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PRACTICE AND TECHNICAL ARTICLE

Teal carbon eelgrass in tidal freshwater estuaries: successful laboratory trial before large-scale field restoration

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Abstract

Introduction: *Vallisneria*, a freshwater angiosperm critical to aquatic biodiversity and teal carbon storage in Great Barrier Reef catchments, has declined due to habitat degradation and poor water quality. Scalable restoration approaches are urgently needed to support ecological and climate outcomes.

Objectives: This study aimed to evaluate two *Vallisneria* restoration techniques—manual transplanting and anchoring—under controlled conditions to determine their viability for broader application in tropical freshwater systems.

Methods: Shoots collected from the Johnstone River (Queensland, Australia) were acclimated and deployed across eight experimental tanks. Growth over 6 weeks was measured using the Plastochrone method, alongside water quality monitoring and carbon sequestration estimates derived from shoot length.

Results: Both methods supported plant survival and rhizome expansion. Transplanting showed higher survivorship (87.5%) than anchoring (66.6%), though shoot growth was comparable: 98.1 cm (SE ± 33.9) for transplanting and 94.2 cm (SE ± 25.8) for anchoring ($F = 2.49$, $p = 0.14$). Estimated carbon sequestration ranged from 0.12 ± 0.10 to 0.22 ± 0.20 g C/m^2 of shoot growth, with total accumulation of 2.42 g C/m^2 over 6 weeks.

Conclusions: Both restoration methods are viable, with anchoring offering a scalable alternative where manual planting is unsafe or impractical.

Implications for Practice: This study aimed to evaluate the effectiveness of two Vallisneria restoration techniques—manual transplanting and anchoring—under controlled laboratory conditions to guide future large-scale field restoration in tropical freshwater systems. Vallisneria shoots were collected, acclimated, and deployed across eight freshwater tanks with standardized conditions. Growth was monitored using the Plastochrone method, and carbon stock was estimated from shoot biomass. Both techniques supported plant survival and rhizome expansion, with higher survivorship in transplanted (87.5%) than anchored (66.6%) shoots. Shoot growth and carbon accumulation were comparable between methods. Anchoring presents a scalable method for Vallisneria restoration. Successful field application will require improved water quality and could support blue carbon initiatives that attract conservation financing.

Key words: biodiversity, blue carbon, climate change, Great Barrier Reef, nature-based solutions

Introduction

Wetlands along the Great Barrier Reef (GBR) coast have faced significant loss and damage due to activities such as agricultural production, residential and industrial expansion, and road construction (Waltham et al. 2019). These alterations have drastically reduced the extent of wetlands (Canning & Waltham 2021), leaving many of the remaining areas in varying states of degradation, impacted by altered hydrology, invasive species, and poor water quality (Adame et al. 2019). The 2024 GBR Outlook Report emphasizes that coastal ecosystems essential to supporting the reef remain in poor condition, with no significant improvement in recent years. Freshwater wetlands, forested floodplains, grasslands, sedgelands, woodlands, and forests are among the most vulnerable, continuing to experience fragmentation, habitat modification, invasive species impacts, and high pollutant loads. As a result, species reliant on these ecosystems are under severe stress, underscoring the urgent

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need for targeted restoration and repair efforts to reverse this decline (Department of Environment Science and Innovation 2023).

Eelgrass (*Vallisneria nana*; hereafter Vallisneria) is a freshwater plant prevalent in the Australian tropics that sequesters carbon, processes available nutrients in waterways, and provides an essential habitat for fish and crustaceans (Cronk & Fennessy 2016). It is a perennial stoloniferous submerged angiosperm with shoots composed of basal narrow ribbon-like leaves as well as small rhizomes, allowing some shoots to survive and support new growth in the wet season (Rea et al. 2002). In the controlled inland rivers of southeast Australia, species within the Vallisneria taxa were formerly quite prevalent. Unfavorable alterations in water flow and quality are responsible for their demise. A cascade of changes would likely ripple across the food chain if Vallisneria disappeared (Rea et al. 2002). Habitat destruction in coastal catchments and poor water quality from nutrient discharge have shifted conditions away from those optimal for Vallisneria. This decline is likely due to decades of steady environmental deterioration. Reduced populations of species dependent on Submerged Aquatic Vegetation (SAV) beds, such as juvenile fish and invertebrates, are severely impacted. Water quality is further compromised by algal blooms and nutrient loading, leading to increased turbidity and reduced light availability for SAV (Rea et al. 2002).

Teal carbon aquatic plants refer to aquatic and semi-aquatic plant species that contribute to carbon sequestration and storage within coastal wetland ecosystems (Malerba et al. 2022; Adame et al. 2024). These plants are critical components of the “teal carbon” concept, which focuses on carbon dynamics in freshwater and coastal ecosystems such as wetlands, rivers, lakes, and estuaries. Vallisneria is analogous to marine seagrass, it is a teal carbon species, and therefore, like its marine angiosperm relative has the potential to play a significant role in blue carbon sequestration in coastal ecosystems (Comte et al. 2024). Both seagrasses and Vallisneria (likely) emphasize the critical role that submerged macrophytes in nutrient cycling, contributing to climate change mitigation and enhancing blue carbon potential (Serrano et al. 2019). Therefore, restoring and conserving submerged plants like Vallisneria could enhance atmospheric carbon storage and contribute to climate change mitigation strategies, in addition to improving water quality and increasing habitat potential for aquatic species—including in the GBR catchments where this species is found and there is a major opportunity for restoration of blue carbon ecosystems (de Paula Costa et al. 2022; Waltham et al. 2023). Future research is needed to quantify the carbon storage capacity of this plant species and to examine ways to assess their role in broader blue carbon initiatives.

The requirement for more robust and scientifically grounded restoration methodologies is apparent, especially as global environmental changes intensify stressors on aquatic ecosystems (Neeson et al. 2016). The lessons learned from both successful and unsuccessful projects underline the importance of addressing site-specific challenges while also considering broader ecological and socioeconomic contexts (Sheaves et al. 2021). Future research must prioritize innovative techniques, long-term monitoring frameworks, and the development of cost-effective approaches that enable scalability (Selkoe et al. 2015). We used laboratory tanks to trial hand planting and using an anchor system that could be easily deployed from a vessel, which is important in tropical locations where estuarine crocodiles are abundant. Growth rates were measured weekly along with carbon biomass to demonstrate which restoration approach is useful before large-scale field deployment operations could commence in the future.

Methods

Plant Collection and Tank Setup

Vallisneria was collected from the Johnston River, north Queensland, Australia (−17.506°, 145.992°) in June 2024. Vallisneria, consisting of rhizomes and leaf blades (shoots) approximately 10–15 cm in length (for both rhizomes and leaf blades), was removed by hand from a boat at low tide (note that the location of this species in the river is dominated by freshwater conditions, though is tidally influenced by the downstream estuary flushing where saline water is not able to reach the location of the meadows because for freshwater influence from the catchment). Plant material and sand substrate were placed into storage bins filled with water from the river and transported back to the laboratory within 48 hours. In the laboratory, plant material was transplanted to 400 L tubs filled with freshwater on the laboratory recirculatory system and was inserted into sand/gravel sourced from Wadda Mooli Creek, James Cook University Campus, Townsville, Australia (−19.324°, 146.762°). Vallisneria was maintained in the tubs approximately 6 weeks before the restoration trial. Algae and dead leaves on plants were removed, as necessary, during this tank acclimation period.

Eight tanks (80 cm × 60 cm × 60 cm) were setup in the laboratory, consisting of 5 cm of sediment collected at the Wadda Mooli Creek. The tanks were filled with freshwater from the recirculatory system. Above the tanks, approximately one per tank, were Fullgain—2835—78 to 60 mm aquarium lights (12 V) suspended approximately 30 cm above the tanks, producing around 197 Iumen units of light (12 hours day, 12 hours night light cycle). One air stone was placed in each tank to aerate the water but was gentle enough to not cause any problems for plants. All tanks contained six shoots, three hand-planted (transplanted) and three anchored (Fig. 1). The tanks were not connected and are therefore independent of each other. All shoots in tanks were not floating in the first 24 hours after commencing the experiment—care was taken during the experiment to not dislodge the shoots and rhizomes.

Tank Monitoring

Water Quality and Changes. Water quality conditions were checked every week ($n = 6$) using a calibrated Quanta water quality multiprobe between the hours of 09:00 and 13:00. Each time water pH, dissolved oxygen (% saturation), SpCond (mS/cm), and temperature (°C) were measured by lowering the probe into the tank where it was allowed to equilibrate

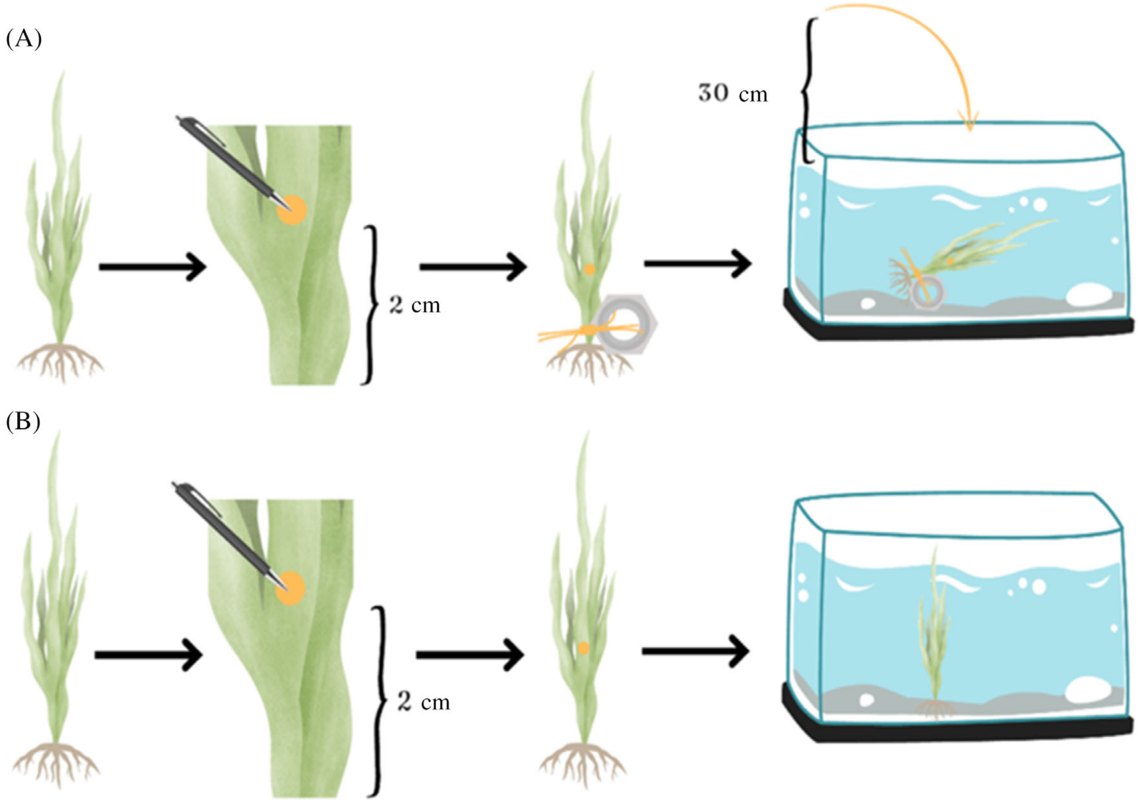


Figure 1. Process of hand transplanting and anchored planting method for Vallisneria: (A) selected Vallisneria shoot with a leaf greater than 1 mm, gently punched a hole with a pen 2 cm above the sheath, gently tying a 150 mm paper tie through 10 mm hex nut and around the shoot, dropping the shoot with the anchor 30 cm above the surface of water in the tank and dropped into the tank (note the anchored plant was not touched during the experiment to similar application in the field); (B) selected Vallisneria shoot with a leaf greater than 1 mm, gently punched a hole with a pen 2 cm above the sheath, and gently planted the shoot making sure the roots are fully submerged within the sediment.

for approximately 10 minutes, after which the results were recorded.

Tank water changes (approximately 80%) were completed every 2 weeks. Any epiphytic algae on shoot blades were removed as necessary to avoid confounding impact on plant growth (note that in the field fish and other crustaceans would likely consume these algae, but not all of it).

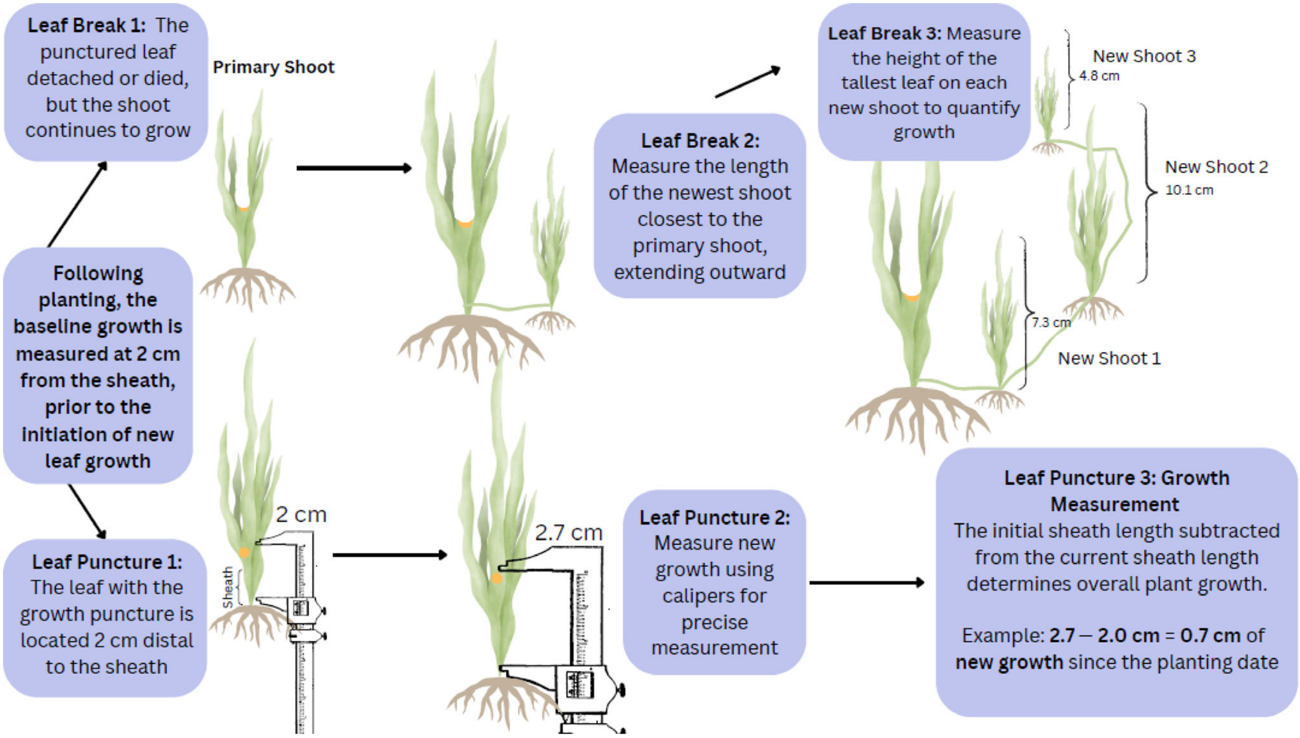
Growth Rate. We used the Plastochrone method here to measure shoot growth (Kentula & McIntire 1986). In short, a pen with a 1 mm nib was used to puncture a hole through one or two of the shoot blades, about 2 cm above the level of the sheath (where all the leaves come together) (Fig. 2). Applying this hole allowed us to measure the segment growth based on the increasing distance between the hole and the sheath. In some instances, new rhizomes emerged with new shoots. In these instances, the new shoot height above the new rhizome was measured, and so on for additional rhizomes. Where the shoot broke or died, new shoot heights were instead measured. In some cases, some plants died during the experiment; they were removed from the experiment.

Carbon Assessment

At the end of the experiment, leaf biomass samples were weighed and oven-dried at 60°C for 48 hours to derive dry biomass estimates. A fraction of the dried biomass was ground in a mortar and pestle, and 0.5 mg of the homogenized biomass samples was used to estimate the total carbon (C) content using an elemental analyzer (Elementar, UNICUBE). Leaf dry biomass (g Dw m^{-2}) was multiplied by the total C content to estimate the leaf biomass C stocks (g C/m^2) per treatment.

Data Analysis

Water quality conditions recorded in experimental tanks were examined using a Levene's *T* test to compare equality of variances. A Levene's *T* test was also performed to compare the growth of Vallisneria in the primary node between transplanted and anchored treatments, and for total rhizome growth (summing second, third, and fourth rhizomes) in experimental tanks. Data analysis was performed using IBM SPSS Statistics v29.0.



Following planting, the baseline growth is measured at 2 cm from the sheath, prior to the initiation of new leaf growth

Leaf Break 1: The punctured leaf detached or died, but the shoot continues to grow

Leaf Break 2: Measure the length of the newest shoot closest to the primary shoot, extending outward

Leaf Break 3: Measure the height of the tallest leaf on each new shoot to quantify growth

Leaf Puncture 1: The leaf with the growth puncture is located 2 cm distal to the sheath

Leaf Puncture 2: Measure new growth using calipers for precise measurement

Leaf Puncture 3: Growth Measurement
The initial sheath length subtracted from the current sheath length determines overall plant growth.

Example: **2.7 – 2.0 cm = 0.7 cm** of **new growth** since the planting date

Figure 2. Measuring growth on Vallisneria after planting primary rhizome. Growth was determined from initial small puncture on leaves (leaf puncture 1). Growth measurements calculated using caliper to determine increasing distance from sheath and puncture (2 and 3). Where the leaf broke and new rhizome growth, height was measured for tallest shoot, and for subsequent rhizomes and shoots, until the end of the experiment.

Results

Tank Conditions

Overall, the water quality conditions among tanks remained similar, with little difference in the range of conditions over the experiment. For water temperature, tanks remained within a narrow range of 21 and 23°C ($F = 3.96$, $p = 0.07$); the same was true for specific conductivity, between 0.28 and 0.38 mS/cm ($F = 0.733$, $p = 0.41$). Dissolved oxygen measured in tanks was between 7.5 and 8.8 mg/L ($F = 1.76$, $p = 0.21$), while pH in tanks was generally neutral with levels between 7.3 and 8.7 ($F = 3.75$, $p = 0.08$) (Fig. 3).

Survivorship and Primary Rhizome Growth for Both Treatments

Following the commencement of the tank trials, Vallisneria survivorship was slightly lower for the anchored treatment (66.6%) compared to transplanting rhizomes by hand (87.5%). Over the 6-week experiment, the growth rate for surviving plants was not significantly different between treatments ($F = 3.04$, $p = 0.09$), though the overall average growth was lower in the anchored treatment (1.59 cm) compared to the transplanted treatment (3.04 cm) (Table S1). It was apparent that there were some differences in growth rates among tanks, with tank 8 generally having the highest growth for both transplanted and anchored (4.1 cm anchored, 4.5 cm transplanted), while tank 2 had the lowest growth (0.4 cm anchored, 1.4 cm transplanted) (Table 1).

New Rhizome Growth for Both Treatments

Only plants transplanted in tank 4 did not produce new rhizome growth, which was different from plants with the anchored treatment in tank 4 producing new rhizome growth. All other tanks had rhizome and shoot growth for both treatments, with at least a first rhizome node of growth, and some replicates producing a second rhizome, and a third rhizome in some tanks. Only tank 8 had three rhizome nodes in both transplanted and anchored treatments. In this study, while growth was a few centimeters over the course of the experiment for the primary rhizome nodes for both transplanted and anchored plants in tanks (see above), growth in shoots at rhizomes 1, 2, and 3 was far greater, with up to 103 cm in transplant plants in tank 5 (the highest individual replicate had total shoot growth of 105.6 cm; Table S2), which is far greater when compared to other tanks, for example, as little as 1.7 cm of growth was recorded for anchored plants in tank 2. When the shoot growth of plants in all tanks is summed, there was 11.05 m linear meters of plant shoots in anchored plants and 11.8 m linear meters of shoots for transplanted plants, with an overall combined sum of 22.8 m linear of shoots across tanks over the 6-week experiment. This means overall that while there was little shoot growth in the primary node after planting, for replicates that spread new rhizomes continued to grow shoots, tending to grow rapidly with large shoots extending in several cases over a meter. Overall, there was no significant difference in the growth of shoots between planting treatments, with an average tank shoot growth of 94.2 cm (SE ±25.8 cm)

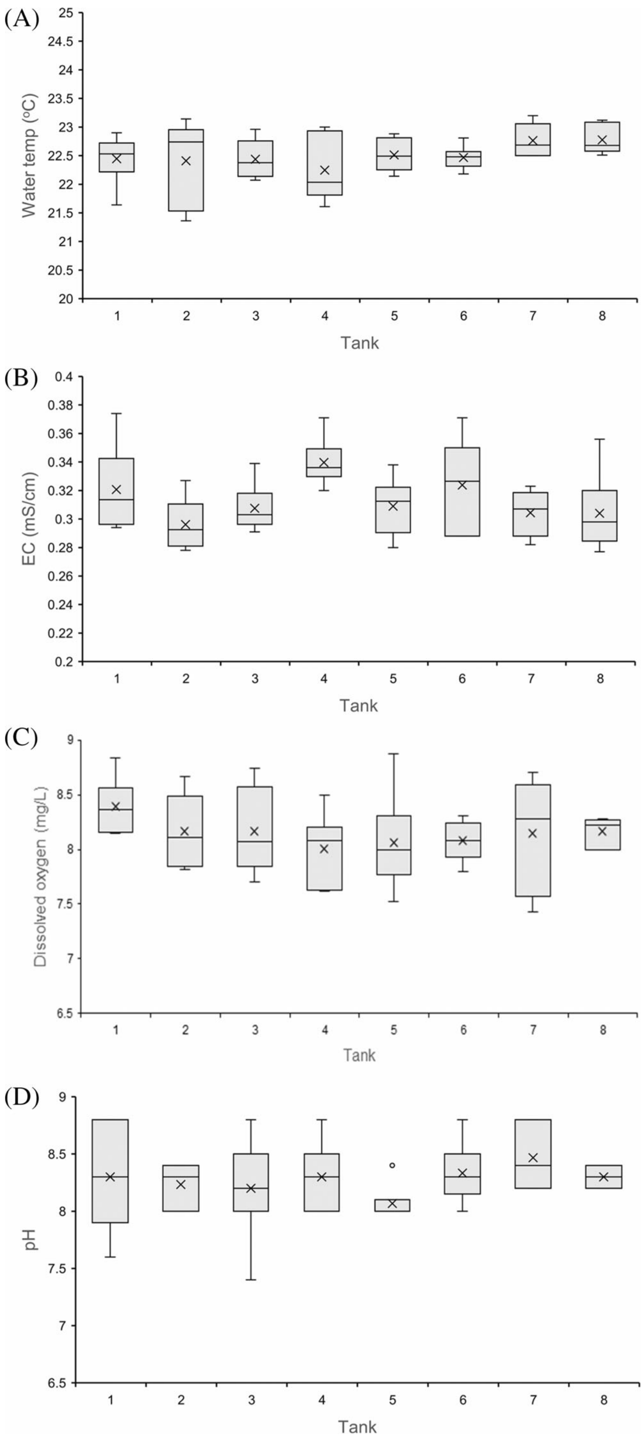


Figure 3. Box plots of water quality conditions recorded in experimental tanks. (A) water temperature; (B) electrical conductivity (mS/cm); (C) dissolved oxygen (% saturation); and (D) pH. Box plots illustrate the distribution of results for each water quality parameter ($n = 6$). Whiskers present the maximum and minimum, boxes are the upper and lower quartiles, solid line is the average, (x) is the median, and outlies are (o) also included (only for pH, Site 5).

per rhizome node for anchored and 98.1 cm (SE ±33.9 cm) of shoot growth per rhizome node for transplanted plants ($F = 2.49$, $p = 0.14$).

Carbon Stock

The shoot biomass C stocks were not significantly different ($F = 2.66$, $p = 0.14$) across the treatments. However, in most of the tanks, the transplanted shoots had higher biomass C stocks compared to the anchored shoots, except for tanks 6 and 7 (Fig. 4). The shoot biomass C stocks of the anchored shoots ranged from 0.021 to 0.086 g C/m^2, whereas that for the transplanted shoots ranged from 0.033 to 0.13 g C/m^2 (Fig. 4). This equates to a total (anchored; 1.01 g C + transplanted; 1.41 g C) of 2.42 g C/m^2 in our tanks over the 6-week period.

Discussion

The surprising growth rates here were accomplished in controlled experimental tanks that were under constant water quality settings with regular changes in a controlled laboratory room. The rivers and estuaries of northern Queensland, draining to the GBR lagoon, are dynamic transitional waterways influenced by seasonal rainfall patterns, which result in considerably variable water quality conditions (Brodie et al. 2010; Kroon et al. 2012). Conditions include strong diel (day/night) cycling of water quality, most notably dissolved oxygen and water temperature, which can vary strongly depending on biological and physiological processes or settings (Arthington et al. 2015; Waltham & Fixler 2017). Because of extensive expansion of floodplain and lowland development, mostly for agricultural production including sugar cane (Bunn et al. 1997; Bramley & Roth 2002), water quality run-off from this land use to the GBR lagoon is a major challenge and has been ongoing for several decades (Bainbridge et al. 2024). Excessively high nutrient and sediment loads are regularly recorded during the wet season (Brodie et al. 2012). It is not clear how the anchoring approach in rivers and tidal freshwater estuaries of northern Queensland experiences these water quality conditions (Kroon et al. 2012). Presumably, under high sediment/turbidity periods, growth rates would be abridged, but with improving conditions, the high nutrient availability might achieve added growth rates beyond those observed in the experimental tanks. A notable concern would be whether trace levels of pesticides and herbicides (Hook et al. 2024) in rivers and estuaries would compromise the growth possible using available high nutrients— this requires further examination.

In addition to water quality conditions, growth rates may also be influenced directly by predators consuming Vallisneria. In analogous restoration approaches, planting seagrass is advancing to conserve and protect this essential coastal habitat for the range of services it provides (Bell et al. 2008; van Katwijk et al. 2016). While seagrass restoration has been successful in some places, it has not in others because of direct consumption from local aquatic species, particularly sea turtles and dugongs in the GBR, which are known to feed extensively on seagrass species (Scott et al. 2021). Trials to understand the direct consumption of these species on seagrass have incorporated feeding exclusion cages, designed to keep out these grazers, with results showing conclusively that feeding from these species is

Table 1. Mean (SE) shoot growth (cm) of Vallisneria over the 6-week experiment in each tank for each treatment (anchored and transplanted) at each of the three rhizome nodes. The measured lengths were totals summed for the experiment. Rhizome (RH) nodes (1, 2, and 3) lengths are shoot lengths that extended from the initial deployment rhizome. (—) are no data. No SE indicates that only a single measurement was possible because other replicates died during the experiment.

Tank	*Treatment*	*Treatment growth*	*RH1 shoot growth*	*RH2 shoot growth*	*RH3 shoot growth*
1	Anchored	2.95 (1.2)	57.4 (0.8)	—	—
	Transplant	1 (0.7)	6.8	—	—
2	Anchored	0.4	103.2	1.7	—
	Transplant	1.4 (1.1)	27 (23.4)	—	—
3	Anchored	1.1 (0.6)	14.1	—	—
	Transplant	4.2 (1.2)	88.6 (34)	72.1 (23.6)	38.2
4	Anchored	1.4 (0.7)	128.4	84.6	39.1
	Transplant	3.3	—	—	—
5	Anchored	2.4 (1.6)	24.1 (21.1)	35	—
	Transplant	4.25 (2.3)	103.9 (1.7)	79.2	21.5
6	Anchored	3.1 (1.1)	50.9 (4.4)	38.2 (4.5)	32.3 (15.4)
	Transplant	3.3 (1.1)	17.3 (5.2)	21.8 (4.7)	—
7	Anchored	0.7	47.7	—	—
	Transplant	6.1 (1.7)	—	51.3	30
8	Anchored	4.2 (1.0)	38.7	42.2	18.3
	Transplant	4.5 (2.0)	27 (0.4)	129	69.7

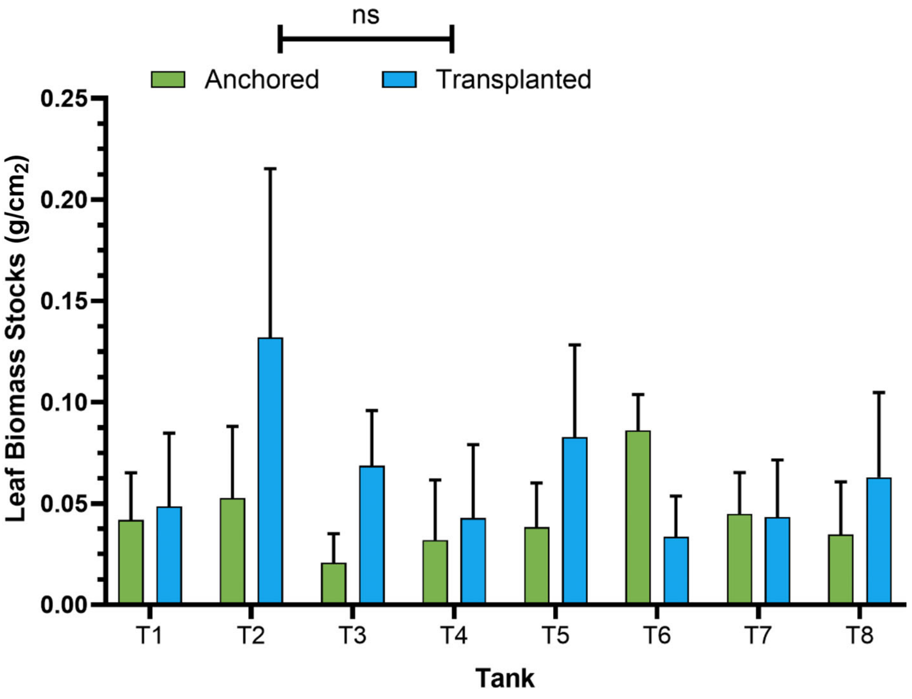


Figure 4. Mean + SD of leaf biomass carbon stocks (g C/m^2) in the aquarium tanks across both treatments. Statistical significance was derived from unpaired *t* test (ns, not significant).

substantial, which has major consequences on seagrass restoration potential (Janiak et al. 2020; Scott et al. 2021). For Vallisneria, there is no known freshwater equivalent species that could have direct grazing pressure. There are some herbivorous fish species associated with this freshwater plant, such as the short-tailed river pipefish (*Microphis brachyurus*) (Rayner et al. 2009), which feeds presumably on epiphytic algae on Vallisneria shoots—based on diet from the analogous *M. deocata* in the United States (Saikia et al. 2024). Further investigation is needed—through both laboratory and field trials—to assess whether fish grazing on restored Vallisneria limits restoration success or whether other factors are responsible for restoration failure, such as the need for heavier weights (or more environmentally friendly weight products) to deal with the challenge of flow and Vallisneria being easily dislodged and washed away.

The emerging focus on restoration of coastal wetland ecosystems, including mangroves, seagrass, and tidal marshes, for the benefits of carbon storage as a natural climate change prospect, has recently turned to aquatic plant species on floodplains and freshwater ecosystems (Malerba et al. 2022; Adame et al. 2024). This is the first study to examine the carbon-positive benefits of Vallisneria, with our estimated mean rates close to 0.12 ± 0.10 g C to 0.22 ± 0.20 g C for each meter of growth in anchored and transplanted shoots, respectively. These results are promising, and we advocate the need to trial restoration, using our technique here, in the field, to examine the benefits. In addition to carbon, Vallisneria provides habitat for a range of fish and crustacean species (N. Waltham unpublished data), including juveniles of commercial species such as barramundi (*Lates calcifier*) and mangrove jack (*Lutjanus argentimaculatus*) (Russell & Garrett 1983). As an intertidal/subtidal plant species, this species also seems to provide sediment scar protection—at least in the study catchment where the three large meadows support expansive sandy habitat. Protection and restoration of this aquatic species is critical, particularly when considering government policies relating to ecosystem protection and conservation, within a setting where these targets are considered ambiguous given the extent of coastal agricultural development that has occurred over the past few decades (Pogonoski et al. 2002). Restoring this aquatic plant species, while possible in light of the findings in this study, still requires broader catchment-wide protection, particularly in relation to reducing sediment and nutrient run-off to levels that are below thresholds that are critical for Vallisneria. Without broader whole-of-ecosystem conservation and repair, long-term restoration success will be challenging.

With global restoration and conservation targets set under the Kunming Montreal Global Biodiversity targets, the United Nations Decade on Ecosystem Restoration, and a range of local and national conservation and biodiversity targets in the GBR lagoon, the development of new and cost-effective restoration projects is now more than ever necessary (Department of Environment Science and Innovation 2023). The outcomes here unveil a way toward large scale restoration for teal blue aquatic plants in transitional waterways, where there are also multiple environmental benefits. These results are important considering the ongoing challenge of legal approval for restoration projects in Australia (Bell-James et al. 2024), which, if resolved, could pave the way for large scale restoration projects (Saunders et al. 2024). Development of new approaches and technology is a major decisive factor in the success of restoration ecology. The results here are promising and now require field trials ahead of large-scale application.

Acknowledgments

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Shoot growth of Vallisneria over the 6-week experiment in each tank for the anchored (A) and transplanted (T) first rhizome only.

Table S2. Shoot growth of Vallisneria over the 6-week experiment in each tank for the anchored (A) and transplanted (T) at all rhizome nodes.

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