

## Note

# Mesocosm evaluation of growth differences between *Vallisneria* taxa

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### INTRODUCTION

Aquatic macrophytes are an integral part of aquatic ecosystems, improving water quality, providing soil stabilization, and providing food and habitat to aquatic fauna. Invasion by weedy species can threaten biodiversity and reduce the ecological and economical services that diverse communities of aquatic macrophytes can provide. Restoration and the presence of native plants can promote biodiversity, reduce invasion potential and the spread of invasive/exotic species, and improve ecosystem health (Petruzzella et al. 2020). *Vallisneria americana* (Michx.) has commonly been chosen as a desirable species for restoration and revegetation efforts in the eastern United States because of its benefits to aquatic biodiversity including soil stabilization, water quality improvement, and serving as habitat to aquatic fauna (Korschgen et al. 1987, Gettys and Haller 2013).

Recent proposed taxonomic revisions and identifications of new *Vallisneria* taxa complicate comparisons with previous studies on *V. americana* (Wasekura et al. 2016, Gorham et al. 2021, Martin and Mort 2023, Les et al. 2008). Several exotic taxa have been identified in the United States: *V. australis* (S.W.L. Jacobs & Les) in California and *V. × pseudorosulata* (S. Fujii et M. Maki) in the Southeast (CDFA 2021, Gorham et al. 2021). *Vallisneria × pseudorosulata* is the proposed hybrid of *V. spiralis* Makino and *V. denseserrulata* L., first identified in Japan and suggested to be hybridized in the aquarium trade because of the lack of overlap in the geographic distribution of the parent species (Wasekura et al. 2016). There is also no definitive understanding of how long these exotic taxa have been present in their invaded ranges, and previous studies may have accidentally attributed differences between taxa as differences between populations or biotypes if genetic testing was not performed.

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DOI: 10.57257/JAPM-D-24-00022

*Vallisneria americana* produces ribbonlike leaves in a rosette and unbranched roots from the base of the rosette (McFarland and Schafer 2008). This species is dioecious, reproducing through pollination at the water surface or asexually via rhizomes and stolons (Korschgen et al. 1987, McFarland and Shafer 2008). Additionally, plants in northern latitudes have been observed to produce overwintering buds (also referred to as turions or tubers) that were not observed in southern populations or taxonomic guides for the Southeast (McFarland and Shafer 2008). *Vallisneria americana* has also historically been split between narrow-leaved and broad-leaved variants or northern and southern biotypes (McFarland and Schafer 2008, Gettys and Haller 2013, Beets et al. 2019). There is evidence for a taxonomic revision of *V. americana* into two species: *V. americana* and *V. neotropicalis* Vict., which may correspond to some previously observed biotype or geographic differences (Martin and Mort 2023).

There is little documentation on the growth of the newly identified exotic *Vallisneria* taxa in their home range or in the populations that are spreading in the United States. In contrast, hydrilla (*Hydrilla verticillata* (L.f. Royle)) is one of the most widespread aquatic weeds in the United States and has been thoroughly studied. Native *Vallisneria* populations are also commonly in competition with hydrilla in water bodies, so revisiting growth differences with an improved taxonomic understanding can aid restoration and management efforts.

Previous studies on *V. americana* have noted differences in growth, morphology, and response to herbicides among variants or biotypes (McFarland and Schafer 2008, Gettys and Haller 2013, Mudge 2013, Beets et al. 2019). However, it is unclear if previous research tested true *V. americana* or other taxa since recent taxonomic revisions of the genus include exotic species that are easily misidentified. Many species in the genus share morphological characteristics such as vertical ribbonlike leaves in rosettes and the discerning characteristics such as leaf striations can be hard to distinguish in field conditions and without close examination (Wasekura et al. 2016, Martin and Mort 2023). Additionally, it is unclear what influence environmental or seasonal conditions may have on these characteristics. Therefore, differential growth rates, phenological patterns, and herbicide response among *Vallisneria* taxa must be revisited to inform future revegetation efforts, ecosystem evaluations, and aquatic plant management programs as *Vallisneria* taxa are becoming increasingly

problematic in the United States. The objective of this study was to provide a small-scale view of differences in growth among four *Vallisneria* taxa.

## MATERIALS AND METHODS

Greenhouse mesocosm experiments were conducted in summer of 2023 (run 1 in June and run 2 in July) at North Carolina State University in Raleigh, NC (35°48'37.0002"N, 78°43'18.1704"W). Plant material of the four *Vallisneria* taxa was sourced from the following sites: *V. americana* from the Connecticut River, CT (41°28'59.988"N, 72°30'23.6154"W), *V. neotropicalis* from Lake Gaston, NC (36°29'52.512"N, 77°50'37.356"W), *V. australis* from Lake Mattamuskeet, NC (35°31'12.468"N, 76°6'31.68"W), and *V. × pseudorosulata* from Wheeler Lake, AL (34°37'28.416"N, 86°59'0.924"W). Monoecious hydrilla was included as reference of a widespread invasive species and was originally sourced from an impoundment in Granville County, NC (36°8'13.1742"N, 78°47'41.9238"W).

We performed species identification using the internal transcribed spacer (ITS) following the methods of Gorham et al. (2021) and Martin and Mort (2023). A neighbor-joining tree with the ITS sequences was constructed in MEGA X (Kumar et al. 2018) using the Kimura two-parameter model of substitution, complete deletion of gaps and missing data, and 1,000 bootstrap replicates. Samples were identified to taxon on the basis of their relationship to reference samples in the resulting phylogeny, and in most cases were identical to one or more previously published GenBank accessions. In the case of *V. × pseudorosulata* (*V. spiralis* × *V. denseserrulata*; "rockstar"), obvious biparental polymorphisms were evident (see Martin and Mort 2023 figure 1 for illustration). The following GenBank reference accessions were used: *V. americana* (EF142958, EF142959, EF142960, EF142961, EF526407, and AY335964); *V. neotropicalis* (EF142990, EF142991, and EF142992); *V. australis* (EF142967 and EF142973); *V. spiralis* (EF142996 and EF694962); and *V. denseserrulata* (EF142976 and EF142977).

A single ramet of each *Vallisneria* taxon was planted in a 0.52-L pot containing topsoil with 3 g kg<sup>-1</sup> of slow-release fertilizer<sup>1</sup> and covered with a sand cap to reduce nutrient leaching into the water column. A single sprouted apical stem of hydrilla was planted in a 0.09-L cup with the same amended topsoil. Pots were placed in a 16-L mesocosm containing 12 L of water, with each mesocosm serving as an experimental unit in a common garden study design. Blocking was established on the basis of plant size 3 wk after planting, allowing an acclimation period. Each run of four replicates per taxon was harvested 6 wk after study initiation (WAI), co-occurring with a herbicide efficacy trial (Beets et al. 2024). Above- and belowground biomass were separated and presence of vegetative propagules was noted. Biomass was dried in a forced-air dryer at 65 C for 48 h before weighing.

Above- and belowground biomass data were square-root transformed to meet assumptions of normality and homogeneity of variance. Above- and belowground biomass data were subjected to a mixed-model ANOVA with taxa as a fixed effect and block and run as random effects. Runs were

TABLE 1. ABOVEGROUND BIOMASS, BELOWGROUND BIOMASS, AND RELATIVE GROWTH RATE OF *VALLISNERIA* AND HYDRILLA 6 WK AFTER STUDY INITIATION. VALUES (MEAN ± SE) FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT (TUKEY HONESTLY SIGNIFICANT DIFFERENCE,  $\alpha = 0.05$ ). RESULTS FROM TWO TRIALS WERE POOLED ( $n = 8$ ).

Taxa	Aboveground Biomass (mg)	Belowground Biomass (mg)	Relative Growth Rate (mg mg <sup>-1</sup> d <sup>-1</sup> )
<i>V. americana</i>	523 ± 115bc	147 ± 32b	52 ± 6a
<i>V. australis</i>	1,439 ± 62a	265 ± 31a	32 ± 1bc
<i>V. neotropicalis</i>	759 ± 65b	246 ± 35a	25 ± 3c
<i>V. × pseudorosulata</i>	582 ± 61bc	106 ± 14b	43 ± 2ab
Hydrilla	418 ± 62c	17 ± 4c	51 ± 4a

considered random because of the lack of significant differences between each run. Similarly, block run did not have a significant effect on above- or belowground biomass or relative growth rate (RGR) ( $P > 0.05$ ). Means were separated by a Tukey honestly significant difference test ( $\alpha = 0.05$ ). RGR was calculated using Equation 1 for each taxon using mean total biomass 6 WAI and subjected to a mixed-model ANOVA with the same parameters as above:

$$\text{RGR} = \frac{\ln(\text{Final}) - \ln(\text{Initial})}{\text{Time since planting}} \quad [1]$$

where ln is the natural log and final is the final total dry biomass of each replicate. Initial is the mean initial total biomass of four replicates of each taxon harvested at study initiation.

## RESULTS AND DISCUSSION

*Vallisneria australis* exhibited greater aboveground biomass than the other *Vallisneria* taxa and hydrilla. Comparisons with a thoroughly studied invasive species such as hydrilla can allow for extrapolation of observed growth rates and potential for spread of new exotic invaders. Six weeks after study initiation, *V. australis* had 2× more biomass than *V. neotropicalis* and 2.6× more biomass than *V. americana* (Table 1). *Vallisneria australis* had 2.4× more biomass than the hybrid taxon *V. × pseudorosulata*, placing it between the two native taxa. *Vallisneria australis* and *V. neotropicalis* were the only *Vallisneria* taxa that produced more aboveground biomass than hydrilla (3.2× and 1.6× respectively). Additionally, *V. neotropicalis* had 1.3× more biomass than the other native taxon, *V. americana*.

Similar trends were observed in belowground biomass compared with aboveground biomass. *Vallisneria americana* belowground biomass did not differ from *V. × pseudorosulata* but was 1.5× less than *V. australis* (Table 1). *Vallisneria neotropicalis* belowground biomass was not significantly different from *V. australis*. Hydrilla had lower belowground biomass than any *Vallisneria* taxa. One replicate of *V. americana* was observed to produce overwintering buds (turions) in the first run and three replicates produced them in run 2. These were incorporated into belowground biomass calculations and no other *Vallisneria* taxa produced these overwintering buds.

Hydrilla and *V. americana* exhibited the greatest RGR (Table 1). The RGR of *V. australis* was similar to that of *V. × pseudorosulata* 6 WAI but was 1.6× smaller than hydrilla and

*V. americana*. *Vallisneria neotropicalis* exhibited the lowest RGR, 2× lower than *V. × pseudorosulata* and 2.5× lower than hydrilla and *V. americana*.

Comparisons with previous studies on the growth of *V. americana* are complicated by the recent proposed taxonomic revisions and presence of cryptic exotic taxa with no definitive indication of how long these exotic taxa have been present in the United States. The results of this study indicate that in addition to potential population-level differences between the native *Vallisneria* taxa, *V. neotropicalis* is a more robust plant than *V. americana*, although it appears to have a lower RGR. These differences likely influence historically observed differences between *Vallisneria* in different parts of the United States. It is important to note that the plants in this study were grown together in a single mesocosm as part of a common garden study design, so competitive interactions for light and space may have influenced some of the observed differences between aboveground biomass, but also allow for a comparison of plants grown in similar environmental conditions. Plants originating from different environments may have adaptations better suited to specific environments (e.g., water temperature, light availability, photoperiod); therefore, more research is needed on multiple populations of each taxon in distinct environmental conditions to identify potential influences on existing distribution and potential spread of the exotic taxa.

Although we attempted to utilize uniformly sized plants, differences in initial ramet size were a confounding factor. The large initial size of propagated *V. australis* ramets may have contributed to its higher aboveground biomass during the study, but despite the greater initial aboveground biomass, its RGR was lower than that of other species that initiated with less than half of the biomass of *V. australis*. Competition trials and future growth studies could incorporate multiple size classes of ramets to account for some of these differences. Van et al. (1999) found that *V. americana* could outcompete hydrilla in low soil nutrient conditions, and the fertilizer addendum in this experiment (3 g kg<sup>-1</sup> of soil) was higher than the highest fertility level tested by Van et al. (1999). The low aboveground biomass of *V. americana* in shared growth conditions indicates that this species may be more susceptible to invasion by the nonnative taxa of *Vallisneria* than *V. neotropicalis*.

Overwintering bud production was only observed in *V. americana*. Historically, it was thought that winter bud production of *V. americana* was temperature or biotype dependent (or both); however, this may also be a differentiating characteristic among taxa (McFarland and Schafer 2008, Gettys and Haller 2013). One method to investigate this could be reciprocal planting of multiple populations of *V. americana* and *V. neotropicalis* in northern and southern climates to determine if winter production is limited to specific populations or taxa or is dependent on environmental conditions. Subterranean vegetative propagules can complicate management actions as many are not responsive to chemical management and necessitate repeat years of management (Van et al. 1999, Mudge 2013). If they are unaffected by chemical management and are only present in a desirable native species, they also may serve as a means of recovery and restoration after management.

Male and female flowers were observed in *V. americana* and *V. neotropicalis*, corresponding to previous observations (Korschgen et al. 1987, McFarland and Schafer 2008, Gettys and Haller 2013, Martin and Mort 2023). However, only female flowers were observed in *V. australis* and *V. × pseudorosulata*. Male flowers have been observed in *V. australis* in Japan, whereas only female flowers have been observed in *V. × pseudorosulata* (Wasekura et al. 2016, Gorham et al. 2021, Martin and Mort 2023). Further investigation is needed into flowering characteristics and seed viability, as hybridization could lead to hybrid vigor or plants with adaptive traits, further complicating management and the spread of exotic and weedy species.

The RGRs observed in this study were comparable with those observed in *Vallisneria* and hydrilla in previous studies. Spencer and Anderson (1986) observed a RGR of 33 to 41 mg mg<sup>-1</sup> d<sup>-1</sup> in monoecious hydrilla 8 WAI, whereas we observed an RGR of 51 mg mg<sup>-1</sup> d<sup>-1</sup> 6 WAI. Similarly, Van et al. (1999) observed RGR in *V. americana* between 40 and 50 mg mg<sup>-1</sup> d<sup>-1</sup>; this corresponds most closely with the RGR we observed in *V. americana* and *V. × pseudorosulata* 6 WAI. The higher RGR of *V. × pseudorosulata* compared with *V. neotropicalis* may partially explain reports from the field of this taxon becoming increasingly problematic across the southeastern United States and potentially displacing populations of native *Vallisneria*. Previous studies also observed differences in RGR dependent on soil fertilization and photoperiod, and additional investigation is needed to identify how these factors affect the growth of each of these newly identified *Vallisneria* taxa.

Further research is needed on the growth differences between native and exotic taxa of *Vallisneria* in the United States. This initial documentation in a small-scale mesocosm setting can provide a basis of understanding for future mesocosm research; however, only one population was tested of each taxon at a small scale. Understanding the growth, reproduction, and life cycle of native and invasive taxa can inform integrated management decisions including proper application timing, improving efficacy, resistance management, and reduction of nontarget effects. Previous studies have discovered differences in growth on the basis of nutrients and salinity levels between ecotypes of a single species (Gettys and Haller 2013). With the new proposed taxonomic revisions these studies should be revisited to better understand potential population-level differences between and within the *Vallisneria* taxa present in the United States. This can also provide a better understanding of differences among *Vallisneria* taxa and potential differences among populations. Understanding these differences can help inform and guide management and restoration decisions to promote healthy and functional aquatic ecosystems.

## SOURCES OF MATERIALS

<sup>1</sup>Osmocote Smart Release 15-9-12<sup>®</sup>, Scotts, Marysville, OH 43041.

## ACKNOWLEDGEMENTS

The authors thank Kara Foley, Andrew Howell, Michael Punt, Delaney Davenport, and Logan Wilson for their assistance with plant propagation and harvesting. The manuscript was reviewed in accordance with U.S. Army Engineer Research and

Development Center policy and approved for publication. Citation of trade names does not constitute endorsement or approval of the use of such commercial products. The content of this work does not necessarily reflect the position or policy of the U.S. government, and no official endorsement should be inferred. The U.S. Department of Agriculture is an equal opportunity employer and provider.

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