COASTAL RECREATIONAL FISHING LICENSE  

FINAL PERFORMANCE REPORT

Recipient: Dr. Maurice Crawford (PI) and Dr. Margaret Young (co-PI)  
Department of Biology and Marine Environmental Science (renamed  
Department of Natural Sciences)  
Elizabeth City State University

Grant Award #: 4055 (DENR Task Order Number)

Grant Title: Genetic Diversity of Submerged Aquatic Vegetation

Grant Award Period: July 1, 2011 – June 30, 2014

Performance Reporting Period: July 1, 2011 – June 30, 2014

Project Costs:

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<th>Category</th>
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<td>Indirect</td>
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<td>81561</td>
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Total Cumulative Expenditures: 81561
Total Remaining Balance: 353
Summary for Managers

Overall genetic diversity among the species and locations sampled in this study were fairly low. We did not find any evidence of large clonal plants spreading in a bed and therefore are unable to recommend a minimum sampling distance for collecting plugs from a donor bed. In addition, the comparison of seeds vs. mother plant (leaf) did not indicate a high level of genetic diversity. This may be because there is inherent limited genetic diversity within the populations or may warrant more sophisticated molecular techniques.

Future research could include increasing the sample size and using more sophisticated molecular biology technique (SNPs – single nucleotide polymorphisms). This however, would warrant increased monies (sequencing).

Issues

The field season for 2013 was not as extensive as we had initially planned. During the summer we lost our technician [he was awarded a full research scholarship (PhD) in plant genomics from North Carolina State University, partially on technical expertise because of this grant]. We had anticipated his leaving and had another technician lined up; but at that last moment she found work elsewhere. Thus we were without technical assistance on the grant during a crucial collecting period which delayed collections and subsequent analyses. In addition, a new technician had to be trained in quality DNA extraction (absence of contamination) and genetic analyses. She subsequently left after at the beginning of March, 2014; and then we had to train another technician for the remainder of the grant.

Description of Work

Measuring Genetic Variation

Genetic diversity can play an important role in the functioning of submerged aquatic vegetation (SAV) (Reusch and Hughes 2006). Studies have linked greater levels of genetic diversity in eelgrass (Zostera marina) to enhanced recovery after grazing by geese (Hughes and Stachowicz 2004); and better survival in high water temperatures (Reusch et al. 2005; Ehlers et al. 2008). In addition, experiments conducted by Williams (2001) indicated that restoration of SAV beds (eelgrass: Zostera marina) from plants that have reduced genetic diversity resulted in plants with lower germination rates, fewer leaves and less flowering shoots (Williams 2001).

Wild celery (Vallisneria americana) and redhead grass (Potamogeton perfoliatus) reproduce asexually (through rhizomes and tubers) but they also produce seeds through sexual reproduction (McFarland 2006; and Kantrud 1990). The levels of genetic diversity in these populations in the Albemarle and Currituck Sounds have not been examined to our knowledge. There is also very little information regarding the natural pollination (self- or outcrossing) rates of these plants. By measuring levels of genetic diversity among wild populations we will gain a better understanding of how these
populations are structured and connected; and be able to recommend how far apart donor plants should be collected in order to maintain natural levels of genetic diversity.

We collected leaves and seeds from six SAV beds in Currituck and Albemarle Sounds (Figure 1). The sites were chosen based on the natural populations of wild celery and redhead grass. In Currituck Sounds samples were collected from the upper, mid and lower sections of the Sound. In Albemarle Sound the collection site was towards the west near Edenton, NC. These sites provided a wide geographic cover in this area. At each site we collected plant material of each species from points along a 20 m transect (0, 2.5, 5, 10 and 20 m) (extended to 40 m in 2012 sampling period). We also collected three core samples at each site to estimate species composition and biomass.

Plant genomic DNA was be extracted from collected seeds and leaves using the MasterPure™ Plant Leaf DNA Purification Kit (Epicentre Biotechnologies). We quantified the DNA for concentration and purity, adjusted to 25 ng/µL and stored at -20°C until use (along with 4 replicate samples). Additionally, unextracted plant material was placed at -80°C for long term storage. For random amplified polymorphic (RAPD), the arbitrary ten base primers in the Operon 10-mer kit (Qiagen) were used and intersimple sequence repeat (ISSR) primer kits purchased from the University of British Columbia, Canada.

Initially, 50 primers from both techniques will be chosen and DNA will be amplified from the different plant materials (seeds and leaves) from both plants. The reaction mix will contain 1 µM primer, 200 µM of each dNTP and 1 U Taq DNA polymerase and varying concentrations of DNA template and MgCl₂. PCR conditions will be from Wang et al. 2007 (RAPD) and Lakshmanan et al. 2007 (ISSR) and the amplified DNA will be subjected to standard electrophoresis. If amplifications are not ideal, optimization of reaction mix concentrations as well as PCR conditions will be conducted. Once ideal conditions are met, PCR amplifications will be conducted on all extracted DNA from both plants (including 3 replicate samples). For both techniques, reproducible fragments (markers) from the primers will be scored as present or absent. The number of scorable markers for each primer will be assessed and used to calculate the percentage of polymorphic bands.
Work Accomplished

SAV Collections

**Boldface type:** sites with complete collections at points along the proposed 20m and 40m transects

**Target species:**
- Wild celery *Vallisneria americana* **Family:** Hydrocharitaceae
- Widgeon grass *Ruppia maritima* **Family:** Ruppiaceae
- Redhead grass *Potamogeton perfoliatus* **Family:** Potamogetonaceae

<table>
<thead>
<tr>
<th>Date (2011)</th>
<th>Site</th>
<th>Transects Completed</th>
<th>Biomass Cores</th>
<th>Species Collected</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 25</td>
<td>Poplar Branch</td>
<td>No</td>
<td>No</td>
<td><em>Vallisneria &amp; Ruppia</em></td>
<td>These were kept in an aquarium in the lab</td>
</tr>
<tr>
<td>Sept 6</td>
<td>Albemarle Sound near Route 32 bridge</td>
<td>No</td>
<td>No</td>
<td><em>Potamogeton</em></td>
<td>Found floating in the water on the southern shore of Albemarle Sound. In the aquarium in the lab</td>
</tr>
<tr>
<td>Sept 13</td>
<td>Poplar Branch</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Vallisneria &amp; Ruppia</em></td>
<td>Plants collected on a Tuesday, DNA extracted on Thursday but poor quality DNA (repeated on Sept 22nd)</td>
</tr>
<tr>
<td>Sept 22</td>
<td>Poplar Branch Site 1</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Vallisneria &amp; Ruppia</em></td>
<td><em>Vallisneria</em> at 0 m was free floating</td>
</tr>
<tr>
<td>Sept 24</td>
<td>Pine Island</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>No SAV found except for a bit near the marsh fringe (<em>Ruppia</em>)</td>
</tr>
<tr>
<td>Oct 8</td>
<td>Back Bay Site 2</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Vallisneria &amp; Potamogeton</em></td>
<td>A very lush SAV bed</td>
</tr>
<tr>
<td>Oct 13</td>
<td>Kitty Hawk Bay and Buzzard Bay</td>
<td>No</td>
<td>No</td>
<td><em>Ruppia</em></td>
<td>Collected some SAV that looks like <em>Ruppia</em> from 3 locations but not sure of identification. Extremely sparse SAV</td>
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<tr>
<td>Oct 15</td>
<td>Whalehead Club Site 3</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Vallisneria &amp; Ruppia</em></td>
<td><em>Vallisneria</em> collected was floating in the water.</td>
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<tr>
<td>Oct 20</td>
<td>Sandy Point east of Route 32 bridge</td>
<td>No</td>
<td>No</td>
<td><em>Vallisneria, Potamogeton Ruppia</em></td>
<td>Collected plants floating along the north shore. <em>Ruppia</em> taken but just a sprig</td>
</tr>
<tr>
<td>Oct 29</td>
<td>Bells Island bridge Site 4</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Ruppia</em></td>
<td>Collected plants in a ditch feeding into Coinjock Bay.</td>
</tr>
<tr>
<td>Nov 3</td>
<td>Sandy Point Site 5 west of Route 32 bridge</td>
<td>Yes</td>
<td>Yes</td>
<td><em>Potamogeton</em></td>
<td>Collected plants on north side of Albemarle Sound. Sparse patches of <em>Potamogeton</em> and <em>Najas</em></td>
</tr>
<tr>
<td>Date (2012)</td>
<td>Site</td>
<td>Transects Completed</td>
<td>Biomass Cores</td>
<td>Species Collected</td>
<td>Comments</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>---------------</td>
<td>--------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>July 17</td>
<td>Poplar Branch Site 1</td>
<td>Yes</td>
<td>No</td>
<td>Vallisneria and Ruppia</td>
<td>Plants in good condition</td>
</tr>
<tr>
<td>August 9</td>
<td>Whalehead Club Site 3</td>
<td>Yes</td>
<td>Yes</td>
<td>Vallisneria and Ruppia</td>
<td></td>
</tr>
<tr>
<td>August 12</td>
<td>Back Bay VA Site 2</td>
<td>Yes</td>
<td>Yes</td>
<td>Vallisneria, Ruppia and Potamogeton</td>
<td></td>
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<tr>
<td>August 21</td>
<td>Kitty Hawk Bay Site 6</td>
<td>Yes</td>
<td>Yes</td>
<td>Ruppia</td>
<td>New site that wasn’t sampled in 2011 with small patches of <em>Ruppia</em> just offshore</td>
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<td>August 23</td>
<td>Bells Island Campground Site 4</td>
<td>Yes</td>
<td>Yes</td>
<td>Vallisneria, Ruppia and Potamogeton</td>
<td>A different location than 2011</td>
</tr>
<tr>
<td>Sept 4</td>
<td>Sandy Point Site 5</td>
<td>Yes</td>
<td>Yes</td>
<td>Vallisneria, Ruppia and Potamogeton</td>
<td>Plants in poor condition</td>
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</table>

<table>
<thead>
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<th>Date (2013)</th>
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<th>Species Collected</th>
<th>Comments</th>
</tr>
</thead>
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<td>Sept. 19</td>
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<td><em>Vallisneria</em> and <em>Ruppia</em></td>
<td>Collected both plants and seeds</td>
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<td>Oct. 3</td>
<td>Bells Island Campground Site 4</td>
<td><em>Vallisneria</em> and <em>Potamogeton</em></td>
<td>Collected 12 <em>Vallisneria</em> plants and seeds (both); plus some <em>Potamogeton</em> but no seeds.</td>
</tr>
<tr>
<td>Oct. 12</td>
<td>Kitty Hawk Bay Site 6</td>
<td><em>Ruppia</em></td>
<td>Collected <em>Ruppia</em> but no seeds.</td>
</tr>
</tbody>
</table>

Figure 1: Map of SAV Collection Sites.
Introduction to Analyses

We collected three species of submerged aquatic vegetation (SAV) during the study: *Ruppia maritima* (widgeon grass), *Vallisneria Americana* (wild celery), and *Potamogeton perfoliatus* (redhead grass). After collecting samples from each designated site and transect locations within each site, we extracted and purified the DNA for each sample. During the 2011 sample collections, poor weather conditions resulted in deteriorated samples yielding low concentrations of DNA and low purity levels. However, the samples from 2012 were healthier plants with higher DNA concentrations. The 2013 collections focused on finding wild celery plants and seeds.

Once the DNA was purified, it was subjected to an amplification process known as polymerase chain reaction (PCR) which multiplied the DNA sample to a sufficient amount for visible analyses. By using different primers (small manufactured DNA sequences that will bind with the plant’s DNA) during this process, several parts of the DNA were amplified so that a wide array of data could be generated for comparison. For this study, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) primers were used (all conditions have been optimized). These types of primers are commonly used for genetic diversity studies because they do not amplify the sequences of genes (which make proteins). By studying smaller, uncommon sequences, a greater chance for finding differences between populations can be found. If the most common genes were studied, it is not likely that any differentiation would be seen because most plants need these genes to survive (these sequences remain the same/similar amongst plants). By using primers designed to amplify discrete portions of DNA in each plant, a virtual fingerprint can be generated for each sample and each population.

Once the DNA was amplified it underwent gel electrophoresis. This process allows the amplified DNA fragments to be separated by size (measured in base pairs) and examined visually. The amplified DNA appears in small bands and many samples can be processed along-side one another for comparison (Figs. 1 & 2). When every sample has DNA bands that are the same size and line up together horizontally across, then there is no difference among them. However, when some samples have DNA bands not present in other samples of the same species, those bands are evidence of some level of genetic diversity. Those DNA bands that are not common throughout all samples are termed polymorphic. The greater the percentage of polymorphic DNA bands present, the greater the level of diversity among samples or populations.
Figure 2: Example of gel electrophoresis from intra-site study of site 3 with *Ruppia maritima* where each lane represents a different distance along the transect within the site. The first lane is a marker lane designed to be a reference for the size of the sample bands.
Figure 3: An example of a site to site comparison for *Ruppia maritima*. Any bands present in one sample lane but not found in others indicates some level of genetic diversity between these sites. The first lane is a marker lane designed to be a reference for the size of the sample bands.
To analyze the gel electrophoresis results with consistency, they were converted into a binary format where 1 represents band presence and a 0 represents no bands present. This allowed the gel pictures to be formatted so statistical analyses could be performed. An example of this format is shown in Figure 3 with DNA bands considered polymorphic highlighted in yellow.

<table>
<thead>
<tr>
<th>Band #</th>
<th>0 m</th>
<th>2.5 m</th>
<th>5 m</th>
<th>10 m</th>
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<td>0</td>
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<td>1</td>
<td>0</td>
<td>1</td>
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</table>

**Figure 4:** An example of a gel electrophoresis picture after converting to binary format for analyses. All yellow entries represent polymorphic DNA bands that are indicative of genetic diversity. The row of red entries show DNA that is common throughout all samples within the transect (not polymorphic).

**Information about Statistical Analyses**

The data generated by the gel electrophoresis of the amplified DNA provides the framework for multiple types of comparisons. Initially, five RAPD primers and five ISSR primers were used to amplify DNA from each species and compared for differences. As expected, many differences were present among the species examined, confirming that each primer could distinguish between species, avoiding possible confusion in later analyses. The same primers were then used to compare samples of the same species that were sampled from different sites. By using a total of 10 different primers, 10 different profiles for each comparison can be used, each with their own specific differences. This provides an in depth and well-rounded analyses of each comparison. Each successful comparison had to be replicated a minimum of three times and up to five times to get accurate results. Not only was each species compared by site but they were also compared within each site. This comparison was designed to determine if there was genetic diversity within a site or if it was a population of clones.
The same 10 primers and number of replications were also used for the intra-site analyses.

Several types of analyses were performed using GenAlEx, an Excel based genetic analysis program. The first analysis determined the percentage of polymorphic loci (PPL) among populations. This is a direct indicator of diversity because it reveals what portion of the DNA present in a species is different from one location to the next. The higher the percentage the greater the level of diversity between the samples studied.

Another measure of diversity used was the analysis of molecular variance (AMOVA). This analysis provides a visual representation of the proportion of variance (difference) within the population studied and among several populations. It also provides the statistic \( \Phi_{pt} \), and this value classifies genetic differentiation on a scale from little to large differences (ranges from 0 to 1).

Nei-Li genetic distance is yet another measure of diversity between populations. By examining each gel and comparing each population, an idea of each population’s differences is presented in a matrix. The Nei-Li formula will assign populations that are very similar a value near 0 and populations that are dissimilar are assigned a value near 1.

Principle coordinate analysis (PCoA) was also used to provide a visible representation of similarities and differences between populations. This analysis uses the genetic distance to generate a representative scatter plot (2 or 3 dimensional chart) that displays how far apart each population is from the next in terms of their genetic structure.

**2011 Data Analyses**

The analysis of the samples of SAV from season one revealed that there was significant genetic differentiation among several sites for *R. maritima* and *V. americana*. Indications of minimal variation within a single site were also present for *R. maritima* but not *V. americana*. *Potamogeton perfoliatus* was not present in sufficient numbers or at sufficient sites to compare for diversity.

**Ruppia maritima (widgeon grass)**

*Ruppia maritima* was present at sites 1, 3 and 4 and compared for differences (Fig. 1). An AMOVA analysis revealed a variance of 81% among sites 1, 3 and 4 (Fig. 5). The \( \Phi_{pt} \) for this comparison was 0.809 which shows a large degree of genetic difference among the three sites. When comparing sites the mean PPL ± SE was 15.38 ± 4.44% (SE is standard error of the mean).
Figure 5: Blue area represents diversity of *Ruppia maritime* among sites 1, 3 and 4, while the red area represents the diversity within each sample studied.

Principle coordinate analysis (PCoA) shows each site in a separate quadrant representing differences among sites 1, 3 and 4 (Fig. 6).

Figure 6: Principle coordinate analysis representing the genetic difference among sites 1, 3 and 4 for *Ruppia maritime*.

For *R. maritima* the greatest differences were seen between sites 3 and 4 with a genetic distance of 0.215 (Table 1). This suggests that although site 1 has DNA in common with both sites 3 and 4, sites 3 and 4 differ from each other moderately at a genetic level.
Table 1: Genetic distance between sites 1, 3 and 4 for *Ruppia maritima*

<table>
<thead>
<tr>
<th></th>
<th>Site 1</th>
<th>Site 3</th>
<th>Site 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 3</td>
<td>0.945</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Site 4</td>
<td>1.169</td>
<td>0.215</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Genetic differentiation was also discovered within a single site for *R. maritima*. At site 3 variations from 13-56% were present depending on the primer used to amplify the DNA (Fig. 7). The PPL for this site ranged from 20.0 ± 9.35% to 37.5 ± 10.46%.

Figure 7: Variations of genetic diversity of *Ruppia maritima* within site 3 as shown by using three different ISSR primers.
**Vallisneria americana** (wild celery)

*Vallisneria americana* was also present in three of the five sites sampled (sites 1, 2 and 3). Analysis with one primer revealed a 63% variance among sites 1, 2 and 3 (Fig. 4). The $\Phi_{pt}$ for this study was 0.625 which represents a large degree of genetic diversity among the three sites (Fig. 8). When comparing sites, the mean PPL ± SE was 18.52 ± 3.70% which was less than with *R. maritima*, but was more consistent through each site.

![Percentages of Molecular Variance](image)

**Figure 8:** Blue area represents diversity of *Vallisneria Americana* among sites 1, 2 and 3, while the red area represents the diversity within each sample studied.

As with *R. maritima*, PCoA analysis of *V. americana* placed each site in different quadrants representing diversity among sites 1, 2 and 3 (Fig. 9).

![Principal Coordinates](image)

**Figure 9:** Principle coordinate analysis representing the genetic difference among sites 1, 2 and 3 with *Vallisneria americana*

The genetic distance found between sites for *V. americana* revealed much greater diversity between all sites investigated (Table 2). Differences were seen among all three sites, the greatest of which was between sites 2 and 3 with a genetic distance of 0.222.
Table 2: Genetic distance among sites 1, 2 and 3 for *Vallisneria americana*

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0.284</td>
<td>Site 1</td>
</tr>
<tr>
<td>0.284</td>
<td>0.000</td>
<td>Site 2</td>
</tr>
<tr>
<td>0.337</td>
<td>0.222</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Unlike *R. maritima*, *V. americana* did not exhibit much differentiation within each site investigated. One primer did reveal a 3% difference but this was not considered significant. Although the PPL for each comparison was anywhere from 20 – 40% in these analyses, it is thought to be related to the small amount of samples available for analysis. Once consolidated with season two data, a more precise comparison should be available.
2012 Data Analysis

The samples collected in 2012 were available in greater numbers and in more locations than those of 2011. *R. maritima* was found in site 1 and sites 3-6, *V. americana* was present in sites 1-5 and *P. perfoliatus* was found at sites 2, 4 and 5. Each species was submitted to the same PCR amplification used for season one (2011) samples. Three RAPD and three ISSR primers were used for each species. Each species was organized by site when analyzed and no less than six samples were used from each site. When possible the six samples from each site include one sample from each transect location within the site (0 meters, 2.5 meters, 5 meters, etc.). When samples were not available from all transect locations other samples from the same site were used (Figure 10).

![Sample picture of gel electrophoresis of DNA from Vallisneria americana. Six samples from each site were compared side by side for genetic differences.](image)

Figure 10: Sample picture of gel electrophoresis of DNA from *Vallisneria americana*. Six samples from each site were compared side by side for genetic differences.
Ruppi maritima

*R. maritima* was available at all sample sites except site 2. AMOVA showed a mean level of diversity among the sample sites to be 16% with a high standard deviation. The highest level of variation was observed at 28% (Fig. 11) while the lowest was 8%. *R. maritima* had the highest average PPL of 76.76%.

**Figure 11:** Representation of the highest amount of variation revealed by AMOVA among sites of *Ruppi maritima* using one primer

Principle coordinate analysis (PCoA) shows that although the populations (sample sites) tend to segregate genetically there are still many instances where samples from separate populations are nearly identical to one another (Fig. 12).

**Principal Coordinates**
Nei-Li’s genetic distance did not exceed 0.2 using any of the six primers which showed that there are differences among the separate populations (Table 3). These data correlate with the AMOVA findings shown previously. There is diversity among the populations of *R. maritima* in the region however these findings do not show that it is a substantial level of diversity.

*Table 3: Nei’s genetic distance among sites 1, 3, 4, 5 and 6 for *Ruppia maritima* for a single primer*

<table>
<thead>
<tr>
<th>Pairwise Population Matrix of Nei Genetic Distance</th>
<th>Site 1</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
<th>Site 6</th>
</tr>
</thead>
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<td>0</td>
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</tr>
<tr>
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<td>0.085</td>
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</table>
**Vallisneria americana**

*V. americana* was also present in 5 of the 6 sample sites. AMOVA revealed a much more consistent level of diversity amongst populations of *V. americana*. The lowest level of molecular variance seen was 15% but the greatest was only 19% (Fig. 13). This indicates a low level of diversity among these populations of *V. americana*.

![Percentages of Molecular Variance](image)

**Figure 13:** Representation of the largest amount of variance among six populations as shown by AMOVA for *Vallisneria americana* using a single primer

Principle coordinate analysis of the populations of *V. americana* illustrates a greater degree of similarity than seen with *R. maritima*. Samples from each population cluster near samples from other populations representing low diversity (Figure 14). This supports the AMOVA results for *V. americana* as well.
Figure 14: Principle coordinate analysis of *Vallisneria americana* with each sample site being represented by a different color. The lack of clear segregation by the color coded populations shows a low level of genetic diversity.

Nei-Li's genetic distance for *V. americana* did not exceed 0.3 which gives similar results to those seen *R. maritima* (Table 4). This implies that there is genetic diversity amongst the populations of *V. americana*; however, the overall level of diversity appears to be low.
Table 4: Genetic distance among sites 1-5 for *Vallisneria americana* using a single primer

<table>
<thead>
<tr>
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<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
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</table>

*Potamogeton perfoliatus*

Unlike *R. maritima* and *V. americana*, *P. perfoliatus* was only found at 3 of the 6 sample sites. There were however, a sufficient number of samples from each site for full analysis of the populations. *P. perfoliatus* had the lowest level of diversity according to the AMOVA results. A maximum level of variance was observed at 27% while the lowest level was 0% (Fig. 15), this lead to a mean level of diversity at 14%. *P. perfoliatus* also showed more fluctuation from the mean than seen in the other two species.

![Percentages of Molecular Variance](image)

**Figure 15:** Representation of the highest level of diversity among sites according to AMOVA for *Potamogeton perfoliatus* with one primer
Principle coordinate analysis of P. perfoliatus reveals distinct similarities within each population which results in tighter clustering of the populations. These results were not as clear with every primer for P. perfoliatus. Similar to R. maritima there is distinct segregation of populations with some overlap (Fig. 16).

Figure 16: Principle coordinate analysis of Potamogeton perfoliatus with each population being represented by a different color

Nei-Li’s genetic distance for P. perfoliatus did not exceed 0.118 which is similar to the results for V. americana and R. maritima (Table 6). In all three cases the Nei-Li’s genetic distance was low which implies some level of genetic diversity.

Table 6: Genetic distance among sites 2, 4 and 5 for Potamogeton perfoliatus using a single primer
Seed DNA Analysis

If the plants are outcrossers (pollinated not by their own pollen), the seeds will exhibit some genetic variability, when they are compared to the mother plant. In fall 2012, seeds were collected for analysis from all three species under investigation. Seeds from *R. maritima* were available in the greatest numbers and were found at sites 1 and 3. Neither site had seeds available at every transect location. *P. perfoliatus* seeds were only found at site 2 and not at every transect location. *V. americana* seeds were also collected from site 2.

*R. maritima* and *P. perfoliatus* seeds were able to produce enough DNA upon extraction to amplify the DNA using primers used in the leaf tissue study. The seed DNA was compared for differences just as in the leaf tissue study. Also the seed DNA was compared to that of the mother plant for each individual to provide more insight into the methods of reproduction used by these species. Initial results indicated few differences between mother plant (leaf) and seeds.

DNA extractions from *V. americana* seeds were optimized to produce viable amounts of pure DNA. The timing of sample collection as well as the method of DNA extraction requires optimization in order to provide more data. Not enough DNA was extracted from a single seed to amplify using the RAPD and ISSR primers used for the leaf tissue DNA.

In fall 2013, seeds were collected from *R. maritima* at sites 1 and 6 (seven plants total); and from sites 1 and 4 for *V. americana* (37 plants total). No sites had *P. perfoliatus* plants that were producing seeds. No other sites had plants of any species that were producing seeds. The DNA extraction protocol was optimized for each plant’s seeds; and sufficient DNA was first amplified using primers for the RUBISCO gene (chlorophyll gene that is found in all green plants). DNA was also extracted from the leaves of fresh plants (same plants that produced seeds); and in a few cases, from the dried plants (all plants are mounted for a herbarium collection). This will also allow for comparison of DNA between the seeds and the mother plant (leaf DNA) for each individual to provide more insight into the methods of reproduction used by these species.

*V. americana* seeds are minute and are produced in a pod. Attempts to use a single seed for DNA extraction were unsuccessful (not enough DNA could be extracted that could be quantified). Therefore, approximately 50 seeds from a pod were used for DNA extraction. Multiple seed pods on an individual mother plant were treated separately. Individual seeds could be used for DNA extraction for *R. maritima*. A decision was made to concentrate on *V. americana* since this species had the highest number of fertile plants (producing seeds).

Five ISSR and 5 RAPD primers were used to identify genetic differences amongst the seeds and between the seeds and the leaves from the same plant. These were the same primers that were identified to produce the largest numbers of polymorphisms from the leaf DNA/species data. PCR was conducted at least twice for each plant (seed and leaf:}
and visual differences were observed between the leaf of the mother plant and seed for all primers. In addition, four additional ISSRs primers were used for genetic diversity studies (9 in all).

Fig. 17: Example of gel electrophoresis from intra-site study of site 4 with *Vallisneria americana* where each lane represents either DNA extracted from the leaf (L) of the mother plant or the seed (S). The first lane is a marker lane (M) designed to be a reference for the size of the sample bands. Four mother plants are represented (L1 – L4). Lanes with same number but different lowercase letters represent different seeds from the same plant e.g. S2a and S2b. W represents the control (water instead of DNA).

For the five RAPD primers, the lowest level of molecular variance seen was 7% and the greatest was 34% (Fig. 13). This indicates a fairly high level of diversity between the seeds and the mother plant for one primer (OPA17: Fig. 18).
Figure 18: Representation of the highest level of diversity among sites according to AMOVA for *V. americana* with the RAPD primer, OPA17

For the nine ISSR primers, the lowest level of molecular variance seen was 0% (no differences) and the greatest was 28% (Fig. 13). This again indicates a fairly high level of diversity between the seeds and the mother plant for one primer (836: Fig. 19).

Figure 19: Representation of the highest level of diversity among sites according to AMOVA for *V. americana* with the ISSR primer, 836

Nei-Li’s genetic distance for the seed compared to the mother plant (leaf) for *V. americana* were variable and dependent on the primers used. The Nei-Li’s genetic distance values for the ISSRs were much lower (e.g. 0.083) than that of the RAPDs, (e.g. 0.219) implying that the ISSRs were able to better distinguish some level of genetic diversity.
**SAV Biomass Characteristics**

At each sampling site three random points were selected along the transect. At each of those points we used a 10.16 cm corer (0.0081 m$^2$) pushed down to a depth of 10 – 20 cm to collect a core of SAV in order to find the mean biomass at that site. The contents of the core were rinsed in the field. In the laboratory the sample was sorted by species, placed in a drying oven at 60°C for three days and the dry weight calculated. Data are standardized to square meter.

**SAV Biomass - Site 1 (Poplar Branch)**

![Graph showing SAV biomass at Site 1 for Sept. 2011 and Aug. 2012]

**SAV Biomass - Site 2 (Back Bay, VA)**

![Graph showing SAV biomass at Site 2 for Oct. 2011 and Aug. 2012]
References


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Williams, SL. 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecological Applications 11: 1472-1488