### 1. Applicant and Proposal Information Summary Sheet

<table>
<thead>
<tr>
<th>Council Member:</th>
<th>State of Alabama</th>
<th>Point of Contact:</th>
<th>Hank Burch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phone:</td>
<td>(251) 625-0814</td>
<td>Email:</td>
<td><a href="mailto:Hank.Burch@dcnr.alabama.gov">Hank.Burch@dcnr.alabama.gov</a></td>
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</tbody>
</table>

### Project Identification

<table>
<thead>
<tr>
<th>Project Title:</th>
<th>Alabama Submerged Aquatic Vegetation Restoration and Monitoring Project</th>
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<tbody>
<tr>
<td>State(s):</td>
<td>AL</td>
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<tr>
<td>County/City/Region:</td>
<td>Mobile and Baldwin Counties, Alabama</td>
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<tr>
<td>General Location:</td>
<td>Projects must be located within the Gulf Coast Region as defined in RESTORE Act. (attach map or photos, if applicable)</td>
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### Mobile and Baldwin Counties, Alabama

#### Project Description

**RESTORE Goals:** Identify all RESTORE Act goals this project supports. Place a _P_ for Primary Goal, and _S_ for secondary goals.

- _P_ Restore and Conserve Habitat
- _S_ Replenish and Protect Living Coastal and Marine Resources
- _S_ Replenish and Protect Living Coastal and Marine Resources
- _S_ Enhance Community Resilience
- __ Restore and Revitalize the Gulf Economy

**RESTORE Objectives:** Identify all RESTORE Act objectives this project supports. Place a _P_ for Primary Objective, and _S_ for secondary objectives.

- _P_ Restore, Enhance, and Protect Habitats
- _S_ Promote Community Resilience
- _S_ Promote Natural Resource Stewardship and Environmental Education
- _S_ Improve Science-Based Decision-Making Processes

- _S_ Restore Water Quality
- _P_ Restore and Restore Living Coastal and Marine Resources
- _S_ Protect and Restore Living Coastal and Marine Resources
- _S_ Protect and Restore Living Coastal and Marine Resources
- __ Restore and Enhance Natural Processes and Shorelines

**RESTORE Priorities:** Identify all RESTORE Act priorities that this project supports.

- √ Priority 1: Projects that are projected to make the greatest contribution …
- __ Priority 2: Large-scale projects and programs that are projected to substantially contribute to restoring …
- √ Priority 3: Projects contained in existing Gulf Coast State comprehensive plans for the restoration …
- √ Priority 4: Projects that restore long-term resiliency of the natural resources, ecosystems, fisheries …

**RESTORE Commitments:** Identify all RESTORE Comprehensive Plan commitments that this project supports.

- √ Commitment to Science-based Decision Making
- √ Commitment to Regional Ecosystem-based Approach to Restoration
- √ Commitment to Engagement, Inclusion, and Transparency
- √ Commitment to Leverage Resources and Partnerships
- √ Commitment to Delivering Results and Measuring Impacts

**RESTORE Proposal Type and Phases:** Please identify which type and phase best suits this proposal.

- _X_ Project
- _X_ Planning
- _X_ Technical Assistance
- _X_ Implementation
- __ Program

### Project Cost and Duration

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<th>Project Cost Estimate:</th>
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<th>Project Timing Estimate:</th>
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<td>Date Anticipated to Start: 06/15, or when awarded</td>
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<tr>
<td>Time to Completion: 5-7 years</td>
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<td>Anticipated Project Lifespan: 5-7 years</td>
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II. Executive Summary

Submerged Aquatic Vegetation (SAV or seagrass) is an important natural resource in freshwater, brackish water, and marine ecosystems that provides food for waterfowl and other animals, as well as habitat for ecologically and economically important species (Manlove et al., 2002). It is estimated that over 70% of recreationally and commercially important fish and invertebrates in the Gulf of Mexico spend some portion of their lives in seagrass systems in near shore subtidal and intertidal zones (Florida Fish and Wildlife Commission, 2003). SAV has also been widely recognized for its importance in the sustainability and ecological function of aquatic habitats (e.g. Kenworthy et al., 1988; Fredette et al., 1990).

Growth and extent of SAV is highly dynamic and can be affected by climate (e.g., tropical events, drought), hydrological processes (e.g., currents and turbidity), and anthropogenic activities (e.g., increased nutrient runoff, sedimentation, boating impacts). Gulf wide, there has been a documented downward trend in the extent of SAV over the last 50 years, with most estuaries showing loss rates ranging from 20% to 100% (Handley 1995). While improvements in water quality in recent decades have allowed SAVs to rebound in certain areas, restoration and protection efforts are still needed.

Because of the dynamic nature of SAV, the cost of aerial imagery collection and the dependence of such aerial data collection on achieving the appropriate range of environmental parameters (e.g., low turbidity and minimal cloud cover during peak growing season), access to consistently collected, large scale data covering a long time frame is limited. As such, status and trends information is also quite limited. In coastal Alabama, there have been several recent SAV mapping and monitoring efforts, including surveys conducted in 1980, 1987, 1994, 2001, 2008 and 2009 (Vittor and Assoc, 2004 & 2009). Additionally, the Mobile Bay NEP conducted a historical analysis of SAV coverage in Mobile Bay based on 1940’s imagery and current mapping data (Vittor and Assoc., 2005). These studies and mapping efforts show wide scale losses in SAV coverage in Mobile Bay, especially along the eastern and western shorelines of Mobile Bay. However, the data also indicates large fluctuations in SAV coverage along upper Mobile Bay and Mississippi Sound over relatively short time spans. Further, an increase in sea grass coverage in lower Perdido Bay over the last 10 year has been noted through interpretation of recent imagery and SAV mapping data. Zolczynski and Shearer (1997) noted that many gains in SAV in upper Mobile Bay and the lower Mobile-Tensaw River Delta are the result of rampant growth of exotic species (e.g., Eurasian watermilfoil and hydrilla).

The Alabama Submerged Aquatic Vegetation Restoration and Monitoring Project seeks to utilize RESTORE Act funds to further the State’s comprehensive efforts to sustain and restore SAV in coastal Alabama. These efforts advance the goals and objectives of the Comprehensive Conservation and Management Plan (CCMP) of the Mobile Bay National Estuary Program (MBNEP 2014) and supports ongoing efforts of the NOAA funded Alabama Coastal Zone Management Program. While the proposed projects are targeted to the State of Alabama, SAV losses are documented throughout the Gulf (Handley, 1995). Alabama proposes the development of a model SAV restoration and monitoring program that is foundational in nature and can be applied Gulf wide.
Implementation: This project would be implemented by the Alabama Department of Conservation and Natural Resources (ADCNR) in partnership with the Dauphin Island Sea Lab (DISL), Mobile Bay National Estuary Program (MBNEP), The City of Orange Beach and The Nature Conservancy (TNC). The proposed timeframe of 5 years builds upon over 30 years of effort to measure and monitor SAV in coastal Alabama. Specific project components include:

- **Lower Perdido Bay Sea Grass Protection and Restoration:**
  Lower Perdido Bay contains some of Alabama’s most extensive seagrass beds, but it is also one of the most popular areas for recreational boating along the coast. In the last decade, the State has implemented no wake zones around the grassbeds and a 2006 Gulf of Mexico Program Community Based Restoration Grant (GOMF-CRP 2006) supported prop scar restoration efforts and education outreach in the area. RESTORE funds will be used to add additional bird stakes to the area, which are a proven method for prop scar restoration (Kenworthy et al., 2000). Additional navigational signage advising the public of the boundaries of no-motor zones and the presence of sea grasses will also be placed. Educational signage describing the importance of SAVs will also be placed at strategic locations around the adjacent waterways and educational brochures will be printed and distributed.

- **Upper Mobile Bay and the lower Mobile/Tensaw River Delta SAV Restoration Project:**
  SAV seeds (particularly *Vallisneria sp.*) will be collected in order to be sown in areas known to historically have *Vallisneria* present (based on 1994 and 2002 mapping) but which have not recovered since tropical storm events and drought of 2004-2007. Seeds may also be utilized to grow SAV plugs for planting in such areas.

- **Submerged Aquatic Vegetation Monitoring Program:**
  It has been five years since the last comprehensive mapping of SAV in coastal Alabama. In 2014, the National Fish and Wildlife Foundation announced that Gulf Environmental Benefit Funds will be awarded to update SAV maps in Alabama. Alabama seeks to leverage this effort to collect 2 additional SAV measurements over the next 5-7 years, each 2-3 years apart. In each case, aerial imagery will be analyzed to determine SAV coverage and create a GIS shapefile of SAV coverage, with species composition noted for each polygon. Imagery acquisition, imagery analysis and field work to ground-truth SAV species and coverage will be conducted in accordance with NOAA protocols in order to maintain data consistency between collections. A report on SAV coverage, species composition and status & trends will be developed from each monitoring event.

Uncertainties and risks associated with the project or program

The ADCNR and its project partners have experience in seagrass restoration, seed collection, and monitoring. *Vallisneria* seed collection and sowing projects have been successful in other locations. The ADCNR recently funded a pilot *Vallisneria* seed collection project conducted by the DISL which was successful at germinating collected seeds in a controlled setting, and there is a viable, readily available seed source in the *Vallisneria* beds of upper Mobile Bay. Finally, the ADCNR and the MBNEP have successfully completed SAV mapping projects in 2001, 2008 and 2009. Based on these factors, the uncertainties and risks associated with this project are relatively low.
III. Proposal Narrative

Introduction and Background
The Alabama Submerged Aquatic Vegetation Restoration and Monitoring Project will protect and restore submerged aquatic vegetation (SAV) resources in coastal Alabama. This project is part of a larger effort being undertaken in Alabama and around the Gulf to protect and restore critically important SAV resources. Based on available data and existing literature, the Gulf of Mexico and adjoining estuaries have experienced widespread SAV losses compared to historical acreages. While improvements in water quality in recent decades have allowed SAVs to rebound in certain areas, restoration and protection efforts are still needed.

In coastal Alabama, SAV mapping efforts were conducted in 1980, 1987, 1994, 2001, 2008 and 2009 (Vittor and Assoc., 2004 & 2009). Additionally, the Mobile Bay NEP conducted a historical analysis of SAV coverage in Mobile based on 1940’s imagery and current mapping data (Vittor and Assoc., 2005). These studies and mapping efforts show wide scale losses in SAV coverage in Mobile Bay, especially along the eastern and western shorelines of Mobile Bay. However, the data also indicates large fluctuations in SAV coverage along upper Mobile Bay and Mississippi Sound over relatively short time spans. Further, an increase in sea grass coverage in lower Perdido Bay over the last 20 year has been noted through interpretation of recent imagery and SAV mapping data.

Implementation
This project will be implemented by the Alabama Department of Conservation and Natural Resources in partnership with the Dauphin Island Sea Lab (DISL), Mobile Bay National Estuary Program (MBNEP), The City of Orange Beach and The Nature Conservancy (TNC). In order to address SAV losses in coastal Alabama and to help gain a further understanding of SAV status, trends and causes of the apparent fluctuations in SAV coverage, the Alabama Submerged Aquatic Vegetation Restoration and Monitoring Project proposes three efforts: 1) implement restoration efforts in Lower Perdido Bay, Baldwin County, AL; 2) Conduct seed collection and sowing in Upper Mobile Bay and Lower Mobile-Tensaw Delta waters; and 3) continue and sustain the Alabama coastal SAV monitoring efforts that date back to 1980. Each of these efforts is described in detail below.

Lower Perdido Bay Sea Grass Protection and Restoration:
Lower Perdido Bay has the most extensive acreage of homogeneous beds of shoal grass (*Halodule wrightii*) in coastal Alabama. These beds can be found along the shorelines of Cotton Bayou, Robinson Island, Walker Island, Ono Island and on the surrounding shallow shoals. As noted above, mapping data and aerial imagery interpretation points to an overall increase in coverage of shoal grass in lower Perdido Bay in the last 10 years. However, there have been impacts to these beds, mainly from prop-scarring by motorized vessels.

In response to these impacts, the ADCNR partnered with the City of Orange Beach, TNC and DISL to implement a seagrass protection and restoration project in lower Perdido Bay (Gulf of Mexico Foundation 2006). As part of this project, bird stakes, a proven method for prop scar
restoration (Kenworthy et al., 2000), were placed in prop-scars, facilitating SAV recovery.

Additionally, certain areas have been designated as “No Motor” zones. In these zones, which are marked by navigational signage advising the public of the boundaries of no-motor zones and the presence of sea grasses, the use of internal combustion engines is not permitted. Boaters may enter the zones, but only under trolling motor power or by use of paddles, poling, etc. These restrictions are enforced by the Alabama Marine Police. Field observation indicates that these efforts have been successful in reducing the amount of prop scarring in the designated zones and that previously existing prop scars have healed. However, as with any signage placed on open waters, navigation signs associated with the zones are occasionally lost to storms, vandalism and other factors. Additionally, even with the signage in place, prop-scarring still occasionally occurs.

In order to address these ongoing issues, Alabama proposes the use of RESTORE Act funds to accomplish annual maintenance and replacement of zone signage as well as the installation of new bird stakes in prop scars as they are identified. Further, educational signage describing the importance of SAV will be placed at strategic locations around the adjacent waterways, including boat launches in the area. An education brochure previously developed by TNC, DISL and the City of Orange Beach will also be re-printed annually for distribution to tourist and residents at varying venues around the City.

Both DISL and TNC currently hold USACE Nationwide Permits for this type of activity in this area. Additionally, this restoration project is considered a Permissible Use under Alabama Coastal Area Management Program. As such, implementation funding is requested.

**Upper Mobile Bay and the lower Mobile/Tensaw River Delta SAV Restoration Project:**
SAV mapping conducted by the MBNEP and the ADCNR in 2002 and 2009 indicates that during the interval between the two mapping events, the acreage of SAVs on upper Mobile Bay and the lower Mobile/Tensaw River Delta (Delta) was significantly reduced. While the cause of this reduction cannot be positively determined, the loss appears to be linked to the active tropical storm seasons of 2004-2005 and the drought conditions that followed in subsequent years.

However, since a return to more normal rain patterns, accompanied by a lack of tropical storm events, field observations indicate that SAV coverage has significantly increased in most areas of the Upper Bay and Delta. That said, some areas are very slow to recover and/or show little signs of recovery at all. A prime example of this is Grand Bay in the Delta, which historically had over 1,000 acres of mixed SAV beds. Recent aerial imagery indicates that there is still less than 100 acres of SAV in Grand Bay. It is believed that the primary reason for this lack of recovery is a lack of seed source.

In response to a concern of the lack of SAV recovery in some areas, during fiscal year 2011, the ADCNR provided funds to DISL to investigate the feasibility of gathering and sowing *Vallisneria* seeds in order to speed the recovery of SAV beds following large scale losses like those seen during 2004-07. During this study, the DISL determined that there is a large seed source in the...
Vallisneria beds of the upper Mobile Bay and that that these seeds are viable. Additionally, literature reviewed showed that this technique has been successfully used in other areas of the southeastern US (Dauphin Island Sea Lab 2012, unpublished; See full report in Section IX-Appendices).

In order to speed the recovery of areas like Grand Bay, the ADCNR, in partnership with the DISL, is proposing to gather SAV seeds (Vallisneria sp.) during the late summer. These seeds will then be stored and over-wintered in the laboratory. In the early spring, the seed will then be sown in marked plots in areas known to historically have Vallisneria present (based on 2002 mapping) but which have not since recovered. These plots will then be monitored to determine project success. Seeds may also be utilized to grow SAV plugs for planting in such areas. The ADCNR proposes to conduct this effort each year for 5 years.

In Alabama, the gathering of SAV seeds in a non-destructive manner is not subject to regulatory requirements. Additionally, this restoration project is considered a Permissible Use under Alabama Coastal Area Management Program. No USACE permit is required for seed gathering or sowing. Seeding plots will be marked in a non-permanent manner using biodegradable natural materials, which will not require USACE permits.

**Submerged Aquatic Vegetation Monitoring Program:**

As mentioned in the executive summary, it has been 5 years since Alabama’s SAV mapping has been updated. In 2014, the National Fish and Wildlife Foundation (NFWF) announced that funds from the Deepwater Horizon Gulf Environmental Benefit Fund would be awarded to the State of Alabama to conduct extensive habitat mapping throughout Coastal Alabama, to explicitly include updates to SAV maps. Given the dynamic nature of SAV, such mapping would ideally be updated every 2-3 years to provide better insight on status and trends. A unique opportunity exists for the RESTORE Council to leverage funds with efforts undertaken by NFWF. As such, Alabama seeks RESTORE funds to complete 2 additional SAV mapping cycles by 2024.

This component will monitor the coverage and species composition of submerged aquatic vegetation (SAV) in Coastal Alabama. This data will provide critical status and trends information to help determine the aerial extent and species composition of SAV in coastal Alabama, as well as changes over time. It will also help inform decisions on SAV restoration, protection and regulatory and policy changes. This data will also help inform regional resources managers on the status and trends in SAV coverage of the Gulf of Mexico as a whole.

Aerial digital ortho-imagery of coastal waters will be obtained on a regular interval, approximately every 2-3 years, depending on suitability of water conditions, weather, and tides during the SAV growing season (July-September). Aerial imagery will be analyzed to determine SAV coverage and create a GIS shapefile of coverage, with species composition noted for each polygon. Imagery acquisition, imagery analysis and field work to ground-truth SAV species and coverage will be conducted in accordance with NOAA protocols. A report on SAV coverage, species composition and status & trends will be developed from each monitoring event. Two (2) sampling events are proposed, which will be conducted at an interval of 2-3 years.
depending on water clarity, tides and meteorological conditions during aerial imagery acquisitions window. SAV monitoring and mapping is not subject to any permitting or regulatory requirements.

The ADCNR will issue a request for proposals for professional serviced to conduct this portion of the project. A Scope of Service and a contractual agreement will be drafted and executed between the ADCNR and the selected qualified contractor. The ADCNR will monitor the contractor to insure project deliverables are completed in accordance with contractual requirements. ADCNR’s Coastal Section, which administers the NOAA-funded Coastal Zone Management Program in Alabama, has provided funding and oversight for past SAV mapping efforts.

**Monitoring and Measures of Success**

A successful program will result in the implementation of SAV restoration and protection measures in Coastal Alabama and a corresponding increase in SAV coverage in restored areas. SAV mapping will acquire vital SAV status and trends data, allowing further investigation to the factors that influence historic SAV loss as well as observed fluctuations in SAV coverage. Additionally, mapping will provide critical SAV extent and species composition data for resource managers and regulatory agencies, better informing regulatory decisions, future restoration efforts and the need for future SAV mapping, protection and restoration efforts.

**Uncertainties and risks associated with the project or program**

The ADCNR and its project partners have successfully implemented a seagrass protection and restoration project in lower Perdido Bay. The proposed lower Perdido Bay project will build on these efforts. Additionally, *Vallisneria* seed collection and sowing projects have been successful in other locations. The ADCNR recently funded a pilot *Vallisneria* seed collection project conducted by the DISL which was successful at germinating collected seeds in a controlled setting. Additionally, it appears that there is a viable readily available seed source in the *Vallisneria* beds of upper Mobile Bay. Finally, the ADCNR and the MBNEP have successfully completed SAV mapping projects in 2001, 2008 and 2009. Based on these factors, the uncertainties and risks associated with this project are relatively low.

**Proposal project/program benefits**

SAV restoration and protection will provide additional critical nursery habitats for many commercially and recreationally finfish and shellfish species and their prey items. SAV also serves to improve water clarity by reducing sediment re-suspension. Healthy SAV beds also provide recreational fishing and snorkeling opportunities, potentially improving the local economy.

While this proposal identifies projects specific to Alabama, every Gulf state faces loss of SAV habitat. The proposed effort could serve as a pilot for a coordinated, Gulf-wide effort to monitor and restore SAV resources.

**Outreach and education opportunities:**
As noted above, the Lower Perdido Bay project will include the placement of educational signage describing the importance of SAV at strategic locations around the adjacent waterways. Additionally, an educational brochure previously developed by TNC, DISL and the City of Orange Beach will be re-printed annually for distribution to tourist and residents at various venues around the coast.

Further, the ADCNR and its project partners will promote the importance of SAV at events such as the DISL Discovery Day, Earth Day Mobile Bay, Delta Woods and Waters Expo, National Shrimp Festival, at the DISL Estuarium, at TNC education and outreach events and in the City of Orange Beach’s Environmental Education Program. Existing outreach resources will be utilized (e.g., Mobile Bay National Estuary Program; Weeks Bay National Estuarine Research Reserve).

**Leveraging of resources and partnerships:**
This project will leverage off of previous efforts to map, restore and protect SAVs, as described above and will spring from an initial SAV mapping update to be conducted with funding from the NFWF Gulf Environmental Benefit Fund. Additionally, each of the project partners brings to the project a unique set of skills and technical expertise that will add value to the project as a whole.
IV. Location Information

Figure 1. Typical SAV Mapping Data on Aerial Imagery for the Upper Mobile Bay/Lower Mobile-Tensaw Delta. The raised bridge I-10 corridor runs east-west in the center of the image:
Figure 2. SAV Coverage Changes in Grand Bay showing extensive SAV loss since 1987. Grand Bay is in the southwest corner of the Mobile-Tensaw Delta immediately north of I-10.
Figure 3. Changes in SAV Coverage in Lower Perdido Bay, showing increase in SAV coverage between 2002 and 2009.
## V. High-Level Budget Narrative

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<th>Project Component</th>
<th>Yearly Funding</th>
<th>Total 5 Year Funding Request</th>
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<tr>
<td><strong>Lower Perdido Bay Seagrass Protection &amp; Restoration</strong>&lt;br&gt;Includes:&lt;br&gt;  - Replacement of Signage and Marker Buoys  &lt;br&gt;  - Bird Stake Placement in Prop Scars  &lt;br&gt;  - Printing of Educational Brochures  &lt;br&gt;  - Placement of Education Signage</td>
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### VI. Environmental Compliance Checklist

**Gulf Coast Ecosystem Restoration Council**  
**Environmental Compliance Checklist**

Please check all federal and state environmental compliance and permit requirements as appropriate to the proposed project/program.

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VII. Data / Information sharing plan

a. Types of environmental data and information that will be created:
The Alabama SAV Restoration and Monitoring Project will collect the following types of data during the course of the project:
- Digital Aerial Ortho-imagery (2 collections over 5 years)
- Mapping of SAV extent and species composition
- Restoration project monitoring data

b. Standards to be used for data/metadata format and content:
Aerial imagery and SAV mapping data will be collected in a manner consistent with previous collections so that datasets can be compared for status and trends analysis. Geospatial data and metadata will adhere to the standards of Federal Geographic Data Committee (FGDC) or the current federally mandated metadata format.

c. Policies addressing data stewardship and preservation:
All data generated by this project will be stored by the ADCNR in accordance with applicable record and data retention policies.

d. Procedures for providing access, sharing, and security:
All data generated by this program will be made available through the DISL, ADCNR, TNC and other project partners. Data are available to the public upon request. Where feasible, data will be made available through existing online portals. Updates on project status as well as data gathered will be posted on ADCNR's [www.alabamacoastalrestoration.org](http://www.alabamacoastalrestoration.org) website. Where feasible, data gathered will be made available through existing online portals at ADCNR or with partnering agencies and, even where not feasible, all data gathered will be available to the public upon request.
VIII. Reference list of literature cited in the proposal


Mobile Bay National Estuary Program. 2013. Respect the Connect. Comprehensive

IX. Other Documents (if applicable)

Appendix I. Photos: The following photos are included to provide additional background information on previous efforts to preserve and restore SAV in the Lower Perdido Bay area.

Photo 1. Typical No-Motor Sign used to alert boaters to the presence of grassbeds. These are enforced by Alabama Marine Police

Photo 2. Typical Education and Outreach Signs
Photo 3. Example of bird stakes placed in existing prop scars

**Appendix II.** Copy of Dauphin Island Sea Lab 2012 Report to ADCNR: SAV Restoration, Coastal Alabama & Northern Gulf of Mexico. As referenced in this proposal, this unpublished report describes DISL’s recent success with seed collection and dispersal of Vallisneria. 18pp.
Final Report to  
Alabama Department of Conservation and Natural Resources:  
State Lands Division

Project Title:  
Dauphin Island Sea Lab- SAV Restoration, Coastal Alabama & Northern Gulf of Mexico

Contract Number: DISL-CZM-309-11-1

Principal Investigator(s):  
Dr. Ken L. Heck Jr., Dauphin Island Sea Lab (DISL) and USA

Project period:  October 1, 2010 - March 15, 2012

Funding for this project provided by the Alabama Department of Conservation and Natural Resources, State Lands Division, Coastal Section, in part, by a grant from the National Oceanic and Atmospheric Administration, Office of Ocean and Coastal Resource Management, Award # 10NOS4190206.

Introduction:

The economic significance of submerged aquatic vegetation (SAV) habitat is great, owing to their highly valued ecosystem services (nearly $19,000/hectare/year, according to Costanza et al. 1997), and their importance as nursery areas for a wide variety of valuable finfish, shrimp and crabs (Heck et al. 2003). Many factors, both natural and anthropological, contribute to SAV decline, including the addition of wastewater and excess nutrients to coastal waters, dredging, coastal construction and direct damage caused by boat propellers. Yet, despite the recognized importance of SAV meadows, we do not fully understand the specific suites of factors that sustain successful meadows in different geographical regions nor are we able to predict where losses may occur or to halt losses once they begin.

Globally, SAV is declining (Orth et al. 2006a), and losses in the Gulf of Mexico have occurred in every state, with areal declines ranging from 20-100% (Handley et al. 2006). A recent survey conducted by Vittor and Associates (2009) for the Mobile Bay National Estuary
Program (MB NEP) found an approximate 15% loss of SAV in upper Mobile Bay when compared with a 2002 survey. Coastal areas have also experienced local losses (e.g. Coffee Island acreage in 2008 versus 2009); although overall, Vittor and Associates (2009) found an increase in coastal SAV acreage in 2009 compared with the 2002 survey. Submerged aquatic vegetation in these areas are known for their high degree of temporal variability due to changing physical environmental conditions (e.g. wave-exposure, turbidity, freshwater outflow and hurricanes; Vittor and Associates, 2009; Byron and Heck, 2006) with losses and gains occurring frequently and without prediction.

Due to these large losses of SAV, coastal resource managers often look toward restoration projects to re-establish previously lost meadows in order to reclaim the ecosystem services these areas once provided. Once conditions become more favorable for SAV reintroduction, whole plant transplants are commonly used for restoration projects but often meet with limited success with many restored sites not surviving for long periods of time (Fonseca et. al. 1996). Many factors may interact to prevent successful restoration and we do not fully understand which factors are most important in promoting successful SAV restoration once conditions become favorable for natural reintroduction or restoration (eg. improvements in water clarity and other water quality conditions). Often times, restored areas cannot overcome the initial transplanting stress to expand and proliferate and result in restored sites not meeting their restoration goals. Furthermore, transplanting adult shoots has inherent problems as adult shoots must be taken from a donor seagrass bed, this can stress the donor bed and may result in loss of the donor habitat as well.

Restoration efforts of the temperate SAV species *Zostera marina* (eelgrass) in Chesapeake Bay have switched from transplanting adult plants to broadcast spreading of seeds,
which is less labor intensive and has seen greater success (Orth et al. 2006b). Here, we examine whether there exists sufficient reservoirs of viable SAV seeds that could be harvested with minimal damage to existing meadows, and, if so, whether restoration by seed planting is likely to be a viable strategy for restoring lost SAV acreage in the Northern GOM.

The two species of interest for Alabama’s waters are *Halodule wrightii* (shoalgrass), a relatively opportunistic mesohaline to polyhaline species which grows rapidly and can establish itself by seed and vegetative fragments (Hall et al. 2006) and *Vallisneria americana* (wild celery or tapegrass), the dominant species of SAV in oligohaline portions of estuaries in the northern Gulf of Mexico, including Mobile Bay.

*Halodule wrightii* is known to produce copious amounts of seeds in the Gulf of Mexico, with estimates as high as 3000 seeds m$^{-2}$ in Texas (McMillan 1981). Yet, to date, there is no study of which we are aware that has determined *H. wrightii* seed reserves in the northern Gulf. A recent paper documented that around 8% of shoalgrass core samples near Bayou La Batre, Alabama contained developing fruits (McGovern and Blankenhorn 2007), thereby providing evidence that sexual reproduction is occurring in local stands of *H. wrightii*. As *H. wrightii* seeds are produced the base of the shoot, they are likely to be buried in place as there is little opportunity for seed transport unless a significant physical event (e.g. a hurricane) disrupts the sediments. Additionally, seeds of *H. wrightii* have a significant dormant period, yet remain viable for as long as 3 years and 10 months (McMillan 1991). This dormancy period may help explain the recent appearance of numerous patches of shoalgrass along several miles of the northwestern shore of Dauphin Island (Vittor and Associates, 2002). These patches were observed during a 2002 survey (Vittor and Associates, 2004), were absent during subsequent surveys in 2004 and 2005 (see Byron and Heck 2006) but have since reappeared (exact year of
reappearance is unknown, personal observation), documenting the highly variable nature of this species. The probability that these patches started from vegetative fragments seems low, owing to their number, spatial extent and distance from stable populations. We believe that Hurricane Katrina in August 2005 may have exposed a previously buried seed bank and improved water clarity via a new breach in Dauphin Island. This breach, which increased the amount of clear Gulf of Mexico water to the area, may have increased the germination success and survival of newly emerging *H. wrightii* shoots. This suggests that substantial seed banks may exist and that seeds buried in local sediments remain viable for periods of nearly four years. Due to the dormancy of *H. wrightii* seeds (McMillian 1981, McMillian 1991), if such a seed reserve exists, additional experiments need to be conducted to determine whether restoration by seed planting is a locally viable seagrass restoration strategy, similar to what is being successfully done with *Z. marina* seeds in the Chesapeake Bay.

*Vallisneria americana*, which, in large part reproduces from overwintering tubers in the northern part of its range (Jarvis and Moore 2008), instead relies on seed banks in lower latitudes. Restoration activities conducted in Mobile Bay in the late 1980s, found some success using *V. americana* transplants in shallow water with highest restoration success found in waters equal to 0.5m depth, documenting the importance of proper site selection for restoration activities (Stout and Heck, 1990). However, transplants are highly destructive to the parent bed and can be labor and cost intensive to plant, with Stout and Heck (1990) estimating each planting unit costing approximately $0.75, based on 1990s prices. Because *V. americana* is known to produce copious amounts of seeds, between 150 to 500 per seed pod (McFarland, 2006), with as many as 20% of the seeds viable after one year (Jarvis and Moore 2008), broadcast seeding may be a viable restoration strategy. Seed pods can easily be harvested and transported to areas that
have experienced SAV loss or stored for future restoration projects; thus, seed based restoration techniques may be more attractive to coastal managers.

Because the reproductive biology of *H. wrightii* and *V. americana* is similar to that of *Z. marina*, the only one to date that has been successfully restored by seeding appropriate areas (Orth et al. 2006b, Moore et al. 2010), we believe that restoration efforts using this simply, lower intensity method of broadcast seeding may be a viable strategy along the northern Gulf of Mexico. To determine if seed based restoration would be appropriate for these two species in the northern Gulf of Mexico, we investigated:

1) whether sufficient reservoirs of SAV seeds existed and could be harvested with minimal damage to existing meadows,

2) whether harvested seeds were viable, and

3) whether harvested seeds could be successfully germinated.

By completing these three goal, we can provide recommendations to local coastal resource managers about the viability of seed based restoration for recovering lost SAV acreage in the northern Gulf of Mexico.

**Methods:**

*Halodule wrightii seed reserves*

To evaluate the potential seed reserves of *Halodule wrightii*, sediment cores were taken over a two year period (2010 – 2011) in variety of locations along the northern Gulf of Mexico where *H. wrightii* beds are found. To increase the likelihood that a sizeable seed reserve would be found, the extent of sampling ranged from Choctawhatchee Bay, FL to the Chandeleur Islands, LA, during 2010 and from St. Joseph Bay, FL to the Chandeleur Islands, LA during 2011.
Pooling resources from a variety of projects, we were able to expand our range to cover this large area of the northern Gulf Coast. During this preliminary investigation for a viable seed reserve, we focused primarily on areas where *H. wrightii* was present. If a substantial seed reserve was found in a particular area, we planned to expand and refocus our sampling efforts to those locations, collecting additional cores from the unvegetated swales and depressions near shoalgrass meadows at each site or locations where shoalgrass had previously been reported but not longer existed. Sampling within the unvegetated areas could be important, because Inglis (2000) found most *Halodule unervis* seeds (up to 114 seeds cm\(^{-2}\)) in low points such as the swales of sand flats or in depressions caused by biological disturbance (e.g., ray feeding pits).

At each location sediment core samples were taken with either a 7.6cm (inner diameter) corer or a 15.2cm corer. All cores taken in 2011, excluding those from the additional project samples described below, were taken with the larger coring device to increase the chances of finding seeds. Cores were push into the sediment to a depth of approximately 10 -15 cm to collect all below-ground biomass and sediment was passed through a 0.5mm sieve. All remaining plant material, sediment and seeds were stored in Ziploc bags and brought to the Dauphin Island Sea Lab for processing. Due the large number of cores collected, samples were stored at -18°C until processing could occur. Samples were sorted under a magnifying lamp and all seeds were identified and enumerated.

Additionally, to examine the seed reserves over a smaller spatial and continuous temporal scale, we documented the presence of SAV seeds in 1) SAV and infauna cores taken as part of the ERDP: Finfish and Shellfish Restoration project along the northeast shore of Point aux Pins, Alabama and 2) SAV cores taken as part of an NGI funded project examining the potential impact of the Deepwater Horizon oil spill in Grand Bay, Alabama on juvenile fishes. Samples
taken in conjunction with the ERDP project were taken at permanently established stations at restored reef or control sections of the shoreline on transects located at three distances: 0.5 m seaward of the first occurrence of *Spartina* at the marsh edge, 1-m inshore of the shoreward edge of the reefs or at a similar distance offshore at the control sites (approximately 110 m offshore), and mid-way between the reef and shore zone stations (approximately 55 m offshore). SAV, specifically *Halodule wrightii* and *Ruppia maritima* has been well documented along these transects since 2008 and have been expanding since reef creation in 2009. Core samples (i.d. 7.6 cm), collected monthly from April 2011 to June 2011, were examined for SAV seeds following the above processing protocol. Samples taken in conjunction with the NGI project were taken at three locations within Grand Bay, Alabama (West Point aux Pins, Marsh Island and Long Island) in documented *H. wrightii* and *R. maritima* meadows. Three cores (i.d. = 7.6 cm) collected monthly from March 2011 to November 2011, were examined for SAV seeds following the above processing protocol.

**Vallisneria americana seed reserves**

On June 16, 2010, as part of a baseline data collection of SAV in the Mobile Delta in advance of the Deepwater Horizon oil spill incident, we collected 10 cores in a variety of SAV habitats, although predominantly those dominated by *V. americana*, throughout the Mobile Delta. SAV and sediment core samples (i.d. 15.2 cm) were collected by pushing the coring device approximately 10-15 cm into the sediment. The samples were then passed through a 0.5 mm sieve and all plant material and remaining sediment and seeds were stored in Ziploc bags and brought to the Dauphin Island Sea Lab for processing. Samples were stored at -18°C until
processing could occur. Samples were sorted under a magnifying lamp and all seeds were identified and enumerated.

On July 8, 2011 we targeted six sites across the Mobile Bay Delta and took triplicate core samples (i.d. 15.2 cm) and collected visible seed pods from *Vallisneria americana* meadows in the Mobile Bay Delta (Figure 1). *Vallisneria americana* has a large reproductive output during the summer and collection of seed pods is relatively simple (Figure 2). Seed pods were brought to the Dauphin Island Sea Lab and placed in a ten gallon aerated aquarium until viability and germination experiments could begin. To minimize any salinity effects on seed viability, the aquarium was filled with water collected from the Delta on the above sampling day. Cores were sorted under a magnifying lamp and all potential seeds were removed from the core and verified under a dissecting scope.

On July 28, 2011 we removed the seeds from ten seed pods and placed them in a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium-chloride (following methods of Lakon, 1948). The seeds were allowed to soak in the tetrazolium solution for 24 hours after which they were observed under a dissection scope to determine if they had turned red (indicating viability)(Figure 3). All viable seeds were planted into small cups filled with beach sand and then carefully placed back into the aquarium (Figure 4) On August 9, 2011 we re-examined the seeds to see how many had germinated.

We observed that as the seed pods began to mature, they sank to the bottom of the aquarium (Figure 4: see left hand side of aquarium). To test if there was a difference in the viability of seeds within floating versus sunken pods, we removed and stained the seeds of five floating and five sunken seed pods. Seeds were left in the tetrazolium solution for 48 hours and then all stained seeds were counted. We also collected an additional 10 floating and 10 sunken
pods for cold storage. Half of each set of pods were placed in a refrigerator and the other half were placed within a -18°C freezer. In early spring of 2012, we re-examined the seeds for viability, planting all viable (ie. stained) seeds to determine germination success. These tests were performed to determine the best procedures for collecting, handling and storing *V. americana* seeds that would lead to successful germination and provide individuals that could later be planted in the field for restoration efforts.

**Results:**

**Halodule wrightii seed reserves**

A total of 108 cores were taken between June and October 2010 and a total of 189 cores, excluding those taken as part of the ERDP project and the NGI project, were taken between May and September 2011 (Table 1). Seeds were found in approximately 17% of the cores taken in 2010 and approximately 20% of the cores taken in 2011.

<table>
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<tr>
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<th>Sample Site</th>
<th># of cores taken</th>
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<td>34</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>2011 Louisiana</td>
<td>20</td>
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Table 1: Number of coring sites and the number of cores with seeds present. *The Alabama coastal sites in 2011 includes the samples taken as part of other projects.*

When examining the cores taken at permanent locations over a continuous temporal scale (from early spring to summer and late fall), we did not find as high a percentage of cores to contain seeds. In a total of 216 cores (monthly n=72) taken as part of the ERDP project, less than 1% of
the cores had seeds present. These seeds were only found in the May 2011 samples and of the 72 core samples taken, only 2 had seeds present. In a total of 90 cores taken across Grand Bay (monthly n =9), no seeds were found. These cores were taken in the same area that McGovern and Blankenhorn (2007) found evidence of sexual reproduction from developing fruits and where we expected, based on their work, to find seeds.

Additionally in those cores where seeds were present, the numbers of seeds was small, often limited to only one or two seeds. Only three cores over the two years sampled had substantial numbers of seeds present, with 36 the maximum number of seeds found in one core. Since so few *Halodule wrightii* seeds were found across both the large and small spatial scale, no germination work was completed.

*Vallisneria americana* seed collection and germination

Cores taken in both 2010 and 2011 in the Mobile Delta had a higher proportion with seeds compared to other areas surveyed. In 2010, of the 10 cores taken 60% showed evidence of seeds or reproductive structures (eg. seed pods). In 2011, approximately 44% of the 18 cores collected contained evidence of seeds. Again, as with most of the cores sampled, seed presence was low and most cores contained only one or two seeds. Additionally in 2011, evidence of seeds was most often found not to be of intact seeds but of seed coats from germinated seeds. Two locations, East Apalache River East and Tensaw Point had one or two germinated seeds.

In 2011 we examined seed pods collected from reproductive plants. These seed pods contained an average of 149 seeds, and approximately 30% of the seeds from each pod, when treated with the tetrazolium solution, were determined to be viable. Of those seeds stained with the tetrazolium solution, approximately 27% germinated (Figure 5). However, some seeds that
did not stain also germinated and after 3 months, we had 534 small seedlings, increasing the germination success to 33%. When we examined the difference between immature seed pods (or those that floated) versus mature seed pods (or those that sunk and were beginning to disintegrate), 20% of the seeds within the floating pods were viable, whereas 42% of seeds within a sunken pod were viable. This indicates that waiting until seed pods had matured would results in the greatest germination success and thus the greatest restoration success.

Because of a methodological error, investigations into the long term storage of seed pods were unsuccessful and seed pod tissues disintegrated before observations on the proper storage technique could be made. However, Jarvis and Moore (2008) found that 20% of V. americana seeds they studied were viable after one year, and we believe it likely that we would have found similar results for seeds collected from the Mobile Delta.

**Conclusions:**

There is a very urgent need to stop the global decline of submerged aquatic vegetation (Orth et al. 2006a), and to develop means of improving conditions that promote the health of SAV meadows. In addition, there is a great desire to restore SAV meadows. Unfortunately, to date seagrass restoration has generally had limited success, and has primarily relied on transplanting adult plants from a healthy bed to an unvegetated area. This not only damages the healthy bed but usually results in poor survival in the transplanted areas (Fonseca et al. 1998). However, restoration of temperate SAV meadows by seeding unvegetated areas that contain conditions suitable for SAV growth has proven to be cost effective and more successful than traditional transplanting methods (Orth et al. 2006; Moore et al. 2010).
Our results suggest that seed based restoration is not a viable strategy for *Halodule wrightii*, as there is a limited and difficult to find seed reserve available. However, seed based restoration by harvesting seed pods would likely be relatively easy and extremely valuable for *Vallisneria americana*. When testing viability of *V. americana* seeds using the tetrazolium methods, we found that approximately 30% of seeds within collected seed pods were viable. However, because only live tissues are stained after tetrazolium treatment, any tissues protected by the seed coat may not have been exposed to the solution and would not be stained. We found evidence of this as we had a higher number of germinated seeds than we did of stained seeds. Longer treatment of the tetrazolium solution (ie. longer soak times) may show a higher proportion of viable seeds, although we have not tested this hypothesis at this time.

Future proposed work will expand upon the results provided here and will examine the effectiveness of restoration using *V. americana* seedlings reared in the laboratory versus broadcast spreading of seeds from mature seed pods collected during the reproductive season.
Figure 1: Map of coring and seed pod collection locations within the Mobile Delta
Figure 2: Vallisneria americana female flowering structures, immature and mature seed pods visible on the water's surface.

Figure 3: Tetrazolium stained Vallisneria seeds. The last seed on the right has germinated.
Figure 4: Photo of aquarium setup. Stained seeds were planted into silo cups and allowed to germinate.

Figure 5: Stained and germinated seeds
**Publications/Presentations** (list the citations for all publications and presentations arising from this project, including those submitted, in press, or published):

None to date

**Outreach/education activities** (summarize any public presentation, media interviews, extension work, or educational activities related to your oyster research):

**Dauphin Island Sea Lab's "Boardwalk Talks" Series** (May 25, 2011) A series of public, informal conversations about current scientific activities at the DISL. Presented by the Northern Gulf Institute and hosted by The Estuarium, DISL's public aquarium. “Seagrass Meadows: A Global Crisis for Our Coastal Nursery Habitats” Talk presented by Ken Heck
Literature:


Orth, RJ, ML Luckenbach, SR Marion, KA Moore and DJ Wilcox. 2006b. Seagrass recovery in the Delmarva Coastal Bays. Aquatic Botany, 84: 26-36


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1. Does the project aim to restore and/or protect natural resources, ecosystems, fisheries, marine and wildlife habitat, beaches, coastal wetlands and economy of the Gulf Coast Region?

- YES  - NO

Notes:

Project seeks to sustain and restore submerged aquatic vegetation in coastal Alabama.

2. Is the proposal a project?

- YES  - NO

If yes, is the proposed activity a discrete project or group of projects where the full scope of the restoration or protection activity has been defined?

- YES  - NO

Notes:
3. Is the proposal a program?

- YES
- NO

If yes, does the proposed activity establish a program where the program manager will solicit, evaluate, select, and carry out discrete projects that best meet the program's restoration objectives and evaluation criteria?

- YES
- NO

Notes:

4. Is the project within the Gulf Coast Region of the respective Gulf States?

- YES
- NO

If no, do project benefits accrue in the Gulf Coast Region?

- YES
- NO

Notes:
Eligibility Determination

ELIGIBLE

Additional Information

Proposal Submission Requirements

1. Is the project submission overall layout complete? *Check if included and formatted correctly.*

A. Summary sheet ✔
B. Executive summary ✔
C. Proposal narrative ✔
D. Location information ✔
E. High level budget narrative ✔
F. Environmental compliance checklist ✔
G. Data/Information sharing plan ✔
H. Reference list ✔
I. Other ✔

If any items are NOT included - please list and provide details
2. Are all proposal components presented within the specified page limits (if applicable)?

☐ YES  ☐ NO

Notes: