Knowl. Manag. Aquat. Ecosyst. 2021, 422, 23
© P. Manolaki *et al.*, Published by EDP Sciences 2021
https://doi.org/10.1051/kmae/2021020

www.kmae-journal.org

Knowledge &
Management of
Aquatic
Ecosystems

Journal fully supported by Office
français de la biodiversité

SHORT COMMUNICATION OPEN ACCESS

Investigating emergent macrophytes establishment rate and propagation towards constructed wetlands efficacy optimization

Paraskevi Manolaki[1,2,*], Annica Olesen[1,3], Bjørg Graves Hvidt[1], Torben L. Lauridsen[3,4,5] and Tenna Riis[1,5]

[1] Department of Biology, Ole Worms Allé 1, Aarhus University, Aarhus C 8000, Denmark
[2] School of Pure & Applied Sciences, Open University of Cyprus, 56 Yiannou Kranidioti Avenue, Latsia 2220, Nicosia Cyprus
[3] Department of Bioscience, Vejlsøvej 25, Silkeborg 8600, Denmark
[4] Sino-Danish Centre for Education and Research, Beijing, PR China
[5] WATEC, Aarhus University Center for Water Technology, Ny Munkegade 120, Aarhus C 8000, Denmark

Received: 19 March 2021 / Accepted: 29 April 2021

Abstract – Constructed wetlands have become a widely used tool for reducing nutrient loading from agriculture drainage water running to aquatic ecosystems. To ensure a high nutrient removal efficiency, it is often suggested to use macrophytes to retain or remove nutrients via uptake and through the denitrifying biofilm. In Europe, *Phragmites australis* and *Typha* spp are the most commonly used aquatic plants in constructed wetlands (CWs) with free surface flow, and these species often form monocultures in the wetlands. In order to achieve a more diverse vegetation, there is a need to introduce more plant species. Creating a mass production of plant material reduces both handling time and the risk of depleting and disturbing vegetation in natural habitats such as streams or lakes. However, a successful and continuous production of such material during growing seasons requires knowledge of the selected species' establishment and propagation. We examined the relative growth rate (RGR) of six emergent macrophyte species collected from streams and small lakes located in Mid Jutland (Denmark), in seasonal experiments from March to October in order to determine the most efficient time period for their propagation. We found that all species had highest RGR in June, and that several species showed high growth efficiency from April to August. The results showed that it is possible to have a full production of emergent macrophytes throughout the growing season, and therefore, we suggest to propagate plants for use in constructed wetlands in order to enhance biodiversity and ecosystem functioning.

Keywords: Aquatic plants / drainage water / relative growth rate

Résumé – Étude du taux d'établissement et de la propagation des macrophytes émergées en vue d'optimiser l'efficacité des zones humides construites. Les zones humides construites sont devenues un outil largement utilisé pour réduire la charge en nutriments des eaux de drainage agricoles s'écoulant vers les écosystèmes aquatiques. Pour garantir une efficacité élevée de l'élimination des nutriments, il est souvent suggéré d'utiliser des macrophytes pour retenir ou éliminer les nutriments par absorption et par le biofilm dénitrifiant. En Europe, *Phragmites australis* et *Typha spp* sont les plantes aquatiques les plus couramment utilisées dans les zones humides construites (ZH) à écoulement superficiel libre, et ces espèces forment souvent des monocultures dans les zones humides. Afin d'obtenir une végétation plus diversifiée, il est nécessaire d'introduire davantage d'espèces végétales. La création d'une production de masse de matériel végétal réduit à la fois le temps de manipulation et le risque d'appauvrir et de perturber la végétation dans les habitats naturels tels que les cours d'eau ou les lacs. Cependant, une production réussie et continue de ce matériel pendant les saisons de croissance nécessite une connaissance de l'établissement et de la propagation des espèces sélectionnées. Nous avons examiné le taux de croissance relatif (RGR) de six espèces d'hélophytes collectées dans des cours d'eau et des petits lacs situés dans le centre du Jutland (Danemark), dans des expériences saisonnières de mars à octobre, afin de déterminer la période la plus efficace pour leur propagation. Nous avons constaté que toutes les espèces avaient le RGR le plus élevé en juin, et que plusieurs espèces présentaient une efficacité de croissance élevée d'avril à août. Les résultats ont montré qu'il est possible d'avoir une production importante de macrophytes émergés tout au long de la saison de

---

*Corresponding author: `paraskevi.manolaki@ouc.ac.cy`

croissance, et donc, nous suggérons de propager les plantes pour une utilisation dans les zones humides construites afin d'améliorer la biodiversité et le fonctionnement de l'écosystème.

Mots clés : Plantes aquatiques / eau de drainage / taux de croissance relatif

1 Introduction

Constructed wetlands are likely to become more widely used as a tool to reduce the transport of nutrients from agriculture to aquatic environments by increasing water residence time which enhance the nutrient retention and removal from the water. Aquatic plants have been effectively used in constructed wetlands (CWs), as they enhance retention and removal of nitrogen (N) and phosphorus (P) via multiple direct and indirect pathways including direct macrophyte assimilation, positively linked to plant biomass and denitrification in the sediment (*e.g.* Bachand and Horne, 2000; Levi *et al.*, 2015). Moreover, macrophytes serve as habitat for bacteria and algae, which also assimilate nutrients and perform denitrification (Srivastava *et al.*, 2017).

The most common vegetation in European CWs with free surface flow consists of monocultures of *Phragmites australis* or *Typha latifolia* (Vymazal, 2013). However, recent studies suggest that higher biodiversity both in terms of species number and of species growth forms (such as more emergent species, floating leaved and submergent species) can significantly increase nutrient removal from a system due to important differences in their nutrient uptake strategies (*e.g.* Bouchard *et al.*, 2007; Choudhury *et al.*, 2018; Manolaki *et al.*, 2020) via niche complementarity (Choudhury *et al.*, 2018; Olesen *et al.*, 2018) and the extension of the nutrient period across seasons (Manolaki *et al.*, 2020).

In Denmark, a plan is underway to create a thousand new constructed wetlands until 2021 in order to reach the N reduction target of the EU Water Framework Directive WFD; (European Commission, 2000). Species propagation and biomass accumulation rate are among the most important parameters that affect the efficacy of CWs in nutrient removal (Chambers and McComb, 1994). Therefore, information about the propagation of tolerant species in relation to species establishment, survival rate and size suitability for planting, will support the high number of plants required for the new CWs. Still, their survival and establishment rate can vary among plant species during the growing season, with some species being most productive in spring, summer and/or autumn (Manolaki *et al.*, 2020).

Seasonal variations in establishment and reproduction influenced primarily on temperature, day length and light quality (Warwick and Brock, 2003). Therefore, plant adaptations to these parameters affect their productivity (Lambers *et al.*, 2008) with some species being more productive than others at some point during the growing season. In order to optimize the efficacy of CWs in nutrient removal by increasing the plant functional diversity, it is important to ensure high establishment success for a number of alternative species. Therefore, it is necessary to know the most suitable time for planting in order to ensure high propagation success.

In this study, we test the establishment and propagation rate of six emergent macrophytes suitable for use in constructed wetlands in five outdoor experiments from March to October in order to identify the most efficient time period for planting based on the plants' growth rate. The results will support a more diverse, efficient and successful planting of constructed wetlands when using the selected species.

2 Materials and methods

Six emergent macrophyte species collected from streams and small lakes located in Mid Jutland (Denmark) were used in this study namely: *Nasturtium officinale* R. Br., *Glyceria fluitans* (L.) R. Br., *Berula erecta* (Huds.) Coville, *Veronica beccabunga* L., *Butomus umbellatus* L. and *Ranunculus hederaceus* L. The species were chosen based on their presence in early spring and their potential for water purification through nutrient uptake. All species have a vegetative growth form. For all of them, shoots with at least two nodes were included in the study. Shoot length depended on the species morphology, as shoots from *e.g. B. umbellatus* were naturally longer than shoots from *B. erecta* (Tab. 1).

The outdoor growth experiment for all species was repeated five times during the growing season. Monthly experiments were performed in March (7 March–5 April; 29 days), April (19 April–16 May; 27 days), June (31 May–28 June; 28 days), August (8 August–4 September; 27 days) and October (19 September–24 October; 35 days). Healthy and green plant material was collected from the field one day prior to each experiment. To make sure that the shoots were approximately at the same age and growth stage, the collection of flowering shoots was avoided. The shoots were rinsed and then planted in 16 partially buried tubs (90 L plastic tubs; 67 cm wide and 53 cm high) which consisted of 24 L washed beach sand sediment (5 cm sediment depth) and tap water to a water level of approximately 5 cm. Each day at noon, 0.3 L of water were added to each tub through a drip tube to compensate for evaporation. For each species there were 10 replicates of shoots distributed in 10 tubs. Individuals from each species were planted in different tubs to avoid pseudo replication and thus, the number of shoots in each tub varied between one and six. There was enough distance (>25 cm) between shoots to avoid competition (Olesen *et al.*, 2018). In total 17 tubs were used in the experiment. Shoots were gently planted in a depth of 5 cm approximately. After planting, three slow releasing fertilizer pellets (5–6 months) were added to the sediment (OsmocotePlus Tablet; 15 N – 4 P – 10 K – 1.2 Mg + Mikro) to ensure enough nutrients for plant growth without excessive algae growth.

Once a week, dissolved oxygen concentration and % saturation was measured in the water in each tub (OxyGuard probe). Data for solar radiation and air temperature was collected from a data logger (CR1000) on a climate station close to the study site (Dynamet Weather Station, Dynamax Inc) (Tab. 2). During the experiment conducted in June, water samples of 15 ml were taken every week from each tub and

Table 1. Range of shoot length (cm) and number of replicas for shoots used in the experiments for the six studied species.

Species	Days	Size range (cm)	Number of replicas	
		Start	Start	End
March: 7 March–5 April	29			
Nasturtium officinale		10–15	10	10
Glyceria fluitans		20–30	10	10
Berula erecta		10–15	10	10
Veronica beccabunga		10–15	10	10
Butomus umbellatus		2–5	10	10
Ranunculus hederaceus		10–15	10	10
April: 19 April–16 May	27			
Nasturtium officinale		15–25	10	10
Glyceria fluitans		30–40	10	10
Berula erecta		15–20	10	10
Veronica beccabunga		10–15	10	10
Butomus umbellatus		30–40	10	10
Ranunculus hederaceus		10–20	10	10
June: 31 May– 28 June	28			
Nasturtium officinale		20–25	10	10
Glyceria fluitans		30–70	10	9
Berula erecta		25-50	10	10
Veronica beccabunga		20–25	10	10
Butomus umbellatus		80–90	10	10
Ranunculus hederaceus		15–20	10	10
August: 8 August–4 September	27			
Nasturtium officinale		20–25	10	10
Glyceria fluitans		30–60	10	10
Berula erecta		25–50	10	10
Veronica beccabunga		20–35	10	10
Butomus umbellatus		50–60	10	7
Ranunculus hederaceus		10–20	10	7
October: 19 September–24 October	35			
Nasturtium officinale		25–35	10	10
Glyceria fluitans		30–60	10	10
Berula erecta		35–50	10	10
Veronica beccabunga		25–40	10	10
Butomus umbellatus		40–80	10	0
Ranunculus hederaceus		10–20	10	10

were analyzed for nutrient concentrations of phosphate-P (PO_4^{3-}-P), ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) on a flow injection analyzer (QuikChem FIA+ 8000 Series, Lachat instruments). The water supply was similar to all experiments and thus, the water quality was also assumed to be similar (Tab. 2). Once a week, and after intensive rainfall, the water level was manually lowered or increased to approximately 5 cm depending on the present water level.

At the end of the growth experiments, one shoot per replicate (6 species × 10 replicates) was taken up and rinsed before being oven-dried (60 °C). The dry weight (DW) for each individual was measured and the relative growth rate was calculated as RGR (day^{-1}) = (ln DW_{end} − ln DW_{start})/time (day). The starting DW for each shoot was estimated in relation to its initial wet weight (WW) and a DW/WW ratio, which was calculated based on 5–10 shoot-replicates similar to the shoots used in the experiment. The doubling time was calculated as DT = ln(2)/RGR (Mitchell, 1974). Relative growth rate (RGR) was used as a measure of propagation success.

We used one-way ANOVA to compare RGR for each species in different months and *post-hoc* Tuckey-HSD test to search for significant differences among growth periods. Data was log10 transformed when necessary for better achievement of variance homogeneity.

3 Results

The survival rate of shoots was generally high (>70%) for all species during each monthly experiment. However, all 10 shoots from *B. umbellatus* died in October. Average negative growth rates (=decay rates) appeared in March for *N. officinale* and in April for *B. umbellatus* (Fig. 1).

Table 2. Monthly average values per experiment for weather and climate data. Data were collected from a climate station located near the study site (solar radiation and air temperature) and from an oxygen probe. Solar radiation and air temperature were logged every half hour, and oxygen was measured once a week. Day values are calculated as an average from 07:00 to 18:30 h, and night values are calculated from 19:00 to 06:30 h. Nutrient concentration was only measured in summer but is from the same source and with similar amount of nutrient pellets in the other seasons.

Abiotic conditions	Month				
	March	April	June	August	October
Water physicochemical parameters					
PO_4^{3-}-P ($mg\,L^{-1}$)	NA	NA	0.015 ± 0.017	NA	NA
NH_4^{+}-N ($mg\,L^{-1}$)	NA	NA	0.333 ± 0.131	NA	NA
NO_3^{-}-N ($mg\,L^{-1}$)	NA	NA	0.852 ± 1.509	NA	NA
Oxygen (water)					
% saturation	NA	NA	75.1 ± 14.2	108.8 ± 16.5	101.5 ± 16.7
ppm	NA	NA	7.0 ± 0.6	10.2 ± 1.4	10.7 ± 1.3
Weather and climatic conditions					
Solar radiation ($kW\,m^{-2}$)					
Day	0.20 ± 0.17	0.34 ± 0.22	0.37 ± 0.22	0.29 ± 0.18	0.13 ± 0.14
Air temperature (°C)					
Day	7.48 ± 4.03	10.1 ± 4.01	16.5 ± 2.78	17.5 ± 2.64	13.1 ± 2.62
Night	4.65 ± 3.12	6.20 ± 3.53	12.9 ± 2.46	13.4 ± 2.44	11.1 ± 2.78
Climate in Denmark[1]					
Average temperature (°C)	3.5	7.7	14.3	16.7	9.8
Total hours of sunshine (h)	146	211	240	187	102
Precipitation (mm)	40	30	64	99	83

[1] Information about the climate in Denmark from DMI (the Danish Meteorological Institute) and is reference values for the period 2006–2015.

There were significant seasonal differences in RGR for all species except for *G. fluitans* (Fig. 1). All species had the highest RGR in June and followed the same bell-shaped distribution of rise and fall in RGR.

N. officinale reached a maximum RGR of 0.090 day^{-1} (doubling time, DT of 7.7 days) and showed high growth efficiency from April to October. *V. beccabunga* had high growth efficiency from April to August with a maximum RGR of 0.076 day^{-1} (DT of 9.2 days). Both *B. erecta* and *R. hederaceus* showed highest growth efficiency from April to June, with a maximum RGR of 0.068 day^{-1} (DT of 10.2 days) and 0.106 day^{-1}(DT of 6.5 days), respectively. *R. hederaceus* showed a significant decrease in RGR in August and October and *B. erecta* showed high variance in RGR in October. *B. umbellatus* had the lowest maximum RGR (0.030 day^{-1}, DT of 22.8 days) and showed only good growth efficiency in June (Fig. 1).

4 Discussion

The results showed differences in relative growth rate (RGR) among species during the growing season with almost all species having the highest growth rate in June and also varying in the range of 0.002–0.106 day^{-1}. The relative growth rate of the specific emergent species used in this study are somewhat in the same range as 0.003–0.13 day^{-1} found by Manolaki *et al.* (2020) and within the range of relative growth rates (0.007–0.109 day^{-1}) found by Nielsen and Sand-Jensen (1991) for 14 submerged species. However, the growth capacity of submerged species is much lower than the one recorded in relation to the emergent species (Manolaki *et al.*, 2020).

We intended to identify at which moment during the growing season the selected plant species achieved the highest growth efficiency and therefore achieved the highest biomass accumulation in the shortest amount of time. This information is important to ensure high propagation rate and establishment success when planting into a constructed wetland, especially when fast growing species like *Phragmites australis* are also present. Our results showed that *G. fluitans* was the only species suitable for mass propagation in March. Even though, *G. fluitans* showed the lowest RGR during summer period (June), as it was also illustrated by other studies (Manolaki *et al.*, 2020), it is generally suitable for propagation during the whole growth period (from early spring to autumn) due to low seasonal variation in its growth efficiency.

Species with the highest growth rates were *N. officinale, B. erecta, V. beccabunga* and *R. hederaceus.* All of them are suitable for propagation from April to June with the highest growth rates observed in the middle of the growing season. Amphibious species like *N. officinale, B. erecta* and *V. beccabunga* did not stored underground organs like other emergent species *e.g. G. fluitans*, which can translocate the stored dry mass from the beginning of the growing season and show an early high RGR (Howard-Williams *et al.*, 1982). Species that lack underground reserves showed bell-shaped RGR curve, which might causean extension of the growth period. Especially for *N. officinale*, the RGR curve showed high relative growth rates both in August and

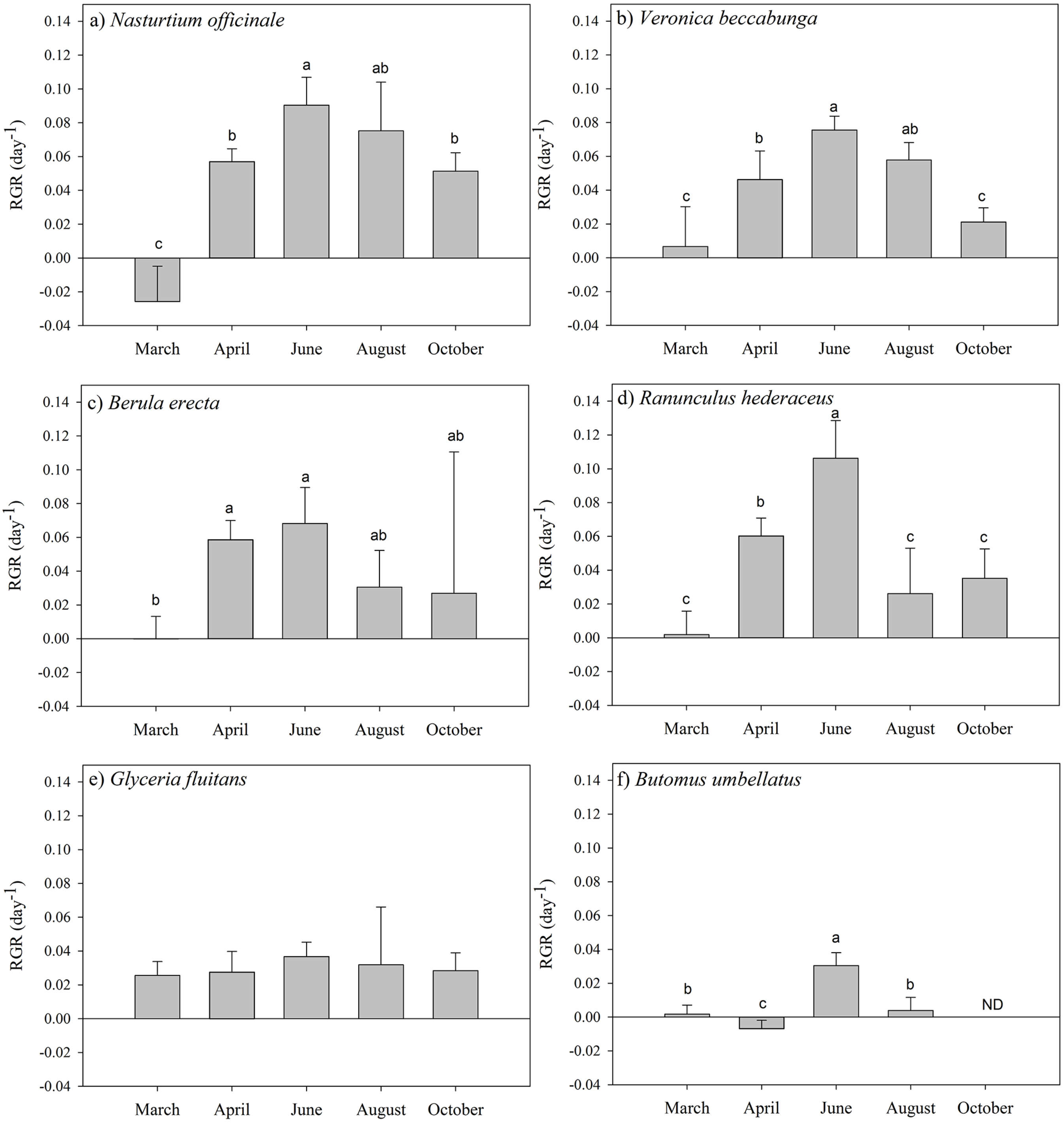

Fig. 1. Box plots with standard deviation (±SD) of the mean monthly values of the relative growth rates (RGR) for the six species. Different letters indicate significant differences ($p < 0.05$). ANOVA results: *N. officinale*: $F(4,45) = 55.83$, $p < 0.0001$, *V. beccabunga*: $F(4,45) = 34.93$, $p < 0.0001$, *B. erecta*: $F(4,45) = 4.49$, $p = 0.0039$, *R. hederaceus*: $F(4,42) = 34.160$, $p < 0.0001$, *G. fluitans*: $F(4,44) = 0.48$, $p = 0.75$, *B. umbellatus* $F(3,33) = 59.92$, $p < 0.0001$.

October, indicating that this species is appropriate for propagation throughout the whole growing season. This might also suggest that the species adaptations and preferences for light, temperature and day length are similar, as they all show highest propagation potential in June, when the temperature and solar radiation is high. However, *N. officinale* and *G. fluitans* showed potential for propagation in autumn (October), suggesting that these species can be planted later in the growing season, since they are better adaptable to lower temperatures and lower light conditions and as such, they are also very useful for constructed wetlands.

The results showed that all species had the highest RGR in June. However several species showed good growth efficiency from April through to August. Hence, it is possible to have a full production of emergent macrophyte species throughout the whole growing season. Based on these results, we suggest the propagation of certain plant species, such as *N. officinale* and *G. fluitans*, for enhancing biodiversity and ecosystem functioning in constructed wetlands.

Acknowledgements. We thank the Danish Ministry of Environment and Food for the financial support through the Green Development and Demonstration Project (GUDP), PLANT-NAT. We thank Prof. Ioannis Vogiatzakis and Dr. Christina Kkona for English language editing.

References

Bachand PAM, Horne AJ. 2000. Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. *Ecol Eng* 14: 17–32.

Bouchard V, Frey SD, Gilbert JM, Reed SE. 2007. Effects of macrophyte functional group richness on emergent freshwater wetland functions. *Ecology* 88: 2903–2914.

Chambers JM, McComb Arthur J. 1994. Establishing wetland plants in artificial systems. *Water Sci Technol* 29: 79–84.

Choudhury MI, McKie BG, Hallin S, Ecke F. 2018. Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Sci Total Environ* 635: 1436–1443.

European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Off J Eur Commun L* 327: 1–72.

Howard-Williams C, Davies J, Pickmere S. 1982. The dynamics of growth, the effects of changing area and nitrate uptake by watercress *Nasturtium officinale* R. Br. in New Zealand stream. *J Appl Ecol* 19: 589–601.

Lambers H, Chapin III FS, Pons TL. 2008. Growth and allocation. In: Plant Physiological Ecology, Publisher: Springer-Verlag New York, Year: 2008 pp. 321–375.

Levi PS, Riis T, Alnøe AB, Peipoch M, Maetzke K, Bruus C, Baattrup-Pedersen A. 2015. Macrophyte complexity controls nutrient uptake in lowland streams. *Ecosystems* 18: 914–931.

Manolaki P, Mouridsen MB, Nielsen E, Olesen A, Jensen SM, Lauridsen TL, Baattrup-Pedersen A, Sorrell BK, Riis T. 2020. A comparison of nutrient uptake efficiency and growth rate between different macrophyte growth forms. *J Environ Manage* 274: 111181.

Mitchell DS. 1974. Aquatic vegetation and its use and control. Unesco, Paris: pp. 135.

Nielsen SL, Sand-Jensen K. 1991. Variation in growth rates of submerged rooted macrophytes. *Aquat Bot* 39: 109–120.

Olesen A, Jensen SM, Alnoee AB, Baattrup-Pedersen A, Lauridsen TL, Sorrell BK, Riis T. 2018. Nutrient kinetics in submerged plant beds: A mesocosm study simulating constructed drainage wetlands. *Ecol Eng* 122: 263–270.

Srivastava JK, Chandra H, Kalra SJS, Mishra P, Khan H, Yadav P. 2017. Plant–microbe interaction in aquatic system and their role in the management of water quality: a review. *Appl Water Sci* 7: 1079–1090.

Vymazal J. 2013. Emergent plants used in free water surface constructed wetlands: a review. *Ecol Eng* 61: 582–592.

Warwick NWM, Brock MA. 2003. Plant reproduction in temporary wetlands: the effects of seasonal timing, depth, and duration of flooding. *Aquat Bot* 77: 153–167.

Cite this article as: Manolaki P, Olesen A, Graves Hvidt B, Lauridsen TL, Riis T. 2021. Investigating emergent macrophytes establishment rate and propagation towards constructed wetlands efficacy optimization. *Knowl. Manag. Aquat. Ecosyst.*, 422, 23.