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Targeting Macrophytes: Optimizing Vegetation Density to Enhance Water Quality within Constructed Wetlands

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Abstract: This study of constructed wetland design investigated relationships between macrophyte species selection and planting density for water quality improvement. A lab-scale wetland was compared against a pilot-scale wetland in San Antonio, Texas, at Mitchell Lake to measure differences in effluent water quality improvement using three native macrophyte species. Using a novel, two-phase method, a targeting macrophyte was identified from among Olney's bulrush (*Schoenoplectus americanus*), hardstem bulrush (*Schoenoplectus acutus*), and California bulrush (*Schoenoplectus californicus*), based on its marked capability for improving water quality factors, then it was planted in varied majority densities to compare differences in treatment effectiveness. The results showed that the planting density with 50% giant bulrush, 25% Olney's bulrush, and 25% hardstem improved conductivity removal by 34% and increased dissolved oxygen by 3713% as compared to the Mitchell Lake pilot-scale results. The 70% and 90% majority density plantings (giant bulrush) were not shown to be as effective for the tested parameters, indicating diminishing returns as the vegetation density increasingly becomes a monoculture within the system. The results of this study showed that this complementary approach to wetland design displayed significant improvement in certain treatment parameters than the evenly planted species distribution of the pilot study. These findings demonstrate that the constructed wetland design can be optimized by selecting and planting macrophytes based on their effectiveness in targeting site-specific water quality concerns by capitalizing on their individual traits within complex wetland systems.

Keywords: constructed wetland; aquatic macrophyte; water quality; nature-based solution; wastewater remediation

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1. Introduction

Nature-based solutions (NBSs) are divided into two major categories based on usage—natural features, or those involving conserving or rehabilitating natural ecosystems, and nature-based systems that use the enhancement or creation of natural processes in modified or artificial ecosystems [1]. While often used in conjunction with other types of environmental actions, NBS practices differ from other types of "green" infrastructure initiatives in that they mimic or even fully utilize natural processes, unlike other man-made infrastructure such as wastewater treatment facilities that require consistent human intervention to continue their functionality [2]. The defining feature of a wetland NBS, however, is not whether the ecosystem used is "natural" but whether natural processes are being proactively managed to achieve a water-related objective [3]. Applied biological remediation methods have been shown to treat synthetic wastewater and even contaminated lake waters [4,5].

Pilot-scale constructed wetlands were built for Mitchell Lake (San Antonio, TX, USA) to remediate the hypereutrophic water quality after decades of being used for dumping local wastes [6]. A constructed wetland is an area of land that has been modified into

a functional approximation of a natural wetland ecosystem to serve a specific purpose, such as improving water quality [7]. Kadlec and Wallace (2009) discussed how wetlands have a high rate of biological activity which encompasses the natural mechanisms to treat common water pollutants without the need for additional expensive operational equipment and energy requirements. They also note that in addition to the many effects that vegetation has on chemical processing and removal in treatment wetlands, the physical functionality of wetland vegetation directly relates to the plant density and vegetation type (i.e., transpiration, flow resistance, and particulate trapping). Kadlec and Wallace (2009) also highlight the interrelated complexity of wetland systems and that wetland design influences performance, such as hydraulics and internal biogeochemical cycling, which become paramount in understanding how treatment wetlands function [8].

Two main types of constructed wetland design, free water surface (FWS) flow and subsurface flow (SSF) systems, are based on water flow hydrology and several criteria such as the presence/absence of flowing surface water, macrophytes present, and direction of flow [9]. The ability of macrophytes, or large aquatic plants, to assist the breakdown of human- and animal-derived wastewater and remove disease-causing microorganisms and pollutants can be harnessed through these wetland applications [10–13]. Aquatic macrophytes are categorized into four groups: floating-leaved, free-floating, emergent, or submerged [8] Emergent macrophytes, like those analyzed in this study, differ from their counterparts in that a typical FWS-constructed wetland with emergent macrophytes (plants with their roots in submerged soils while growing up and out beyond the surface of the water) consists of a shallow, sealed basin (or sequence of basins), containing 20–30 cm of rooting soil, with a water depth of 20–40 cm, and densely planted emergent vegetation covering more than 50% of the surface [8]. Emergent macrophytes also have one of two types of root system morphologies, fibrous roots or taproots [7], that allow the plant to obtain resources at different locations in the soil. Fibrous root systems consist of multiple fine roots branching out laterally from the stem, maintaining a shallow soil depth and covering a large surface area, while taproot systems have one main root growing vertically from the stem but reaching a greater soil depth [14]. Both emergent macrophyte root systems serve to secure the plant body, prevent soil erosion, and absorb surrounding water and nutrients [14]. Wahl and Ryser (2000) not only found that the anatomical traits of roots were directly associated with plant productivity but also observed a relationship between a plant's growth characteristics and its root structure [15].

In constructed wetland designs, the growth of macrophytes and their contaminant uptake performance may also be influenced by interactions among pollutants. Zhang et al. (2007) identified nutrient uptake by macrophytes in constructed wetland systems as a major mechanism in which those types of constituents are removed [16]. A small-scale constructed wetland study [17] was conducted with multiple emergent macrophyte species (*Juncus effusus*, *Scirpus validus*, and *Typha latifolia*) and differences were noted between the wastewater effluent quality improvement and constituent removal capabilities of the macrophyte species. Additionally, Weisner et al. (1994) observed macrophytes as directly increasing nitrate removal by facilitating the surface attachment of denitrifying bacteria as well as by affecting the wetland hydraulics which promoted denitrification processes [18]. Therefore, as certain macrophytes facilitate nutrient removal at varied rates and different macrophytes vary in their resource requirements, specific species of macrophytes would be expected to display a higher affinity for nutrient removal and contaminant mitigation compared to other native counterparts even in the same constructed wetland system [7,19–21].

Trait-based macrophyte preselection is supported through the landscape-filter framework in which species functionality within freshwater ecosystems allows for predictive assumptions on its distribution, abundance, and local community composition by focusing on its environmental conditions and site-specific constraints [22]. Environmental conditions impose "filters" through which species in the ecosystem must "pass through" to be present. Empirical examples and models of this filter approach have already been applied

to wetland plants at the local scale [23,24]. A macrophyte with the niche species traits best suited for the harsh and site-specific environment of a constructed wetland could be determined by proactively applying those environmental filters as the general conditions of that niche habitat and assessing the continued presence and impact of that species. In principle, macrophyte planting density should be determined in consideration of species traits wherein the prominent traits of "survival" and "water treatment" are foundational to the goals of all constructed wetlands. Macrophyte species would need to survive in constant wastewater conditions and they would need to positively impact the water treatment process. Therefore, macrophytes with species traits that are best suited to the habitat conditions of treatment wetlands and have the best impact on improving water quality should inform how their planting density should be structured throughout the design. And that connection started bringing all the pieces together—macrophyte species traits represented by their treatment performance, how they perform compared to other species, and how they would perform in uneven multigroup plantings.

While there are numerous interrelated factors associated with the performance of contaminant removal through constructed wetlands (i.e., wetland flora, hydrology, microorganisms, soil infiltration, etc.), the present study's scope was delimited to the effect of a specific wetland plant species as the primary performance factor of contaminant mitigation in relation to its native counterparts [25]. This study applied a novel two-phase testing method to constructed wetland design. By first singling out the individual effectiveness of a *Targeting Macrophyte*, the selected macrophyte species was determined for its water quality improvement traits amongst comparable species, and that species could then be evaluated in majority density plantings for further design optimization as the primary mechanism for targeting specific water contaminants in constructed wetlands.

Through this iterative method of testing prior to the construction of wetland systems, the most efficacious combination of vegetation could be chosen to selectively target the highest priority contaminant factors and optimize the path towards established water treatment goals. From this premise, this study aims to address the following research objectives: (i) Identify differences in effluent concentrations between wetland monoculture flora species for constructed wetlands when similarly designed but assessed individually. (ii) Utilize the targeting macrophyte method for weighted planting and assess the water quality improvement compared to the even species groupings used in the Mitchell Lake wetland pilot project. (iii) Identify ways that constructed wetland design can ultimately be improved with the forethought of knowing which wetland plants can be installed to target contaminates linked to the site-specific water quality concerns for the area.

2. Materials and Methods

2.1. Targeting Macrophyte Method Framework

Three species of native wetland bulrush planted, namely Olney's bulrush (*Schoenoplectus americanus*), hardstem bulrush (*Schoenoplectus acutus*), and California bulrush (*Schoenoplectus californicus*), were selected for the target macrophyte experiments [26]. The same species were used in the pilot wetland project conducted to evaluate the effectiveness of constructed wetland to mitigate the water quality in Mitchell Lake (San Antonio, TX, USA), whose effluent discharges from the lake exceeded water quality limits, prompting the United States Environmental Protection Agency (USEPA) to issue an enforcement action to mandate improving water quality in the lake [27]. These local plant species were chosen for their vegetative health and plant density, presuming that the species that would establish high percent coverage equated to the most desirable plant species to be used for full-scale construction [6].

To investigate the water quality improvement effectiveness of each of the three macrophyte species used in the pilot program, lab-scale constructed wetland models were built to test their individual performance relative to grouped performance. As the pilot study had the macrophytes evenly planted within the cells, there was no way to determine how each of the macrophyte species, as a component of a complex and multivariate wetland

system, contributed to the overall water quality treatment. The lab-scale model was conceptualized by first incorporating the wetland design processes proposed in the pilot study, the macrophyte species utilized in the pilot design, and the same permitted water quality parameters that would need to be tested.

By modeling the experimental design after the existing study and paring down the factors affecting water quality to individual macrophyte species, comparisons could be made between the results of the evenly planted design of the pilot study and designs with other configurations of the three species. The plant species would need to be tested in two phases: Phase I and Phase II (Figure 1). Phase I would test the planted macrophytes as monocultures to ascertain the effectiveness of each species at treating the source water and which parameters were the most improved. The results of Phase I comparing the species would reveal the targeting macrophyte as the most able to address one or more of the measured water quality parameters better than the others.

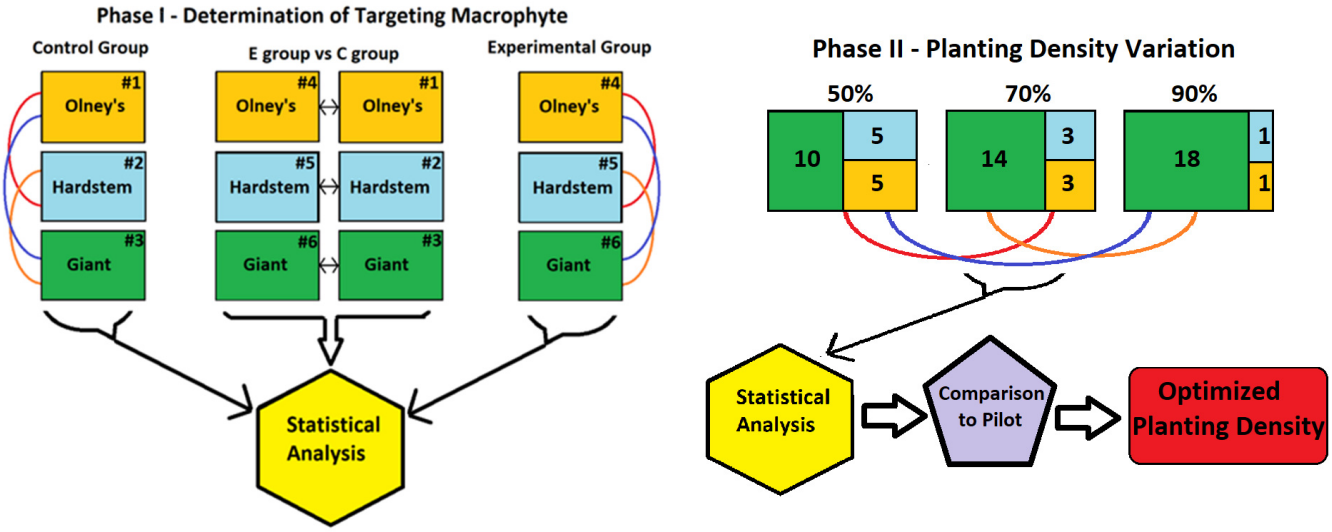

Figure 1. Intra- and intercomparisons of macrophyte species for the control and experimental groups during Phase I (bin #1–6). The majority density planting plan of the targeting macrophyte species in relation to the other two species during Phase II (bin #7–9).

When the results from Phase I indicated the presence of a targeting macrophyte, another 8-week assessment (Phase II) was conducted with a majority planting density of the targeting macrophyte in majority-scaled density plantings (at 50%, 70%, and 90%) along with even plantings of the other two macrophyte species (at 25%, 15%, and 5% each) up to 100% planting density. Given the bin size capping the total number of plantings to twenty per bin, the density plantings of the targeting macrophyte equated to 10 (50%), 14 (70%), and 18 (90%), respectively, with 5 (25%), 3 (15%), or 1 (5%) for each of the other two species. In Phase II, the species would need to be retested using the targeting macrophyte in various majority density plantings to see if one of those configurations leads to the further improvement in the same and/or more water quality parameters. Using the source water as a baseline, comparisons would need to be made not just among the majority density plantings but also between the even design of the pilot and the Phase II density designs.

2.2. Lab-Scale Constructed Wetland

The lab-scale wetland system was constructed based on the FWS overall design of the Mitchell Lake pilot project cell trains (each approximately L 115 ft × W 85 ft) and their relation to the inflow and outflow locations [6]. Each lab setup included a set of two 27-gallon (102-L) totes (H 15.27 in, W 19.61 in, L 28.55 in) with lids: one for water containment representative of the circular inflow from and subsequent outflow back into

Mitchell Lake (reservoir bin) and one for vegetation and soils (growing bin) representative of the dual-cell train design used in the pilot project (Figure 2a). Each growing bin was set at counter height and connected to its corresponding gravity-fed reservoir bin on the floor constituting a paired experimental set and assigned a different numerical designation (each paired set herein referred to as a bin with its corresponding numerical designation i.e., "bin #1").

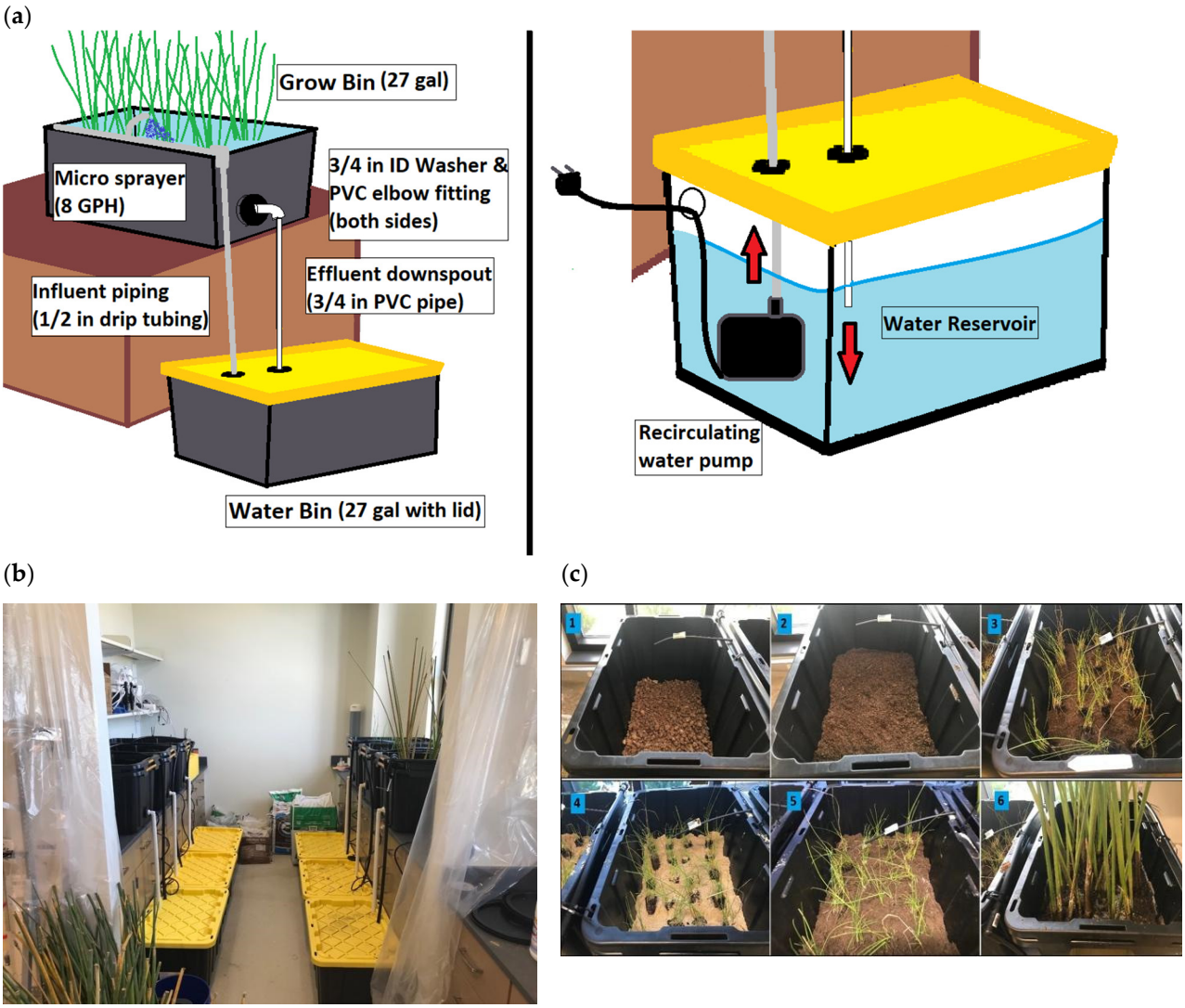

Figure 2. (**a**) Planned lab-scale experimental design of each of the individual wetland systems including an interior perspective detailing the gravity-fed recirculating pump system. (**b**) Setup of the lab-scale experimental design of the individual wetland bins #1–6 within the lab setting. (**c**) Stages of soil layer construction and macrophyte planting within the growing bins: (1) pebble layer, (2) peat moss layer, (3) planting of vegetation, (4) coarse builders' sand layer, (5) Texas topsoil layer, (6) surface water.

The impermeable tote containers had the utility of mimicking the compacted clay lining within the wetland cells used during the pilot project for the growing bins and their black color helped reduce sunlight exposure which might induce algal growth within the reservoir bins. The tote lids were kept on the reservoir bins to reduce the possibility of splashback and evaporation with the exception of small holes for the inflow and outflow piping as well as the outlet plug. A recirculating fountain pump immersed in each water bin was used to pump the effluent from the grow bin back into the inflow location of the growing bin using a fountain pump (rated at 300 gal/h) and ½-inch irrigation drip tubing.

The inflow drip tubing ran up from the recirculating pump in the water bin, attached along the side edge of the grow bin, and was capped at the end with a ½-inch compression end cap. A micro sprayer (8 gal/h) attached to ¼-inch drip tubing using a ¼-inch barbed connector was branched perpendicularly from the ¾-inch drip tubing towards the planted macrophytes at the back of the grow bin. After running through the wetland system, the effluent water passed through a ¾-inch PVC outflow pipe leading from the front of the grow bin into the corresponding water bin below where it was collected and recirculated continuously back into the wetland system.

Six total lab setups (Figure 2b) were constructed using three for the control set (bins #1–3) and three for the experimental set during Phase I (bins #4–6), then they were replanted again for Phase II (bins #7–9). The control setups ran concurrently through both phases and differed from the experimental set in only two ways: (1) the planted vegetation stayed in the monoculture Phase I groupings for 19 straight weeks through both phases and (2) the systems were operated using only tap water and not exposed to any of the collected water samples.

The grow bins were positioned to maximize natural sunlight exposure from the adjacent, north-facing lab windows. The lab environment was maintained at a consistent room temperature throughout both testing phases.

2.3. Vegetations and Planting

The three species of wetland plants were purchased from two regional plant nurseries. The samples of Olney's bulrush and hardstem bulrush were obtained from Aquatic and Wetland Nursery, L.L.C (Fort Lupton, CO, USA), and the giant bulrush was obtained from Southwest Aquatic Services (Altair, TX, USA). The Olney's bulrush and hardstem bulrush arrived in smaller rooted funnels while the giant bulrush arrived larger and more developed. The plant specimens not installed during Phase I were maintained as unplanted with water access in the lab environment until Phase II.

The soil layer-by-layer packing of the grow bins included an eight-inch soil layer composition (Figure 2c), with two inches each of coarse pebbles, followed by a two-inch layer of peat moss, then coarse builders' sand, and then finally a layer of local Texas topsoil [28]. The materials used to recreate the individual soil layers were procured from local hardware stores using commercially available gardening supplies.

2.4. Water Samples

The water samples were taken from a creek receiving the outflow of the Michell Lake water approximately 0.5 miles southeast of the lake's pilot project. The effluent quality limits measured for the pilot program (daily average BODs limit of 30 mg/L, a daily average TSS limit of 90 mg/L, a pH limit between 6.0 and 9.0, and a minimum DO concentration of 4.0 mg/L) were used as the goal metrics when evaluating the water quality of each testing sample but were not the only and/or same factors measured during this study [6]. The water quality factors that were measured over both Phases I and II were pH, turbidity, dissolved oxygen (DO), conductivity, temperature, ammonia, *E. coli*, total coliform, and chemical oxygen demand (COD).

The EasyGel Coliscan testing kits were used on samples of the collected water to show the presence of *E. coli* and/or total coliform. None of the natural water samples taken for Phase I and Phase II experiments showed a detectable presence of *E. coli* or total coliform.

The initial water quality measurements for the other parameters were conducted for Phase I and Phase II on the collected water samples as the baseline water quality values are listed in Table 1.

Table 1. Initial water quality measurements of collected water for Phases I and II.

	Phase I	Phase II
Turbidity (NTU)	3.87	28.2
Conductivity (µS/cm)	3779	3616
pH	7.32	6.99
Temperature (°C)	20.1	28.4
DO (mg/L)	16	0.6
COD (mg/L)	49.2	97
E. coli *	ND	ND
Total Coliform *	ND	ND
Ammonia	UR	10.415

At 70 °F and 1 atm, DO % saturation = 8.9 mg/L DO in water. * Coliform and *E. coli* with EasyGel. ND = Not Detectable/UR = Under Range.

2.5. Water Quality Analysis

COD testing was performed using a Hach Digital Reactor Block 200 (DRB 200) with potassium dichromate as the strong oxidizing agent. Water samples (2.0 mL) were placed in heated vials (preheated reactor at 150 °C) containing mercuric sulfate, which could eliminate chloride interference up to a maximum Cl concentration of 2000 mg/L. The results of the COD testing are defined as the mg of O_2 consumed per liter of sample.

Ammonia was tested with a Hach Digital Reader (DR 1900) portable spectrophotometer. Water samples (10 mL) were tested using the Permachem Nitrogen-Ammonia, Salicylate Method (0.50 mg/L) with Ammonia Salicylate Reagent Powder (10 mL) and Ammonia Cysturate Reagent Powder (10 mL). Additional high-range ammonia testing was conducted using the TNTplus®-Method 10205 with TNT 832 Nitrogen and Ammonia tests.

Ion chromatography was measured using a Thermo Scientific-DIONEX Aquion unit and corresponding autosampler (DIONEX As-DV) for water chemistry analysis. Ion chromatograph calibration curves were made quantifying fluoride, chlorite, bromate, chloride, nitrite, bromide, nitrate, phosphate, and sulfate concentrations. Three test water samples were recorded during Phase I and one during Phase II. A raw sample of the source water was collected on 20 October 2023 and used as a comparison to the recorded test samples. A handheld probe (YSI ProDSS) was used to measure the pH, DO, and conductivity of the water samples.

2.6. Statistical Analysis for Phase I and Phase II Studies

Effluent concentrations and removal of the constituents were analyzed using SPSS software (Version 27.0) through one-way ANOVA analysis ($p < 0.05$) including post hoc Tukey honestly significant difference (HSD) procedures to further assess pairwise comparisons between the species where significant effects were verified [29]. All normality assumptions for the analysis of variance were met. This analysis was conducted separately for the control group and then for the experimental group. Repeated independent sample *t*-test analysis then compared each of the measured water quality factors between the Phase I experimental and control groups for the same species (e.g., bin #1 to #4, bin #2 to #5, and bin #3 to #6). The species were then ranked according to their effectiveness among the significantly impacted water quality factors. A linear trend analysis compared the water quality factors between the experimental and control groups for bins #1–6 using Microsoft Excel. Recorded observations and photos of the general health and growth of the three different species made over the Phase I testing period also factored into the determination of their effectiveness. These combined evaluations formed the determination of the targeting macrophyte species out of the group.

The experimental group from Phase II (bins #7, #8, and #9) was analyzed for constituent removal and overall water quality using one-way ANOVA analysis ($p < 0.05$) including post hoc Tukey honestly significant difference (HSD) procedures to further assess pairwise comparisons between the species where significant effects were verified [29]. A linear trend analysis compared the water quality factors between the experimental and control groups

for bins #1–9 using Microsoft Excel. Finally, parameter mean differences [29] were used to determine if there was a percentage increase in effectiveness between the Phase II group water quality factors and those recorded in the wetland pilot study.

3. Results and Discussion

The water quality data collected for bins #1–9 was compiled and compared to each other utilizing each phase as a comparable 8-week timeline (Figure S1). The initial water quality measurements were compared between the control group (bins #1–3) and both experimental groups (bins #4–6 and bins #7–9). While the temperature, turbidity, DO, and pH factors were within their acceptable permit ranges, the other water quality factors (conductivity and COD) exceeded the discharge limits. Only bin #7 appeared to be trending down towards acceptable levels of conductivity compared to any of the other experimental bins in either Phase I or Phase II.

3.1. Phase I Results

3.1.1. Control Group

Ammonia (NH_3-N) testing was conducted during week 4 of Phase I. The values were tested at ranges 0.01–0.5 ppm, 2–47 ppm, 47–130 ppm, and up to 1500 ppm. Levels of ammonia were not detectable at any of these ranges. During the following week 5 testing, none of the ammonia ranges for the control group or experimental group were able to be recorded from 0.2 ppm to 1500 ppm.

A one-way ANOVA ($p < 0.05$) was conducted after Phase I to evaluate the effectiveness between the three different macrophyte species from the control group (bin #1, bin #2, and bin #3) in improving the measured water quality over the eight-week time period. The turbidity, temperature, and conductivity factors were not found to be significant compared to the control group.

The pH results from the control group indicated a significant main effect between the species ($F_{2,21} = 31.702$, $p < 0.000$). Post hoc Tukey honestly significant difference (HSD) tests were performed to assess pairwise differences between species (Supplementary Materials Section S1). The comparisons of bin #1 to bin #2 ($p = 0.002$), bin #1 to bin #3 ($p < 0.000$), and bin #2 to bin #3 ($p = 0.002$) were found to be statistically significant. Examination of the mean values revealed that bin #1 ($M = 7.663$, SD =.060) exhibited the highest effectiveness at maintaining neutral pH, followed by bin #2 ($M = 7.798$, $SD = 0.062$) and bin #3 ($M = 7.936$, $SD = 0.132$). Therefore, bin #1 (*S. americanus*) was identified as the most effective species, followed by bin #2 (*S. acutus*) and bin #3 (*S. californicus*).

The DO results from the control group indicated a significant main effect between the species ($F_{2,21} = 80.974$, $p < 0.000$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the organisms. The comparisons bin #1 to bin #2 ($p = 0.002$), bin #1 to bin #3 ($p < 0.000$), and bin #2 to bin #3 ($p = 0.002$) were found to be statistically significant. Examination of the mean values revealed that bin #1 ($M = 52.723$, $SD = 4.967$) exhibited the lowest DO percentage, followed by bin #2 ($M = 74.763$, $SD = 8.304$) and bin #3 ($M = 90.700$, $SD = 3.763$). Therefore, bin #3 was identified as the most effective species, followed by bin #2, and bin #1.

The COD results from the control group indicated a significant main effect between the species ($F_{2,21} = 16.363$, $p < 0.000$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the species. The comparisons bin #1 to bin #2 ($p < 0.000$) and bin #2 to bin #3 ($p < 0.000$) were found to be statistically significant. Examination of the mean values revealed that bin #2 ($M = 276.25$, $SD = 33.470$) exhibited the highest COD concentration, followed by bin #1 ($M = 218.125$, $SD = 18.527$) and bin #3 ($M = 212.875$, $SD = 18.735$). Therefore, bin #3 was identified as the most effective species for this factor, followed by bin #1 and bin #2. The largest effect size (η^2) found for the control group's significant factors was DO at 0.885, followed by pH at 0.751 and COD at 0.609, indicating large effects for each of those factors.

3.1.2. Experimental Group

A one-way ANOVA ($p < 0.05$) was then conducted to evaluate the effectiveness between the three different macrophyte species from the experimental group (bin #4, bin #5, and bin #6) in improving the measured water quality during Phase I. The significance value of the Levene statistic based on a comparison of means for pH was 0.035 and 0.038 for COD. This is a significant result, which means the requirement of homogeneity of variance was not met. However, their F-values and their related *p*-values were alternatively validated for both pH ($F_{2,21} = 5.004$, $p = 0.017$) and COD ($F_{2,21} = 14.555$ $p < 0.000$), respectively. As the assumption of homogeneity was violated, a second one-way ANOVA ($p < 0.05$) with a Welch test was performed, showing significance for the temperature, DO, and COD factors, concluding that mean water quality factor comparisons are not equal over all bins. Post hoc Games–Howell testing showed that only bin #4 and bin #6 had mean water quality factors that differed significantly [29]. The turbidity and conductivity factors were not found to be significant compared to the experimental group.

The pH results from the experimental group indicated a significant main effect between the species ($F_{2,21} = 5.004$, $p = 0.017$). Post hoc Tukey HSD tests were performed to assess pairwise differences between species. The comparison of bin #4 to bin #6 ($p = 0.013$) was found to be statistically significant. Examination of the mean values revealed that bin #4 ($M = 7.511$, $SD = 0.145$) exhibited the highest effectiveness at maintaining neutral pH, followed by bin #5 ($M = 7.585$, $SD = 0.083$) and bin #6 ($M = 7.710$, $SD = 0.144$). Therefore, bin #4 (*S. americanus*) was identified as the most effective species, followed by bin #5 (*S. acutus*) and bin #6 (*S. californicus*).

The temperature results from the experimental group indicated a significant main effect between the species (F2,21 = 3.955, $p = 0.035$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the organisms. The comparison of bin #4 to bin #6 ($p = 0.029$) was found to be statistically significant. Examination of the mean values revealed that bin #4 (M = 19.900, SD = 0.590) exhibited the highest effectiveness at maintaining room temperature (20 °C), followed by bin #5 (M = 20.175, SD = 0.520) and bin #6 (M = 20.650, SD = 0.504). Therefore, bin #5 was identified as the most effective species, followed by bin #4 and bin #6.

The DO results from the experimental group indicated a significant main effect between the species (F2,21 = 17.511, $p < 0.000$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the organisms. The comparisons bin #4 to bin #6 ($p < 0.000$) and bin #6 to bin #5 ($p = 0.001$) were found to be statistically significant. Examination of the mean values revealed that bin #4 (M = 68.025, SD = 7.656) exhibited the lowest DO percentage followed by bin #5 (M = 74.087, SD = 9.771) and bin #6 (M = 89.888, SD = 4.530). Therefore, bin #6 was identified as the most effective species, followed by bin #5 and bin #4.

The COD results from the experimental group indicated a significant main effect between the species (F2,21 = 16.363, $p < 0.000$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the species. The comparisons bin #6 to bin #5 ($p < 0.000$) and bin #5 to bin #4 ($p = 0.001$) were found to be statistically significant. Examination of the mean values revealed that bin #5 (M = 218.50, SD = 12.387) exhibited the highest COD concentration, followed by bin #4 (M = 196.88, SD = 12.088) and bin #6 (M = 192.25, SD = 4.921). Therefore, bin #6 was identified as the most effective species for this factor, followed by bin #4 and bin #5. The largest effect size found for the experimental group's significant factors was DO at 0.625, followed by COD at 0.581, pH at 0.323, and temperature at 0.274 η^2.

3.1.3. Control Group vs. Experimental Group

Repeated independent samples *t*-tests ($p < 0.05$) were performed to evaluate the effectiveness of the three different macrophyte species from the control group compared to the same species planted in the experimental group (bin #1 to bin #4 (*S. americanus*), bin #2

to bin #5 (*S. acutus*), and bin #3 to bin #6 (*S. californicus*) in improving the measured water quality during Phase I.

The first *t*-test compared the measured water quality factors between bin #1 and bin #4 (*S. americanus*) (Supplementary Materials Section S2). Turbidity ($p = 0.410$) was the only water quality factor that did not show a significant difference between the paired species comparisons.

The results indicated that experimental group bin #4 (*S. americanus*) ($M = 4499.25$, $SD = 704.351$) had significantly higher conductivity than the control group bin #1 ($M = 765.63$, $SD = 122.528$, $t(14) = -14.771$, $p < 0.000$). Similarly, the DO in bin #4 ($M = 68.025$, $SD = 7.656$) was also significantly higher than the control group bin #1 ($M = 52.725$, $SD = 4.9667$, $t(14) = -4.742$, $p < 0.000$). However, the pH factor in bin #4 ($M = 7.511$, $SD = 0.144$) was significantly lower than bin #1 ($M = 7.663$, $SD = 0.060$, $t(14) = 2.737$, $p = 0.016$). The temperature factor in bin #4 ($M = 19.900$, $SD = 0.590$) was also significantly lower than bin #1 ($M = 20.713$, $SD = 0.372$, $t(14) = 3.293$, $p = 0.005$). Lastly, the COD in bin #4 ($M = 196.88$, $SD = 12.088$) was significantly lower than bin #1 ($M = 218.13$, $SD = 18.527$, $t(14) = 2.737$, $p = 0.017$).

The second t-test compared the measured water quality factors between bin #2 and bin #5 (*S. acutus*). Turbidity ($p = 0.500$) and DO ($p = 0.884$) were the only water quality factors that did not show a significant difference between the paired species comparisons. The results indicated that experimental group bin #5 (*S. acutus*) ($M = 4026.625$, $SD = 939.879$) had significantly higher conductivity than the control group bin #2 ($M = 703.350$, $SD = 190.174$, $t(14) = -9.802$, $p < 0.000$).

However, the pH factor in bin #5 ($M = 7.585$, $SD = 0.083$) was significantly lower than bin #2 ($M = 7.798$, $SD = 0.062$, $t(14) = 5.816$, $p < 0.000$). The temperature factor in bin #5 ($M = 20.175$, $SD = 0.520$) was also significantly lower than bin #2 ($M = 20.688$, $SD = 0.380$, $t(14) = 2.251$, $p = 0.041$). Lastly, the COD in bin #5 ($M = 218.50$, $SD = 12.387$) was significantly lower than bin #2 ($M = 276.25$, $SD = 33.470$, $t(14) = 4.577$, $p < 0.000$).

The third *t*-test compared the measured water quality factors between bin #3 and bin #6 (*S. californicus*). Turbidity ($p = 0.425$), DO ($p = 0.702$), and temperature ($p = 0.687$) were the only water quality factors that did not show a significant difference between the paired species comparisons.

The results indicated that experimental group bin #6 (*S. acutus*) ($M = 3827.250$, $SD = 511.311$) had significantly higher conductivity than the control group bin #3 ($M = 641.050$, $SD = 109.189$, $t(14) = -17.237$, $p < 0.000$). However, the pH factor in bin #6 ($M = 7.710$, $SD = 0.144$) was significantly lower than bin #3 ($M = 7.936$, $SD = 0.082$, $t(14) = 3.860$, $p = 0.002$). Lastly, the COD in bin #6 ($M = 192.25$, $SD = 4.921$) was significantly lower than bin #3 ($M = 212.88$, $SD = 18.735$, $t(14) = 4.577$, $p = 0.009$).

3.1.4. Determination of Targeting Macrophyte

The targeting macrophyte determination was conducted after evaluating the statistical analysis from Phase I and the recorded observations from the ongoing week #1–8 photos documenting the health and growth of the plant specimens. The plant species were ranked 1 to 3 based on their performance in the control and experimental groups, where rank "1" was the most effective species for that water quality factor and "3" was determined as the least effective (Table 2). From the one-way ANOVA testing for each group, giant bulrush (*S. californicus*) received the highest ranking overall with four instances of a #1 ranking but particularly for the factors with some of the largest effect sizes: dissolved oxygen and chemical oxygen demand.

Table 2. Ranking of macrophyte species among the various measured water quality factors to determine the most effective "targeting macrophyte" species from the control and experimental testing groups ("1" as the most effective and "3" as the least effective, "X" indicates data were not significantly different).

Control Group				
Species Parameter	Olney	Hardstem	Giant	Effect size
Turbidity (NTU)	X	X	X	X
Conductivity (µS/cm)	X	X	X	X
pH	1	2	3	0.751
Temperature (°C)	X	X	X	X
DO (%)	3	2	1	0.885
COD (mg/L)	2	3	1	0.691
Experimental Group				
Species Parameter	Olney	Hardstem	Giant	Effect size
Turbidity (NTU)	X	X	X	X
Conductivity (µS/cm)	X	X	X	X
pH	1	2	3	0.323
Temperature (°C)	2	1	3	0.274
DO (%)	3	2	1	0.625
COD (mg/L)	2	3	1	0.581

3.2. Phase II Results

Giant bulrush (*S. californicus*) was determined as the targeting macrophyte during Phase I and was planted in 50%, 70%, and 90% majority planting densities for Phase II. Bin #7 contained 50% planting density, bin #8 had 70%, and bin #9 had a 90% planting density. Initial water quality measurements were conducted for Phase II on the collected water samples as the baseline water quality values (see Table 1). Source water was first collected for Phase II on 12 August 2023. The ammonia (NH_3-N) was analyzed three days after sampling from that collection. The value recorded was initially 8.51 ppm, which then gradually changed to 8.73 over 24 h. The ammonia range was recorded at 12.32 ppm on the following water collection date of 16 August 2023.

A one-way ANOVA ($p < 0.05$) was conducted after Phase II to evaluate the effectiveness of the three different density planting groupings of the targeting macrophyte experimental group (bin #7, bin #8, and bin #9) at filtering the measured water quality parameters over the eight-week period.

The turbidity ($p = 0.986$), pH ($p = 0.054$), temperature ($p = 0.345$), and COD ($p = 0.330$) factors were not found to be significant compared amongst this experimental group (Supplementary Materials Section S3).

The conductivity results from the experimental group indicated a significant main effect between the bins ($F_{2,21} = 3.840$, $p = 0.038$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the planting densities. The comparisons bin #7 to bin #8 ($p = 0.038$) and bin #7 to bin #9 ($p = 0.014$) were found to be statistically significant. Examination of the mean values revealed that bin #7 ($M = 3549.438$, $SD = 1374.360$) exhibited the lowest conductivity measurement followed by bin #9 ($M = 3904.125$, $SD = 852.679$) and bin #8 ($M = 5010.875$, $SD = 1007.847$). Therefore, bin #7 (50% planting density) was identified as the most effective species, followed by bin #9 (90% planting density) and bin #8 (70% planting density).

The DO results from the experimental group indicated a significant main effect between the bins ($F_{2,21} = 5.313$, $p = 0.014$). Post hoc Tukey HSD tests were performed to assess pairwise differences between the planting densities. The comparison of bin #7 to bin #9 ($p = 0.014$) was found to be statistically significant. Examination of the mean values revealed that bin #9 ($M = 52.775$, $SD = 20.476$) exhibited the lowest dissolved oxy-

gen measurement followed by bin #8 (M = 71.025, SD = 8.342) and bin #7 (M = 76.263, SD = 14.066). Therefore, bin #7 (50% planting density) was identified as the most effective species, followed by bin #8 (70% planting density) and bin #8 (90% planting density).

The water quality data collected for bins #1–9 were compiled and compared to each other utilizing each phase as a comparable 8-week timeline and then the experimental groups were compared to their respective initial water quality measurements (Table 3). The calculated difference between influent water quality and the experimental effluent for each parameter was recorded as the percent removal. While the temperature, turbidity, and pH factors were within their acceptable permit ranges, the other water quality factors (DO, conductivity, and COD) all exceeded their limits. Only bin #7 appeared to be trending down towards acceptable levels of conductivity compared to any of the other experimental bins in either Phase I or Phase II. This could possibly be due to the low water levels in the reservoir bin reported during Phase II week #8 that were not similarly reported for bin #8 or bin #9.

Table 3. Comparisons of the water quality parameter means across Phase I, II, and the Mitchell Lake wetland pilot project findings.

PHASE I *							
Water quality parameter	Influent	Bin #4	#4 % Removal	Bin #5	#5 % Removal	Bin #6	#6 % Removal
Turbidity (NTU)	3.87	1.22	68.44	2.39	38.37	2.46	36.43
Conductivity (µS/cm)	3779	4499.25	−19.06	4026.63	−6.55	3827.25	−1.28
pH	7.32	7.51	NA	7.59	NA	7.71	NA
Temperature (°C)	20.1	19.90	NA	20.18	NA	20.65	NA
DO (mg/L)	16	68.03	325.16	74.09	363.05	89.89	461.80
COD (mg/L)	49.2	196.88	−300.15	218.50	−344.11	192.25	−290.75
E. coli	ND	ND	NA	ND	NA	ND	NA
Total Coliform	ND	ND	NA	ND	NA	ND	NA
Ammonia	UR	UR	NA	UR	NA	UR	NA
PHASE II *							
	Influent	Bin #7	#7 % Removal	Bin #8	#8 % Removal	Bin #9	#9 % Removal
Turbidity (NTU)	28.2	1.11	96.06	1.00	96.45	1.13	96.00
Conductivity (µS/cm)	3616	3549.44	1.84	5010.88	−38.58	3904.13	−7.97
pH	6.99	7.55	NA	7.50	NA	7.37	NA
Temperature (°C)	28.4	20.04	NA	19.55	NA	19.91	NA
DO (mg/L)	0.6	76.26	12,610.42	71.03	11,737.50	52.78	8695.83
COD (mg/L)	97	287.13	−196.01	260.13	−168.17	259.00	−167.01
E. coli	ND	ND	NA	ND	NA	ND	NA
Total Coliform	ND	ND	NA	ND	NA	ND	NA
Ammonia	10.415	UR	NA	UR	NA	UR	NA
PILOT COMPARISON **							
	Pilot Effluent (mean)	#7 mean difference	% Diff	#8 mean difference	% Diff	#9 mean difference	% Diff
Turbidity (NTU)	NA	NA	NA	NA	NA	NA	NA
Conductivity (µS/cm)	5364.76	1815.32	33.84	353.89	6.60	1460.64	27.23
pH	7.42	−0.13	−1.74	−0.08	−1.05	0.06	0.79
Temperature (°C)	21.00	0.96	4.58	1.45	6.90	1.09	5.18
DO (mg/L)	2.00	74.26	3713.13	69.03	3451.25	50.78	2538.75
COD (mg/L)	NA	NA	NA	NA	NA	NA	NA
E. coli	NA	NA	NA	NA	NA	NA	NA
Total Coliform	NA	NA	NA	NA	NA	NA	NA
Ammonia	NA	NA	NA	NA	NA	NA	NA

Notes: * The calculated difference between influent water quality and the experimental effluent was recorded as the percent removal. ** The calculated difference between the pilot effluent water quality and the experimental effluent was recorded as the percent difference. ND = Not Detectable/UR = Under Range/NA = Not Applicable.

The mean difference between those factors (i.e., conductivity, pH, temperature, and DO) was derived from a comparison to the results from Phase II. The calculated difference between the pilot effluent water quality and the experimental effluent for those four parameters was recorded as the percent difference. While all three bins had positive improvements compared to the pilot study results, bin #7 had the highest percent increase in conductivity removal (+33.84%), followed by bin #9 (+27.23%) and bin #8 (+6.60%). The pH levels improved in bin #9 (+0.06%), while bin #8 (−1.05%) and bin #7 (−1.74%) worsened. The DO measurements were greater for all three bins compared to the pilot study with bin #7 having the largest difference (+3713.13%). The temperature measurements for the pilot study were also higher than any of the bins; however, this is likely accounted for due to the difference in study locations as the bins are in a closed laboratory with a regulated thermostat setting while the pilot project is located outdoors.

3.3. Validity of Targeting Macrophyte Method

Phase I of the targeting macrophyte method is a step to determine a targeting macrophyte species from among multi-species plantings. This method hypothesized that there would be a species standout from among a group. Additionally, the configurations of planting density with that species standout impacted overall water quality and were determined for treatment optimization. That hypothesis was supported by [17] who also conducted a small-scale study that investigated wetland plant monocultures against multi-species plantings of three macrophytes. Their analysis not only concluded that the increases in contaminant removal were significant between the three tested species but that the evenly planted, mixed groupings of the species had a "consistently greater effect on effluent quality" than the planted monocultures, which shows that species differences impacted design optimization when those traits were assumed to be equal and not capitalized upon. They also remarked that one of their plant species, *Typha*, disproportionately outperformed the other two species and they referred to it as "the apparent winner in competition", laying the theoretical groundwork for deriving a targeting macrophyte as demonstrated in this study.

This work also suggests that wetland species selection should coincide with that species' treatment capability of the identified site-specific conditions. Ref. [17] came to the same conclusion when they stated that site-specific differences in influent composition would likely vary a species' treatment capability. This targeting macrophyte method goes further to include other environmental factors, not just influent, that would affect their species traits and, therefore, their overall treatment capabilities.

3.4. Applicability to Wetland Design

The selection of native vegetation when planning constructed wetland design should not solely focus on growth density but should also be based on their individual effective capacity for constituent removal and overall effect on water quality. By focusing on the combined chemical, biological, and/or physical interactions of individual species of the native wetland flora that improve water quality within the wetland system, the vegetation chosen when designing constructed wetlands can thus be tailored to optimize specific contaminant removal of known water conditions more selectively in addition to addressing overall water quality. This alternative approach would continue the NBS plan for improving water quality through naturally occurring wetland processes and would incorporate better informed operative considerations of macrophyte selection during the initial constructed wetland design.

The results from this study both align with and differ from comparable studies that analyze the impacts of plant species on water quality treatment within wetland systems. This study recorded large increases in dissolved oxygen (+2538% to +3713%) compared to the Mitchell Lake pilot study. Zurita et al. (2006) tested the performance of a lab-scale wetland using five different species of tropical plants to treat domestic wastewater and noted similarly large percentage increases in DO (from 0.175 mg/L to 5.8 mg/L, +3314%) for their effluent. This was a significant improvement over the pilot study DO effluent

measurements. If this method was applied to a larger design, it could avoid the pilot programs' stated need for "additional aeration downstream of the full-scale wetland and upstream of the outfall" [30].

Although some of the water quality parameters in the study improved, the pH and COD parameters worsened compared to the initial samples, and the conductivity removal improved for bin #7 but worsened for bins #8 and #9. This was not the case in the Coleman and Zurita studies, as many of the same water quality parameter measurements were all generally improved [6,31]. More research would need to be conducted to assess why these differences occurred.

Much of the existing literature [13,32,33] describes the role of wetland plants as a type of necessary bulk component whose role is to maximize the hydrodynamic processes rather than highlighting each species' contribution in addressing water remediation goals. However, with the variance in water quality issues that can be regional and/or contaminant-specific, the selection of native vegetation when planning constructed wetland design should not solely focus on growth density but should also be based on their individual effective capacity for constituent removal and overall effect on water quality. This study shows that through this *targeting macrophyte* method, the vegetation selection and planting density's impact on water quality can be evaluated—not as a substitute for existing approaches to constructed wetland design, but as a complement to other site-specific design needs.

3.5. Study Limitations

Natural wetlands are large, outdoor systems of water, plants, and media that take time to become fully developed. An indoor, lab-scale constructed wetland, however, does not experience the same natural effects; namely, similar sun, wind, weather events, insect, bird, and other animal interactions, that would occur in an outdoor setting. As the control bins were positioned closer to the window than the experimental bins, the access to sunlight exposure could have impacted the photosynthetic processes and growth patterns between the groups. In addition, the effluent samples were all tested during the day and during a certain time of year. Further research for those water quality parameters tested during the evening or other portions of the year could yield variant results based on those differences.

Vymazal (2018) identifies two major concerns when gleaning results from constructed wetland testing: scalability and study length. He states that the direct comparison to full-scale wetland systems should not be made without taking into consideration the experimental scale. Vymazal gives an example of a microcosm study in which the small wetland area tested (<0.05 m^2) negatively impacted the plants' growth pattern compared to growing in a larger area. When the scale of the lab experimentation is too small, Vymazal contends that the overall system is more subject to *edge effects* and therefore the results from such studies could never be applied to full-scale wetland design guidelines. These edge effects are understood as microenvironmental gradient differences between the interior and exterior zones of a habitat that affect the vegetation and associated species [34]. Thus, if the habitat area is large, then the proportion of the "edge" to the interior is small, but if the habitat area decreases, the proportion of the edge increases, and the differences between the interior and exterior zones are more pronounced. However, Báldi concluded that the distinction of an interior zone for a reedbed habitat area was only present for habitat areas with a 15 m or larger radius. Therefore, concerns about edge effects would not apply to a study area of this size as there would be no distinction between an interior zone and an exterior zone to compare vegetation differences.

Additionally, Kadlec and Wallace (2009) separate constructed wetland sizing into four categories with decreasing levels of direct applicability to full-scale wetlands: field-scale, pilot-scale, mesocosm, and microcosm. This study design would be categorized as a mesocosm-scale project—larger than Vymazal's microscale example and would consequently have results which, by his own interpretation, would be more applicable to a larger system. Even Kadlec et al. (2005) documented a Lakeland, Florida, case study of a full-scale

wetland showing first-order, exponential declines in nitrate that had lab-derived values that were comparable to the field values showing that smaller, lab-scaled results can mirror those of the larger-scale design [35].

Lastly, aspects of any complex system can and should be analyzed before constructing the full-scale design as component factors within the system exhibit known traits before construction. For example, when constructing a bridge, materials testing is conducted separately on bridge components (steel beams, concrete, etc.) before they are assembled, and those tested results inform the properties of the whole design. In terms of ecological design, the aggregate complexity of the system is determined by the many interrelated relationships of those different system components [36]. While the efficacy of any study of complex systems can be improved with scale, interpolations can be made by utilizing smaller-scale experimentation methods like those in this study.

In terms of study length, Vymazal (2018) criticizes short-term studies as having ecological systems too juvenile to exhibit the full complexity of a mature wetlands ecosystem where extrapolations to large-scale projects could lead to costly design mistakes, noting that issues like clogging take time to present themselves, sometimes longer than the study itself allows [31]. While the data collection from the present study was limited to two months per stage, that timescale is not too short to allow for significant and useful findings for larger systems. For example, Kadlec and Wallace (2009) reported exchanges for SSF treatment wetlands (four mesocosm trains and one field-scale wetland containing well-established bulrushes, *Schoenoplectus tabernaemontani*, with another unvegetated field-scale wetland) that ran for only 24 days. Also, the wetland plant species used in this study were live root specimens rather than from seed which mitigated the distortion of water treatment results from waiting on underdeveloped plants to establish root systems.

4. Conclusions

This lab-scale study tested the performance of a novel targeting macrophyte method wherein macrophyte species that were the most effective at facilitating the improvement in water quality parameters within a wetland system were tested again with majority density plantings for further improvement in those parameters. From the two-phase testing conducted, it was shown that the planting density with 50% giant bulrush (*Schoenoplectus californicus*), 25% Olney's bulrush (*Schoenoplectus americanus)*, and 25% hardstem bulrush (*Schoenoplectus acutus*) improved conductivity removal by 34% and increased dissolved oxygen by 3713% as compared to the Mitchell Lake pilot-scale results. The 70% and 90% density plantings (bin #8 and bin #9, respectively) were not shown to be as effective for the tested parameters, indicating diminishing returns as the vegetation density increasingly becomes a monoculture within the system. These results highlight the applicability of this method to improve upon constructed wetland design by first identifying species whose traits would further facilitate the water improvement goals and then comparing their majority density plantings for optimized configurations.

Few studies have compared the effectiveness of wetland plant species for effluent wastewater treatment, let alone the use of macrophyte planting densities to target the removal of specific known factors and contaminants. More research is needed to more thoroughly examine the multitude of factors present within natural wetland systems in relation to vegetative species density to better be able to optimize the design of future constructed wetland projects. The results of this study demonstrate that there were significant differences between macrophyte species' effectiveness at removing water contaminants and that certain treatment parameters could be improved by incorporating this targeting macrophyte method as part of the overall consideration process for wetland design and vegetation selection.

We demonstrated that the *targeting macrophyte* approach using lab-scale systems can be a cost-effective method to optimize the selection of plants in a complex constructed wetland system. This is significant because the pilot-scale and full-scale performance evaluation of constructed wetlands for water quality remediation is typically cost- and

time-intensive. Our proposed method, based on its effectiveness in targeting site-specific water quality concerns, can significantly reduce the costs and time needed at the initial design and optimization phase.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16162278/s1, Figure S1. Constituent removal comparison between Bins #1–9 in either the Control Group or Experimental Groups; Section S1. Data Analysis for Phase I—Control Group and Experimental Group; Section S2. Data Analysis for Phase I—Control Group and Experimental Group; Section S3. Data Analysis for Phase II—Target Macrophyte Experiments.

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