States. While many seagrasses worldwide face challenges that are similar to those described above, the charge of this group was to focus on climate impacts on the most common seagrass along the mid-Atlantic/ New England coast, eelgrass (*Zostera marina*), which is distributed across much of the temperate northern hemisphere. A major objective was to learn about methods being used in other systems that could help eelgrass build resiliency in general and in particular develop resiliency to thermal stressors. Appendix A provides additional background on eelgrass and outlines the workshop's purpose and charge.
 Appendix B contains a glossary to support a common and consistent interpretation of terms used during the

workshop. Other appendices include the workshop agendas, list of attendees, presentation slide decks, survey results, flip chart notes, discussion notes, and suggested references.

Eelgrass Restoration Workshop

Workshop Purpose

The workshop's main purpose was to learn from others who have experience with enabling **species resilience** (the ability to recover from disturbance) and **species resistance** (the ability to withstand disturbance) to changing climate. We sought to understand the limits and potentials of methods to assist eelgrass resilience and resistance, and discussed what approaches may be most suitable for large-scale deployment across eelgrass's Mid-Atlantic/New England range. In many ways, this workshop was a discussion about species resiliency rather than eelgrass restoration.

Objectives

- · Foster a space to learn from the work of others.
- Generate wide discussion around the possible approaches available.
- Forge a path forward for the restoration and management community to consider as we contend with climate change impacts on eelgrass, with a particular focus on thermal stresses.



Desired Outcomes

- Potential tractable and implementable pathways to promote resiliency, to be presented at upcoming conferences and meetings in order to further the discussion on eelgrass restoration.
- Potential partners to draft a manuscript that captures the workshop discussions and is distributed widely to aid seagrass (and other coastal) restorations.
- Specific, implementable actions and near-term next steps, particularly to communicate, fundraise, test methods, and build awareness/support for eelgrass restoration.

Workshop Structure and Design

Schedule and Session Topics

The workshop sessions were held virtually in June 2022 and divided into three sessions to systematically understand, explore, and develop promising pathways for eelgrass resiliency.

- Session 1 Thursday, June 2: Workshop kick-off, understanding the challenge, panel discussion
- Session 2 Tuesday, June 7: Identify and explore potential pathways
- Session 3 Tuesday, June 28: Build out pathways, implementation approaches, moving forward

Appendix C provides the agendas for each session.

Planning and Design

A steering committee was formed to organize the workshop that included practitioners and subject matter experts across the mid-Atlantic and New England region: The Nature Conservancy, Stony Brook University, Northeastern University, Smithsonian Institution and MarineGEO Program, Ocean Sewage Alliance,

and the U.S. Environmental Protection Agency (EPA). The steering committee received additional planning support from the Consensus Building Institute, a neutral-party organization providing facilitation services. **Appendix D** lists steering committee representatives and support.

The steering committee began planning in winter 2022 to outline the purpose and scope of the workshop. The group identified a range of perspectives and associated potential experts to participate in the workshop. The steering committee specifically invited non-eelgrass experts to foster broader learning and creative ideas. At the start of the workshop, the steering committee encouraged participants to share all ideas, regardless of effort or cost, as options to explore. The workshop sessions included several opportunities for participants to learn and share ideas verbally and in writing via panel discussions, breakout groups, virtual flip charting/ brainstorming, informal polls, etc. Participants could also share ideas and provide feedback via online surveys. **Appendices E** through I include the presentation slide decks, discussion and poll/survey outputs, and other ideas/resources shared during the workshop series. **Figure 1** outlines the workshop design and approach.

All The Options	Learn From Elsewhere			
SURVEY	EXPERT PANEL DISCUSSION			SMALL GROUP DISCUSSION
What <u>Could W</u> e Do?	What Has Worked? What Are the Risks?	What <u>Should </u> We Do?	What Was Most Promising?	How Should It Be Done?
 Assisted Migration Gene Editing Selective Breeding 	Explicitly Non-Seagrass • Terrestrial & Marine	How would this work with eelgrass? Who would need to	Where should we focus?	Milestones Action Steps
 Assisted Gene Flow Environmental 	Agriculture Aquaculture	be engaged?		Considerations
Hardening • Microbiome Manipulations • Surveys of Genetics • Reduce Other	• Corals • Trees • Oysters	What risks are there?		
Threats				

Figure 1: Eelgrass Restoration Workshop Process Workflow that we followed as we narrowed a broad set of options to the most promising approaches and pathways that the restoration community should consider.

Participants

There were 38 participants in the workshop (refer to **Table 1** for a list of participants and **Appendix D** for participants' bios). The workshop included a panel of experts from a broad set of fields, including forestry, aquaculture, genomics, tropical coral ecology, agriculture, and climate change biology, who participated in a panel and group discussion about the tools and methods that

have been developed and used in other systems. Another set of experts included estuary program and resource managers and researchers with backgrounds in plant genetics and eelgrass. These experts had special insight into eelgrass management and restoration and provided feedback on what approaches were transferrable or applicable to the eelgrass system.

Table 1

WORKSHOP PARTICIPANTS

Name	Affiliation and Title
Aldo Croquer	The Nature Conservancy, Marine Program Conservation Manager for the Central Caribbean, Dominican Republic
Andrew Jacobs	Wampanoag Tribe of Gay Head (Aquinnah), Laboratory Manager
Betsy Stoner	Bentley University, Assistant Professor
Brandon Lind	Northeastern University, Research Fellow
Cayla Sullivan	U.S. EPA Long Island Sound Office, Life Scientist
Collin Timm*	Johns Hopkins University, Applied Physics Laboratory
Cynthia Hays	Keene State College, Professor, Biology Department
Dina Proestou*	USDA ARS, Research Geneticist
Eric Sotka	College of Charleston, Professor of Biology
Erin Shields	CBNERR-VA/VIMS, Lead Marine Scientist
Hollie Putnam*	University of Rhode Island, Associate Professor of Biological Sciences
Holly Plaisted	National Park Service Biologist
J. Brooke Landry	Maryland Department of Natural Resources, Natural Resource Biologist; Chair, Chesapeake Bay Program SAV Workgroup
Jennifer Ruesink	University of Washington, Professor of Biology
Jessie Jarvis	University of North Carolina - Wilmington, Associate Professor
Jillian Dunic	Simon Fraser University, PhD Candidate
Jonathan Grabowski	Northeastern University, Professor
Jonathan Puritz	University of Rhode Island, Assistant Professor
Joyce Novak	Peconic Estuary Partnership, Executive Director
Katie DuBois	Bowdoin College, Postdoctoral Scholar
Katie E. Lotterhos*	Northeastern University
Kelly Racette*	The Nature Conservancy, Sustainability Scientist, Ag & Food Systems
Natalie Cosentino-Manning	NOAA, Marine Habitat Restoration Specialist
Stefanie Simpson	The Nature Conservancy, Coastal Climate Program Manager
Tay Evans	Massachusetts Division of Marine Fisheries, Fisheries Habitat Program, Scientist and Environmental Analyst
Thomas G. Whitham*	Northern Arizona University, Regents Professor Emeritus
W. Judson Kenworthy	Albemarle National Estuary Partnership, North Carolina

* Session 1 Panelists

WORKSHOP STEERING COMMITTEE AND SUPPORT

Name	Affiliation and Title
Adam Starke	The Nature Conservancy, Coastal Scientist
Bennett Brooks	Consensus Building Institute, Senior Mediator/Facilitator
Boze Hancock	The Nature Conservancy, Senior Marine Habitat Restoration Scientist
Bradley Peterson	Stony Brook University, Community Ecologist
Chris Nadeau	Northeastern University, Climate Change Biologist
Christopher Clapp	Ocean Sewage Alliance, Executive Director
Jonathan Lefcheck	Smithsonian Institution, Coordinating Scientist, MarineGEO
Phil Colarusso	U.S. EPA, Marine Biologist
Randall Hughes	Northeastern University, Evolutionary Ecologist
Stephanie Horii	Consensus Building Institute, Senior Associate Facilitator
Stephen Heck	Stony Brook University, PhD Candidate in Marine Sciences

Summary Outline

The following parts of the report summarize the steps we followed to develop the most innovative and promising pathways for eelgrass restoration. They include key takeaways from the discussions and participants' input on potential pathways.

- **Part 2** focuses on the initial work and discussions to identify and explore promising pathways during Sessions 1 and 2.
- **Part 3** goes into greater detail on Session 3 discussions regarding building out the most promising pathways: assisted gene flow, selective breeding, and environmental hardening.
- Part 4 outlines key next steps and a call to action.

For more details, refer to the appendices:

- A. Workshop Brief
- B. Glossary of Terms
- C. Session Agendas
- D. List of Attendees / Bios
- E. Presentation Slide Decks
- F. Survey Results
- G. Session 2 Discussion Flip Chart Notes
- H. Building Out Potential Pathways: Discussion Notes, Conceptual Models, and Implementation Actions/Tasks
- I. Suggested References



PART 2 Ideas for Promising Pathways

Pre-Workshop

Participants were asked to review a workshop brief (**Appendix A**), glossary of terms (**Appendix B**), and additional key background readings (**Appendix I**) to familiarize themselves with the issues prior to the workshop. To prime their creative thinking, participants were also asked to share one innovative method that they thought could increase eelgrass resiliency to climate change. Their responses appear in **Figure 2**.

The steering committee used the responses from the pre-workshop survey to generate an initial list of potential approaches/pathways. These were presented and refined during the Session 1 discussion.

Environmental Hardening

Selective Breeding

Identify Resilient Populations/Genotypes Eelgrass Resilience

Nurseries Donor Beds

Modify Existing Conditions

Reduce Non-climate Threats

Map Genetic Diversity ats Microbiome ^{Se}

Microbiome Seed Banks Manipulation

Figure 2: Word cloud of pre-workshop survey ideas, in response to the question "What is the most innovative method you have considered that could increase eelgrass resiliency to climate change?"

The steering committee used the responses from the pre-workshop survey to generate an initial list of potential approaches/pathways. These were presented and refined during the Session 1 discussion.

Session 1: Understanding the Challenge

Session 1 kicked off the workshop with presentations that explained the current status and challenge that eelgrass faces along the East Coast. The group then engaged in a panel discussion led by moderators to uncover the approaches that have been used in other systems and the experiences with those approaches. Refer to Appendix E for the Session 1 presentation slide deck.

Learning From Other Systems - Panel Discussion

A panel of non-eelgrass experts (**Table 2**), shared their experiences and lessons learned working in other systems and how different tools or methods might apply to eelgrass restoration.



Pre-workshop activities and Sessions 1 and 2 laid the groundwork for developing innovative and promising pathways for eelgrass restoration. These initial activities focused on learning, brainstorming, and identifying the most promising pathways (which are described in greater detail in **Part 3**).

PART 2 | IDEAS FOR PROMISING PATHWAYS

Table 2

SESSION 1 PANELISTS

Name and Affiliation	Expertise
Dina Proestou, USDA Agricultural Research Service	Oyster Selective Breeding
Kelly Racette, The Nature Conservancy, Sustainability Scientist	Agriculture & Food Systems
Thomas G. Whitham, Northern Arizona University	Assisted Migration in Trees
Katie Lotterhos, Northeastern University	Genetic Offset Methods for Population Restoration
Hollie Putnam, University of Rhode Island	Reef-building Coral Restoration
Collin Timm, Johns Hopkins University	Plant-Microbe Interactions and Plant Crop Production

Discussion Takeaways

The following key insights from panelists were important for discussions in subsequent sessions:

Goal Setting and Planning

- The sense of urgency is extremely high for addressing the drastic loss in eelgrass beds. Given that urgency, a reasonable near-term and primary goal might be to halt the decline, while the long-term and aspirational goal is to improve eelgrass system health.
- It is extremely important to define clear restoration and resiliency objectives and goals and develop robust monitoring designs that can help identify the success of an intervention, exit ramps, or indicators that alternative methods and approaches may be needed.
- Resilience and resistance are unique ecological processes; the goal is to achieve either or, ideally, both.
- There are multiple stressors affecting the ecosystem. Acknowledge and work with them in mind. Understanding the variability in different systems/locations (e.g., environmental, genetic) informs our targets (e.g., X amount of species diversity) and overarching goals (e.g., healthy systems similar to the past or goods/services beneficial to humans).
- A regional framework is needed for working across geographies and jurisdictions.
- As we think through implementation, we must have strategic outreach and engagement plans to reach a broader audience and widen support. The sense of urgency is not reaching many in the general public who can be strong potential partners.
- Common garden experiments, an agriculture and forestry research tool in which plants from various

environmental gradients/ecotones are relocated to a common area and observed, was quickly identified as an effective tool for identifying resilient populations.

Potential Pathways

(Also see the "Potential Pathways" subsection below.) Many of the suggested pathways were overlapping and similar. Multiple strategies will be needed.

- · Assisted gene flow/managed adaptation
 - This strategy is being used in riparian zones in the southwestern United States, where stocks are sourced from areas 2–3°C offset (generally from southern latitudes in riparian systems with cottonwoods).
 - Moving plants within their predicted range tended to have limited (but not non-existent) regulatory roadblocks.
 - For common garden experiments, we need broad and long-term stakeholder support (e.g., a 50-year lease on lands for terrestrial common gardens).
 Priority areas for common gardens could be areas anticipated to need restoration adjacent to large landowners who are potential partners.

Hybridization/selective breeding/artificial selection

- It is important to know the genetic background of the target species (one example raised was that of an oyster that had lower hybrid vigor when two distinctly different but polymorphic species were hybridized). Potential hybrids could backcross (or show introgression) with remaining parental species.
- ~ Selection for breeding will reduce overall genetic variation, which could increase vulnerability to other stressors.

Microbiome manipulation

- There has been little exploration of microbiome manipulation (introducing or selecting for specific species) in the marine environment, although it is a fairly common strategy used in terrestrial systems.
- Field trials are an important part of the solution to supplement lab trials. The lab/nursery microbial community likely differs from the field. Field trials are also needed to better estimate scalability.

Environmental hardening

The scalability of environmental hardening (exposing seeds to higher temperatures before planting them in the wild) depends on the hardening mechanism, and the duration of the hardening can vary (e.g., changing environmental cues could switch on/off desired genetic characteristics). It has worked well in terrestrial systems (annual row crops) when seeding is done on a frequent basis. It is not a "one-size-fits all" or "one and done" strategy, but it can have short-term beneficial results (e.g., could support species resilience during times of high mortality).

Genetic editing

- ~ Genetic editing may be promising in the early stages, but it is not necessarily transferrable across all species.
- ~ Genetic editing has become important for building resiliency in species that have become highly homogeneous (e.g., many agricultural crops) and can offer many lessons about strategies and important outreach and messaging. Eelgrass is fairly genetically diverse, even among local populations.

Potential Pathways

The ideas generated in the pre-workshop survey and the session 1 discussions formed the following potential pathways:

- Identify resilient populations/genotypes. Use an array of possible methods, ranging from laboratory and observational studies to genetic offsets, to identify resilient populations/genotypes.
- Increase genetic diversity/assisted gene flow. Source plants from multiple populations to increase genetic diversity in a vulnerable population or

restoration, which might also include moving individuals from populations that are identified as resilient. It may not be necessary to understand the exact mechanism of resistance/resilience, as long as the outcome is effective.

- **Perform selective breeding/hybridization/artificial** selection. Breed resilient genotypes (i.e., selective breeding) or species (i.e., hybridization) in the lab/nursery to produce individuals with desired traits.
- **Perform microbiome manipulation.** Modify microbial associates (e.g., soil microbes) that can alter resilience.
- Conduct environmental hardening of seeds/seedlings. Expose seeds/seedlings to higher temperatures in the lab/nursery before planting them in the wild.
- Modify existing conditions/reduce non-climate threats. Alter the environment (e.g., soil, wave breaks) to increase eelgrass production or reduce existing stressors (e.g., improve water quality).
- **Perform genetic editing.** Modify the genome directly to introduce genes that confer resilience (e.g., using CRISPR).



Session 2 – Identifying Potential Pathways

Session 2 built on Session 1 to consider lessons learned from other systems that directly pertain to eelgrass restoration (refer to **Appendix E** for the Session 2 presentation slide deck). Toward the end of the session, participants were asked where to focus the Session 3 discussion. **Figure 3** displays the informal polling results:

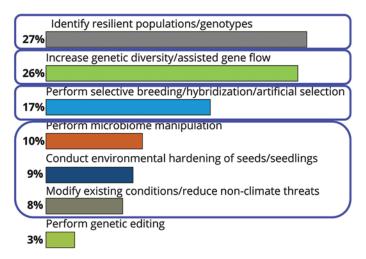


Figure 3: Results of the poll asking "Where should we allocate our attention for future workshop discussion?"

Based on these results, we created four breakout groups, each focused on a particular pathway: **resilient populations, assisted gene flow, selective breeding**, and a broad "**catch-all**" group.

Participants circulated through all four topics/breakout groups to identify and explore possible approaches related to implementing the overarching pathways. Steering committee members and support personnel moderated/facilitated discussions and captured high-level ideas on a virtual flip chart (refer to **Appendix G** for detailed notes from each breakout group). Participants could add comments to expand on ideas and share helpful references/studies.

In plenary and utilizing Mentimeter polling, participants indicated how promising these possible approaches seemed and the degree to which they warranted further discussion in Session 3. Refer to **Appendix F** for Session 2 Mentimeter poll results, including the range of participants' responses. **Table 3** summarizes the suggested approaches and Mentimeter poll results.

Table 3 Session 2 list of suggested approaches for respective promising pathways and results of the Mentimeter poll: "Rate how promising/interesting for future workshop discussion."

Pathway	Approach	Promising? More Discussion?
	A. Validate genetic offsets	3.5
Resilient Populations/	B1. Multi-stressor experiments [Lab]	3.1
Genotypes	B2. Multi-stressor experiments [Field]	4
	C1–C2. Baseline field observational studies	4.2
	A. Target a single resilient population	2.5
Genetic Diversity / Assisted Gene Flow	B. Combine multiple resilient populations or resilient and other populations	4.5
	C. Increase diversity without targeting resilient populations	2.8
	A. Selective breeding	3
Selective Breeding /	B. Intraspecific hybridization	2.6
Hybridization /	C. Artificial selection	2.7
Artificial Selection	D. Calling out the overlap of the idea Selective Breeding and Artificial Selection by some	2.8
Catch-All	A. Microbiome manipulation	2.5
	B. Environmental hardening of seeds/seedlings	2.8
	C. Modify existing conditions (e.g., hydrodynamics)	3.4
	D. Assisted migration of subtropical species	2.4

PART 2 | IDEAS FOR PROMISING PATHWAYS

Note that the Promising "scores" reflect the group's general interest in further discussion and not necessarily the level of feasibility, effectiveness, etc.

Table 3. Session 2 list of suggested approaches forrespective promising pathways and results of theMentimeter poll: "Rate how promising/interesting forfuture workshop discussion."

Discussion Takeaways

During the plenary discussion, the group raised the following issues:

- Collaboration is critical for success. Build on other existing, large research networks that have already laid the groundwork to more easily disseminate information, identify more promising opportunities, and foster novel ideas into greater maturity.
- Develop a common shared goal, with collaboration, coordination, and standardized approaches. This will be essential for sharing information and advancing region-wide efforts.
- Aspirational goals (e.g., functional system diversity) may lack traction in the near term but are still worth exploring and outlining potential pathways for others to pick up in the future.
- We need a multi-pronged approach with some activities in parallel, some in series dependent upon each other.
- Coordinate to create a strategic experimental design framework to avoid multiple, haphazard approaches.

- Coordinate a monitoring framework that identifies multiple meadow-health and project-related metrics to track success and challenges and evaluate thermal tolerance.
- Consider how ecosystem services will change depending on the approach, particularly if we focus too much on short-term, lower-effort strategies.
 Perennial eelgrass systems offer year-long habitats for fish and other aquatic organisms, which may be lost if they are replaced with annual populations.
- Intraspecific hybridization is more likely to be successful (and ethical) than hybridizing different seagrass species.
- Transplanting (adults or seed), followed by monitoring of performance, may be a lower-effort immediate option that does not require prior investment in research.
- Natural selective events (heat waves, changes to tidal flushing) may help identify resilient populations/ genotypes by eliminating all except the resilient plants.

Thoughts for Session 3 and Beyond

- Restoration can be extremely expensive, as well as labor and resource intensive. The cost and level of effort need to be considered when prioritizing which pathways to advance over the next several years.
- Pathways that received less discussion during the workshop (e.g., gene editing, microbiome manipulation) were still mentioned multiple times; therefore, others may wish to further explore them at a future time and in parallel with implementing the approaches here.



PART 3 Building Out Innovative Pathways



During Sessions 1 and 2, participants selected the three most promising pathways for building eelgrass resiliency and resistance to thermal stress. These were **assisted gene flow, selective breeding, and environmental hardening**. These three pathways were considered the "big and bold" ideas that would benefit most from in-depth workshop discussion to map out meaningful pathways toward implementation. (Refer to **Appendix E** for the Session 3 presentation slide deck.)

Session 3 breakout group discussions focused on 1) mapping "theory of change conceptual models" that described major milestones for the three pathways over the next 10 years, and 2) identifying key actions or tasks to get to and through the milestones (e.g., research and monitoring, coordination/collaboration, communication and outreach, funding, regulatory review, risks).

Participants were considerably more interested in the assisted gene flow pathway than in selective breeding or environmental hardening. Therefore, participants were split into four breakout groups:

- Group A Assisted Gene Flow
- Group B Assisted Gene Flow
- Group C Selective Breeding
- · Group D Environmental Hardening

These breakout groups focused on the development of the flow and sequence of conceptual models for each of the pathways. Participants remained in their breakout groups during the entire session to complete substantial discussion on their respective topic. Participants were assigned to breakout groups based on a survey administered before Session 3 that asked for their top two preferred topics.

The facilitators of each breakout group responded to a series of questions to capture important takeaways. While the discussions within the groups varied, the conceptual models were developed with the understanding that multiple different actions could (and might have to) progress in parallel within each pathway. There was a general consensus that many overlapping aspects of each of the three pathways would need to be accomplished to pursue any of them. There was also a general sentiment that progress could be, and should be, made on all three pathways as soon as possible.

The following is a compilation of the takeaways from all four breakout groups.



Sessions 1 and 2 laid the groundwork for detailed discussions during Session 3 on the most innovative and promising pathways. Here, we comprehensively describe the ideas that the group shared regarding those pathways.

Overlapping Pathway Components

All four groups described components that they viewed as essential to developing their pathway. The steps for all groups generally reflect simultaneous incremental lab experiments and field experiments (common garden experiments).

Compilation of existing data and standardized data collection during future monitoring

All four groups described the need to compile existing data on the distribution of eelgrass, along with environmental data throughout its range along the eastern seaboard of North America. Existing data capture the current status of eelgrass populations and also help to identify populations that may be particularly resilient and resistant to thermal stress.

The assisted gene flow and selective breeding groups also viewed any existing information on genetics and physical traits as essential. Such data can help us select from populations for assisted gene flow and maintain genetic diversity. One of the assisted gene flow groups also discussed the value of consolidating existing data in order to inform the design and distribution of a common garden network. Furthermore, existing genetics data can help us understand the risks involved in moving eelgrass genotypes across locations of varying distance.

All four groups decided that developing plans to collect standardized data in the future would be essential to assessing the status of eelgrass populations (to answer questions such as: are populations continuing to decline, is the rate of decline slowing, and is the decline reversing) and justifying the need for action. Furthermore, these data would allow us to pivot from calculated and cautious efforts to more aggressive efforts if the status of eelgrass worsens. This monitoring was deemed essential to understanding whether restoration efforts were effective at local and regional scales and whether shifts to more aggressive methods were necessary.

Coordination

All four breakout groups emphasized the need for an entity to coordinate efforts within and among the three pathways (assisted gene flow, selective breeding, and environmental hardening). Without significant coordination throughout these pathways, including standardized monitoring approaches, an online data repository, and a website for coordination, the power of common gardens (and other aspects of each pathway) would be reduced significantly. All groups identified the need for a full-time coordinator as a very high priority.

Regulatory review and stakeholder engagement

The assisted gene flow and selective breeding groups stated that discussion with local regulators to understand and establish the legal framework for conducting this work needs to be initiated immediately, since the process will likely take a substantial amount of time. This discussion is particularly necessary for these two pathways, since their success may necessitate transferring eelgrass seeds or plants between different estuaries and across state lines.

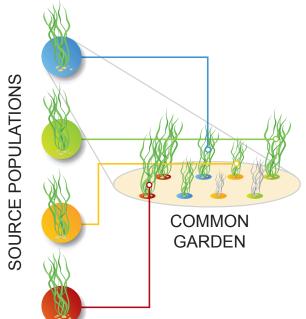
The current policy surrounding the interstate movement of eelgrass seeds and plants is unknown to the group, and it is unclear whether such movement is unregulated or against existing state policy in most locations. Participants in multiple groups shared that they have had pushback from regulators when they have mentioned moving eelgrass from one region to another. For example, a participant in one of the assisted gene flow groups stated that they are unable to move genotypes within Long Island Sound from New York to Connecticut. The regulations likely differ significantly among states and regulations may also be highly localized (e.g., city-level conservation commissions) or interpreted differently across agencies or jurisdictions. A regulatory review is needed to better understand the regulatory roadblocks that might limit assisted gene

flow, selective breeding, and environmental hardening, although the concerns with environmental hardening are substantially lower than those for the other two approaches. It is necessary to learn how regulations might be revised to overcome these roadblocks. This effort should be started immediately, since regulatory changes will take time.

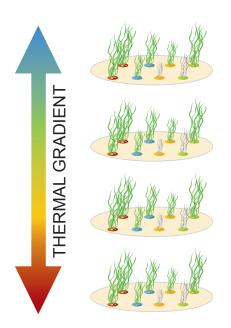
Although the environmental hardening group did not discuss regulatory hurdles, this approach would require the large-scale harvesting of seeds, seedlings, and shoots, which would need to comply with existing regulations under the Clean Water Act and various state wetland laws. These regulations are not new and are well known, so it is not necessarily a new hurdle, but depending on the scale and the state, it could be problematic. All four groups identified early stakeholder engagement and education as essential to the process. Involving all partners in the collective design of the work was also seen as vital to the success of any of these pathways. Further, fundraising efforts may be able to access funding allocated for improving coastal resilience in general, perhaps in conjunction with the restoration of oyster or salt marsh habitats.

Development of common gardens

All four groups focused a substantial amount of time on the design and implementation of common gardens to learn and test resilient transplants (seed and shoots). All groups indicated that common gardens would be an valuable tool in the effective deployment of their pathway.



WHAT IS A COMMON GARDEN?



- A simple yet powerful experimental design used to gain insight to a plant's ability to adapt to the environment.
- Plants from several different source populations are grown under shared conditions.
- Comparing the performance of the plants within the common garden can provide insight into the plants' phenotypic plasticity, its heritable traits and can be used to test populations being considered for assisted gene flow, selective breeding or hardened seeds.
- Replicating these common gardens, in a coordinated fashion, across a broad geography or thermal gradient, will improve our understanding of how adaptive populations are as well as potentially identify those populations that are more resilient.
- An investigation seeking thermal resilient populations should collect source plants or seed from across a thermal gradient or a latitudinal range.

Both assisted gene flow groups emphasized that a common garden network should be developed to identify genotypes that are resilient to high temperatures and to evaluate the effects of multiple stressors. All of the aforementioned background research should be compiled and viewed as essential to the proper design of the common garden approach. There was a general consensus among the four groups that in the near future we need to carefully design and start implementing common gardens within the region along a latitudinal (or temperature) gradient. Dr. Whitham, who drew on his extensive experience with common gardens in forests, suggested that this should occur over the next 3-5 years. Given that latitudinal temperature gradients are very different in aquatic systems than they are in terrestrial systems, the number of common gardens needed for such a network needs to be discussed in further detail with Dr. Whitham and others. All groups discussed the need for a clear articulation of guestions that should be answered using the common garden approach to enhance their usefulness. Since this process will take a significant amount of time, it should be started as soon as possible. Furthermore, all groups felt that the development of partnerships between scientists, restoration practitioners, and regulators was important for a common garden network to be effective. This is also true of all three pathways in general.

Assisted Gene Flow—Emphasized using a series of replicated common gardens to test the resilience of eelgrass from different source populations. Common gardens should have well-characterized and monitored environments. Further discussion is needed to ensure that results from common garden experiments can help deploy the assisted gene flow approach.

Selective Breeding—Generally viewed common gardens as an important tool to identifying founder populations for selective breeding that have a high resistance and resilience to thermal stressors. The group also discussed the utility of common gardens to test selectively bred genotypes to evaluate their performance in the field under a range of environmental conditions.

Environmental Hardening—Did not discuss common gardens as much as the other three groups; they were viewed as a route to test hardened eelgrass seeds and plants in the field. There was universal agreement within this group that the co-development of a common

garden experiment on a regional scale is necessary to advance quickly in our ability to identify which genotypes to migrate northward.

The benefits of the common garden approach are numerous and well tested in other systems and show a lot of promise for use in developing and testing eelgrass resiliency, particularly when deployed across a wide geography and across environmental gradients.

Pathway-Specific Thoughts

While both of the assisted gene flow groups spent a lot of time discussing the logistics of common gardens, they did not spend time discussing the actual steps of experimental assisted gene flow. Furthermore, neither of the groups talked much about scaling up the process of assisted gene flow in terms of transporting seeds from one area to another, or associated risks, such as pathogen introduction. The selective breeding and environmental hardening groups only briefly touched on the logistics of scaling up selective breeding or environmental hardening if either pathway proves to be effective at developing resistance to thermal stress and other stressors.

The selective breeding group felt that this approach may ultimately prove to be an important strategy in parallel to assisted migration and environmental hardening to halt the decline and assist in restoring eelgrass coverage to its historical distribution. Many steps within the selective breeding pathway, including the identification of populations of eelgrass that exhibit resilience and resistance to thermal stress, are likely necessary for the other pathways to succeed, especially assisted migration, and can be followed in parallel. However, other steps must be taken before a formal selective breeding program can be established, and given that it will likely take a decade to establish a successful breeding program and place selectively bred individuals in the field, these steps should begin right away. For instance, we need to conduct experiments to see if it is possible and feasible to grow eelgrass in tank systems and induce it to flower, cross-pollinate, and produce seed. These methods need to be established before any selective breeding in tanks can be done. If such experimentation is not feasible from a logistical or cost standpoint, our efforts may need to pivot to focus solely on assisted migration or other approaches.

To our knowledge, there are no published reports of successfully breeding eelgrass at a large scale in a mesocosm environment. If cultivation and breeding of eelgrass in tanks prove to be possible, selection experiments in tanks should run in parallel to field experiments in common gardens, since the viability of plants in tank systems may be quite different from their viability in the field. Furthermore, if selection experiments in tanks are effective at producing plants with higher thermal tolerances and adapted for future environmental conditions, this information could help convince regulators to allow transportation of eelgrass genetic material (plants or seeds) across state lines in service of assisted migration and selective breeding.

The environmental hardening group noted that it was first necessary to identify the trigger(s) that we expect to

induce climate resistance in seeds, seedlings, and/or adult plants, such as warm (and/or cold) exposure. They then proposed to design lab (controlled trials) and field (transplants from lab to field) experiments to test the potential of these triggers to induce resistance and promote resilience. The results of these experiments can help identify resilient individuals from the field for use in lab trials. Further experiments may test the mechanism behind resilience (if present), such as identification of specific genetic paths, epigenetic activation, and/or interactions with the microbiome. Longer-term field transplants would have to be monitored for several years at a minimum to determine whether and how long climate resistance from hardening would last. The final challenge is scaling up hardening in restoration efforts, perhaps in conjunction with the selective breeding program outlined above.

Breakout Group Discussions

In the following section, we summarize each breakout group's discussion focus, critical questions tackled, unresolved questions and key next steps.

Assisted Gene Flow (Group A)

This group's conversation focused primarily on the short-term steps needed to pursue this pathway: gathering knowledge, conducting standardized surveys, and establishing common gardens, and the specifics that would be needed at each of those steps. They did not spend much time on the multiple stressors that eelgrass faces or the actual steps of experimental assisted gene flow. The group discussed the importance of coordination, communication, and consistent and standardized data collection and reporting, which are essential to learning from assisted gene flow efforts. They recognized that there are trade-offs regarding the need to collect standardized information that is not currently available versus the need to act now to address eelgrass losses, and how to balance those needs. The value of identifying 'tipping points' that would indicate a shift toward greater action is needed (e.g., if rates of loss increased) was discussed, though none were identified. This group was very interested in figuring out what characteristics of natural populations are indicators of resilience and resistance to stress (e.g., are the

lushest, healthiest beds the most resilient, or the ones that are persisting in stressful areas?).

Assisted Gene Flow (Group B)

This group focused most on the design and implementation of common gardens. It was clear from their discussion that the value of a distributed common garden network comes from significant coordination among sites and participants. It was also apparent that the work needed for coordination was no small task. The primary outcome of their discussion was that a full-time common garden coordinator is needed to pursue assisted gene flow as a strategy. In the very near term, the group thought that it would be highly useful to consolidate existing information on eelgrass along the Atlantic coast of North America to better understand the species' status, distribution, and genetics. This information was seen as necessary for designing common gardens and could also justify the need for a distributed common garden network by documenting the current and future decline of eelgrass.

The group questioned what regulations might restrict the movement of seeds or adult shoots within and among states. They decided that a regulatory review would be necessary to understand what regulatory roadblocks could hinder the implementation of a distributed common

garden network and ultimately assisted gene flow. They also questioned who would coordinate a common garden network. They discussed several groups that have an interest in doing this, including EPA LISS (Cayla Sullivan), Alliance for SAV Enhancement (PEW), World Seagrass Association, Chesapeake Bay Program's SAV Workgroup (Brooke Landry), and National Estuaries Programs. The group discussed how these organizations, along with the workshop steering committee, might form another steering committee to determine how to fund and locate a coordinator. They agreed that it would be very important to clearly articulate the questions that we hope to answer with a distributed common garden before planning and implementation occur. Once the question is clearly articulated, it will be possible to choose the location of a large number of common gardens and the source populations to be planted in each garden. They emphasized that this alone is a substantial task.

Selective Breeding (Group C)

This group concentrated on the need to identify the goals of this approach, whether genetic material could be sourced from local eelgrass meadows for selective breeding directed at restoring eelgrass within the same area, and what research would need to be conducted to develop an effective selective breeding program. The question of whether genetic material could be sourced from local areas for restoration in the same general area was important; if the local genetic pool has a large enough thermal tolerance range from which to select, it may be preferable to confine selective breeding to localized areas. Harvesting from local populations for selective breeding and restoration in the same area is believed to be beneficial, as these plants are likely to have adaptations to other local environmental conditions that may increase survival. Further, the group noted that if eelgrass can be sourced from relatively local areas, there will be no need to address the regulatory hurdles regarding interstate movement of plant genetic material.

The group extensively discussed the process of selective breeding and the differences in approaches between blunt (mass selection) breeding and precision (quantitative) breeding. Overall, they decided that a blunt approach to selective breeding is likely the first place to start, since it is less expensive and less labor intensive than a precision approach. The group felt that it would be ideal to selectively breed for future forecasted conditions (e.g., 50 years from now), but that a good starting point was to breed for conditions forecast for the near future (within the coming decade).

The group generally felt that while selective breeding takes longer to get started than assisted migration and is likely more expensive, it is worth pursuing since assisted migration alone may not produce the desired adaptation to a changing climate. Since many of the key first steps in selective breeding are the same as those for assisted migration and environmental hardening (e.g., identifying resilient populations, developing a common garden program) the group felt all three pathways could be started in parallel. However, a major unanswered question was whether eelgrass can be successfully grown and cross-pollinated in tanks. Experiments to answer this guestion need to be started immediately to see whether this route is worth pursuing. The group also talked about the importance of establishing the legal framework to allow selective breeding to happen, especially if the interstate transportation of eelgrass seeds is necessary. This was viewed as work that should be started immediately since the process is prerequisite to a number of subsequent tasks.

Environmental Hardening (Group D)

This group focused primarily on the need for lab and field experiments to demonstrate that environmental hardening can in fact lead to short- or long-term resistance and resilience to high temperatures. They also discussed putative mechanisms, such as epigenetics; whether additional experiments would be necessary to elucidate those; and at what point it would be necessary to do so (i.e., can we demonstrate the value of hardening before we understand it?).

The group discussed many questions that would ultimately be critical components of using environmental hardening as a pathway to develop eelgrass resilience and resistance to thermal stress (and other stressors). These included whether hardening could be tied into other efforts (e.g., assisted gene flow or selective breeding) as a complementary tactic, and whether this is a secondary line of inquiry to these other pathways or parallel. The

group was interested in whether eelgrass individuals could be exposed to multiple stressors (e.g., thermal and light stress) to produce resistance to multiple stressors simultaneously. The group noted the importance of understanding whether the effects of environmental hardening would be intragenerational or intergenerational. They also talked about the importance of establishing research goals (e.g., improving flowering rates, seed production, or seed germination). An additional goal would be to use environmental hardening to improve these metrics within a single generation or sustain climate resistance across multiple generations. The largest lingering research question centered around whether it was even possible to use environmental hardening to facilitate resilience and resistance of eelgrass to a changing climate across many generations.

Like the other groups, the environmental hardening group identified the need for funding to achieve these goals. They discussed developing a proposal that would include all three pathways to pitch to funders, since all three are intertwined. They also brought up the question of how environmental hardening can be tied into and/or inform assisted migration and selective breeding. They also discussed whether there are synergies in field monitoring (for example) to identify resilient genotypes that can further benefit from hardening experiments.

Breakout Group Summaries

The following are the "elevator pitch" summaries from the facilitators of each group.

Assisted Gene Flow (Group A)

Seagrass conservation on the East Coast of the United States is at a pivotal point. Although there have been some improvements in water quality and localized restoration successes in recent years, the practitioner and academic communities agree that co-development at a macro scale is needed to address the threat of rapidly increasing water temperatures. There is a clear need for a single collaborative network with dedicated resources and personnel to coordinate efforts, facilitate information sharing in near–real time, and continually adjust best practices based on current data. Substantial fundraising dedicated to this effort may be the last and best possible option for ensuring that this critical habitat can persist into the next generation.

Assisted Gene Flow (Group B)

A distributed network of common gardens is an efficient and proven method to rapidly identify the resilient genotypes needed to save eelgrass from climate change. However, effectively designing and implementing a distributed common-garden network requires many partners in multiple states, standardization, a website, a centralized database, regulatory oversight, and long-term management. Failure to coordinate these many logistics will result in an inefficient use of resources and time, which could make learning slow and threaten the project's goals. Hiring a full-time coordinator will ensure that we learn quickly, which is essential to saving eelgrass in many locations. For the cost of a single full-time employee, we might be able to efficiently save a species that provides benefits to fish, wildlife, and people along much of the Atlantic coast.

Selective Breeding (Group C)

While assisted migration is a promising pathway to facilitate the halting and reversal of eelgrass decline in the face of rising sea surface temperatures, selective breeding may be a necessary component to increase the rate of adaptation of this important foundation species to heightened thermal stress. It is likely to take nearly a decade to establish a viable selective breeding program. However, many of the initial steps are relatively low cost and could be conducted within 3 or 4 years to evaluate whether this is a practical approach.

Environmental Hardening (Group D)

Environmental hardening is a super straightforward approach: expose some adult shoots, seedlings and/or seeds to stress; do not expose some others; and examine whether the survivorship of the two groups differs. This can be done at a single site with minimal logistics and cost, but scaling across the region would prove more challenging.

Post-Workshop Discussion on the Conceptual Models

At the World Seagrass Conference & International Seagrass Biology Workshop (ISBW) in Annapolis, Maryland (August 7–12, 2022), a broader community of eelgrass experts provided feedback and further explored the promising pathways from the June Eelgrass Restoration Workshop. Refer to Appendix H for the ISBW Conference conceptual models on assisted gene flow and selective breeding.

PART 4 Next Steps and Call to Action

Next Steps Overview and Timing

The steering committee reviewed and compiled the three pathways to propose an overall gameplan for eelgrass restoration (**Figure 4**).

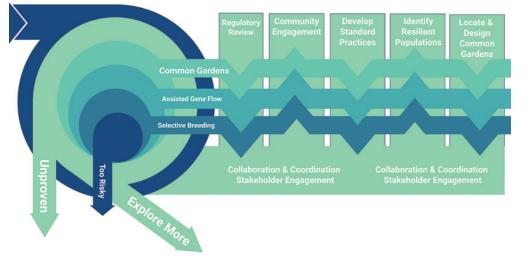


Figure 4: Building Out Eelgrass Restoration Pathways: Over the course of the three-day workshop, a number of potential pathways were discussed, some of which were potentially risky, were unproven or needed more research to be considered. Workshop participants began narrowing in on two pathways, assisted gene flow and selective breeding, and one specific tool, common gardens, that can help test the fitness of populations and that was identified as a required first step for any of the pathways discussed. There are many overlapping considerations to these pathways, and a need for coordination and collaboration across and within the pathways.

Near-Term Implementation Opportunities

Based on the workshop discussions, the steering committee identified key opportunities for immediate and near-term implementation.

Immediate Next Step: Coordination

Coordination is key. Funding, program and project management, and data management need to be well aligned and coordinated throughout the geography to avoid duplicating efforts or competing for funds, and to ensure that data are collected and experiments are run with standardized approaches and measurements. The following steps must be taken in the next 3 months to one year:

 Create a new steering committee (that will serve as a successor to the June 2022 workshop steering committee) to oversee the process. The new committee should represent diverse geographies, skills, and sectors. Scientific, regulatory, practitioner, management, and other types of experience are needed



Climate change-driven thermal stress poses urgent, irreversible threats to the **Mid-Atlantic and** New England seagrass meadows that demand a strategic and coordinated plan, and implementation must begin now. The June 2022 Eelgrass **Restoration Workshop** offered a unique opportunity for eelgrass and non-eelgrass experts to develop creative and implementable pathways to address these critical challenges.

PART 4 | NEXT STEPS AND CALL TO ACTION

(e.g., government relations/finance/fundraising). The committee will need defined roles and terms such as support and oversight of coordinator, fundraising, strategic guidance, etc.

- a. Develop a detailed 3-5 year workplan.
- b.Convene regular meetings to address standardizing methodologies and measurements.
- c. Convene a workgroup specifically focused on raising and addressing regulatory concerns.
- 2. Create an organizational entity and find a fiscal sponsor. A new entity (e.g., new organization or network) and fiscal sponsor (e.g., Multiplier), potentially modeled after the Ocean Sewage Alliance or the Reef Resilience Network within The Nature Conservancy, or theZostera Experimental Network.
- **3. Hire a full-time coordinator.** This person should preferably have post-doc/graduate-level experience with strong project management and communication skills.
- **4. Build a platform for the work to be accessed.** Create a website, Slack (or similar) account and channels for specific workstreams to allow open and transparent discussions.

Other Next Steps

The workshop steering committee identified additional important next steps:

- 1. Fundraise. The new steering committee should identify a workgroup that will be dedicated to identifying public funds (such as National Estuary Program funds), direct congressional line items, and private funds.
- 2. Compile existing data and normalize standardize it.
 - a. Create a database or leverage existing databases that can be searched and added to.
 - b. Useful data relate to genetics, physical parameters, and biological parameters.
- 3. Identify resilient populations.
 - a. Identify populations to be included in the common garden experiments.
 - i. Develop standard metrics for assessing a plant or population's resilience or resistance.
 - ii. Develop standard metrics for selecting populations for inclusion in and location of common garden.
- 4. Create a standardized approach and best practices for seed material collection, handling, and moving.
- 5. Begin common garden and mesocosm-type experiments to test for resilient genotypes and functional groups.
- 6. Begin selective breeding demonstration experiments to confirm the approach will work for *Zostera*.
 - a. Identify necessary infrastructure needs.
 - b. Develop/secure said infrastructure.



Conclusion

Seagrass meadows are essential, but often underappreciated and unrecognized ecosystems. Seagrasses provide food and shelter for diverse organisms, enhance coastal protection, improve water quality, sequester carbon, and reduce disease. This workshop was convened to discuss how to avoid the loss of these irreplaceable habitats and services. We envisioned a future where these habitats can survive the threat of climate change, and shared ideas, concerns, and opportunities. The discussion explored a number of potential pathways that restoration practitioners and resource managers could follow to aid this important habitat in adapting to a changing climate.

We hope that the points captured in these proceedings will prompt a longer conversation among many stakeholders and that they establish a foundation of collaboration and coordination to unify the community of practice around restoration and management of this important habitat.

The 2022 June Eelgrass Restoration Workshop gathered diverse experts to map out promising pathways and milestone objectives to tackle within this decade.

This workshop report not only outlines the gameplan over the course of the next 10 years, it identifies specific activities to be implemented now. The resource management and restoration community must now lead and enhance broader and larger-scale coordination and partnerships to avert catastrophic losses of our treasured seagrass meadows.



Figure 5: Conceptual model of full workshop process that led participants from co-learning about the challenges and opportunities of the issue toward a pathway for restoration.

PART 5 List of Appendices

Workshop Brief

AT A GLANCE

In the coastal bays along the eastern coast of the United States, eelgrass (*Zostera marina*) grows on the bottom, creating meadows that serve several critical roles. Eelgrasses and other seagrasses are of fundamental importance to world fisheries production (<u>Unsworth, Nordlund et al. 2019</u>). They offer shelter, feeding and nursery grounds, as evidenced by the high diversity and abundance of fauna within them (<u>Sievers, Brown et al. 2019</u>); they improve water quality by filtering, cycling and storing nutrients and pollutants through uptake by their leaves and roots (<u>Sandoval-Gil, Alexandre et al. 2016</u>); they are a significant carbon sink at the global scale (<u>Nelleman, Corcoran et al. 2008</u>); and they play an important role in protecting coastal areas from erosion, flooding and storm surges (<u>Ondiviela, Losada et al. 2014</u>). However, this seagrass, like all others across the globe, has been declining worldwide. In the western Atlantic, eelgrass has been retreating across its range. In some areas, less than 10% remains of what was there less than a century ago. To preserve eelgrass, many restoration efforts have been made with varying degrees of success. The vast majority of these efforts fall into the category of long-term failure.

Over the years, efforts have been made to better understand the threats to this plant. The top threats according to the literature, aside from direct removal of eelgrass through physical disturbances, appear to be the combined impacts of warmer temperatures and eutrophication-related light stress. As a direct response to the Clean Water Act, various actions are underway to reduce nutrient loads to many of the coastal estuaries. Unfortunately, in many cases, large improvements in water quality have not led to subsequent increases in eelgrass, nor to increased restoration success.

Monitoring data from several areas along the East Coast of the United States clearly shows eelgrass retreating from the interior of bays and only persisting near areas of ocean exchange. On Long Island, the Peconic Estuary shows a clear line where eelgrass is no longer present that coincides with the boundary of cumulative hours of water temperatures that exceeds a threshold for temperature stress. Similar declines are occurring across much of the western Atlantic coast, with particular concerns at the southern extent of eelgrass. The Chesapeake Research Consortium recently held a workshop entitled "Rising Watershed and Bay Water Temperatures – Ecological Implications and Management Responses," which concluded that eelgrass may be entirely extirpated from Chesapeake Bay in two decades.

Eelgrass is a clonal plant that also reproduces sexually with flowers and seeds. Eelgrass can exhibit considerable plasticity, and many beds have high levels of genetic diversity, which suggest the capacity for adaptation, yet it appears that the pace of increasing water temperature in the western Atlantic may be beyond the ability of the species to respond. This problem may be exacerbated by the current low level of genetic exchange between regional populations and across estuaries.

TEMPERATURE EFFECTS ON EELGRASS

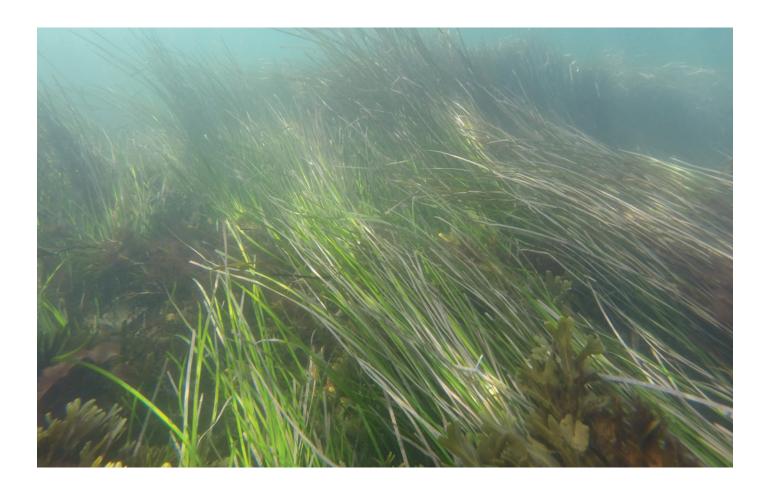
Z. marina is a temperate species whose optimal water temperature is approximately 10–20 °C, with 16–17 °C being an optimal range for seedling growth (<u>Niu, Zhang et al. 2012</u>). Colder temperatures are tolerated and plants remain healthy at 5 °C. At these colder temperatures, growth is slowed but photosynthesis:respiration ratios are maximized. Eelgrass growth rates increase linearly from 5 to 25 °C. Beyond this temperature, however, deleterious effects emerge. High temperatures of 25–30 °C depress rates of photosynthesis and growth and dramatically

PART 5 | A. WORKSHOP BRIEF

increase mortality. Marsh and colleagues (<u>Marsh, Dennison et al. 1986</u>) determined that at temperatures above 30 °C, *Z. marina* has a negative net carbon balance, photosynthesis becomes overwhelmed by increasing rates of respiration, and plants decline rapidly. Hammer and colleagues (<u>Hammer, Borum et al. 2018</u>) found that high temperatures (30 °C) negatively affect eelgrass growth, tissue integrity, nitrogen metabolism, and protein/enzyme synthesis. The impact of elevated temperatures can be worse in low light. Kaldy (<u>Kaldy 2014</u>) showed that temperature-induced increase in eelgrass respiration can be problematic even at temperatures between 10 and 20 °C when light is limiting photosynthesis. In theory, eelgrass could escape deleterious temperatures by retreating to deeper, cooler waters. Increasing colonization depth, however, is not likely to be a successful strategy for adapting to future climate change, as the lower depth of eelgrass is restricted by light penetration. The poor tolerance of eelgrass to elevated temperatures suggests a challenging future for the species.

The impacts of thermal stress have already been observed in the Chesapeake and neighboring coastal bays in Delaware, Maryland, and Virginia. Extended warm periods, such as those occurring in the 1980s and 1990s, have been linked to population declines of eelgrass in the eastern Atlantic (<u>Glemarec, Le Faou et al. 1997</u>). Acute warming from summertime heat waves has triggered shoot mortality and population declines.

Eelgrass diebacks in the Goodwin Islands and York River Chesapeake Bay National Estuarine Research Reserve in Virginia during 2005 were attributed to a greater frequency and duration of water temperatures above 30 °C (<u>Moore and Jarvis 2008, Moore, Shields et al. 2014</u>). These authors noted a tipping point at 23 °C; changing eelgrass cover from 2004 to 2011 was linked with temperatures below and above 23 °C.



PART 5 | A. WORKSHOP BRIEF

Although a variety of other factors influence the thermal tolerance of *Z. marina*, it is clear that temperatures above 25 °C or, more generally, increases of 1–5 °C above normal summertime temperatures, can trigger large-scale die-off of eelgrass. For example, it is predicted that: (1) short-term exposures to summer temperatures 4–5 °C above normal will "result in widespread diebacks that may lead to *Z. marina* extirpation from historically vegetated areas"; (2) longer-term average temperature increases of 1–4 °C are predicted to "severely reduce or eliminate" *Z. marina*; and (3) "an increase in the frequency of days when summer water temperature exceeds 30 °C will cause more frequent summer die-offs" and is likely to trigger a phase change from which "recovery is not possible" (<u>Carr, D'Odorico et al. 2012</u>).

Long-term observations and research have also shown that temperature is an important environmental factor that controls the germination, growth, reproduction, and mortality of eelgrass. These effects will become even more important in the future with the continued thermal increase in the coastal zone. An abundance of evidence suggests that the outlook is poor for eelgrass, a cool-water species, in a steadily warming western Atlantic.

WORKING GROUP CHARGE

This workgroup will convene a diverse set of scientists, practitioners, managers, and stakeholders who will brainstorm possible options for human intervention to assist eelgrass survival along the East Coast of the United States. We will wrestle with four questions:

- 1) Is this something we should attempt? 3) What are the relative risks of action and inaction?
- 2) What could we possibly do? 4) How do we actually go about doing these things?

We will discuss these issues in rapid iterations to evaluate the feasibility of finding functional genotypes for thermal tolerance, the efficacy of assisted migration of these genotypes, the potential of selectively breeding for these species to adapt to the rapidly changing temperatures, and engineering super clones for heat tolerance. In response to the rapidly changing climate, similar efforts are already underway with coral reef species in an effort to save the world's reefs. We believe that we are facing a similar situation for eelgrass in much of the western Atlantic, and it is time for us to begin a serious conversation about how we should respond to this crisis.

Eelgrass management and restoration has largely ignored genetic intervention, beyond recommendations to enhance genetic diversity within restoration areas by including genets from multiple source sites. Discussions have begun to pursue identifying functional genotypes for thermal tolerances. Recently, the first investigation of induced acclimation occurred for a Mediterranean seagrass (<u>Pazzaglia, Badalamenti et al. 2022</u>). To our knowledge, eelgrass managers have not pursued assisted population migration, assisted range expansion, or assisted species migration as scientists have done for the terrestrial counterparts of eelgrass, nor have they tried selective breeding.

To close, this workshop series is meant to be a space to learn from the work of others, generate discussion around the approaches that are available, and forge a path forward for the restoration and management community to consider as we contend with climate change impacts on eelgrass, with a particular focus on thermal stresses. The learnings from these workshops will be presented to the attendees of the International Seagrass Biology Workshop at the World Seagrass Conference in Annapolis, Maryland, in August 2022, as a way to continue the discussion with the international community. The steering committee is already committed to drafting a manuscript that captures all of these discussions, which will be available for review and open to contributions from all the participants of these workshops.

Glossary of Terms

Pathway	Approach
artificial selection	A change in species traits caused by inadvertent or intentional human choices. For example, hatchery fish are often artificially selected to spawn early in the season because of logistical difficulties in catching late-spawning fish in the wild.
assisted evolution	"A conservation strategy that involves manipulating the genes of organisms in order to enhance their resilience to climate change and other human impacts. The potential for impacted or vulnerable species to genetically adapt to handle changing environmental conditions depends on the standing genetic variation in the population and how quickly new genetic changes are incorporated. Assisted evolution strategies aim to accelerate the rate of naturally occurring evolutionary processes. Such measures include moving resilient individuals to vulnerable margins of their species distribution, or genetically modifying wild species to promote recovery or increase their capacity to resist stressors" (Filbee-Dexter & Smajdor 2019). The term assisted evolution encompasses many of the other terms defined in this list, including selective breeding, artificial selection, conservation breeding, and assisted gene flow. (Described in van Oppen et al. 2015)
assisted gene flow	"Intentional translocation of individuals within a species range to facilitate adaptation to anticipated local conditions." (Aitken and Whitlock 2013)
assisted migration	"The intentional translocation of individuals within or outside the natural range of a species." (Aitken and Whitlock 2013)
conservation breeding program	"A program for restoring or preserving species at high risk of extinction that involves breeding management in captive or semi-wild settings (e.g., predator-free islands) and may include conservation translocations." (Kosch et al. 2022)
conservation translocation	"The deliberate movement of organisms from one site for release in another that must yield a measurable conservation benefit at the levels of a population, species, or ecosystem, and not only provide benefit to translocated individuals." (IUCN 2013, quoted in Bradley et al. 2022)
CRISPR	Clustered Regularly-Interspaced Short Palindrome Repeats. A genetic engineering tool used in gene editing to directly modify genetic composition. (Discussed in depth in Lino et al. 2018)
evolutionary rescue	"Reversal of the demographic decline of a population through adaptation to new environmental conditions." (Aitken and Whitlock 2013)
foundation species	"A species that plays an important role in structuring and creating habitat within a community." (Aitken and Whitlock 2013)
gene editing	Using molecular techniques to intentionally alter specific genes within the genome of a living organism, by inserting, replacing, or deleting sequences of DNA.
gene flow	"Movement of individuals or gametes (e.g., pollen) between populations that results in successful introduction of migrant alleles." (Aitken and Whitlock 2013)
gene-targeted conservation	"Using our understanding of the genetic basis of fitness-related traits to advance the conservation of biodiversity. A fundamental requirement of gene-targeted conservation is identifying the loci underlying variation in fitness-related traits, including those that are detrimental. When such loci are identified, gene-targeted approaches have the potential to advance conservation." (Kardos and Shafer 2018)

PART 5 | B. GLOSSARY OF TERMS

Pathway	Approach
genetic rescue	"An increase in population fitness due to immigration alleviating inbreeding depression and increasing genetic diversity." (Aitken and Whitlock 2013)
genomic offset	"The distance between the current and required genomic composition in a set of putatively adaptive loci under a future/changed environment. The latter can be understood in a spatial or temporal perspective." (Rellstab 2021)
hybridization	The interbreeding of individuals from two distinct species or populations of the same species that are distinguishable by heritable characteristics. For clarity, it is important to distinguish between inter- and intra-specific hybridization.
local adaptation	"Higher fitness of local than nonlocal populations resulting from divergent selection among environments." (Aitken and Whitlock 2013)
mitigation translocation	"A subgroup of conservation translocation" that is usually "implemented in response to legislation or governmental regulation, with the intent of reducing a development project's effects on animals or plants inhabiting the site." (Defined by Germano et al. 2015, quoted in Bradley et al. 2022)
natural selection	The differential survival and reproduction of individuals due to differences in phenotype. If phenotypic differences have a genetic basis, then natural selection can result in adaptation via evolution.
outbreeding depression	"Reduction in fitness resulting from mating individuals from different populations." (Aitken and Whitlock 2013)
phenotypic plasticity	"Ability of a genotype to produce different phenotypes under different environmental conditions." (Aitken and Whitlock 2013)
resilience	The rate or degree to which an organism recovers from a disturbance event (Pimm 1984, Westman 1978).
resistance	The ability of an organism to remain unaffected by a disturbance event (Pimm 1984).
selective breeding	Choosing individuals with particular characteristics to breed together with the intention of producing offspring that exhibit specific characteristics.
targeted gene flow	"A conservation approach that involves translocation of individuals with favorable traits." (Kosch et al. 2022)
targeted genetic intervention	"Approaches that increase fitness in the presence of intractable threats by changing occurrence or frequency of targeted alleles in a populationThese approaches should aim to preserve the natural characteristics of the species. This involves collecting baseline phenotypic data (e.g. microbiome, fitness, behavior) that can be monitored as the breeding program progresses." (Kosch et al. 2022)

Session Agendas

Session 1: June 2, 2022

1:00–1:15 p.m. Kickoff for Workshop Defining our purpose Community Agreements

1:15-1:30 p.m. Understanding the Challenge

1:30-1:45 p.m. Lightning Round Brainstorm

1:45-2:45 p.m.

Learning Session (part 1)

Panel Discussion with:

- Collin Timm
- Dina Proestou
- Hollie Putnam
- Katie Lotterhos
- Kelly Racette
- Tom Whitham

2:45-3:05 p.m.

Break

3:05-4:05 p.m.

Learning Session (part 2)

Panel Discussion with:

- Collin Timm
- Dina Proestou
- Hollie Putnam
- Katie Lotterhos
- Kelly Racette
- Tom Whitham

4:05-4:45 p.m.

Open Discussion: Initial Takeaways

4:45-5:00 p.m.

Looking Ahead

5:00 p.m. Session Close

Session 2: June 7, 2022

1:00-1:05 p.m.

Welcome, Logistics & Community Agreements Session 1 Recap Session 2 Objectives

1:05-1:25 p.m.

Identifying Potential Pathways Overview of process Discussion of pathways

1:25-1:30 p.m.

Exploring Potential Pathways World Café Instructions

1:30-2:10 p.m.

World Café Round 1 Break (10 min)

2:20-2:55 p.m.

World Café Round 2 Quick Break (5 min)

3:00-3:30 p.m.

World Café Round 3 Quick Break (5 min)

3:35-3:55 p.m.

World Café Round 4 Break (10 min)

4:05-4:15 p.m.

Report Outs

4:15-4:55 p.m. Discussion: Takeaways

4:55-5:00 p.m.

Next Steps and Closing What to expect for Session 3

Session 3: June 28, 2022

1:00-1:05 p.m.

Welcome, Logistics & Community Agreements

1:05-1:25 p.m.

Session Objectives, Structure, and Output Session 2 takeaways Approach to Session 3 Session outputs

1:25-1:35 p.m.

Building Out an Innovative Pathway: Overview Overview of process Developing pathway flow and sequence

1:35-2:25 p.m.

Exploring Potential Pathways: Part 1 Developing pathway flow in breakout groups

2:25-2:40 p,m.

Report Outs Break (15 min)

2:55-3:55 p.m.

Exploring Potential Pathways: Part 2 Designing an implementation approach

3:55-4:15 p.m.

Report Outs Break (15 min)

4:30-5:00 p.m.

Moving Forward Post Workshop

Happy Hour

List of Attendees / Bios

Christopher Clapp

- Ocean Sewage Alliance, Executive Director
- I have worked on ecosystem-based approaches to eelgrass and shellfish restoration in past iterations of my career. The data from those efforts has lead me towards working on nutrient pollution reduction knowing it was unlikely we could change the temperature stress.

Phil Colarusso

- US EPA, Marine Biologist
- I have been conducting monitoring, research, regulating and conserving eelgrass in New England for over 30 years.

Natalie Cosentino-Manning

- · NOAA, Marine Habitat Restoration Specialist
- I have worked at the NOAA Fisheries Restoration Center for 20 years. I specialize in the restoration of rocky intertidal, nearshore, and estuarine habitats. I have also have experience in assessing impacts from contaminant releases to SAV, and subtidal habitats in CA and the Gulf of Mexico.

Aldo Croquer

- The Nature Conservancy, Marine Conservation Program Manager
- I am a coral reef ecologist, with particular interest in ecological processes that shape the structure and function of these ecosystems using experimental approaches.

Katie DuBois

- · Bowdoin College, Postdoctoral Scholar
- I have nine years of experience studying eelgrass response to warming using both field-based experiments and mesocosm-based experiments. My research focuses on how local adaptation, genotypic diversity, and plasticity determine eelgrass response to temperature stress.

Jillian Dunic

- Simon Fraser University, PhD Candidate
- I am a marine ecologist and have focused on understanding the effects of multiple pressures on coastal ecosystems. Recently I have worked on synthesis of global seagrass trends, modelling the effect of temperature and light on eelgrass growth rates, and surveys of eelgrass morphology and disease prevalence.

Tay Evans

- MA Division of Marine Fisheries, Fisheries Habitat Program, Scientist and Environmental Analyst
- I am a senior member of the Ma DMF Fisheries Habitat Program where I conduct seagrass research, monitoring and restoration, perform technical environmental reviews and other fisheries habitat related work. I earned a MS at University of New Hampshire in 2002 working on eelgrass restoration.

Jonathan Grabowski

- Northeastern University, Professor
- I have worked on the fish and mobile invertebrate communities associated with sea grass habitat and restoration throughout my career. This has included field work in New England and the Mid Atlantic, and syntheses throughout the U.S.

PART 5 | D. LIST OF ATTENDEES / BIOS

Boze Hancock

- · The Nature Conservancy, Senior Marine Habitat Restoration Scientist
- Background in fisheries science in Western Australia, NOAA's Restoration Center and TNC Global Oceans Program. Primary focus is applying the science we have to the restoration of critical coastal habitats (reefs- coral and shellfish, seagrass, salt marsh, mangrove, and kelp) globally.

Cynthia Hays

- Keene State College, Professor, Biology Department
- I am an evolutionary ecologist; my research focuses on local adaptation (especially micro geographic adaptation) and the consequences of mating system variation and gene flow on the genetic structure and demography of seaweeds and seagrasses.

Stephen Heck

- · Stony Brook University PhD candidate in Marine Sciences
- I am a Ph.D. candidate working in the Peterson Marine Community Ecology Lab. My research has included studying trophic cascades driven by black sea bass in eelgrass meadows, long-term eelgrass and bivalve population monitoring, and applied eelgrass and bivalve restoration efforts.

Randall Hughes

- Northeastern University, Professor
- My overall goal is to inform effective and equitable marine conservation and management practices that promote resilience to climate change. I have worked extensively with eelgrass, demonstrating the ecological importance of plant genetic diversity for productivity and response to disturbance.

Andrew Jacobs

- · Wampanoag Tribe of Gay Head (Aquinnah); Laboratory Manager
- I have been working for the Wampanoag Tribe's natural resources department for 14 years and have experience with fish monitoring, shellfish propagation, eelgrass restoration efforts, and water quality monitoring.

Jessie Jarvis

- · University of North Carolina Wilmington, Associate Professor
- I am a seagrass ecologist who currently works with eelgrass in North Carolina at its southern limit. I investigate its response to climate stressors and interactions with other species. I have studied eelgrass seed ecology in the Chesapeake Bay and worked with seagrasses in the Great Barrier Reef.

W. Judson Kenworthy

- · Albemarle National Estuary Partnership, North Carolina
- My main research and management interests are focused on seagrass ecology and integrating ecological knowledge into best monitoring and managing practices for the benefit of seagrass conservation.

J. Brooke Landry

- · Maryland Department of Natural Resources, Natural Resource Biologist
- Chair, Chesapeake Bay Program SAV Workgroup
- I'm an SAV ecologist with 20+ years experience in SAV research (with an emphasis on watershed impacts), conservation, restoration, and management. I've studied SAV from the Caribbean to the Chesapeake where I currently work with the Maryland Department of Natural Resources and run the Chesapeake Bay Program's SAV Workgroup. The SAV Workgroup serves the broader Chesapeake Bay community by providing technical

expertise and applying research findings to issues impacting SAV in the Bay and by guiding managers on the protection and restoration of SAV. I also led the development of and coordinate the CBP's SAV Watchers volunteer SAV monitoring program and SAV Sentinel Site Program.

Jonathan Lefcheck

- · MarineGEO, Smithsonian Institution, Coordinating Scientist
- I am a marine community ecologist, biodiversity scientist, and seagrass biologist. I have worked on seagrass ecosystems for over 10 years, including on one of the largest restorations of eelgrass in Virginia's coastal bays.

Brandon Lind

- Northeastern University, Research Fellow
- My focus is evolutionary genomics and climate adaptation in conifers, predicting climate vulnerability and evaluating existing methods. Currently a postdoc with Dr. Katie Lotterhos

Katie E. Lotterhos

- Northeastern University
- I have expertise in population genomics, evolutionary modeling, and adaptation.

Chris Nadeau

- Northeastern University
- I am a climate change biologist studying assisted migration/gene flow in multiple systems, including active experiments with subalpine plants, aquatic crustaceans, and eelgrass.

Joyce Novak

- · Peconic Estuary Partnership, Executive Director
- A coastal paleoceanographer by education, I am now the Executive Director of the Peconic Estuary Partnership, the National Estuary Program on the East End of Long Island, NY. We operate at the intersection of science, policy, and community engagement and work closely with partners to carry out strategies for clean water and healthy habitats in the Peconic watershed. Our Comprehensive Conservation Management Plan (CCMP) dedicates Action 30 to protecting eelgrass and enhancing damaged and decimated beds.

Bradley Peterson

- Stony Brook University
- I am a community ecologist working in seagrass habitats often looking at plant-animal interactions and how organisms can act as resource providers to the plants.

Holly Plaisted

- National Park Service Biologist
- My expertise lies within nearshore and intertidal marine ecosystem science, monitoring and management; estuarine/marine ecology and water quality; seagrass biology, conservation, genetics, and restoration

Dina Proestou

- USDA ARS, Research Geneticist
- Geneticist with the USDA Agricultural Research Service. Goal to link phenotype with genotype for eastern oyster traits important to breeders and farmers. Developed genetic tools for the eastern oyster, evaluated performance of selected oyster lines in diverse environments, and addressed using hatchery seed in restoration.

Jonathan Puritz

- · University of Rhode Island, Assistant Professor
- My lab investigates how natural and anthropogenic processes affect the evolution of marine populations through the lens of larval dispersal. We combine multi-stressor larval exposure experiments with genomic surveys of natural populations, analyzing selection and migration using seascape genomics.

Hollie Putnam

- · University of Rhode Island, Associate Professor of Biological Sciences
- I am an integrative molecular ecophysiologist, with a focus on marine invertebrates. Our work seeks to provide a deeper mechanistic understanding of biological adaptations that promote physiological homeostasis and ecological persistence of organisms under a changing environment.

Kelly Racette

- The Nature Conservancy, Sustainability Scientist, Ag & Food Systems
- I am a scientist embedded within TNC's corporate engagement team, providing scientific leadership to our work with agricultural companies. I received my PhD in plant breeding and agronomy from Univ. of FL and represent TNC in multi-stakeholder initiatives related to the application of synthetic biology and advanced breeding techniques for conservation outcomes.

Jennifer Ruesink

- University of Washington, Professor of Biology
- 20 years in Washington state estuaries, carrying out field experimental tests of Zostera marina responses to factors such as density and damage manipulations and transplantation among sites. Recent collaborative work on *Z. marina* genome and transcriptome (related to flowering).

Erin Shields

- · CBNERR-VA/VIMS, Lead Marine Scientist
- I have been at VIMS/CBNERR-VA for 16 years working on seagrass and water quality monitoring, research, and restoration. I currently run the seagrass program at CBNERR-VA where I manage several research and monitoring projects involving eelgrass response to stressors, documenting multiple die-off events in response to water temperature.

Stefanie Simpson

- The Nature Conservancy, Coastal Climate Program Manager
- I support our country and state programs to scope and develop blue carbon market projects, including an eelgrass restoration project in Virginia. I can offer lessons learned from the VA eelgrass restoration carbon market project.

Eric Sotka

- College of Charleston, Professor of Biology
- I am an evolutionary ecologist that roots my work in field observations and manipulative experiments and places these results within the context of biogeographic and evolutionary perspectives. I have worked with Zostera marina in NC and MA for over a decade.

Adam Starke

- The Nature Conservancy, Coastal Scientist
- I've worked in the marine and coastal restoration/conservation space primarily focused on shellfish and salt marshes. More recently, I've been focused on ways in which we can help both nature and people adapt to climate change.

Betsy Stoner

- · Bentley University, Assistant Professor
- My research focuses on understanding the role that benthic organisms play in nearshore seagrass beds. Most recently, we are beginning a project focused on evaluating the ecological memory and potential resiliency of sub-tropical seagrasses (in South Florida and The Bahamas) to multiple disturbances.

Cayla Sullivan

- · US EPA Long Island Sound Office, Life Scientist
- Received MS in Soil and Water Sciences from University of Florida (Dr. Laura Reynolds). Currently, I am the Long Island Sound Office lead for habitat restoration where I am concentrating on progressing our eelgrass extent target. Working with partners to prioritize embayment's for management and future restoration.

Collin Timm

- · Johns Hopkins University, Applied Physics Laboratory
- My research focuses on plant stress detection and mitigation, primarily focused on microbiome modulation to mitigate specific plant stresses. My work includes microbiology and plant microbe interactions, microbiome studies and analyses, and plant phenotype analyses.

Thomas G Whitman

- · Northern Arizona University, Regents' Processor Emeritus
- My focus is in community ecology, genetics, and climate change mitigation. I use studies in the wild and reciprocal common gardens along latitude and elevation gradients to identify plant genotypes and populations that will survive a given level of climate change. We have a bill pending in Congress to fund an experimental forest/common garden network to identify how trees are locally adapted and to identify which genotypes and populations should be planted for current and future conditions. The same approach should apply to seagrass and many other foundation species that largely define their respective communities/ecosystems. I would like to work on broad initiatives to make this a reality in diverse systems.

FACILITATORS

Bennett Brooks

- Senior Mediator, Consensus Building Institute
- Bennett Brooks is a Senior Mediator at the Consensus Building Institute. He has facilitated dozens of complex and highly contentious collaborative dialogues on issues related to coastal adaptation, water resource conflicts, fisheries, and ecosystem restoration throughout the U.S.

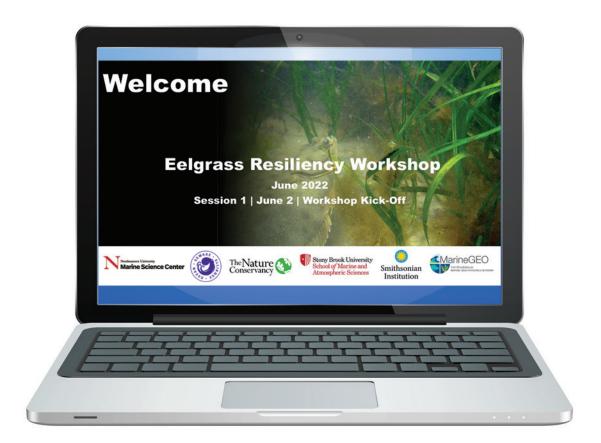
Stephanie Horii

- Senior Associate, Consensus Building Institute
- Stephanie Horii is a Senior Associate at the Consensus Building Institute with extensive experience in facilitation services and marine sciences/environmental management. She is a seasoned professional in multi-party facilitation and collaborative problem-solving.

Presentation Slide Decks

AT A GLANCE

Workshop 1 - June 2, 2022 Workshop 2 - June 7, 2022 Workshop 3 - June 28, 2022



Survey Results

Eelgrass Workshop, Session 1 | June 2, 2022 | Running List of Ideas from Menti Polling

#	B. O. Group	Short Name (for Menti Poll) [% Jun 2 Menti results]	Description / Notes
1.	GROUP A	Identify resilient populations/ genotypes [27%]	Identify resilient populations/genotypes. Use an array of possible methods, ranging from observational studies to genetic offsets, to identify resilient populations/genotypes.
2.	GROUP B	Increase genetic diversity/ assisted gene flow [26%]	Increase genetic diversity/assisted gene flow. Source plants from multiple populations to increase genetic diversity in a vulnerable population or restoration, which might also include moving individuals from populations identified as resilient.
3.	GROUP C	Conduct selective breeding/hybrid- ization/artificial selection [17%]	Conduct selective breeding/hybridization/artificial selection. Breed resilient genotypes (i.e., selective breeding) or species (i.e., hybridization) in the lab/nursery or use selection in the lab/nursery to produce individuals with desired traits.
4.		Perform microbiome manipulation [10%]	Perform microbiome manipulation. Modify microbial associates (e.g., soil microbes) that can alter resilience.
5.	GROUP D	Perform environmental hardening of seeds/seedlings [9%]	Perform environmental hardening of seeds/seedlings. Expose seeds/seedlings to higher temperatures in the lab/nursery before planting them in the wild.
6.		Modify existing conditions/reduce non-climate threats [8%]	Modify existing conditions/reduce non-climate threats. Alter the environment (e.g., soil, wave breaks) to increase eelgrass production or reduce existing stressors (e.g., improve water quality).



PART 5 | F. SURVEY RESULTS

Eelgrass Workshop, Session 2 | June 7, 2022 | Breakout Group Approaches Menti Polling Results

Modifying existing conditions/reduce non-climate threats Very promising! Let's Explore! Selective breeding/artificial selection Increasing genetic diversity/assisted gene flow Gene editing Microbiome manipulation Environmental hardening of seeds/seedlings Put head in the sand and hope it gets better

Rate the following - Promising/Interesting?

Not promising

Resilient Populations/Genotypes: Which seem most promising now?

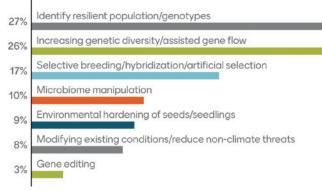
A. Validate Genetic Offsets Very promising! What's next? B1. Multi-stressor experiments - Lab B2. Multi-stressor experiments - Field C1-C2. Baseline Field Observational Studies D. Integrated Approach

Not promising

Selective Breeding/Hybridization/Artificial/ Selection: Which seem most promising now?



100 points: Where should we allocated our attention?

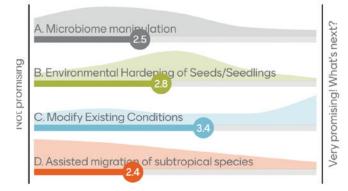


Genetic Diversity/Assisted Gene Flow: Which seem most promising now?



Very promising! What's next?

Catch All Group: Which seem most promising now?



Session 2 Discussion Flip Chart Notes

Eelgrass Breakout Group Notes | GROUP A: Identify Resilient Populations/Genotypes

OVERVIEW & INSTRUCTIONS

Facilitation Team

- Moderator/Reporter: Chris Clapp
- Notes/Flip Charter: Boze Hancock
- Facilitator: Stephanie Horii
- Panelist(s): Katie Lotterhos
 +Brandon Lind

Instructions for Participants

- Community Agreements: Share Space | Stay on Topic | Ask Questions of Each Other | Not Seeking Consensus
- **NOTE:** The Flip Charter will capture high-level ideas shared verbally (left side of the table). Please avoid writing in this area.
- Written ideas:
 - ~ In the top right corner, confirm you have the green "Suggesting" mode active
 - ~ Add general comments in the COMMENTS column
 - Comment on a specific item click and hold to highlight a section or sentence. Click on one of the bubbles on the right side of the page to add a comment or suggestion. Add your comment and click post comment to save.
- Other Ideas: We want to focus discussions to the particular breakout group topic; however, feel free to capture other ideas in the "parking lot/tide pool" at the bottom of this document

(**POST MEETING NOTE:** These Google Docs will be open for additional comments until **June 17**)

Glossary of terms

World Cafe / Breakout Session Game Plan

- Round 1 (40 Minutes)
 - ~ Welcome and Process Review (~5 Minutes)
 - ~ Open Discussion (35 Minutes)
- Round 2 (10 min break and 35 min discussion)
 - ~ Transition (+10 min break)
 - ~ Welcome and Process Reminder (~2 Minutes)
 - ~ Review ideas from Round 1 (~3-5 minutes)
 - ~ Open Discussion (~25 minutes)
- Round 3 (Bio Break and 30 min discussion)
 - ~ Transition+Bio Break, Welcome, and Process Reminder (5 Minutes)
 - ~ Review ideas from Rounds 1 & 2 (~3-5 minutes)
 - ~ Open Discussion (20 minutes)
- Round 4 (Bio Break and 20 min discussion)
- ~ Transition+Bio Break, Welcome, and Process Reminder (5 Minutes)
- ~ Review ideas from Rounds 1 & 2 (~2 minutes)
- ~ Open Discussion (~15 minutes)
- Break and Full Group Report Out and Discussion (10 min break, 10 min report out, 40 min discussion)
 - ~ Back to Plenary and 10 min break; then moderators report back top 2-3 takeaways (10 min)
 - ~ Full group facilitated discussion (~40 min)

Links to the Other Groups

- Group A
- Group B
- Group C
- Group D

Eelgrass Breakout Group Notes | GROUP A: Identify Resilient Populations/Genotypes

Identify resilient populations/genotypes: use an array of possible methods, ranging from observational studies to genetic offsets, to identify resilient populations/genotypes.

	FOR PARTICIPANTS			
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?
A. Validate Genetic Offsets (prediction of how a genotype will perform in a multivariate environment relative to other genotypes)	 Relates to (informs) assisted gene flow and choosing genotypes for restoration Get both genetic info and environmental distance. Can use individual genotype in clonal reproduction environment. May not need to know the "genes that matter" 	 Implicit assumption is genotypes are specifically adapted to where they are Predictions are only as good as the input data Same risks associated with experiments/ transplants Can be biased by complex demography 	 Potential to identify regional/ coastwide areas for field verification Can set parameters around how much variability is explained in order to apply prediction All of the below need should be integrated Standardized genetic data / environmental data collection 	 Jeanine Olsen paper. Repopulation following last genotype; (Olsen et al. 2016) Gather range wide information on genotypes and phenotypes? Do we need more baseline functional genomic information?
B1. Multi-stressor experiments - Lab. Greenhouse / Mesocosm or testing one population against another	Able to be deliberate about the stressors tested	 Ignoring important stressor Might not be representative of what is happening in the field such as currents, facultative relationships etc Difficult to fund (with restoration \$\$, potentially EPA) 	Limited # genotypes	 (Zimmerman et al. 2017) (Palacios and Zimmerman 2007)
B2. Multi-stressor experiments- Field. Common garden type experiments/ reciprocal transplants	 Get real life (field) scenario Important for testing suitability prior to scaling. There are gradients within estuaries that can inform transplants (DuBois experiment). (DuBois et al. 2022) 	 Low risk of introgression if transferring into large populations Not as controlled Stresses in some common gardens may overshadow temperature adaptation (e.g., disease) Risk of overharvesting the donor beds. Risk of too many genotypes dying at warm sites Funding requires multi-pronged approach- action beyond experimentation. Less difficult to fund 	• Guidelines for how to handle moving material from one region to the next.	 New Whitlock paper on low risk of introgression - (Grummer et al. 2022) Establishing population-based differences (in eg local adaptation, disease resistance/ tolerance) will be crucial for seed sourcing. Will also be important in determining if genetic offset (relative population performance in a given environment) predictions will perform well. If there is a baseline of observational data, it is possible to use a major storm, ECE, etc as a stressor and evaluate the legacy effects of this stressor (instead of needing to manipulate temp, for instance) compared to the baseline population
C1. Baseline Field Observational Studies - Material -> ID Genetic Structure: Collection of material from the field and identifying genetic structure	 Get information directly relative to the conditions it is experiencing. Takes into consideration the phenotype 	Harvesting from stressed system	 Need standardized environmental data to integrate with genetics Standardized genetic data collection Minimum genetic data to inform restoration at scale. 	Include genotype?

PART 5 | G. SESSION 2 DISCUSSION FLIP CHART NOTES

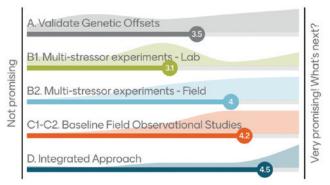
	FOR FLIPCHARTER ONLY						
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?			
C2. Baseline Field Observational Studies - Demographic -> ID Resilience - Demographic field data identifying resilience	 No translocation risk Have good environmental data from many beds These are required in order to generate fundable projects. 	Resource intensive	 Lots of background data exists Allows moving to scale Need standardized measures 	 Across temporal & spatial scales; Standardized environmental data collection! 			
D. Integrated approach.		 Perfection being the enemy of action Requires time bound action plan 	 To scale the identification of resilient genotypes these ideas all need to be integrated. Funding requires this integration. 				

OTHER IDEAS AKA "PARKING LOT" or "TIDE POOL"

- Must monitor systems to identify resilient pops (both the meadows extent and conditions and the environmental conditions) and publish the data/reports!
- All ideas here will affect pros/cons of assisted gene flow and practicality of application
- Link to recent paper regarding practicality (and risks) of assisted gene flow with large local population sizes in mind
- Need for a community of practice with standardized approach
- Erik Sotka mentioned RADseq study that revealed high structure - I wondered if this was reinforced by flowering time/reproductive isolation
- How difficult / time-intensive / expensive are the genetic offset experiments? Do they require strong loci to describe multivariate phenotypes? How strong do the genotype-phenotype correlations need to be?
- Really curious if the models are going to work with the highly restricted gene flow conditions most Zostera marina populations experience
- Need to get the information we have in one place.
- Could sampling for the flowering pathway be easily incorporated in existing monitoring programs?

- Just a leaf tip to test for FT expression, which cues transition of shoot apical meristem from vegetative to flowering
- Will resilient populations warrant more aggressive/restricted conservation/management approaches? MPAs?
- Are there correlated traits that tend to be associated with temperature resilience (e.g., shorter shoots, larger rhizomes, smaller blades), that could be indicators of and/or problematic for tolerance of additional stressors like light limitation, sulfide, physical damage?

Resilient Populations/Genotypes: Which seem most promising now?



PART 5 | G. SESSION 2 DISCUSSION FLIP CHART NOTES

Eelgrass Breakout Group Notes | GROUP B - Increasing genetic diversity/assisted gene flow

OVERVIEW & INSTRUCTIONS

Facilitation Team

- Moderator/Facilitator: Chris Nadeau
- · Notes/Flip Charter: Randall Hughes
- Panelist(s): Hollie Putnam, Tom Whitham

Instructions for Participants

- Community Agreements: Share Space | Stay on Topic | Ask Questions of Each Other | Not Seeking Consensus
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(**POST MEETING NOTE:** These Google Docs will be open for additional comments until **June 17**)

Glossary of terms

World Cafe / Breakout Session Game Plan

- Round 1 (40 Minutes)
 - ~ Welcome and Process Review (~5 Minutes)
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Links to the Other Groups

- Group A
- Group B
- Group C
- Group D

Group B: Increasing genetic diversity/assisted gene flow | NOTES TABLE

Identify resilient populations/genotypes: use an array of possible methods, ranging from observational studies to genetic offsets, to identify resilient populations/genotypes.

	FOR PARTICIPANTS				
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?	
A. Target a single resilient population: Restoration with single population that is resistant/resilient to temperature (single pop assisted migration)	 Addresses urgent need to respond to temp; could supplement later with additional donor populations Lower probability of trans- mitting disease/parasites/ microbes from a single population vs multiple populations 	 This requires a priori knowledge of resilience / resistance == Time If we move a single population everywhere (across a landscape scale), it may not be a safe long-term strategy, because that single pop will likely not be able to handle all conditions / environments; lowers genetic diversity May reduce functional variation May be more susceptible to disease / herbivory / other stressors (tradeoffs with other phenotypes) Potential impacts to donor site (more plants needed from a single site than if collecting from multiple populations), particularly if collecting adult shoots Association of temp resilience and life history (annual vs perennial) may lead to reduced/absent cover in some parts of the year Potential for maladaptation to cold (particularly if moving from farther south) 	 Time and expense of collecting from multiple populations Time and expense of identifying temp resilient / resistant populations Could have sites that are genetic repositories / nurseries; number of breeders could be greatly reduced Are there limits on availability of biomass / density of particular genotypes or populations State / regulatory boundaries as they pertain to moving populations Potential risk of having to re-seed annual populations yearly if they don't set enough seed to regrow (if / until they switch to perennial) 	 Low risk to moving a single population, given generally high within-population genetic diversity High risk to sourcing outside of US Atlantic coast, even if thermal tolerance exists on Pacific side Eelgrass Transplant Experiment, population from pristine site dies completely at all other sites: (DuBois et al. 2022) 	
B. Combine multiple resilient populations or resilient and other populations. Resto- ration with multiple populations, at least some of <i>which</i> have been chosen because they are resistant/re- silient to temperature (multi pop assisted migration / genetic diversity)	 Potentially hedges our bets for multiple stressors beyond temp Potential for intraspecific hybridization May balance urgency and risk Using this strategy in a field trial framework could inform further targeted efforts 	 Potential for intraspecific hybridization and outbreeding depression; clonal reproduction may compensate for this risk; outbreeding depression relatively low risk for large local populations especially if transplant is relatively low proportion of total individuals Increased potential for transmitting disease/parasites/microbes from multiple source populations May reduce functional variation May be more susceptible to disease / herbivory / other stressors (tradeoffs with other phenotypes) Potential for maladaptation to cold (particularly if moving from farther south) 	 Time and expense of collecting from multiple populations Time and expense and logistics of identifying temp resilient / resistant populations Could have sites that are genetic repositories / nurseries Potential need to treat for disease / pathogens prior to movement State / regulatory boundaries as they pertain to moving populations Using this strategy in a field trial framework could inform further targeted efforts 		

PART 5 | G. SESSION 2 DISCUSSION FLIP CHART NOTES

	FOR PARTICIPANTS			
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?
C. Increase diversity without targeting resilient popula- tions. Restoration with multiple viable source populations not targeted specifically for temperature resistance/resilience (genetic diversity)	 Potentially hedges our bets for multiple stressors beyond temp Captures variation in natural populations Potential for intraspecific hybridization Don't have to have a priori knowledge of resistance/resilience 	 Could have high mortality if much of that diversity is not suited for the site Potential for intraspecific hybridization and outbreeding depression; clonal reproduction may compensate for this risk Increased potential for transmitting disease/parasites/microbes from multiple source populations How do you pick the donor sites? Status quo may be ease of access; similar physical characteristics; proximity; may miss what makes a more resilient meadow 	 Time and expense of collecting from multiple populations Could have sites that are genetic repositories / nurseries Potential need to treat for disease / pathogens prior to movement State / regulatory boundaries as they pertain to moving populations 	

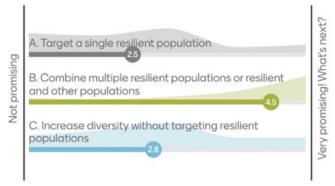
OTHER IDEAS AKA "PARKING LOT" or "TIDE POOL"

- What is the end goal? E.g., coral restoration (will depend on scale)
- Portfolio approach? coral example
- Scale of source will be proportional to scale of restoration
- What is mortality rate in restorations? 30% success rate at site level; can have high mortality initially and then clonal recovery
- Time frame of restoration efforts is not long enough
- Need to know how locally adapted populations are, and at what scale
- · Constraining ourselves to Atlantic coast sources
- Microgeographic variation
- · Selecting on plasticity rather than thermal tolerance?
- Identifying populations field trials can be very useful, and they take time; also, hard to track genotypes of eelgrass through time in the field; feels feasible to plant from multiple populations and using genetic techniques and figuring out who persists; doing while learning
- Restoring from bare sediment| supplementing declining meadows
- Restoration may need a "window of opportunity" for success (stochastic event), meaning that what persists after transplant is more random with respect to temperature than we would wish
- I do wonder if there are differences between Pacific "forests" and Atlantic "meadows" in how we do this.

For instance, mortality rates may change the decision about "throwing lots of populations into a restoration"

- · Is there a risk to using seeds only?
- Site selection (for where you are restoring) is critical for all approaches
- Risk to nurseries / field gardens / repositories is selection that will occur there
- Herbivore movement may be happening faster than plant movement (work with *Thalassia*)
- Consider life history traits of organisms, not just genetic diversity
- Could site-specific seed banks be maintained? That way site-specific restoration could happen during stressful years.
- · Marginal sources may be better than pristine sources

Genetic Diversity/Assisted Gene Flow: Which seem most promising now?



Eelgrass Breakout Group Notes | GROUP C - Selective breeding/hybridization/ artificial selection

OVERVIEW & INSTRUCTIONS

Facilitation Team

- Moderator: Brad Peterson
- Notes/Flip Charter: Steve Heck
- · Facilitator: Adam Starke
- Panelist(s): Dina Proestou, Kelly Racette

Instructions for Participants

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World Cafe / Breakout Session Game Plan

- Group A
- Group B
- Group C
- Group D

[Group C: Selective breeding/hybridization/ artificial selection] | NOTES TABLE

Selective breeding/hybridization/artificial selection: breed resilient genotypes (i.e., selective breeding) or species (i.e., hybridization) in the lab/nursery or use selection in the lab/nursery to produce individuals with desired traits

	FOR PARTICIPANTS			
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?
A. Selective Breeding (Choosing individuals with particular characteristics to breed together with the intention of producing offspring that exhibit specific characteristics.)	 Potentially one piece of a multi-faceted solution that may help produce plants with higher resistance and resiliency to thermal stress. Traits that are selected may also provide other traits beneficial to the plants adaptation. Selecting for eelgrass that can persist provides a wide-array of ecosystem services-possibly selecting for specific traits that can support individual services-can be in line with UN. SDGs and funding therein. If integrating genomic information, additional information on traits may be provided 	 A one-size fits all approach may be risky, since there are different stressors at each site. Potential inadvertent selection against beneficial traits if focus is directed as selecting for higher thermal tolerance. Identifying populations resilient/ resistant to thermal stress and creating selective breeding programs at scale may take a lot of time. Duration is an issue, where would the funding come from long-term? You cannot consume eelgrass, so it is not generally viewed as important as other species (e.g. oysters) from a funding standpoint. Phenotyping is difficult. Lack of knowledge - (it seems) Hard to replicate the number of breeders in the wild. Perhaps more likely to have unintended consequences 	 Cross-pollinating eelgrass plants has never been done before as far as we know. How to do this at scale (if it can be done) may be very challenging. Has been done but small small scale. Daunting - lots of hurdles Challenging and labor intensive, but possible. At least 8-10 years Before we have a result 	 Is there a chance of introducing reproductive incompatibility at the ploidy level (like triploid oysters)? Calling out the overlap of the idea Selective Breeding and Artifical Selection by some
B. Intraspecific Hybridization (Hybridization is the interbreeding of individuals from 2 distinct species or populations of the same species that are distinguible by heritable characteristics. For clarity, it is important to distinguish between inter- and intra-specific hybridization.)	Hybrids might overcome the problem of thermal stress rapidly	 Intraspecific hybridization of populations from the south and north may be viable for 1st generation but subsequent generations may not be viable. Something to figure out before doing this at large scale. Hybrids might be more susceptible to other stressors 		 This is very similar to assisted migration Calling out the overlap of the idea Selective Breeding and Artifical Selection by some
C. Artificial Selection (A change in species traits caused by inadvertent or intentional human choices. For example, hatchery fish are often artificially selected to spawn early in the season because of logistical difficulties in catching late spawning fish in the wild.)	Similar benefits to selective breeding.	 Differences in photoperiod across latitudinal gradients may limit success of southerly populations in northern latitudes. Shift in flowering timing about an hour of photoperiod has occurred in the south Material indirectly selected for hatchery conditions may not perform well in wild 		 Artificial selection for a shift (higher) thermal performance curve (thermal specialist) OR for a broader TPC (thermal generalist) Beyond the 'normal' phenotypic targets that one might think of off top of their head, early flowering time might be an important mechanism by which to avoid heat waves and dieback

OTHER IDEAS AKA "PARKING LOT" or "TIDE POOL"

- Traits that breeders want and select for are usually associated with other genes that are good for climate adaptation
- Termal performance curves (population specific) do they exist for eelgrass?
- Some lessons from tree breeding and maintaining local adaptation to climate: (MacLachlan et al. 2018; MacLachlan et al. 2021)
- · Reproductive output may be something to look into

Eelgrass Breakout Group Notes | GROUP D - Catch All

OVERVIEW & INSTRUCTIONS

Facilitation Team

- Moderator: Phil Colarusso
- Notes/Flip Charter: Jon Lefcheck
- · Facilitator: Bennett Brooks
- Panelist(s): Collin Timm

Instructions for Participants

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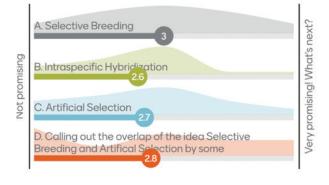
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Links to the Other Groups

Group A
 Group B
 Group C
 Group D

Selective Breedin/Hybridzation/Artificial Selection: Which seem most promising now?



[Group D: Catch All] | NOTES TABLE

Microbiome manipulation: modifying microbial associates (e.g., soil microbes) that can alter resilience.

Environmental hardening of seeds/seedlings: exposing seeds/seedlings to higher temperatures in the lab/nursery before planting in the wild.

Modifying existing conditions/reduce non-climate threats: alter the environment (e.g., soil, wave breaks) to increase eelgrass production or reduce existing stressors (e.g., increasing water quality). **Other?**

	FOR FLIPCHARTER ONLY						
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?			
A. Microbiome manipulation	 Easier to achieve with current technology Increased root biomass, stabilization of seedling, increased reproductive output of seeds to flood the system 	 Don't know identity of beneficial organisms (yet) Difficult to culture, would have to transplant sediments to inoculate meadows How long does the treatment last? Expensive to reapply? Temp not only threat; other sources of mortality Microbiome manipulation benefits might be smaller than host genotype/ phenotype effects Need to better understand gaps in our knowledge 	 Easily scaled but persistence of beneficial microbes may be difficult to achieve long-term (some evidence from PhD dissertation) How to apply to seedling, grown plants, and when? 	 Is there truly a proven causal relationship between microbiome and host health/resilience/resistance? Inoculate flowers with microbes, get beneficial microbes into seeds (example for terrestrial system) Are we talking about inoculating the soil itself with microbes or placing the seeds in a microbe/soil slurry and creating microbe-rich seed bombs? Seed bombs may also help with seed retention and burial, which opens recruitment bottleneck 			
 B. Environmental hardening of seeds/seedlings seed coating 	 Potentially end up with thermally resistant plant Easy to implement 	 Unclear whether hardening passes to next generation Mechanism unclear (does this activate pathways to infer temperature resistance?) Will nature take care of this problem for us with warmer winters during germination? (it hasn't yet) 	 Difficult to expose large enough quantities of seedlings to temperature to support large-scale out plantings 	Hardening seeds vs. seedlings			
 C. Modify existing conditions relieve other axes of stress to improve resistance to rising temperatures stabilize hydrodynamic conditions using co-restoration/artificial structures identify symbiotic organisms (e.g., fungi, lucinid bivalves) to improve sediment conditions living shorelines (e.g., salt marsh) to reduce sediment/nutrient input introduce grazing epifaunal inver- tebrates to reduce overgrowth & promote light accessibility 	 Controllable, demonstrated benefit in some systems Hopefully the ecosystem takes hold and is self-sustaining (one time modification) Living shorelines have shown success to multiple species and reduction in shoreline loss 	 Potentially Expensive Timing is probably important So many to choose from! What is most important/impactful 	 Easily scalable Combine with infrastructure needs (protecting highways, rail, bridges etc.) for example, with oysters 	• Take an ecosystem restoration-based approach as opposed to taxon-specific?			

PART 5 | G. SESSION 2 DISCUSSION FLIP CHART NOTES

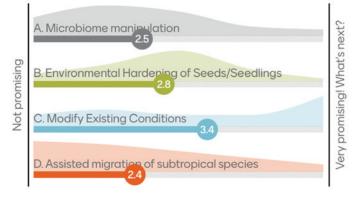
	FOR PARTICIPANTS			
Idea & Short Description (Approach for pursuing this pathway)	Benefits	Risks	Scalability	COMMENTS Expand on the idea. How might this idea work?
D. Assisted migration of subtropical species Halodule wrightii	 Need to be open to considering more within-region options Avoid "missing the chance" for natural range increase/ change Easy to implement, cost-effective; may provide a fix if other alternatives don't work Create mixed beds that support Zostera recruitment & persistence 	 When does this become an invasive species? (ethical concerns) Need regulatory framework for moving plants/seeds/ different species from state to state (across any jurisdictional boundaries) May provide very different ecosystem services May face annual die-offs due to winter temperatures Potential pushback 	• Very scalable	 Need to rethink/refine our regulatory allergy to bringing in other species (revisit restrictions and controls) - need to open up this conversation
 E. Increase belowground carbon stores to reduce stress e.g., plastic sheets focus certain wavelengths to drive buildup of sugars in the roots in winter 	Increases resistance to rising temperatures	 Period of thermal stress continues to increase and period of carbohydrate storage decreases 		
F. Transplant annual eelgrass from southern edge	 Annual population (may be?) less sensitive to extremes between generations Fairly easy to assess 	 Unclear if annual plants are available to test if limited to sourcing on East Coast Unless truly annual populations (not intermixing with perennial) then may revert to perennial on changing conditions Can we even identify true annual populations in NC? Does this sustain ecosystem services? Possibly not May be plastic and require no intervention to switch to annual life cycle as temperatures warm at higher latitudes 	Depends on the number of annual populations to source from	 Look at transcriptome of perennial vs. annual identify genes or pathways to stimulate to get more annual behavior = FT (florigen) gene expression seems to be very important to cuing flowering - can we add florigen to the system to make them more annual like? Annual life histories ("true" in which they germinate and flower within a single growing season) = occur in two general conditions, 1) southern range limits in Japan, Korea, Baja California (probably not NC but check with Jessie Jarvis) where seeds are present during the hottest water temperature seasons, 2) mid-range upper-latitudinal zones where seeds are present during the coldest darkest iciest time of year. May be more valuable to identify mechanisms/ pathways than focus on active movement

OTHER IDEAS AKA "PARKING LOT" or "TIDE POOL"

- Grasspave2 Porous Grass Paver | Invisible Structures | Porous Paving Solutions - plastic based, but would be good to have something biodegradable
- Blake Bextine, PM at DARPA, wanted to control the "annual - perennial transition" - genetic modification was the approach, but what we could do with chemicals/hormones/nutrients
- Can we induce mixing of cold water? Can we pump cold water up to maintain slightly lower temps, is this infeasible?
- Leverage efforts to industrialize seagrass farming as a mechanism for funding for building resilient/resistance populations
- Can we score all of the approaches (groups A, B, C, D) by "time to implementation" against the timeline for losses of existing systems
- Invertebrates clean the leaves, to allow for better light
 can we recruit these inverts to retain health plants (Katie DuBois)

- Site suitability models to identify climate refugia
- Can someone share any mesocosm environment modification studies (in situ) of Zostera?
- Is there a risk of focusing exclusively on seed-based strategies?
- Seed coating to reduce predation, can add microbes or small molecules to enhance germination or make them "taste bad" to crabs and other predators

Catch Group: Which seem most promising now?



References

- DuBois, K., K. N. Pollard, B. J. Kauffman, S. L. Williams, and J. J. Stachowicz. 2022. Local adaptation in a marine foundation species: Implications for resilience to future global change. Global Change Biology 28: 2596–2610. https://doi.org/10.1111/ gcb.16080.
- Grummer, J. A., T. R. Booker, R. Matthey-Doret, P. Nietlisbach, A. T. Thomaz, and M. C. Whitlock. 2022. The immediate costs and long-term benefits of assisted gene flow in large populations. Conservation Biology 36: e13911. https://doi.org/10.1111/ cobi.13911.
- MacLachlan, I. R., T. K. McDonald, B. M. Lind, L. H. Rieseberg, S. Yeaman, and S. N. Aitken. 2021. Genome-wide shifts in climate-related variation underpin responses to selective breeding in a widespread conifer. Proceedings of the National Academy of Sciences 118: e2016900118. https://doi.org/10.1073/pnas.2016900118.
- MacLachlan, I. R., S. Yeaman, and S. N. Aitken. 2018. Growth gains from selective breeding in a spruce hybrid zone do not compromise local adaptation to climate. Evolutionary Applications 11: 166–181. https://doi.org/10.1111/eva.12525.
- Olsen, J. L., P. Rouzé, B. Verhelst, Y.-C. Lin, T. Bayer, J. Collen, E. Dattolo, et al. 2016. The genome of the seagrass Zostera marina reveals angiosperm adaptation to the sea. Nature 530. Nature Publishing Group: 331–335. https://doi.org/10.1038/nature16548.
- Palacios, S. L., and R. C. Zimmerman. 2007. Response of eelgrass Zostera marina to CO2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats. Marine Ecology Progress Series 344: 1–13. https://doi.org/10.3354/meps07084.
- Zimmerman, R. C., V. J. Hill, M. Jinuntuya, B. Celebi, D. Ruble, M. Smith, T. Cedeno, and W. M. Swingle. 2017. Experimental impacts of climate warming and ocean carbonation on eelgrass Zostera marina. Marine Ecology Progress Series 566: 1–15. https://doi.org/10.3354/meps12051.

Building Out Potential Pathways

Discussion Notes, Conceptual Models, and Implementation Actions/Tasks

This appendix contains additional content from the discussions that occurred during the breakout groups, including a synopsis of the major topics that were covered and conceptual models that were developed during the sessions. The conceptual models were reorganized and refined for formatting and inclusion in these proceedings.

Contents

Click on links to jump to a section.

Discussion Notes:

I Session 3 Breakout Groups (Groups A–D)

- a. Discussion Overview
 i. Overlapping Pathway Components
 ii. Pathway-Specific Thoughts
- b. Breakout Group Discussions

II Conceptual Models and Implementation Actions

- a. Group A Assisted Gene Flow
- b. Group B Assisted Gene Flow
- c. Group C Selective Breeding
- d. Group D Environmental Hardening

III ISBW Conference Conceptual Models

a.Assisted Gene Flow b.Selective Breeding

I. DISCUSSION NOTES: SESSION 3 BREAKOUT GROUPS

Discussion Overview

Overlapping pathway components described by all groups

Many components described by all four groups were viewed as essential to developing each of the three pathways. The steps for all groups generally reflect simultaneous incremental lab experiments and field experiments (common garden experiments).

Compilation of existing data and standardized future monitoring data collection

All four groups described the need to compile existing data that are available on the distribution of eelgrass, along with environmental data throughout its range along the eastern seaboard of North America. For all groups, existing data would provide an understanding of the current status of eelgrass populations and also assist in the identification of populations of eelgrass that may be particularly resilient and resistant to thermal stress. For the assisted gene flow and selective breeding groups, any existing information on genetics was also viewed as essential for an array of reasons. Existing data on eelgrass genetics and physical traits were considered to be potentially very important to understanding which populations to select from for use in assisted gene flow, as well as to address issues surrounding the maintenance of genetic diversity. Group B also discussed the value of consolidating existing data to inform the design and distribution of a common garden network. Furthermore, existing data on genetics were viewed as helpful to understanding the risks involved in moving eelgrass genotypes across varying distances. The assisted gene flow, selective breeding, and environmental hardening groups all decided that developing plans for the collection of standardized data into the future would be an essential component of assessing the status of eelgrass populations in the focal region (e.g., are populations continuing to decline, is the rate of decline slowing, is the decline reversing) and justifying the need for action. Furthermore, these data would allow efforts to be pivoted from being calculated and cautious to more aggressive if the status of eelgrass worsens. This was deemed essential to understanding whether restoration efforts were proving to be effective at local and regional scales and whether shifts to more aggressive methods were necessary.

Coordination

Across the board, all four breakout groups emphasized the need for an entity to coordinate the efforts of all three pathways (assisted gene flow, selective breeding, and environmental hardening). Without significant coordination throughout these pathways, including standardized monitoring approaches, an online data repository, and a website for coordination, the power of common gardens (and other aspects of each pathway) would be reduced significantly. All groups identified the need for a full-time coordinator as a very high priority.

Regulatory review and stakeholder engagement

The assisted gene flow and selective breeding groups all brought up that discussions with local regulators to understand and establish the legal framework for conducting this work need to be initiated immediately, since the process will likely take a substantial amount of time. This is particularly necessary for these two pathways, since the success of both may necessitate transferring eelgrass seeds or plants between different estuaries and across state lines. Currently the policy surrounding the interstate movement of eelgrass seeds and plants is murky at best, and it is unclear whether it is illegal or against existing state policy in most locations.

Participants in multiple groups suggested that they have had pushback from regulators when they have mentioned moving eelgrass genotypes from one region to another. For example, a participant in Group B suggested that they are unable to move genotypes within Long Island Sound from New York to Connecticut. However, the regulations likely differ significantly among states, and regulations may also be highly localized (e.g., city-level conservation commissions). A regulatory review is needed to better understand the regulatory roadblocks that might limit assisted gene flow, selective breeding, and environmental hardening, although the concerns with environmental hardening are substantially reduced. An exploration of how regulations might be revised to overcome these roadblocks is necessary and should be started immediately, since regulatory changes will take time.

Although the environmental hardening group (Group D) did not discuss regulatory hurdles, the large-scale harvesting of seeds, seedlings, and shoots to use in environmental hardening efforts would need to meet existing regulations under the Clean Water Act and various state wetland laws. These are not new regulations and are well known, so it is not a new hurdle, but depending on the scale and the state, it could be problematic.

All four groups identified stakeholder engagement and education early on as essential to the process. Further, getting all partners on board to involve them in the collective design of the work was also seen as vital to the success of any of these pathways.

Development of Common Gardens

All four groups also dedicated a substantial amount of time to the design and implementation of common gardens. All groups indicated that common gardens would be essential to the effectiveness of a range of components of the three pathways.

Both assisted gene flow breakout groups emphasized the development of a common garden network to identify genotypes that are resilient to high temperatures as well as to evaluate the effects of multiple stressors. All of the aforementioned existing background research should be compiled and was viewed as essential to the proper design of the common garden approach. There was general consensus among the four groups that in the near future we need to carefully design and start implementing common gardens within the region along a latitudinal (or temperature) gradient. Tom Whitham (in Group B), who drew on his extensive experience with common gardens in terrestrial (forestry) ecosystems, suggested that this should occur over the next 3-5 years. Given that latitudinal temperature gradients are very different in aquatic systems than they are in terrestrial, the number of common gardens that is needed for such a network needs to be discussed in further detail with Tom and others. Tom suggested this number as necessary to determine which genotypes or populations are resilient and how far different genotypes or populations can be moved before the environment becomes unsuitable for them.

All groups discussed the need for a clear articulation of questions that should be answered using the common garden approach to enhance their usefulness. Since this process will take a significant amount of time, it should be started as soon as possible. Furthermore, all groups felt it was important to develop partnerships between scientists, restoration practitioners, and regulators in order for a common garden network to be effective. This is also true of all three pathways in general.

Group A (Assisted Gene Flow) emphasized the importance of using a series of replicated common gardens to test eelgrass from different source populations to identify resilient eelgrass genotypes/populations. The sites for common gardens should have well-characterized environments.

Group B (Assisted Gene Flow) stated that in the long term (8–10 years), the results from the common gardens could be used to identify resilient source populations for experimental assisted gene flow. With that said, Group B did not discuss how the results of the common gardens would be used to inform the assisted gene flow approach. Group B felt that more discussion is needed around the methods used for assisted gene flow to ensure that the common garden approach provides the necessary information.

Group C (Selective Breeding) generally viewed common gardens as an important tool to identifying founder populations for selective breeding that have high resistance and resilience to thermal stressors. Group C also discussed the utility of common gardens in terms of testing out genotypes that were selectively bred to evaluate their performance in the field under a range of environmental conditions. While Group D (Environmental Hardening) did not discuss common gardens as much as the other three groups, they viewed common gardens as a route to test hardened eelgrass seeds and plants in the field.

Pathway-Specific Thoughts

While both assisted gene flow groups (Groups A and B) spent a lot of time discussing the logistics of common gardens, they did not spend a lot of time discussing the actual steps of experimental assisted gene flow. Furthermore, neither group talked much about scaling up the process of assisted gene flow in terms of transporting seeds from one area to another. Group C (Selective Breeding) and Group D (Environmental Hardening) briefly touched upon the logistics of scaling up selective breeding or environmental hardening if either pathway proves to be effective at developing resistance to thermal stress and other stressors.

Group C felt that selective breeding may ultimately prove to be an important strategy in parallel to assisted migration and environmental hardening to halt the decline and assist in restoring eelgrass coverage to historical distributions. It will likely take nearly a decade to establish a successful breeding program and transfer selectively bred individuals into the field, but there are many steps that can be begun right away. Many steps within the selective breeding pathway, including the identification of populations of eelgrass that are already relatively resilient and resistant to thermal stress, are likely necessary for the other pathways discussed, especially assisted migration. There are many components of the selective breeding pathway that overlap with one another and could be (and should be) done in parallel.

However, many steps need to be taken prior to the establishment of a selective breeding program and need to be done in sequence. For instance, experiments must be conducted to see whether it is possible to grow eelgrass in tank systems and get it to flower, cross pollinate, and go to seed. This needs to be done before any selective breeding in tanks can be done. If growing eelgrass in tanks is not feasible from a logistic or cost standpoint, efforts may need to pivot to focus solely on assisted migration. However, the development of a common garden program, as well as ongoing monitoring efforts, should be done for all proposed pathways and can be done almost immediately and run in tandem with other selective breeding efforts.

Each step in the pathway represents a necessary component in the development of a selective breeding program. While there are many parallels with assisted gene flow (e.g., identifying populations of eelgrass that are resilient and resistant to thermal stress, developing a common garden program), the exploration of the logistics and cost feasibility of cultivating eelgrass and breeding it in tanks is very divergent from assisted migration. To our knowledge, there are no published reports of successfully breeding eelgrass at a large scale in a mesocosm environment. If cultivation and breeding of eelgrass in tanks proves to be possible, selection experiments in tanks should run in parallel to field experiments in common gardens since the viability of plants in tank systems may be quite different from in the field. Furthermore, if selection experiments in tanks are effective at producing plants with higher thermal tolerances and adapted for forecasted future environmental conditions, this information could convince regulators to allow transportation of eelgrass genetic material (plants or seeds) across state lines for assisted migration and selective breeding efforts.

Group D (Environmental Hardening) discussed that it was first necessary to identify triggers, such as warm (and/or cold) exposure, that we expect to induce climate resistance to seeds, seedlings, and/or adult plants that the adults can pass along to their offspring. They then proposed to design lab (controlled trials) and field experiments (transplants from lab-to-field) to test the potential of these triggers to induce resistance and promote resilience. There will be feedback from field and lab experiments (e.g., identifying resilient individuals from the field for use in lab trials). Further experiments may test the mechanism behind resilience (if present) such as through genetic paths, epigenetic activation, and/ or interactions with the microbiome. Longer-term field transplants would have to be monitored for several years at a minimum to determine whether and how long climate resistance from hardening would last. The final challenge is how to scale up hardening in restoration efforts.

Breakout Groups Discussions

Each breakout group's discussion focus, critical questions tackled, unresolved questions, and key next steps are summarized here.

Group A (Assisted Gene Flow)

Group A focused primarily on the short-term steps needed to pursue this pathway-gathering knowledge, conducting standardized surveys, and establishing common gardens, and the specifics that would be needed at each of those steps. They did not spend much time on the multiple stressors or the actual steps of experimental assisted gene flow. Group A discussed the importance of coordination, communication, and consistent and standardized data collection and reporting as essential to learning from assisted gene flow efforts. They recognized that there are trade-offs regarding the need to collect standardized information that is not currently available versus the need to act now to address eelgrass losses, and discussed how to balance those needs. They noted the value of identifying 'tipping points' that would indicate a shift is needed toward greater action (e.g., if rates of loss increased), though no such tipping points were identified. This group had a lot of interest in figuring out what characteristics of natural populations are indicators of resilience and resistance to stress (e.g., are the lushest, healthiest beds the ones to target, or should we focus on the ones that are already hanging on in stressful areas?).

Group B (Assisted Gene Flow)

Group B focused most on the design and implementation of common gardens. It was clear from their discussion that the value of a distributed common garden network comes from significant coordination among sites and participants. It was also apparent that the work needed for coordination was no small task. The primary outcome of their discussion was that there will be a need for a full-time common garden coordinator if assisted gene flow is to be pursued. This group discussed how to design and implement a distributed common garden network and what is needed for the coordination of such a network. In the very near term, Group B thought that it would be highly useful to consolidate existing information

on eelgrass along the Atlantic coast of North America to better understand the status, distribution, and genetics. This information was seen as necessary for designing the common gardens, and would also help justify the need for a distributed common garden network by documenting the current and future decline of eelgrass.

The unresolved questions of Group B included what regulations might restrict the movement of seeds or adult shoots within and among states. Stemming from that, they decided that a regulatory review would be necessary to understand what regulatory roadblocks could hinder the implementation of a distributed common garden network and ultimately assisted gene flow. Another large unresolved question for Group B was who would coordinate a common garden network. They discussed several groups that have an interest in doing this, including EPA LISS (Cayla Sullivan), Alliance for SAV Enhancement (PEW), World Seagrass Association, Chesapeake Bay Program's SAV Workgroup (Brooke Landry), and Natural Estuaries Programs. Group B discussed how these organizations, along with the workshop steering committee, might form another steering committee to determine how to fund and locate such a position. They noted that it would be very important to clearly articulate the questions that we hope to answer with a distributed common garden before planning and implementation can occur. Once the question is clearly articulated, it will be possible to decide on the locations of a large number of common gardens and the source populations to be planted in each garden. The group emphasized that this alone is a substantial task.

Group C (Selective Breeding)

Group C concentrated on the need to identify the goals of this approach, talked about whether genetic material could be sourced from local eelgrass meadows for selective breeding directed at restoring eelgrass within the same area, and discussed the research that would need to be conducted to develop an effective selective breeding program. The question of whether genetic material could be sourced from localized areas for restoration in the same general area was an important one; the group centered around this for a long time. If the genetic pool on a local level has a large enough thermal tolerance range, it may be preferable to confine selective breeding to localized areas. Harvesting from local populations for selective breeding for restoration in the same area is believed to be beneficial, as these plants are likely to have adaptations to the local environmental conditions, which may increase survival. Further, the group brought up that if harvesting can be done of relatively local sources of eelgrass, the regulatory hurdles of interstate movement of plant genetic material may not need to be addressed.

The process of selective breeding and the differences in approaches between blunt (mass selection) breeding and precision (quantitative) breeding was extensively discussed. Overall, the group decided that a blunt approach to selective breeding is likely the first place to start since it is less expensive and less labor intensive than a precision approach. The group also discussed how to design selection experiments and agreed that it would be ideal to selectively breed for conditions forecasted into the future (e.g., 50 years from now). Generally, beginning with conditions forecast for the near future (within the coming decade) was viewed as a good starting point.

The group generally felt that while selective breeding is a longer process to get started than assisted migration and likely more expensive, it is a worthy pathway to pursue, since assisted migration alone may not produce the desired adaptation to a changing climate. Since many of the key first steps in selective breeding are the same as assisted migration and environmental hardening (e.g., identifying resilient populations, developing a common garden program) the group felt that all three pathways could be started in parallel. However, one of the major questions remaining unanswered was whether eelgrass can be successfully grown and cross-pollinated in tanks. Since this is the first question that we must answer in order to understand whether selective breeding of eelgrass could be a viable way to foster resiliency and resistance of eelgrass to stressors, the group agreed that this needs to be started immediately to see whether this route is worth pursuing. The group also talked about the importance of establishing the legal framework to allow selective breeding to happen, especially if the

interstate transportation of eelgrass seeds is deemed necessary. This was viewed as something that should be started immediately, since the process is prerequisite to a number of subsequent tasks.

Group D (Environmental Hardening)

Group D (Environmental Hardening) focused primarily on the need for lab and field experiments to demonstrate that environmental hardening can in fact lead to short- or long-term resistance and resilience to high temperatures. There was additional discussion on putative mechanisms, such as epigenetics, whether additional experiments would be necessary to elucidate those, and at what point in time it is necessary to do so (can we demonstrate the value of hardening before we understand it?).

Group D discussed many questions that would ultimately be critical components of using environmental hardening as a pathway to develop eelgrass resilience and resistance to thermal stress (and other stressors). These included whether hardening could be tied into other efforts (e.g., assisted gene flow or selective breeding) as a complementary tactic and whether this is a secondary line of inquiry to these other pathways or parallel. The group was interested in whether eelgrass individuals could be exposed to multiple stressors (e.g., thermal and light) to produce resistance to many axes of stress simultaneously. Further, the group discussed the importance of understanding whether the effects of environmental hardening would be intragenerational or intergenerational. Group D also talked about the importance of establishing research goals. These goals might include improving flowering rates, seed production, and seed germination. A second goal would be to use environmental hardening to improve these metrics within a single generation or sustain climate resistance across multiple generations. The largest lingering research question centered around whether it was even possible to use environmental hardening to facilitate resilience and resistance of eelgrass to a changing climate across many generations.

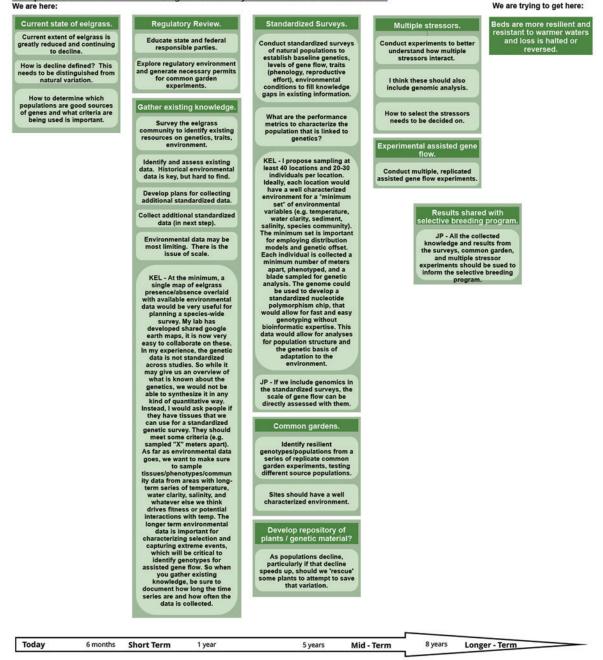
Like the other groups, Group D also identified the need for funding to achieve these goals. Group D also discussed developing a proposal that includes all three pathways to pitch to funders since they are all very intertwined. They also brought up the necessary question of how environmental hardening can be tied into and/or inform assisted migration and selective breeding. Group D also discussed whether there are synergies in field monitoring (for example) to identify resilient genotypes that can further benefit from hardening experiments.



CONCEPTUAL MODELS AND IMPLEMENTATION ACTIONS / TASKS

Group A: Assisted Gene Flow Assisted Gene Flow Conceptual Model

Group A - Assisted Gene-Flow for Eelgrass Resiliency & Resistance to Thermal Stress



Assisted Gene Flow | Implementation Action Tasks Action and Considerations for Milestones of Conceptual Model

Factors Impacting Implementation: Knowledge Gaps, Research Questions, and Monitoring

Immediate (now):

- Deciding scale / location of sites, not exact location, but general targets.
- Establishing criteria for when assisted gene flow should occur. Which meadows, why?
- · Identify sites with environmental data.
- · Identify sites with seagrass beds.
- Identify sites / areas where we have long (-ish) term demographic data for eelgrass (at least presence/absence).
- · Identify common garden sites questions:
 - ~ Seeds vs. shoots?
 - ~ In tanks or in the field?
 - ~ Preventing disease spread or epifauna spread?
 - ~ Scale of population comparison within each common garden?
- Develop consensus on what is considered suitable habitat for setting up common gardens (consistent evaluation of environmental conditions).
- · Figure out how to preserve seeds in a repository.

Near Term (1-4 years):

Standardized surveys:

- Goal: conduct standardized surveys of natural populations to establish baseline genetics, traits (phenology, reproductive effort), environmental conditions to fill knowledge gaps in existing information.
- · Design:
 - ~ 40 locations and 20–30 genets per location.
 - ~ Each individual is collected a minimum number of meters apart from others, phenotyped, and a blade sampled for genetic analysis.
- · Genomic data:
 - Whole genome or standardized SNP chip (would require development).
 - The genome could be used to develop a standardized nucleotide polymorphism chip, that would allow for fast and easy genotyping without bioinformatic expertise. These data would allow analyses of population structure and the genetic basis of adaptation to the environment.

- · Analysis:
 - ~ Gene flow and population structure.
 - ~ Genome-wide association test.
 - Looking for loci contributing to phenotypes.
 - ~ Environmental association tests.
 - Looking for loci that are responding to different environment.
- Outputs:
 - ~ Scale of genomic diversity and gene flow.
 - ~ Genomic loci of interest.
 - Data to send to genetic offsets analysis.
 - ~ Data to inform selective breeding.
- · Common gardens:
 - Identify resilient genotypes/populations from a series of replicate common garden experiments, testing different source populations.
 - ~ Sites should have well-characterized environment.
- Regional tank common gardens run for at least a year.
- Establish field common gardens 3–4 years out? Smaller test scale?

Mid-Term (5-8 years):

• Continuing to monitor natural beds, adaptive timelines, how does urgency or timeline for implementing assisted gene flow change a decade from now?

Long-Term (8-15 years):

Long-term monitoring

Factor Impacting Implementation: Exit Ramps: When do we know that we should stop and pivot?

Immediate (now):

• When 40–50 sites are identified that have the desired minimum set of environmental data overlapping with eelgrass occurrence.

Near Term (1-4 years):

- Common garden: If we only see evidence of plasticity across the source being tested, or a lack of variation, need to pivot to new sources and/or other approaches.
- If rate of decline accelerates, pivot to assisted gene f low experiments with data collection.

Factors Impacting Implementation: Coordination Needs; Collaboration Opportunities

Immediate (now):

- Common gardens; standardized surveys—set up network of researchers, managers working across multiple sites.
- Collaborative network established. Coordinator hired.
- Establish a hub or other shared drive where data, reports, etc. are regularly uploaded.
- Adopt some relevant case studies from other ecosystems—where has this approach been taken and what were the outcomes?

Near Term (1-4 years):

• Annual conferences / working groups.

Factor Impacting Implementation: Regulatory Needs, Strategies

Immediate (now):

- Review and compile coastal state and federal policies and regulations on collecting and transplanting seagrass. What is permitted?
- Consider what are least destructive approaches to satisfy regulators.
- Consider what (if any) regulations and documentation are needed so that it's not the Wild West.
- Convey sense of urgency and loss of habitat outweighs concerns.

Near Term (1-4 years):

 Assess what is allowed/begin dialog to inform regulators on science plans and possibilities for assisted gene flow in practical application.

Mid-Term (5-8 years):

- Establish network of scientists and regulators to sustain dialog on scientific progress.
- Adapt/revise policies and regulations to facilitate application.

Factor Impacting Implementation:

Strategic Communication

Immediate (now):

- Dedicate one person to managing communication for the group (?).
- A shared message is brought to managers, practitioners, and funders.
- Set up a Slack channel or some other communication medium.
- Network communications and regulatory issues and keep message consistent.

Near Term (1-4 years):

- Earned media on release of approach.
- Don't hide failures, focus on lessons learned, keep this through all stages. Celebrate model of try, fail, adapt, tweak approach, etc.
- Netflix documentary film (a la Chasing Corals).

Mid-Term (5-8 years):

• Earned media on progress.

Long-Term (8-15 years):

· Earned media on progress.

Factors Impacting Implementation: Social Equity, Environmental, Cultural, and Other Risks

Immediate (now):

- Identify historical lands of Native American groups from which samples are collected and thoughtfully and authentically bring them in early.
- Expand and diversify who is part of the discussion.
- Get the word out about these efforts.
- · Citizen science monitoring?

Near Term (1-4 years):

• Common gardens: risks of introducing disease, other species as we begin moving plants/seeds around.

Long-Term (8-15 years):

- · Genetic risks of assisted migration:
 - ~ Outbreeding depression.
 - ~ Range-wide genetic homogenization.
 - ~ Reduction in overall and localized effective population sizes.

Factor Impacting Implementation: Funding Needs or Opportunities

Immediate (now):

- Identify funding needs.
- Begin working on Congressional bill and/or other big-ticket and novel funding sources.
- Identify foundational supporter (s) to get program off the ground for 3 years minimum.
- Build a strong case for co-benefits and maintain this as high profile in all stages.

Near Term (1-4 years):

- Write grants and get funding.
- Public funding, NOAA, estuary programs, NERRS, etc. enhance and or provide in-kind services.

Mid-Term (5-8 years):

• Influence carbon credit, acidification credit, and nitrogen credit markets to invest in the program.

Factor Impacting Implementation: Data Organization

Immediate (now):

- Set up database or apps for standardized data collection. Make sure there is a plan for someone to monitor data integrity.
 - ~ KEL: "I've been using AppSheets to develop mobile app for standardized data collection for my lab and it works great!"
- Have a required repository associated with the network (a la BCO-DMO).

Near Term (1-4 years):

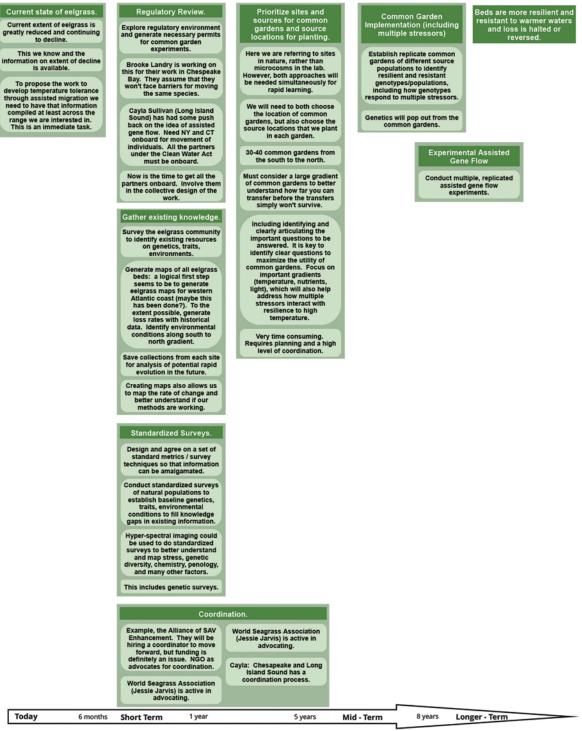
• Need funding to sustain this in the long term.



Group B: Assisted Gene Flow Assisted Gene Flow | Conceptual Model

Group B - Assisted Gene-Flow for Eelgrass Resiliency & Resistance to Thermal Stress We are here:

e are nere.



We are trying to get here:

Assisted Gene Flow Implementation Action Tasks

Action and Considerations for Milestones of Conceptual Model

Factors Impacting Implementation: Knowledge Gaps, Research Questions, and Monitoring

Immediate (now):

- Common garden
 - ~ Research questions:
 - Is there local adaptation to temperature in eelgrass on the Atlantic coast?
 - What is the effect of microbiome on resilience (need to specifically manipulate to quantify)?
 - ~ How many common gardens are needed, and where are they going to be?
 - ~ What are the important source populations to plant in each garden?

Factors Impacting Implementation: Exit Ramps: When do we know that we should stop and pivot?

Near Term (1-4 years):

- Is there local adaptation at all? Probably a very remote possibility that there is no local adaptation. Many other clonal plants are locally adapted on very fine spatial scales.
- It is possible that there are no individuals that can withstand the temperature in Chesapeake Bay or Long Island Sound. If there are no resilient genotypes, but a lot of genetic variation, maybe that's when we move into the genetic engineering route.

Factors Impacting Implementation: Coordination Needs; Collaboration Opportunities

Immediate (now):

- Utilizing EPA Geographic Programs and National Estuary Programs Collective databases.
- · Coordinating bodies identified
 - ~ EPA LISS
 - ~ Alliance for SAV Enhancement (PEW)
 - ~ World Seagrass Association
 - ~ Chesapeake Bay WH (Brooke Landry)
 - ~ NEERS
- Common garden
 - ~ We need a common garden manager who works with managers/owners of the common garden sites.
 - ~ An NGO (e.g.. PEW) could be an excellent coordinator. They may be able to find funding for it.
 - ~ Web space needed for coordination.

- ~ NERRS might be a good location for a coordinated common garden.
- ~ A common database is necessary.
- Standardized approaches
 - ~ Need protocols
 - ~ Need common databases
 - ~ Might need standardized approaches at the international level

Near Term (1-4 years):

- Common garden
 - Once the gardens are established, people will want to get involved and conduct their own experiments. A coordinator is necessary to determine how new users affect ongoing research.

Factor Impacting Implementation:

Regulatory Needs, Strategies

Immediate (now):

- Regulators: NOAA, EPA, NERR, NPS
- Partner with stakeholders from the beginning so that regulations might be easier to overcome.
- A regulatory review would maybe come first (Brooke has done this in Chesapeake Bay for SAV in general). Coordinator (NGO) would create new regulations to formalize the process by getting bills sponsored (via Brooke, regulatory review): <u>https://www.chesapeakelegal.org/guides-resources/report-existing-chesapeake-bay-watershed-statutes-and-regulations-affecting-submerged-aquatic-vegetation/</u>
- Statewide and interstate regulations, but extremely local too

Factor Impacting Implementation:

Strategic Communication

Immediate (now):

• Web space for coordinating common needs (e.g., protocols, data).

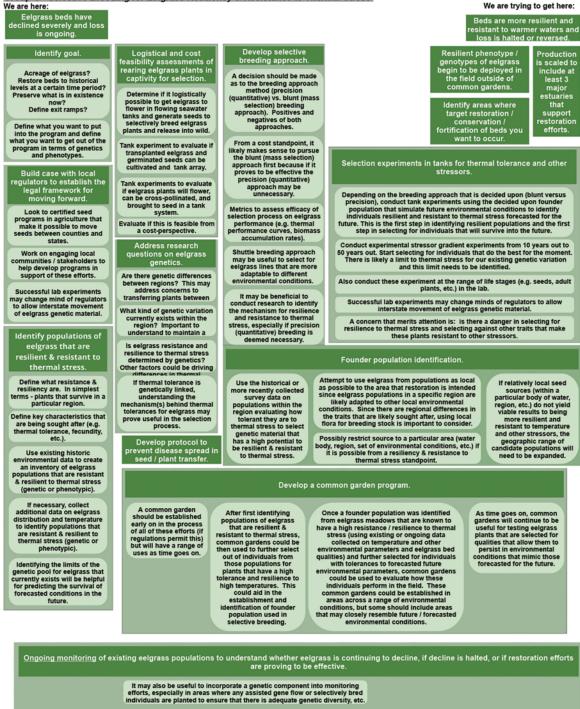
Factor Impacting Implementation: Funding Needs

Immediate (now):

- Common gardens
 - ~ Funding a common garden coordinator: granting agencies will want a coordinator
 - ~ Infrastructure money might be available at NERRS to help get the project started

Group C: Selective Breeding Selective Breeding | Conceptual Model

Group C - Selective Breeding for Eelgrass Resiliency & Resistance to Thermal Stress



Today	6 months	Short Term	1 year	5 years	Mid - Term	8 years	Longer - Term

Selective Breeding | Implementation Action Tasks Action and Considerations for Milestones of Conceptual Model

Factors Impacting Implementation: Knowledge Gaps, Research Questions, and Monitoring

Immediate (now):

- Eelgrass monitoring: Continue existing eelgrass monitoring programs to maintain datasets concerning the status of eelgrass throughout the region.
- Define key characteristics that are being sought (e.g., thermal tolerance, fecundity).
- Define resistance and resiliency. In simplest terms: plants that survive in a particular region.
- Use existing historical environmental data to create an inventory of eelgrass populations that are resistant and resilient to thermal stress (genetic or phenotypic). This is the first step in selecting for a founder population. Ideally, use local eelgrass stock to retain other adaptations specific to that region.
- Decide on common garden locations based on current environmental data across a range of conditions.

Near Term (1-4 years):

- Do tank experiments to determine whether it is logistically possible to cultivate transplanted eelgrass and germinated seeds in a tank array.
- Do tank experiments to evaluate whether eelgrass plants will flower, cross-pollinate, and bear seed in a tank system.
- Evaluate the feasibility of cultivation and selectively breeding eelgrass in tank systems.
- Survey populations within the region to see how tolerant they are to thermal stress, given that eelgrass populations in a specific region are likely adapted to other local conditions (initial founder population selection).
- Do experiments simulating future environmental conditions to identify individuals resilient and resistant to thermal stress forecasted for the future. This is the first step in identifying resilient populations and the first step in selecting for individuals that will survive into the future (further selection of resilient individuals).
- Conduct experiments to understand the thermal tolerance limits of the genetic pool for eelgrass; this is essential for understanding whether it will be possible for eelgrass to adapt to forecasted environmental

conditions (temperature specifically) in 10 years, 50 years, etc.

- Experimental stressor gradient experiments from 10 years out to 50 years out. Start selecting for individuals that do the best for the moment. There is likely a limit to thermal stress for our existing genetic variation.
- Rigorous science and experiments needed to counter issues with selection bottlenecks.
- Forcing into a factorial design doesn't make sense. These stressors are gradients.
- If necessary, collect additional data on eelgrass distribution and temperature to identify populations that are resistant and resilient to thermal stress (genetic or phenotypic).
- Are there genetic differences between regions? This may address concerns about transferring plants between regions from both a regulatory and genetic integrity standpoint.
- What kind of genetic variation currently exists within the region at various scales? This may be critical to maintain a similar level of genetic diversity through the range of restoration efforts.
- Is eelgrass resistance and resilience to high thermal stress determined by genetics? While we assume that this is likely the case, other factors could be driving differences in thermal tolerance.
- A common garden program should be established early on in the process of all of these efforts (if regulations permit this) but will have a range of uses as time goes on. The earliest use will be to aid in the selection of plants from populations that are resilient / resistant to thermal stress. This will assist the selection of a founder population from which to selectively breed.
- Ongoing monitoring of existing eelgrass populations to understand whether eelgrass is continuing to decline, if decline is halted, or if restoration efforts are proving to be effective.

Mid-Term (5-8 years):

 If thermal tolerance is genetically linked, understanding the mechanism(s) behind thermal tolerances for eelgrass may prove useful in the selection process, especially from a precision (quantitative) selection standpoint.

- As time goes on, common gardens will continue to be useful for testing eelgrass plants that are selected for qualities that allow them to persist in environmental conditions that mimic those forecasted for the future. These can also be used to evaluate whether selecting for specific qualities (e.g., thermal tolerance) reduces the performance of eelgrass in other ways.
- If a pivot from a blunt (mass selection) breeding approach to a precision (quantitative) approach is necessary, it is likely necessary to identify the mechanism for resilience and resistance to thermal stress.
- Continue to conduct selective breeding for environmental conditions forecasted for 10 and 50 years out across gradients through a range of life stages (e.g., seeds, adult plants).
- As resilient phenotype / genotypes of eelgrass begin to be deployed in the field outside of common gardens, continued monitoring is needed.
- Ongoing monitoring of existing eelgrass populations is necessary to understand whether eelgrass is continuing to decline, if decline is halted, or if restoration efforts are effective.
- It may also be useful to incorporate a genetic component into monitoring efforts, especially in areas where any assisted gene flow or selectively bred individuals are planted, to ensure that there is adequate genetic diversity, etc.

Long-Term (8-15 years):

- Ongoing monitoring of existing eelgrass populations is necessary to understand whether eelgrass is continuing to decline, if decline is halted, or if restoration efforts are effective.
- It may also be useful to incorporate a genetic component into monitoring efforts, especially in areas where any assisted gene flow or selectively bred individuals are planted, to ensure that there is adequate genetic diversity, etc.

Factor Impacting Implementation: Exit Ramps: When do we know that we should stop and pivot?

Near Term (1-4 years):

- If the cultivation and breeding of eelgrass in tank systems is not possible from a logistical or cost perspective, possibly switch focus and resources to an alternative method such as assisted migration.
- If selective breeding is not effective at arriving at desired traits, maybe eelgrass plasticity or seed hardening is more important.
- Traits that potentially reduce fecundity in a future generation may be a deal-breaker. This was stated as a concern in a previous workshop for individuals from populations that were far apart and that were being crossed.

Mid-Term (5-8 years):

- If the local genetic pool is not supplying individuals with high thermal tolerance, it may be necessary to expand the geographic area from which selection is taking place.
- If blunt (mass selection) breeding approach does not prove effective, a more precision (quantitative) breeding approach may be necessary; however, a more precision approach is much more expensive and complicated to accomplish.

Long-Term (8-15 years):

• If the rate of decline accelerates or the current genetics of eelgrass are not enabling the selection of individuals capable of withstanding temperatures forecasted in the future, CRISPR may become a more appealing option.

Factors Impacting Implementation: Coordination Needs; Collaboration Opportunities

Immediate (now):

- Establish a collaborative organization to continue the momentum from these workshops.
- Develop common gardens across regions.
- Create standardized monitoring efforts to be used across regions, especially within common gardens.

Near Term (1-4 years):

 Certified seed programs in agriculture may be a valuable resource to learn from in terms of what makes it possible to move seeds between counties and states.

Factors Impacting Implementation: Regulatory Needs and Strategies

Immediate (now):

- Depends on the approach and spatial scale in terms of whom to contact; however, we need to start building the case with local regulators at the town, county, or state level to establish the legal framework for moving forward.
- Work on engaging local communities/stakeholders to help develop programs in support of these efforts.

Near Term (1-4 years):

 If experiments in the lab are successful at selecting for eelgrass plants with higher thermal tolerances, this could help change the minds of regulators to allow for the interstate movement of eelgrass genetic material.

Factor Impacting Implementation:

Strategic Communication

Immediate (now):

- Communicate the ecological and economic importance of eelgrass (e.g., nursery habitat, carbon sink, CO2 buffering, sediment stabilization).
- · Identify the goal (this applies to all groups).
- Present the outcomes of this workshop to others in the eelgrass community at conferences and other workshops to get further feedback and buy-in from the rest of the eelgrass community.

Near Term (1-4 years):

 Find a team to make a documentary that conveys the importance and plight of eelgrass to the general public. **Factors Impacting Implementation:** Social Equity, Environmental, Cultural, and Other Risks

Near Term (1-4 years):

- Understanding local genetics may help answer some of the questions related to targeting genotypes, as well as confirm that genetic diversity exists, to help clarify what is at risk for bringing in new genets (helps inform the risk).
- Consider preserving local genetics versus replacing them with transplants from farther away.

Mid-Term (5-8 years):

 Altering the genetic diversity and viability of eelgrass on a local level is a risk, but is likely outweighed by the severity of current eelgrass decline.

Factor Impacting Implementation: Funding Needs or Opportunities

Immediate (now):

• Starting and funding a collaborative group and coordinator is essential to continuing the momentum of this endeavor.

Near Term (1-4 years):

• Continue to fund and maintain a collaborative group to accomplish the goals of selective breeding, assisted migration, and environmental hardening.

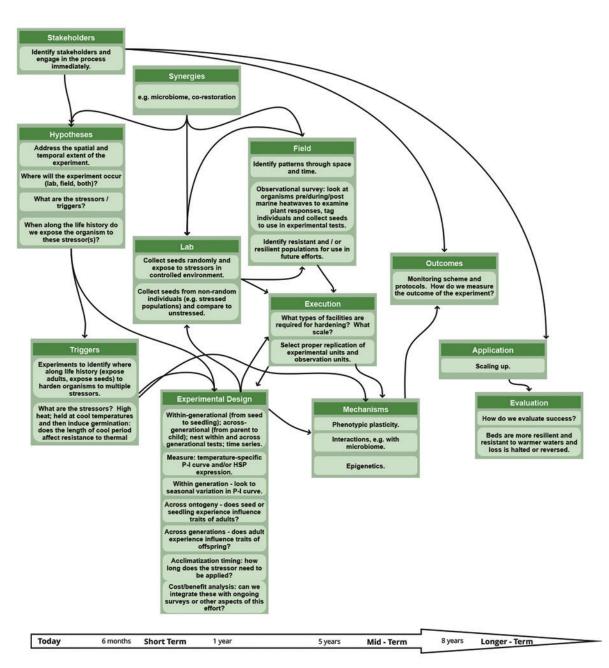
Mid-Term (5-8 years):

• Continue to fund and maintain a collaborative group to accomplish the goals of selective breeding, assisted migration, and environmental hardening.

Group D: Selective Breeding Selective Breeding | Conceptual Model

Group D - Non-Genetics: Thermal Hardening for Eelgrass Resiliency & Resistance to Thermal Stress We are here:

We are trying to get here:



Selective Breeding | Implementation Action Tasks Action and Considerations for Milestones of Conceptual Model

Factors Impacting Implementation: Knowledge Gaps, Research Questions, and Monitoring

Immediate (now):

- · Does thermal hardening work?
- · Acclimatization dynamics
- Triggers
- Seasonal/annual changes
 - ~ A plant in North Carolina may respond differently to changes in temperature through seasons and across years than one in Washington State. To what degree can that database can be broken down to inform disparate efforts geographically, or is that another knowledge gap?
- Longevity
 - ~ How long does hardening take to emerge?
 - ~ How long do the effects of hardening last?
- How predictive are lab experiments for Zostera field performance? Should we skip right to field hardening and mesocosms?
- Are there technical needs? For sediment stabilization, temperature sensing? Seed storage?
- Standardized monitoring
 - ~ Once experiments are designed, plan monitoring protocols to collect variables we want to use in standardized way.
- Discuss ways to collect and store the data. Name a group of people who will be responsible to analyze and socialize the data with other members of this group.
- · Establish key institutional framework to implement.
 - ~ Find easy ways to collect data (e.g., citizen science).
- · Evaluation to address the success of each effort.

Near Term (1-4 years):

- · Amount of phenotypic plasticity in thermal tolerance
 - ~ Use field observations seasonally, and also laboratory tests, to determine how much variation exists within an individual (or genotype) in the thermally specific P-I curve, especially compensation point at high temperatures. If there's not much flexibility, then acclimatization won't be able to help resistance / resilience.

- Analyze existing data
 - ~ Material and environmental legacies: is there actually a signal of climate shifting properties of seagrass ecosystems? Or any nearshore ecosystem?
 - ~ Is there a synthesis group to collate data on this topic?
 - ~ (Allcock et al. 2022)
- · Simulate/model outputs
 - ~ Use Jillian Dunic's model as a jumping-off point and parameterize with existing data to estimate success.
- · Learn from model plants
 - Take some time to consult with plant molecular biologists to learn the molecular pathways by which temperature is known to affect plant traits, such as flowering and photosynthesis. These may include the compounds expressed or circulating in the plant, or what's known at the level of epigenetic change on DNA.
- · Photosynthesis modeling
 - Compile environmental data and plant physiological data in the context of Dynamic Energy Budget or Grasslight (Zimmerman biooptical model, which includes photosynthesis and respiration as functions of T, light, CO₂).
 What parts of this relationship have leverage for increasing net photosynthesis at high T?
- · Evaluation to address the success of each effort

Mid-Term (5-8 years):

· Evaluation to address the success of each effort.

Long-Term (8-15 years):

· Evaluation to address the success of each effort.

Factor Impacting Implementation: Exit Ramps: When do we know that we should stop and pivot?

Immediate (now):

- Experiments don't show much plasticity in thermal tolerance.
- Conversation was more about adaptive management and shifts in direction, not actual exit ramps.

Factors Impacting Implementation: Coordination Needs; Collaboration Opportunities

Immediate (now):

- Better coordination between bioregions.
 - ~ ZEN
 - ~ NERRS
 - ~ NSF RCN
 - ~ Local communities, e.g., NGOs, citizen scientists (Audubon, Ducks Unlimited, CoastWatch)
 - ~ Need to engage regulatory agencies to move plants around.
- Synthesis group?
- Central Zostera coordination, then regional? Should this group support a central website and resource portal?
- Regular meetings and/or workshop with a small targeted group to plan and design specific experiments.

Factor Impacting Implementation: Regulatory Needs and Strategies

Immediate (now):

- · Local, state, and federal approvals
- Coordination among those, researchers, practitioners, and citizens
- Minimal challenges with hardening studies and deployment, potential difficulty with other environmental modification strategies

Factor Impacting Implementation: Strategic Communication Needs

Immediate (now):

- · Engage citizen groups.
 - ~ Where are we heading? Aspirations? How groups can be engaged?

- ~ Audubon, Ducks Unlimited, etc.
- ~ Getting the word out, starting a forum/engagement process.
- ~ Communicating urgency and importance.
- ~ Included linking up with elected officials.
- ~ Ensure outreach pegged to objects—need to consider equity and access.
- ~ Communications: What systems do we need to tie into? Food, coastal restoration, protected lands?
- Collin Timm: During Covid, we were challenged to work with high school interns. We piloted a project where we sent tomato seeds, grow lights, and materials and asked students to choose a stressor to study. It was a great success for a virtual internship, and they learned some scientific methods along the way.

Factor Impacting Implementation: Funding Needs or Opportunities

Immediate (now):

- NERRS/NSF/NSF Earth Cube as possible funding sources
- · Coordination for funding
- Education and outreach grants, STEM, for citizen science components
- Find donors, application for grants, alternatives for funding

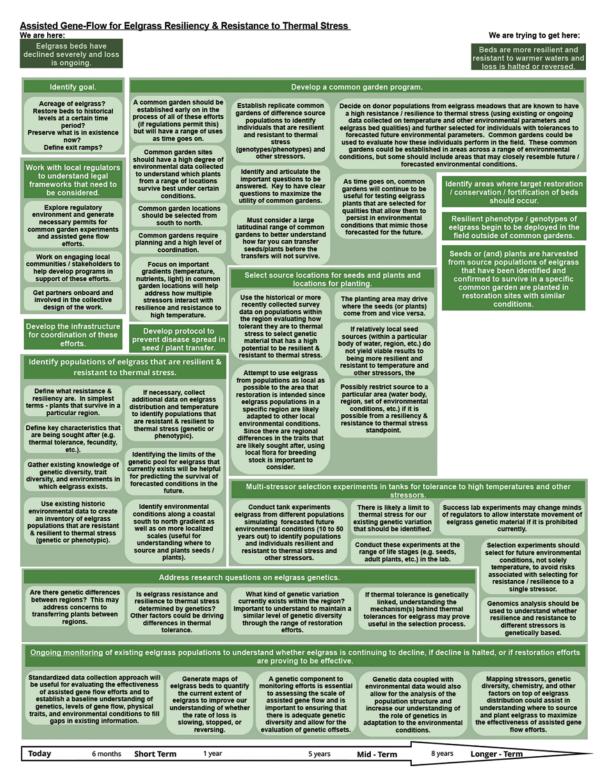
Near Term (1-4 years):

- Establish ways to become self-sustaining
- Avoid depending on getting grants for the continuity of the program

ISBW Conference Conceptual Models

At the World Seagrass Conference & International Seagrass Biology Workshop (ISBW) in Annapolis, Maryland (August 7–12, 2022), a broader community of eelgrass experts provided feedback and further explored the promising pathways from the June Eelgrass Restoration Workshop.

Selective Breeding Conceptual Model



Selective Breeding

Selective Breeding for Eelgrass Resiliency & Resistance to Thermal Stress

Identify goal. Acreage of eelgrass? Restore beds to historical levels at a certain time period? Preserve what is in existence now?	Logistical and cost feasibility assessments of	Develop colective		loss is halted or reversed
Define exit ramps? Define what you want to put into the program and define what you want to get out of the program in terms of genetics and phenotypes. Work with local regulators to understand legal frameworks that need to be considered. Look to certified seed programs in agriculture that make it possible to move seeds between counties and states. Work on engaging local communities / stakeholders to help develop programs in support of these efforts. Successful lab experiments may change mind of regulators to allow interstate movement of elgrass genetic material.	rearing eelgrass plants in captivity for selection. Determine if it logistically possible to get eelgrass to flower in flowing seawater tanks and generate seeds to selectively breed eelgrass plants and release into wild. Tank experiment to evaluate if transplanted seeds can be cultivated and tank array. Tank experiments to evaluate if eelgrass plants will flower, can be cross-polinated, and brought to seed in a tank system. Evaluate if this Is feasible from a cost-perspective. Address research questions on eelgrass genetics. Are there genetic differences between regions? This may address concerns to transferring plants between	Develop selective breeding approach. A decision should be made as to the breeding approach method (precision (quantitative) vs. blunt (mass selection) breeding approach. Positives and negatives of both approach. Positives and negatives of both approach first because if it proves to be effective the procession (quantitative) approach first because if it proves to be effective the proproach may be unnecessary. Metrics to assess efficacy of selection process on eelgrass performance (e.g. thermal performance (e	Selection experiments in tanks for thermal tolerance and other stressors. Conduct tank experiments on founder population simulating forecasted future environmental conditions (10 to 50 years out) to identify individuals resilient and resistant to thermal stress. Start selecting for individuals that do the best for the farthest forecasted conditions that will survive. There is likely a limit to thermal stress for our existing genetic variation that should be identified. Conduct these experiments at the range of life stages (e.g. seeds, adult plants, etc.) in the lab. Success lab experiments may change minds of regulators to allow interstate movement of eeigrass genetic material if it is prohibited currently. Selection experiments should select for future environmental conditions, not solely temperature, to avoid risks associated with	Resilient phenotype / genotypes of eelgrass begin to be deployed in the field outside of common gardens. Product is scaled least is major estarition conservation / fortification of beds you want to occur. Identify areas where target restoration / fortification of beds you want to occur. Product estuarition restoration to be increas in follow years tig facilitation eelgrast meadow
Identify populations of eelgrass that are resilient & resistant to thermal stress. Define what resistance & resiliency are. In simplest terms - plants that survive in a particular region. Define key characteristics that are being sought after (e.g. thermal tolerance, fecundity, etc.). Use existing historic environmental data to create an inventory of eelgrass	understand to maintain a Is eelgrass resistance and resilience to thermal stress determined by genetics? Other factors could be genetics? Other factors could be themail If thermal tolerance is genetically linked, understanding the mechanism(s) behind thermal tolerances for eelgrass may prove useful in the selection process. Develop protocol to prevent disease spread in seed / plant transfer.	survey data on populations within the region evaluating how tolerant they are to thermal stress to select genetic material that has a hich potential to P	selecting for resistance / resilience to one stressor. Founder population iden tempt to use eelgrass from popu s possible to the area that restorat ince eelgrass populations in a spe likely adapted to other local env onditions. Since there are regiona the traits that are likely sought aft lora for breeding stock is importation ossibly restrict source to a particu- ossibly restrict source to a particu- it is possible from a resiliency & thermal stress standpol	lations as local tion is intended crific region are irricular body of wate region, etc.) do not yiel viable results to being more resillent and resistant to temperatur and other stressors, th geographic range of candidate populations w noned to be expanded.
populations that are resistant & resilient to thermal stress (genetic or phenotypic). If necessary, collect additional data on eelgrass distribution and temperature to identify populations that are resistant & resilient to thermal stress (genetic or phenotypic). Identifying the limits of the genetic pool for eelgrass that currently exists will be helpful for predicting the survival of forecasted conditions in the future. Develop the infrastructure for coordination of these efforts.	of all of these efforts (if regulations permit this) but will have a range of uses as time goes on.	After first identifying populations of eeigrass that are resilient & ha sistant to thermal stress, ommon gardens could be neu used to further select out of individuals from those populations for plants that have a high olerance and resilience to high temperatures. This could aid in the establishment and identification of founder	Once a founder program. Once a founder population was idd rom eeigrass meadows that are kn ve a high resistance / resilience to stress (using existing or ongoing collected on temperature and o with tolerances to forecasted fur with tolerances to forecasted fur vironmental parameters, common could be used to evaluate how to individuals perform in the field. toommon gardens could be establi- tareas across a range of environn onditions, but some should inclue / at may closely resemble future / fo environmental conditions.	own to beformal data data data data dividuals ture gardens plants that are selected for persist in environmental conditions that mimic those forecasted for the future. These shed in ental e areas
Ongoing monitoring of ex	It may also be useful to incor efforts, especially in areas wh	o understand whether eelgra: are proving to be eff porate a genetic component into ere any assisted gene flow or sel ire that there is adequate genetic	ective. monitoring ectively bred	decline is halted, or if restoration effor

References

Workshop Pre-reads

- Dunic et al. 2020. Long-term declines and recovery of meadow area across the world's seagrass bioregions.
- Gaitlan-Espitia & Hobday. 2020. Evolutionary principles and genetic considerations for guiding conservation interventions under climate change.
- Kardos & Shafer. 2018. The peril of gene-targeted conservation.
- Van Katwijk et al. 2021. Rewilding the sea with domesticated seagrass.

Workshop Brief References

- Carr, J., et al. 2012. <u>Modeling the effects of climate change on eelgrass stability and resilience: future scenarios and leading indicators of collapse</u>. *Marine Ecology Progress Series* 448: 289–301.
- Glemarec, M., et al. 1997. Long-term changes of seagrass bed in the Glenan Archipelago (South Brittany).
 Oceanologica Acta 20(1): 217–227.
- Hammer, K.J., et al. 2018. <u>High temperatures cause reduced growth, plant death and metabolic changes in eel-</u> grass *Zostera marina*. *Marine Ecology Progress Series* 604: 121–132.
- Kaldy, J.E. 2014. Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. Journal of Experimental Marine Biology and Ecology 453: 108–115.
- Marsh, J.A., et al. 1986. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *Journal of Experimental Marine Biology and Ecology* 101(3): 257–267.
- Moore, K.A., and J.C. Jarvis. 2008. <u>Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: implications for long-term persistence</u>. *Journal of Coastal Research* 55: 135–147.
- Moore, K.A., et al. 2014. <u>Impacts of varying estuarine temperature and light conditions on Zostera marina (eelgrass)</u> and its interactions with *Ruppia maritima* (widgeongrass). *Estuaries and Coasts* 37(S1): 20–30.
- Nelleman, C., et al. 2008. Blue carbon: The role of healthy oceans in binding carbon. UNEP/FAO/UNESCO/IUCN/CSIS.
- Niu, S., et al. 2012. <u>The effect of temperature on the survival, growth, photosynthesis, and respiration of young</u> seedlings of eelgrass *Zostera marina* L. *Aquaculture* 350–353: 98–108.
- Ondiviela, B., et al. 2014. <u>The role of seagrasses in coastal protection in a changing climate</u>. *Coastal Engineering* 87: 158–168.
- Pazzaglia, J., et al. 2022. <u>Thermo-priming increases heat-stress tolerance in seedlings of the Mediterranean</u> <u>seagrass *P. oceanica. Marine Pollution Bulletin* 174: 113164.</u>
- Sandoval-Gil, J., et al. 2016. <u>Nitrogen uptake and internal recycling in *Zostera marina* exposed to oyster farming: <u>Eelgrass potential as a natural biofilter</u>. *Estuaries and Coasts* 39(6): 1694–1708.</u>
- Sievers, M., et al. 2019. <u>The role of vegetated coastal wetlands for marine megafauna conservation</u>. *Trends in Ecology & Evolution* 34(9): 807–817.
- Unsworth, R.K., et al. 2019. <u>Seagrass meadows support global fisheries production</u>. *Conservation Letters* 12(1): e12566.

Session 1 References

Slide Deck

Zoom Chat References

- Lau, J.A., & Lennon, J.T. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences of the United States of America* 109(35): <u>14058–14062. https://doi.org/10.1073/pnas</u> <u>1202319109 https://pubmed.ncbi.nlm.nih.gov/22891306/</u>
- ~ Pennisi, E. "World's Largest Organism Found in Australia." <u>https://www.science.org/content/article/world-s-largest-organism-found-australia</u>
- ~ Hagedorn, M., C.A. Page, K.L. O'Neil, D.M. Flores, L. Tichy, T. Conn, V.F. Chamberland, C. Lager, N. Zuchowicz, K. Lohr, H. Blackburn, T. Vardi, J. Moore, T. Moore, I.B. Baums, M.J.A. Vermeij, and K.L. Marhaver. <u>Assisted</u> <u>gene flow using cryopreserved sperm in critically endangered coral</u>.
- ~ Scholz, V.V., B.C. Martin, R. Meyer, et al. 2021. <u>Cable bacteria at oxygen-releasing roots of aquatic plants: a</u> <u>widespread and diverse plant-microbe association</u>. *The New Phytologist* 232(5): 2138–2151. DOI: 10.1111/ nph.17415. PMID: 33891715; PMCID: PMC8596878.
- ~ Unzueta-Martínez ,A., H. Welch, and J.L. Bowen. 2022. Determining the composition of resident and transient members of the oyster microbiome. *Frontiers in Microbiology* 12:828692. doi: 10.3389/fmicb.2021.828692
- ~ Sogin, E.M., D. Michellod, H.R. Gruber-Vodicka, et al. <u>Sugars dominate the seagrass rhizosphere</u>. *Nature Ecology and Evolution* 6: 866–877. https://doi.org/10.1038/s41559-022-01740-z

Session 2 References

- <u>Slide Deck</u>
- Flip Charts
 - ~ <u>Group A Identify Resilient Populations/Genotypes</u>
 - ~ Group B Increasing Genetic Diversity/Assisted Gene Flow
 - ~ Group C Selective Breeding/Hybridization/Artificial Selection
 - ~ Group D Catch-all
- Zoom Chat References
 - ~ https://drive.google.com/file/d/1LtRNMEJHnu-gJ87NhMBpgx1K6GEpUzl6/view?usp=sharing
 - ~ Wood, M.A., R.N. Lipcius. 2022. Non-native red alga *Gracilaria vermiculophylla* compensates for seagrass loss as blue crab nursery habitat in the emerging Chesapeake Bay ecosystem. *PLoS ONE* 17(5): e0267880. <u>https://doi.org/10.1371/journal.pone.0267880</u>

Session 3 References

- <u>Slide Deck</u>
- Miro Boards
 - ~ Group A Assisted Gene Flow
 - ~ Group B Assisted Gene Flow
 - ~ Group C Selective Breeding
 - ~ Group D Thermal Hardening
- Zoom Chat References
 - ~ Shaver, E.C., E. McLeod, M.Y. Hein, S.R. Palumbi, K. Quigley, T. Vardi, P.J. Mumby, D. Smith, P. Montoya-Maya, E.M. Muller, A.T. Banaszak, I.M. McLeod, and D. Wachenfeld. 2022. A roadmap to integrating resilience into the practice of coral reef restoration. *Global Change Biology* 28: 4751–4764. <u>https://doi.org/10.1111/gcb.16212</u>
 - ~ <u>https://www.chesapeakelegal.org/guides-resources/report-existing-chesapeake-bay-watershed-statutes-and-re-gulations-affecting-submerged-aquatic-vegetation/</u>
 - ~ http://www.asmfc.org/files/Habitat/HMS_MgmtSeries15_SAV_PolicyUpdate_Winter2022.pdf

