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Submerged Aquatic Vegetation Species and Populations Within Species Respond Differently to Environmental Stressors Common in Restorations

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Abstract

Submerged aquatic vegetation (SAV) improves environmental conditions by acting as a sediment stabilizer and nutrient retention tool; therefore, reintroduction of SAV is a common freshwater restoration goal. Initial plant establishment is often difficult in suboptimal conditions, and planting material with specific traits may increase establishment rates. Here we evaluate the variability in plant traits based on collection location. We find consistent differences in traits of plants collected from different natural water bodies, and those differences persist in plants grown from seeds under common garden greenhouse conditions—presumably because of genetic differentiation. In three separate mesocosm experiments, we tested the interactive impacts of collection location and environmental condition (control conditions, reduced light, elevated nutrients, or a combination of reduced light and elevated nutrients) on plant reproduction and on traits that might indicate future restoration success (plant height, number of leaves, and rhizome diameter). In most cases, plant traits at the end of the experiments varied by collection location, environmental condition, and an interaction between the two. The best performing plants also depended on response variable (e.g., plant height or number of new shoots produced). Together these results suggest that unpredictable environmental conditions at restoration sites will make selection of a single high-performing plant source difficult, so we suggest incorporating a diverse set of collection locations to increase the probability of incorporating desirable traits.

Keywords SAV · Potamogeton · Vallisneria · Diversity · Ecosystem restoration

Introduction

Submerged aquatic vegetation (SAV) has the potential to assist in the recovery of water bodies by stabilizing sediment and improving water clarity (Hansen and Reidenbach 2012); by retaining carbon, nutrients, and altering nutrient cycles (McGlathery et al. 2007, Greiner et al. 2013); and by providing physical structure that provides shelter and food for many organisms (Hemminga and Duarte 2000; Looby et al. 2021). These environmental modifications create positive feedback—altering conditions (decreased wave energy and increased light) in such a way that SAV meadows will expand through both vegetative propagation and successful sexual reproduction (Orth et al. 2012). Successful plant establishment can result in improved ecosystem functions (e.g., carbon sequestration and denitrification) with significant value resulting in a positive return on investment (Reynolds et al. 2016a). However, poor

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environmental conditions at restoration sites are often blamed for SAV planting failure (Kupsky and Dornbush 2019). Because restoration occurs in systems that have been degraded, damaged, or destroyed (SER—Society for Ecological Restoration International Science & Policy Working Group (2004)), low initial plant survival under suboptimal environmental conditions acts as a barrier to using SAV restoration as a tool to improve overall ecosystem condition.

The traits of plant source material can impact both initial survival and long-term persistence. The term SAV incorporates many species; and individual SAV species, their genetically distinct populations, and even clones within those populations can all vary in ecologically important traits—clonal reproduction rates, plant height, number of leaves, and resistance to specific disturbances (Hughes et al. 2009; Tomas et al. 2011; Reynolds et al. 2016b; Abbott et al. 2018). If specifically matched to environmental conditions, specific traits can increase initial survival. For example, taller plants extend higher into the water column and have more access to light in turbid or deep environments.

Selected traits should be informed by specific restoration goals and site limits. If fish use is a goal, complexity of leaves may be a desired trait (Crowder and Cooper 1982, Martin and Valentine 2011). If rapid establishment is a goal, a high rate of clonal production is desirable. However, selecting for a specific measurable trait may have unintended consequences. For example, for some species, plants that are taller tend to reproduce clonally at slower rates (Engelhardt et al. 2014), and clones that reproduce quickly are less tolerant of heat events (Reynolds et al. 2016b).

Procuring restoration source material with the appropriate combination of traits is also complex. One approach is purchasing a well-established cultivar or super clone (Pater 1995, Harrison et al. 2001) advertised to have specific traits or tolerances. Typically, these cultivars are chosen to establish well under stress. In this case, we expect that favorable traits shaped by a plant's genetics will result in high performance. Important considerations, however, are availability and plasticity. Cultivar availability will vary with commercial development and by target species and region. SAV tends to be quite plastic and can vary significantly with environmental conditions (Middelboe and Markager 1997, Yang et al. 2004, Ni 2001, French and Moore 2003), meaning that plant traits may change when introduced to a new environment. Collecting from a wild source with environmental conditions well matched to a restoration site is another strategy aimed at selecting traits that will boost performance (the local adaptation approach). This approach combines genetic identity and environmental effects on traits—if plants are performing well under similar conditions, they may be well adapted, and a transition to a different site with a similar environment should not significantly change trait expression. Importantly though, predicting the exact suite of stressors that a plant will experience is difficult since seasons, storms, or other disturbances could alter the suite of stressors present. For example, a large rain event and subsequent runoff could increase nutrient loads in a lake, or a large wind event could resuspend sediments reducing light levels over a short time period. Likewise, plants could be stressed by grazing events or impacts from boat traffic. Predicting responses to multiple, interacting stressors is difficult since the interactive effects of stressors vary and could be antagonistic (stressors cancel each other out), additive (stressor effects are independent), or synergistic (the total impact is greater than the sum of individual effects) (Crain et al. 2008). An additional potential drawback of natural collection is impact to source sites.

Alternatively, if either environmental condition or plant source traits variation and plasticity are not well known, using a diverse (intraspecific and/or interspecific) group of plants with diverse traits will increase the probability of including individuals well matched to environmental conditions (Hughes et al. 2008). This approach has the further benefit that increased diversity may increase long-term persistence since diversity affords the ability to evolve with changing conditions (Hughes et al. 2008). Selection of restoration source material with trait diversity also requires careful consideration. SAV species often vary significantly in their traits, so incorporating trait diversity through the selection of multiple species is relatively straightforward. However, incorporating trait diversity within species (e.g., genetic diversity) into restoration plantings is relatively rare. Despite several studies documenting the positive impact of both species and genetic diversity on restoration outcomes and the increased availability and lower cost of many genetic analyses, most published restoration designs do not assess intraspecific or interspecific diversity (Reynolds et al. 2012; Williams et al. 2017; Hughes et al. 2018) in part due to limitations associated with cost and availability of resources to characterize genetic diversity (Kettenring et al. 2014, Hughes et al. 2018). We hypothesize that for freshwater SAV, collections from multiple hydrologically isolated water bodies may be relatively genetically distinct and act as an effective proxy for incorporating trait diversity related to genetic diversity, although we acknowledge that there are additional biological (e.g., birds) and mechanical (e.g., boat propellers) dispersal agents that could connect plant populations between water bodies. This approach would eliminate many of the barriers to incorporating this theoretical approach into practice.

In this study, we investigate trait diversity in SAV restoration source material. We focus on three specific traits—shoot height, number of leaves, and rhizome diameter since they are linked to performance in restoration. We also consider new shoot production as a response variable. Water bodies targeted for SAV restoration often have sub-optimal light conditions, which suggests that plants with longer leaves reaching higher into the water column will be less stressed and more likely to survive, or that plants with large rhizomes and large carbohydrate stores may survive longer. Plants with more leaves generate complexity and increase habitat quality, and new shoot production may predict increased restoration expansion. We hypothesized that collections of SAV from disconnected water bodies will have different traits resulting from both genetics and environmental variation of source water bodies. To test this, we grew seedlings from disconnected lakes in a greenhouse common garden to determine the underlying genetic driver of trait diversity. We also hypothesized that common stressors associated with SAV restorations would hinder plant growth and reproduction, but the effects of stressors would vary by collection location (i.e., genetics). We tested this in greenhouse experiments, examining the impact of environmental conditions—elevated nutrient concentrations, reduced light, and a combination of the two—on the reproduction and traits of each of these collections.

Methods

For these experiments, we used two common freshwater SAV species—tape grass (*Vallisneria americana*, hereafter *Vallisneria*) and Illinois or shining pondweed (*Potamogeton illinoensis*, hereafter *Potamogeton*). We chose these species because they are widespread SAV species and are used regularly in SAV restoration projects. *Vallisneria* (family *Hydrocharitaceae*) and *Potamogeton* (*Potamogetonaceae*) are frequently found together in Florida lakes (Florida LAKEWATCH 2020) but have different morphology and life histories. *Vallisneria* has long, tape-like, submerged leaves that originate at the base, while *Potamogeton* has elliptical leaves, both floating and submerged, that alternate from stems that can extend through the water column.

For each species, we used purchased nursery-grown plants and field-collected plants from hydrologically isolated water bodies throughout central Florida. We measured *Vallisneria* plant traits of field-collected adult plants and field-collected seeds grown in common garden conditions from Lake Conway, Lake Hartridge, and Lake Mariana. We measured those same traits on *Potamogeton* from Lake Butler, Lake Jessamine, Lake Conway, Lake Hartridge, and Lake Mariana. Environmental effects experiments were conducted with a cultivated *Vallisneria* clone commonly used for restoration in central Florida (Experiment 1) and with *Vallisneria* populations collected from Lake Maude, Lake Tennessee, the Santa Fe River, and a nursery-grown source (different from Experiment 1) (Experiment 2). *Potamogeton* populations used in the environmental effects experiments were collected from the Ichetucknee River, Kirby Pit, and a nursery-grown source (Experiment 3). Collections were performed by commercial collectors and represent material that is available for actual SAV restorations. Water bodies tended to have good environmental conditions (e.g., clear water); however, we do not have environmental data from the collection sources since in many cases, the exact locations of these commercially significant species are proprietary to protect source locations from overharvesting. Approximate locations are mapped in Fig. S1.

Plant Traits Measurements

At purchase or field collection, we evaluated differences in plant traits by collection source. For twenty representative planting units, we measured: (1) maximum shoot height (from base of the stem or leaf to the tip of the tallest leaf) (cm), (2) number of leaves per shoot, and (3) rhizome diameter (mm). Data were checked for assumptions (normality and homogeneity of variance), transformed as needed, and analyzed using ANOVA using the software SAS 9.4. Results were considered significant at $p \leq 0.05$, and individual differences were determined using Tukey Kramer post hoc analyses. To further quantify our estimate of these differences in plant traits by collection source, we calculated the omega squared effect size. Because the plants were from different collection locations and grew under different environmental conditions, the effect size can be assumed to represent effects on traits due to both sources of variation.

At each collection site where sexual reproduction was observed, lake sediment was collected beneath *Potamogeton* and *Vallisneria* beds and spread in shallow, flooded trays in a greenhouse. After germination, *Vallisneria* and *Potamogeton* seedlings were placed in 76 L glass aquaria in the same media in a greenhouse common garden and grown for approximately seven months, at which time twenty surviving individual shoots were randomly collected and traits were measured and analyzed as above. Because seedlings from all collection locations were grown in the same environmental conditions, the calculated effect size can be assumed to represent effects mostly due to different collection locations, and potentially genetic differences between those populations.

Environmental Effects Experiments

We conducted three greenhouse experiments to evaluate the effects of environment on plant traits of two SAV species collected from various locations. The goal of the experiments was to test the performance of various collection sources under both control and stressed (reduced light, increased nutrients, and a combination of the two) conditions. Plant material availability and logistic constraints determined experiment order and timing, and required that results from each experiment be analyzed independently. Experiment 1 ran from February to May 2018. Experiment 2 ran from June to November 2017, and Experiment 3 ran from February to May 2018.

For each experiment, plants were grown in 19 L buckets (30 cm diameter and 26 cm deep). Buckets were randomly assigned one of four manipulated environment treatments: (1) control, (2) elevated (+) nutrients, (3) reduced (−) light, (4) a combination of elevated (+) nutrients and reduced (−) light. We selected reduced light and elevated nutrient load treatments as representative stressors because they are assumed to be among the most common barriers to successful establishment in restoration projects. Nutrients were added to the sediment at the start of the experiment by adding 10 g of slow-release Osmocote ® fertilizer pellets (14-14-14). At fertilization, that amount of fertilizer equated to ~1000 µg TN L^{-1} in unfertilized buckets versus ~74,000 µg TN L^{-1}, although fertilization efficacy was monitored using plant nutrients as described below. Light was reduced by covering each bucket with one piece of window screen, which blocked approximately 70% of photosynthetically active light, which was verified by measuring PAR in all treatments of all experiments. These levels of manipulation were intended to be stressful and to represent conditions experienced by some lakes in central Florida. In Lake Apopka, a highly degraded lake located in Orange and Lake Counties, FL, the mean maximum TN was 126,000 µg TN L^{-1}, while the average of all other lakes in these counties was 811 µg TN L^{-1} (Orange County) and 1173 µg TN L^{-1}(Lake County) (Florida LAKEWATCH 2020). Mean Secchi depths for lakes in the same counties was 1.9 m with some individual lakes having much lower values (e.g., Lake Apopka Secchi depth was ~0.3 m, Florida LAKEWATCH (2020)). Given the instances of extremely low Secchi depths, a 70% reduction in light level is realistic.

The buckets were arranged in a randomized block design to account for possible environmental gradients in the greenhouse. Three shoots of SAV were planted in each bucket in a triangle formation equidistant from one another in approximately 10 cm of clean, commercially available sand, making the sediment consistent amongst treatments but not necessarily representative of the natural environment. For *Vallisneria*, at the beginning of the experiment, all plants were standardized to a height of 30 cm by clipping the leaf tips; however, *Potamogeton* grows from the tips as opposed to the base, so this species could not be clipped to a standardized height. All treatment by source combinations were replicated six times.

The number of new shoots (measured as total shoots minus three, as original shoots could not be discerned at experiment end) and the maximum shoot height was recorded approximately every two weeks. Abiotic conditions were regularly monitored throughout the experiment. Water temperature (°C), salinity measured as specific conductance (ppt), and dissolved oxygen (DO) were measured with a YSI ProDSS, and photosynthetically active radiation (PAR) was measured using a LI-COR spherical quantum sensor just below the water surface. Data were checked for assumptions (normality and homogeneity of variance), transformed as needed, and differences between treatments were analyzed using a repeated measures ANOVA.

At the end of each experiment, plant traits (as described in Plant trait measurements above) from each experimental unit were measured from three haphazardly selected shoots. To assess the efficacy of nutrient additions, dried and ground plant material was analyzed for carbon and nitrogen tissue concentration at the Wetlands Biogeochemistry Lab at the University of Florida. Fertilizer contained both nitrogen and phosphorus, but we measured nitrogen only as a proxy to demonstrate that plants took up nutrients released from fertilizer.

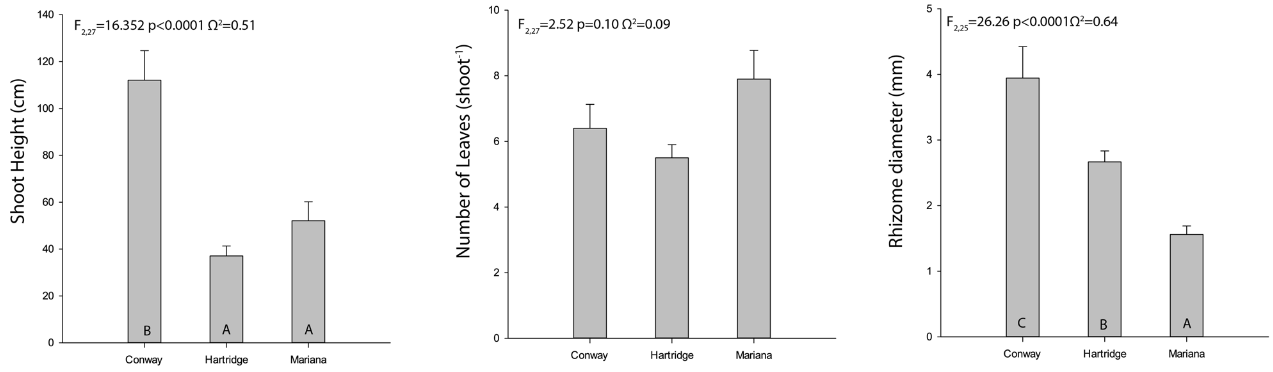
Data were checked for assumptions (normality and homogeneity of variance), transformed as needed, and analyzed using ANOVA (Factors: source, treatment, and source*treatment) using the software SAS 9.4. Results were considered significant at $p \le 0.05$, and individual differences were determined using Tukey Kramer post hoc analyses. Because there were source*treatment interactions, we conducted a second ANOVA for each population source separately to estimate effect size. In these experiments, in which each population was grown under manipulated conditions, effect sizes are estimates of the differences in traits due solely to environment.

Effect sizes in our data sets, as described above, represent the variation in traits due to three potential influences. For the initial plant trait measurements of field-collected plants, effect sizes are estimates of the differences in traits due to both environment *and* population source. For seedlings germinated and grown under greenhouse conditions, effect sizes are estimates of differences in traits due solely to population source. For the environmental effects experiments, in which each population was grown under manipulated conditions, effect sizes are estimates of the differences in traits due solely to environmental effects. For each trait, we compared the effect sizes for each of these influences using an ANOVA approach.

Results

Plant Traits Measurements

At purchase or field collection and prior to experimental manipulation, population sources differed in all traits, Field Collected *Vallisneria*



Vallisinera seedlings

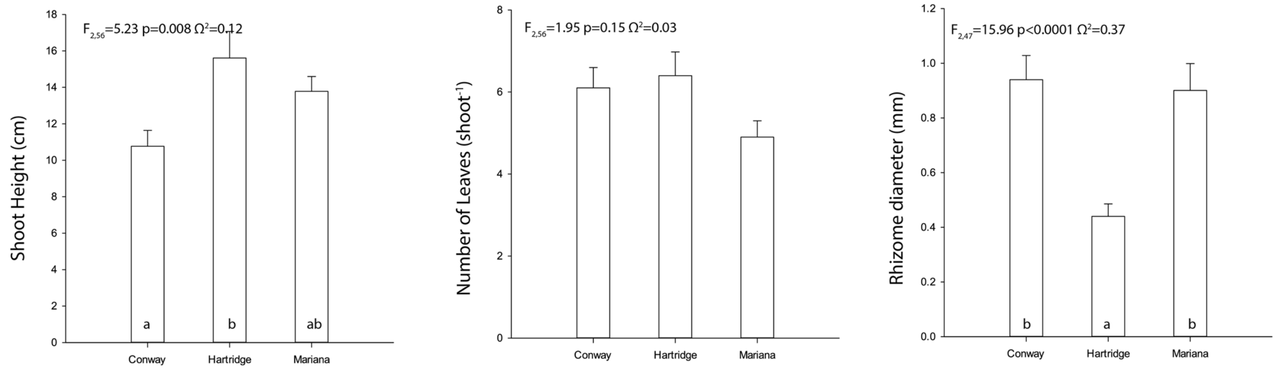


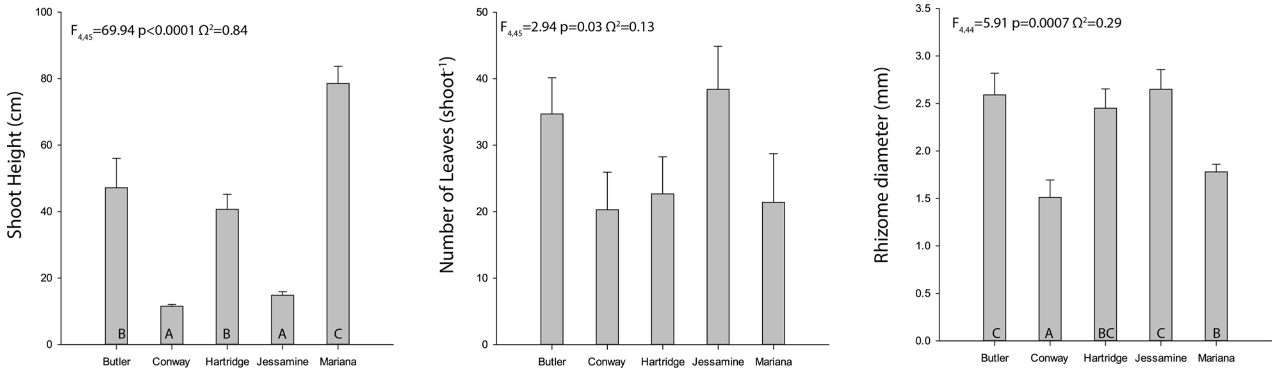
Fig. 1 Traits of *Vallisneria* plants (mean and SE) at field collection (in grey) and traits of seedlings germinated and grown in a greenhouse common garden (in white). Letters represent differences determined using Tukey Kramer post hoc analyses following an ANOVA. Upper and lower case letters represent different analyses. Each graph includes the F statistic and p-value for the main effect of source population on the trait, and omega squared effect size for the variation in the trait explained by source population

except number of leaves. For field-collected *Vallisneria*, Lake Conway plants were the largest with greater shoot height (112 cm) and rhizome diameter (3.9 mm). After germination and growth in the common garden, plants from all sources were generally smaller than at field collection, but populations still differed in ecologically important traits—height and rhizome diameter, with seedlings originating from Lake Conway still tending to have larger rhizomes (0.9 mm), but plants from Lake Hartridge were tallest (15.6 cm) (Fig. 1). For field-collected *Potamogeton*, plants from Lake Mariana were tallest (78.6 cm), but plants from Lake Butler (2.6 mm) and Lake Jessamine (2.7 mm) had the largest rhizomes. After germination and growth in the common garden, plants from all sources were generally smaller, but *Potamogeton* populations still differed in ecologically important traits—height and rhizome diameter, with Lake Hartridge seedlings being the tallest (26.6 cm) and having more leaves (10.0) but Lake Conway (0.69 mm) and Mariana (0.67 mm) seedlings having the largest rhizomes (Fig. 2). No single collection was the most vigorous for all traits, and for many traits, the most vigorous population differed when grown in the field versus in a common garden. Mean effect size of source population of traits upon field collection was (0.48 ± 0.12 SE), which was slightly higher than the effect size of source population on traits after common garden rearing (0.23 ± 0.10).

Environmental Effects Experiments

Environmental conditions during each of the experiments are described in Table S1. Temperature ranged from 12 to 28 °C but did not vary by treatment. Salinity varied little (0.2–0.8 ppt), but in experiments 1 and 3 was significantly higher in elevated nutrient and both elevated nutrient and reduced light conditions. As designed, light was lower in reduced light and both elevated nutrient and reduced light conditions, and foliar C:N was elevated in high nutrient and both elevated nutrient and reduced light conditions compared to the other treatments. Dissolved oxygen was generally close to saturation but was higher in elevated nutrient and both elevated nutrient and reduced light conditions. Manipulated environmental conditions (elevated nutrients, reduced light, or a combination of the two) proved stressful for all plants, as evidenced by a reduction in new shoot production.

Field Collected *Potamogeton*



Potamogeton seedlings

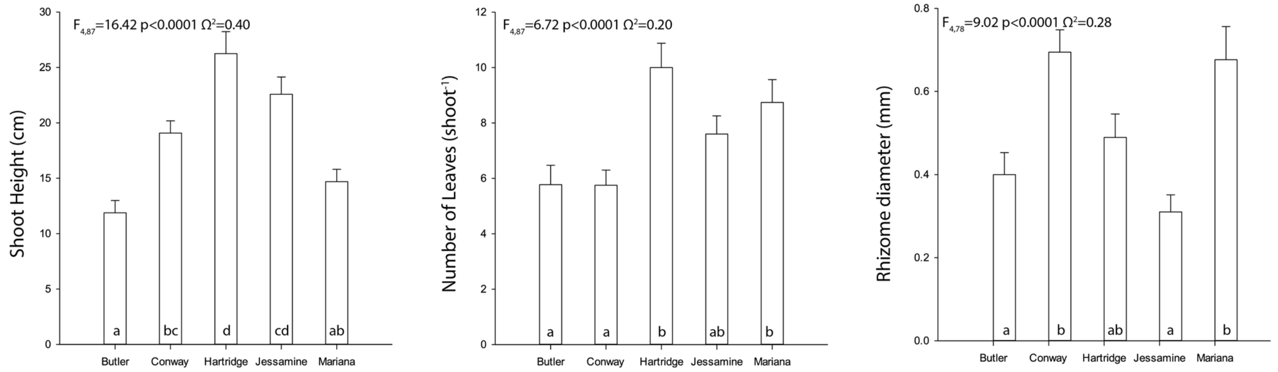


Fig. 2 Traits of plants *Potamogeton* (mean and SE) at field collection (in grey) and traits of seedlings germinated and grown in a greenhouse common garden (in white). Letters represent differences determined using Tukey Kramer post hoc analyses following an ANOVA. Upper and lower case letters represent different analyses. Each graph includes the F statistic and p value for the main effect of source population on the trait, and omega squared effect size for the variation in the trait explained by source population

Experiment 1 – *Vallisneria* Cultivar Variability

The widely used *Vallisneria* cultivar produced fewer new shoots when conditions were manipulated to reduce light and/or increase nutrient concentrations (Fig. 3). Environment also had a significant effect on shoot height and number of leaves, with both traits increasing in the reduced light treatments. Environment did not affect rhizome diameter (Fig. 3). Results were similar whether analyzed as repeated measures over time (Fig. S2, Table 1) or as traits of plants surviving at the end of the experiment (Fig. 3). The effect size of environmental conditions on plant traits ranged from 0 to 0.89 (Fig. 3).

Experiment 2 – *Vallisneria* Source Variability

Overall, new shoot production was highest under control conditions, and Lake Tennessee *Vallisneria* plants tended to produce more new shoots. However, new shoot production varied with both stress and by source, as evidenced by significant source*treatment interaction when analyzed over time (Fig. S3, Table 1). This trend was evident but the interaction was not statistically significant when analyzed only using measurements at the end of the experiment ($F_{8,23} = 1.5$, $p = 0.15$, Fig. 4). Plant height increased under reduced light and reduced light combined with elevated nutrient conditions. Source, treatment, and their interaction explained a significant amount of the variation when analyzed over time (Fig. S4, Table 1) and as measurements at the end of the experiment (Fig. 4). Number of leaves and rhizome diameter were measured only at the end of the experiment. The number of leaves, like shoot height, varied by source, treatment, and interaction between the two, while rhizome diameter only varied by source, with Lake Maude tending to have plants with larger rhizome diameters (Fig. 4). For this experiment, the effect size of environment (within population) on plant traits ranged from 0 to 0.75 (Fig. 4).

Experiment 3 –*Potamogeton* Source Variability

Over time, *Potamogeton* new shoot production and shoot height was greatest under control conditions and

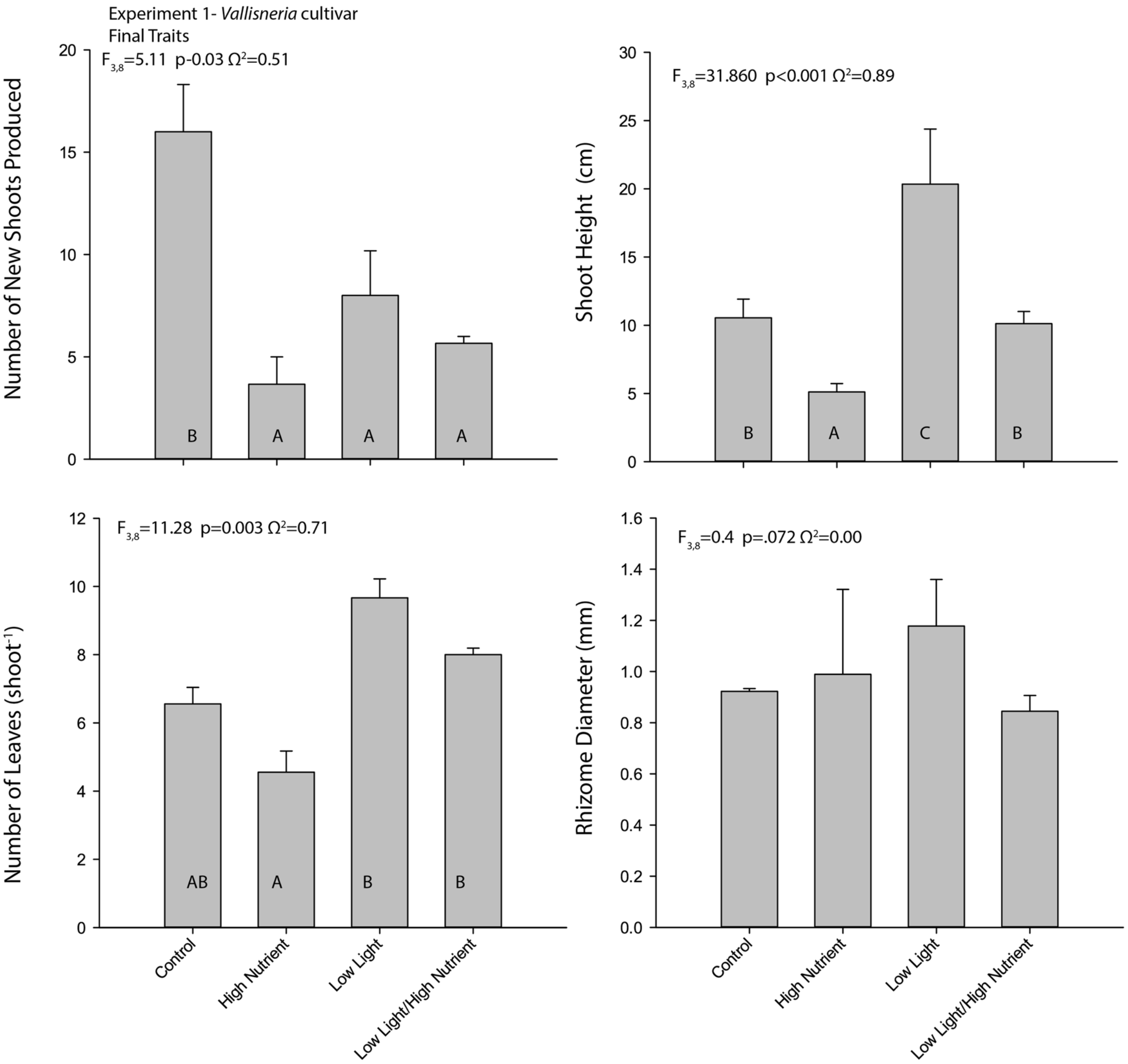


Fig. 3 Shoot production and traits (mean and SE) of the *Vallisneria* cultivar after 16 week incubation under different environmental conditions (Experiment 1). Letters represent differences determined using Tukey Kramer post hoc analyses following an ANOVA. Each graph includes the *F* statistic and *p* value for the main effect of environmental treatment on the trait, and omega squared effect size for the variation in traits explained by environmental treatments

significantly higher in the Kirby Pit and Ichetucknee sources compared with the nursery source (Fig. S5 and S6, Table 1). For all sources, stress-reduced shoot production and shoot height, especially when nutrient levels were elevated. In fact, most plants died in elevated nutrient concentrations and reduced light conditions combined with elevated nutrient concentrations (Fig. S5). The relatively poor performance of the nursery source disappeared under stress conditions, as performance of the other two sources was more impacted by stress. In other words, the best performing source changed with environmental condition—i.e., a significant source*treatment interaction (Table 1).

When traits were analyzed at the end of this experiment, the ANOVA models show that treatment effects were largely not statistically significant. However, we note that statistical power was low for this analysis resulting from high mortality, especially in the elevated nutrient + reduced light treatment (Fig. 5). For this experiment, the effect size of environment (within population) on plant traits ranged from 0 to 0.48 (Fig. 5).

Effect Size

Mean effect size (impacts of population, environment, or a combination of the two on plant traits) varied with response variable, but in many cases the confidence interval included zero (equivalent to "no effect"). Effect sizes did not vary significantly between traits ($F_{2,13} = 1.18$, $p = 0.34$), species ($F_{1,13} = 0.05$, $p = 0.82$), or source of variation (population, environment, or a combination of both $F_{2,13} = 0.72$, $p =$

Table 1 Repeated measures ANOVA results for each experiment

		Experiment 1		Experiment 2		Experiment 3	
		New shoots	Shoot height	New shoots	Shoot height	New shoots	Shoot height
Source	*df*			3,567	3,567	2,226	2,226
	F			35.39	135.36	37.1	4.04
	p			<0.0001	<0.0001	<0.0001	0.0189
Treatment	*df*	3,56	3,56	2,567	2,567	3,226	3,226
	F	21.84	29.1	146.51	2.12	26.16	7.11
	p	<0.0001	<0.0001	<0.0001	0.12	<0.0001	0.0001
Date	*df*	6,56	6,56	13,567	13,567	5,226	5,226
	F	5.2	12.86	20.37	9.46	4.08	4.47
	p	0.0003	<0.0001	<0.0001	<0.0001	0.0015	0.0007
Source*Treatment	*df*			6,567	6,567	4,226	4,226
	F			3.23	6.42	6.74	0.055
	p			<0.0001	<0.0001	<0.0001	0.6957
Source*Date	*df*			14,567	14,567	10,226	10,226
	F			2.27	1.77	1.9	2.42
	p			0.0052	0.0405	0.0468	0.0094
Treatment*Date	*df*	18,56	18,56	26,567	26,567	15,226	15,226
	F	2.15	3.24	15.04	0.74	2.97	1.52
	p	0.0154	0.0004	<0.0001	0.8266	0.0002	0.0998
Source*Treatment*Date	*df*			26,567	26,567	16,226	16,226
	F			0.7	0.65	0.78	0.41
	p			0.8751	0.9212	0.7027	0.9788

Data are presented in the Supplementary figures

*signifies the interaction term

0.50) (Fig. 6), indicating that no one source of variation was a stronger driver of trait variability than the others.

Discussion

These results showcase the high variability in potentially ecologically important traits in two species of submerged aquatic vegetation. We found that traits of plants from different water bodies varied at collection, after germinating and rearing under common greenhouse conditions, and again after experimental incubation under light and nutrient conditions known to stress SAV (Dennison et al. 1993). As expected, low light and elevated nutrients reduced survival and new shoot production. However, effect sizes attributed to source population (i.e., genetics) and to the environment were nearly equal, indicating that both play a role in establishment and growth of SAV. Therefore, both the source population characteristics and their growing environment should guide effective selection of restoration source material.

The impact of stress on trait variation differed both between species and between measured traits. Shoot height and number of leaves varied with stress for *Vallisneria* but not for *Potamogeton*, and rhizome diameter did not vary by environment. Likewise, in many cases, the response of plant traits varied by source, as evidenced by significant source*treatment interactions. These interactions were frequently significant when analyzed over time (Table 1, Figs. S2–S6) but not when analyzed as traits at the end of the experiment (Figs. 3, 4, and 5). This is likely a result of reduced statistical power over time, as mortality reduced sample sizes in stress treatments at the end of the experiment, especially in Experiment 3 *(Potamogeton)*. Importantly, trends, while often not statistically significant, are still evident when analyzed at the end of the experiment. For example, *Vallisneria* plants from Lake Maude produced relatively high numbers of new shoots under control and low light conditions but performed poorly when nutrient levels were high (Experiment 2). This trend is evident under both analysis scenarios, but the interaction is only statistically significant when analyzed over time ($F_{6.648} = 9.12$, $p < 0.0001$) and not at the end of the experiment ($F_{8,12} = 1.5$, $p = 0.15$).

Our data suggest that using the cultivar approach would not increase initial restoration success in this scenario (i.e.,

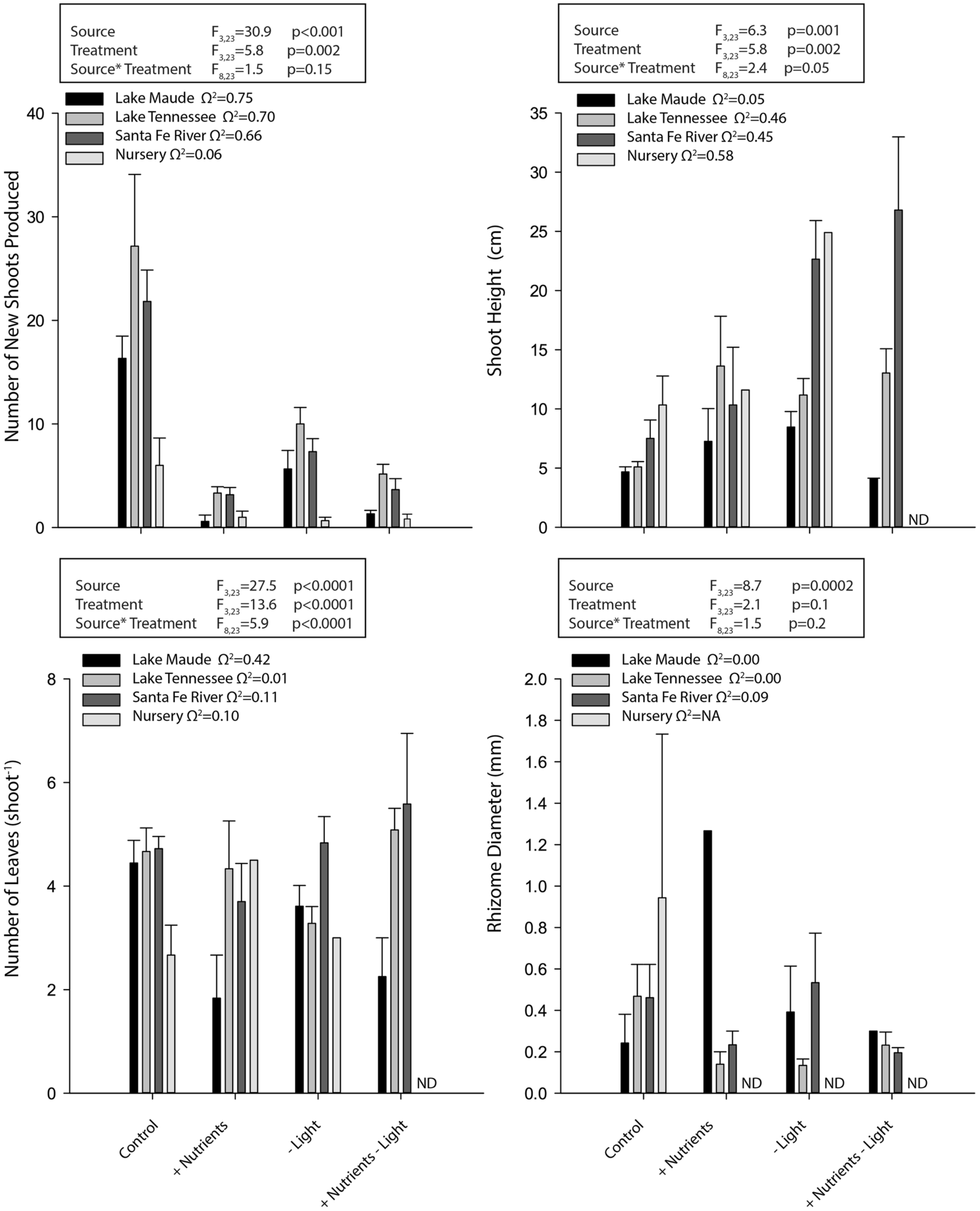


Fig. 4 *Vallisneria* traits (mean and SE) from four different source populations after 20 weeks of incubation under different environmental conditions (Experiment 2). Boxed results are ANOVA results, and because of the significant interactions, omega squared was calculated as the effect size if the population was analyzed alone. ND indicates no data due to high mortality in that treatment

these plant sources and environmental conditions). Cultivars are often used in restoration projects because their desirable traits facilitate rapid establishment under suboptimal conditions (Seliskar 1998; Lesica & Allendorf 1999; Lambert et al. 2011). Importantly, SAV cultivars are available in Florida (Gettys & Haller 2013), propagated at multiple culture facilities (Eric Latimer, pers comm.; Cater Henne, pers comm), and used widely across the state (East Coast of FL: Jordan 2021; West Coast of FL: Save Crystal River Staff (2021), Eric Latimer, pers comm, Central Florida lakes: Eric Latimer, pers comm). We expected smaller differences in performance between control and

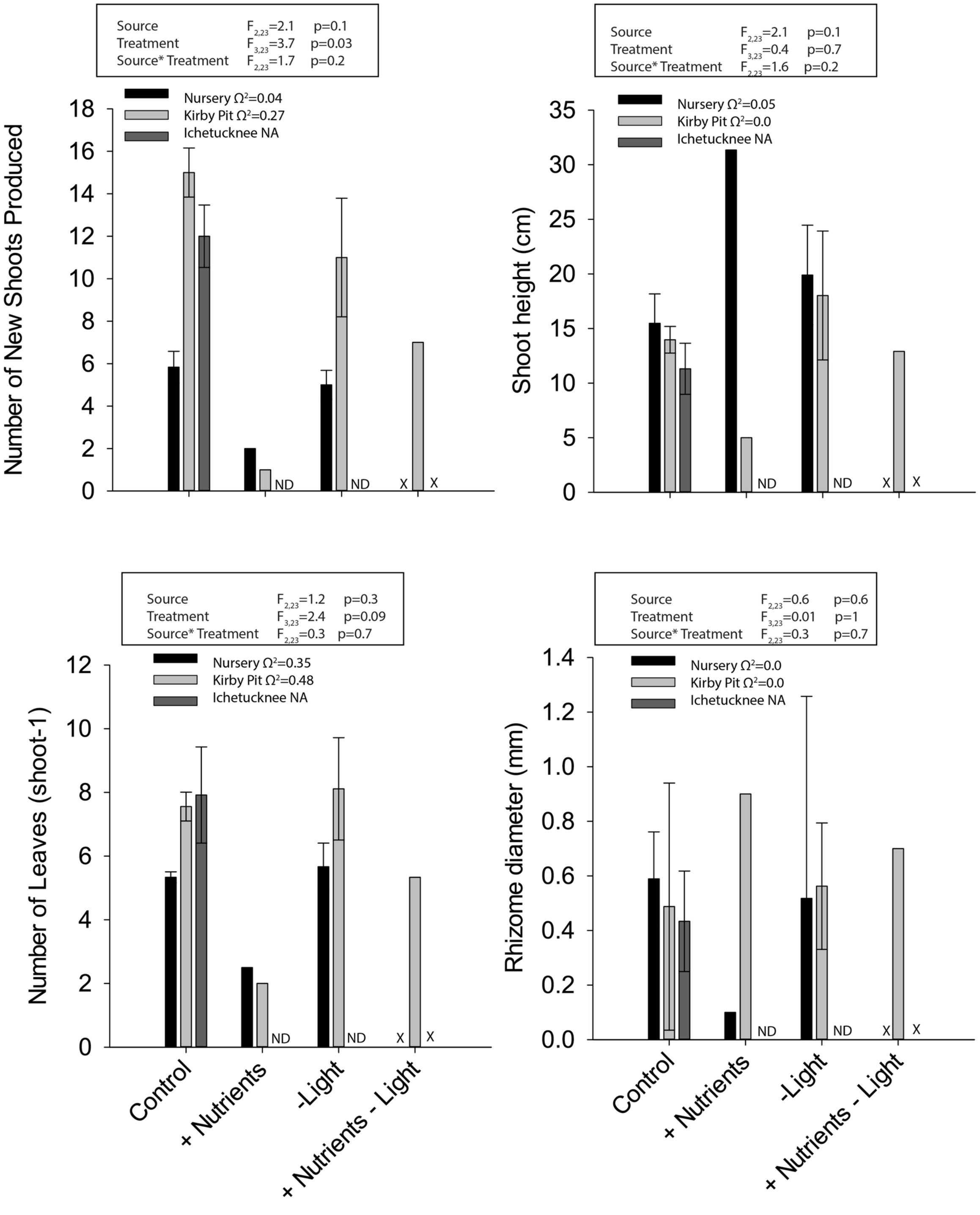


Fig. 5 *Potamogeton* traits (mean and SE) after 16 weeks of incubation under different environmental conditions. Statistical results are from ANOVA. Boxed results are ANOVA results, and omega squared represents the effect size if the population was analyzed alone, which was necessary because of the significant interactions. The Ichetucknee source was only used in the control and +Nutrients −Light treatments (high water levels prevented adequate collection for inclusion in all treatments). ND indicates no data, and X indicates complete mortality

stressed treatments for cultivars as opposed to natural collections. Although not directly comparable because of the timing of experiments, the relative effect of environmental change for the cultivar was not reduced; it did not better resist stressors or establish quicker than natural collections (Experiments 1 and 2). Low light and high nutrient conditions reduced cultivar shoot production (93%) and shoot height (76%) relative to the control, similar to the stress response in nursery and lake collected sources (shoot production reduced 90–122% and height by 44–78% relative to

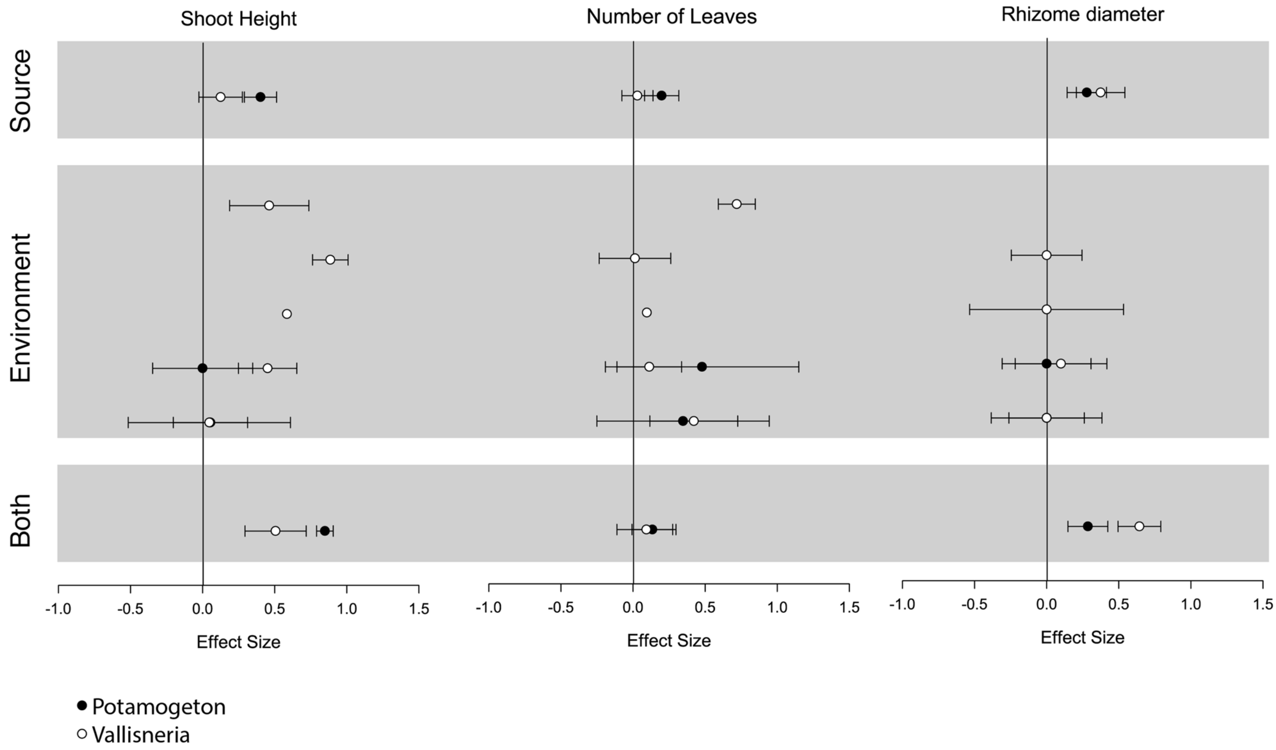


Fig. 6 Omega squared effect sizes explain variation in traits due to environmental effects (collection source-specific analyses of experiments), collection source effects (seed germination in the greenhouse), and a combination of the two (field collection). Effect size statistics are means with 95% bootstrapped confidence intervals

control) (Figs. 3, 4). Effect sizes also reflect this comparatively similar stress response, with new shoot production, plant height, and number of leaves ranging from 0.51–0.89 for the cultivar (Fig. 3) and 0.01–0.75 for natural collections (Fig. 4).

Even if this *Vallisneria* cultivar had performed better than lake collections, there are risks associated with the widespread planting of cultivars in restoration practice (Lesica and Allendorf 1999). *Vallisneria* is dioecious; thus, single clones of *Vallisneria* can spread asexually but will not sexually reproduce (Cook et al. 1974). Therefore, restorations solely using this material lack the ability to adapt to changing conditions through genetic recombination. A *Potamogeton* cultivar could allow for comparatively more adaptation because this species is monoecious and can self-pollinate (Philbrick and Anderson 1987). However, sexual reproduction within a small gene pool increases the probability of inbreeding depression. In the case of either species, adding a large number of genes that are not locally adapted can result in outbreeding depression through sexual reproduction with remnant populations (Hufford and Mazer 2003). Even sourcing plants from a single lake (e.g., closely related clones), as opposed to a single clone, would diminish these issues. The increased genetic variability within natural collections is illustrated by the greater trait variability observed in our experiments. At the end of Experiment 2, plant traits for related *Vallisneria* individuals varied substantially in both shoot production (SE 0.3–6.3) and shoot height (SE 0.5–8.7) (Fig. 4), while the single cultivar varied substantially less (SE 0.7–1.5 new shoots, SE 0.2–2.8 shoot height) (Fig. 3).

Plants from hydrologically isolated lakes had persistent trait differentiation in our experiments, indicating genetic-based differentiation between lakes. The possibility for genetic differentiation is further supported by observations of considerable genetic differentiation of *Vallisneria* even within a connected waterbody (Chesapeake Bay) (Lloyd et al. 2011). Because we reared field-collected seeds for a single generation under the common conditions, there is potential for unaccounted-for contribution of maternal effects (Gutterman 1992). However, eliminating maternal effects in this experiment would likely have led to unrealistic estimates of SAV performance in restoration scenarios since maternal effects may be a part of the natural population differentiation and contribute adaptability (Bischoff and Müller-Schärer 2010).

Apparent genetic differences suggest the potential for local adaptation, and our experimental results also support this theory. Differences in traits from different source collections suggest that there may be some clones or closely related genotypes that withstand a single stressor more effectively than others. In our experiments, frequent significant source*treatment interactions suggest changes in environmental conditions will alter the relative performance of that source population. Importantly, we only tested two different environmental variations—light and nutrients—and our environmental manipulations, while realistic, were harsh and designed to cause stress. Likewise, our plant material—the material available for purchase for restoration—came from mostly clear water bodies. We did not match the collection and restoration condition. Relative performance of sources may differ with more moderate stress levels or under additional untested conditions (e.g., temperature and current velocity) that may impact plant traits alone or combined with other stressors (Kupsky and Dornbush 2019). Given the large suite and unpredictability of potential stressors that impact SAV traits, even small differences in collection and restoration environments may reduce the efficacy of the local adaptation approach. The cultivar approach, likewise, requires complete understanding of the restoration site to select an appropriate cultivar.

The apparent issues with the cultivar and local adaptation approaches suggest that SAV restorations should use diverse restoration material to increase the probability of incorporating plants with desirable traits. A reported barrier to incorporating genetic diversity is the additional cost and logistics of genetic analyses (Hughes et al. 2018). Importantly, the mechanism for the positive effects of genetic diversity is trait diversity (Hughes et al. 2008). While this study did not genetically analyze source plants, we observed substantial trait differences among populations at collection of plant material and also after seed germination and rearing under common conditions, suggesting genetic differences among collection sources and confirming the approach of increasing trait diversity through diverse collections is valid. Given the advantages of locally adapted material and the potential impacts of maternal effects, collecting diverse source material should be paired with the local adaptation approach by selecting multiple water bodies with similar environmental conditions to the restoration site. By combining these strategies, plant selection is most likely to satisfy two common goals in restoration plantings: ecosystem function (via diversity in plant collections) and long-term population persistence (via locally adapted material) (Kettenring et al. 2014).

The diversity approach also offers insurance against poor trait selection (Hughes et al. 2008). In this study, we considered the major traits that may contribute to restoration success—shoot height, number of leaves, new shoot production, and rhizome diameter. There were not enough data to investigate tradeoffs, but collections with the tallest shoots were not always the ones that produced the newest shoots. The literature also indicates potential trait tradeoffs in SAV species—plants that allocate more biomass to belowground storage structures have lower sexual reproduction (Li et al. 2018) and larger plants reproduce clonally at slower rates (Engelhardt et al. 2014). Restoration goals may guide plant trait selection; for example, a practitioner may select a source with high reproduction rates to promote population spread and self-sustainability (Ruiz-Jaen and Aide 2005), or they may choose a source with longer leaves to reach light higher in the water column when a restoration site has sub-optimal light conditions. However, additional unmeasured traits may be more important for restoration success, such as deeper roots that may access more nutrient resources (Hughes et al. 2009) or higher secondary compounds that may resist grazing stressors (Vergés et al. 2007). Prioritizing one trait over the other is difficult, and varies with restoration goals and environment. The diversity approach relies less on proper trait selection and instead aims to incorporate desirable traits through probability.

This work provides recommendations for SAV restoration source selection—we suggest collection from multiple locations with similar environmental conditions to the restoration site—incorporating population and environmental effects. Importantly, it is common practice to use the same planting sources throughout the state (East Coast of FL: Jordan 2021; West Coast of FL: Save Crystal River Staff (2021), Eric Latimer, pers comm, Central Florida lakes: Eric Latimer, pers comm) over similar or in some cases larger distances than the lakes sampled in this study (Fig. S1). While the movement of introduced aquatic plants is regulated, there are no regulatory barriers for moving native aquatic species throughout Florida (FDACS- Florida Department of Agriculture and Consumer Services (2018)), making this approach possible in this region. Our data suggest that for these SAV species in this region, plants with diverse traits can be selected without genetic analyses, which are declining in cost but still relatively expensive. However, genetic analyses, when feasible, will create more certainty in maximizing genetic diversity in source selection. Introducing inappropriate plant material could have negative consequences (Hufford and Mazer 2003), such as poor performance (if the donor and restoration sites are a poor match) or outbreeding depression from breeding between introduced plants and remnant populations (Marsden et al. 2013).

These and other studies of biodiversity influences on ecosystem function suggest that increased diversity in restoration plantings can arm natural and newly restored ecosystems against stressors with variability in ecologically important traits (Hughes et al. 2008). Natural systems often have shifting stress regimes due to changing environmental conditions. Because source populations respond differentially to stressors, higher diversity plantings are most likely to establish and persist in unpredictable future environments. While the three experiments were independent and therefore only explicitly tested within species variability, the consistency of results across experiments (e.g., common source*environment interactions) and the differences in relative species performance (e.g., high *Potamogeton* mortality indicated increased vulnerability to light and nutrient stressors compared to *Vallisneria*) suggest that both genetic and species diversity are important. Given changing environmental conditions, diverse sources will increase the probability of having high performers or survivors. This is documented in both restoration settings (Reynolds et al. 2012) and natural SAV populations. In the Chesapeake Bay, repeated high-temperature events resulted in die-off of the dominant *Zostera marina*; however, co-occurring more resistant *Ruppia maritima* is increasing in abundance reducing the total coverage loss (Moore et al. 2014, Shields et al. 2019). Although some SAV populations or clones can resist specific stressors better than others, it is prudent to choose collection material with potential for local adaptation. Therefore, we recommend collecting material from several water bodies where plants are thriving under conditions similar to restoration sites.

Particularly for large restoration projects in highly degraded systems, appropriate guidance for effective plant selection has a high potential to contribute to maximizing ecosystem recovery. Although our experiments were in controlled conditions which do not approximate a natural lake, this experimental setting allowed isolation of common stressors on these species. Our findings contribute to a growing body of work illustrating the importance of diversity to ecosystem function and therefore restoration goals. Because it requires considerable investment, increasing source material diversity is only logical for species for which gains are well-founded. This information is surprisingly lacking for many commonly implemented revegetation scenarios, creating a critical science gap for ecological restoration. By providing evidence that both genetic and species diversity are important goals, our work fills that gap for these common species used in the restoration of large, degraded lakes.

Data Availability

Data available upon request

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Conflict of Interest The authors declare no competing interests.

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