MethodsX 16 (2026) 103800

Contents lists available at ScienceDirect

MethodsX

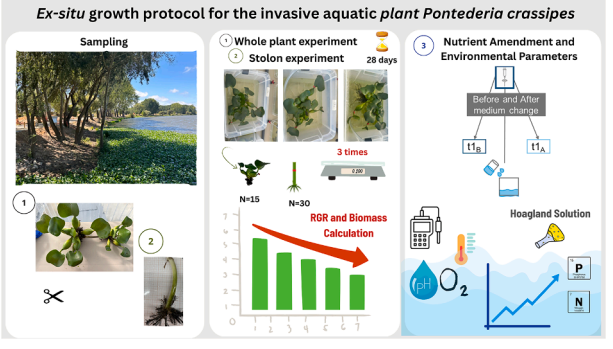
journal homepage: www.elsevier.com/locate/methodsx

Ex-situ growth protocol for the invasive macrophyte *Pontederia crassipes*

Letícia da Silva Brito [a,*], Sidinei Magela Thomaz [b], Heliana Teixeira [a], Ana I. Lillebø [a]

[a] *ECOMARE, CESAM-Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal*
[b] *Departamento de Biologia, Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá, Maringá, Brazil*

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Eichhornia crassipes
Mesocosm
Experimental reproducibility
Growth rate
Invasive alien species

ABSTRACT

Pontederia crassipes is known for its asexual reproduction and rapid growth. Outside its native range, it has been identified as an environmental threat, while it has also been widely used for *ex-situ* phytoremediation. To understand both its invasive potential and its phytoremediation capacity, it is necessary to examine the environmental factors that favor its growth beyond those already described in the literature, such as water temperature and nutrient availability. Previous studies also suggest that alkalinity, conductivity, dissolved oxygen, salinity, water depth and pH. These variables help define the species niche and highlight the importance of distinguishing between its fundamental niche, the full set of abiotic conditions that support growth and its realized niche, which reflects biotic interactions and local constraints. However, the scientific

* Corresponding author.
E-mail address: let.brito@ua.pt (L.S. Brito).

https://doi.org/10.1016/j.mex.2026.103800
Received 31 October 2025; Accepted 12 January 2026
Available online 16 January 2026

literature does not yet provide sufficient description of the *ex-situ* experimental conditions required for the successful cultivation of this aquatic plant in controlled settings. This protocol therefore reports the results and lessons learned from a series of mesocosm experiments. By standardizing procedures and documenting growth outcomes, the protocol enhances reproducibility, facilitates comparisons across studies and supports both basic and applied research on *P. crassipes*.

Specifications table

Subject area	Environmental Science
More specific subject area	Aquatic Ecology / Applied Botany
Name of your protocol	Growth of *Pontederia crassipes* under mesocosm controlled experiment.
Reagents/tools	**Reagents:** Ammonium dihydrogen orthophosphate Potassium nitrate Calcium nitrate tetrahydrate Magnesium sulfate heptahydrate Boric acid Copper (II) sulfate pentahydrate 99 %+ Zinc sulfate heptahydrate 99.5 % Manganese (II) chloride tetrahydrate Molybdenum acid ($\geq$85 % MoO_3) Titriplex® III (ethylenedinitrilotetraacetic acid disodium salt dihydrate) EDTA Potassium hydroxide Ferrous Sulphate **Tools:** Analytical Balance. Conductivity, Temperature, and Salinity portable meters: ProfiLine Cond 3320 (WTW, Weilheim, Germany) pH portable meter: ProfiLine pH 3310 (WTW, Germany) Dissolved Oxygen portable meter: ProfiLine Oxi 3310 (WTW, Germany) Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, Netherlands) for nutrients analysis Air Pump to enhance water mixing
Experimental design	Two independent experiments were conducted using the same design and salinity treatments. In the first experiment, 5 plastic boxes (the experimental units) were used, each containing six stolons (pseudo-replicates). This resulted in a total of 30 stolons. The second experiment used the same design, but with each box containing three plants, resulting in a total of 15 plants. The box was defined as the experimental unit, whereas the individual plants/stolons within each box were considered pseudo-replicates. All plants/stolons were grown in the same source to minimize variation due to substrate.
Trial registration	Not applicable.
Ethics	Due to its invasive behavior, experiments involving this species require prior authorization and safety handling. In the case of Portugal this authorization was required from the Institute for Nature Conservation and Forests (ICNF).
Value of the Protocol	Standardizes the mesocosm experiment for assessing *Pontederia crassipes* biomass growth rate. Enables reproducibility of results by different researchers under varying conditions. Facilitates investigation of environmental parameters (e.g., salinity, pollutants) relevant to specific research questions.

Background

Pontederia crassipes (Mart.), formerly *Eichhornia crassipes* (Mart.) Solms and commonly known as water hyacinth, is a free-floating aquatic plant widely recognized for its rapid growth and high invasive potential [1]. Native to South America, it was first recorded outside its natural range in New Orleans in 1884 [2]. Since then, it has been reported as an invasive species on five continents, excluding Antarctica (EPPO, 2008). It is considered among the 100 world's worst invasive alien species by IUCN [3] and in 2023, it was identified as one of the major threats to global biodiversity [4]. Conversely, it has also been used for phytoremediation purposes, particularly to treat industrial effluents under *ex-situ* conditions [5]. Moreover, its biomass has been utilized for a variety of purposes [6]. Although both sexual and asexual reproduction have been described in the literature, to the best of our knowledge, the enabling conditions for reproduction of this species under *ex-situ* settings are not sufficiently documented (Table 1). Addressing this knowledge gap is essential to ensure experimental reproducibility and thereby advance scientific research following the FAIR (Findable, Accessible, Interoperable, and Reusable) principles of Open Science [7]. To this end, the first step was to analyze the available literature on mesocosm experiments targeting biomass production, including studies on constructed wetlands and phytoremediation. Table 1 summarizes the findings of ten mesocosm studies, which ranged in duration from 22 to 150 days. The table shows the use of different container volumes (100–1000 L) and temperature ranges (5–35 °C). In terms of environmental conditions, the growth medium included artificial nutrient water, well water, pond water, lake water and wastewater, with a retention time mostly ranging from one to six weeks. Nutrient concentrations ranged from 0.05 to 28 mg/L for nitrogen and from 0.05 to 8.86 mg/L for phosphorus. In addition to variability in experimental setup, initial biomass varied from 15 to 680 g dry weight per m^2, while in one experiment, the authors used

Table 1
Comparative summary of mesocosm experiments testing the effects on biomass growth of *Pontederia crassipes* from the literature. NA means absence of information.

Growth indicator	Mesocosm experiment	Duration	Hydrological conditions				Nutrient source	Nutritional conditions			Growth rate			References
		(days)	**Volume** (L)	**Temperature** (ºC)	**Retention time** (weeks)	**Water circulation**		**Nitrogen** (mg L^{-1})	**Phosphorus** (mg L^{-1})	**micronutrients** (mg L^{-1})	**T0** (g DW m^{-2})	**Tf** (g DW m^{-2})	**Rate** (g DW m^{-2} day^{-1})	
Whole plant (Petiolo+lamina+steam+root)														
Net productivity (dry weight)	1	84	300	26ºC-28ºC	1	daily by submersible pump	constructed wetlands	20	5	NA	445	2303	0.0193	Reddy and Tucker [8]
	2	100	1000	NA	1	no information	concret valvus with nutrient medium	10.5	3.1	K: 8.4 Ca: 26.4 Mg: 5.6 Fe: 0.3 Mn: 0.0	680	3300	14.25	Reddy [9]
	3	80	1000	NA	1	12 h by submersible pump	artificial water prepared with nutrients	21	3.1	Fe: 4 Cu: 0.2 Mn: 1.5 B: 0.04 Mo: 0.02 S:	500	2000	0.0173	Reddy and Debusk [10]
	4	83	1000	15ºC-33ºC	1	daily by submersible pump	well water	0.05	3	K: 25 Fe: 4 Cu: 0.2 Mn: 1.5 B: 0.04 Mo: 0.02 S: 3	225	2000	0.0263	Reddy et al. [11]
	5	96	1000	5ºC-35ºC	1	daily by submersible pump	well water	20	0.06	K: 25	225	830	0.0147	Reddy et al. [12]
	6	22 *in two seasons	500–1000	17.8ºC-24.8ºC	48 h and 60 h	NA	domestic water after passing primary sedimentation pond.	20	7.5	K: 16.5	48	225	0.0112	Ho and Wong [13]
	7	56	200	15ºC −25ºC	1	NA	well water	5	0.05	K: 23 Ca: 20 Mg: 5.0 Fe: 0.60 B: 0.51 Mn: 0.52 Zn: 0.05 Cu: 0.02 Mo: 0.01	30	120	0.0248	Xie et al. [14]
	8	105	300	NA	6	NA	constructed wetlands	28	7.7	NA	plant with 20 cm	NA	NA	Jayaweera et al. [15]
	9	42	100	23.5ºC-28.8ºC	1	NA	lake water	0.6	0.05	NA	15	235	0.042	You et al. [16]
	10	150	104	NA	1		pond water	0.58	8.86	NA	260	405.83	0.0112	Kumwimba et al. [17]

plant length (cm) rather than biomass as responsable variable. While most authors report on plant biomass, the description of the included plant organs is not sufficiently clear, with some studies referring to shoots and others only to plants, without providing detailed measurement criteria. Overall, the literature reveals a heterogeneity of protocols, as well as short-term and low representativeness and incomplete reporting of conditions. The lack of standardized reporting with sufficient detail to enable the validation and reproducibility of experimental conditions must be addressed to foster progress in this field of research. The main objective of this protocol is to share a set of *ex-situ* growth experiments with *Pontederia crassipes* and clearly demonstrate the most and least effective methods to support progress in this field.

What is well known: Sexual reproduction in this species occurs through seeds [18], while asexual reproduction occurs via vegetative propagation [19,20]. A small portion of plant's horizontal stem, known as a stolon, may break due to physical or biological agents and act as a propagule capable of producing new individuals. Under favorable conditions, such as optimal temperature and nutrient availability, the plant can cover large areas of the water surface within just a few weeks [1]. This rapid proliferation has negative ecological impacts, including oxygen depletion and consequent fish mortality [21], increased water evaporation [22], and reduced biodiversity due to sunlight blockage and decreased net productivity [23]. In economic terms, it results in high costs associated with control and management strategies [24]. Socially, it affects local communities that depend on aquatic ecosystems [24]. The remaining knowledge gaps lie in determining the optimal conditions for propagation to both manage the spread of *P. crassipes* and explore potential sustainable applications, as well as in developing effective control strategies [25]. For instance, none of the above-mentioned studies addressed the role of stolons or reported the challenges of maintaining this species under controlled mesocosm conditions.

This protocol emphasizes the value of mesocosm experiments as controlled systems for studying the growth dynamics of *P. crassipes*, highlighting the limitations and opportunities these systems offer for advancing basic and applied research on the ecology of invasive species.

Description of protocol

Site collection

Collecting the material

Pontederia crassipes was collected from the Pateira de Fermentelos freshwater lagoon in Aveiro, Portugal (40°34′48″N, 8°31′12″W) in September and October 2024. Two independent experiments were conducted using different *Pontederia crassipes* propagules: stolon and whole plant. Both experiments were initiated separately in late autumn 2024. Separate collections were made for each propagation type. Knowing the plant's life cycle in the collection area is important for planning the collection according to its availability in the ecosystem.

Experimental units were standardized by selecting plants with comparable leaf numbers and sizes. They were then conditioned in plastic bags and transported to the laboratory.

Note: the plants should be carefully placed in water to maintain humidity during transportation to the laboratory.

Material and equipment

Sample

- Stolon and whole plant
- Select and collect healthy plants of similar sizes, measured by fresh weight.
- Plastic transport plants to the laboratory.

Equipment

- Digital precision balance (A&D Company Limited, model FX-5000i; maximum capacity 5200 g; accuracy 0.01 g).
- Conductivity, Temperature, and Salinity portable meters: ProfiLine Cond 3320 (WTW, Weilheim, Germany)
- pH portable meter: ProfiLine pH 3310 (WTW, Germany)
- Dissolved Oxygen portable meter: ProfiLine Oxi 3310 (WTW, Germany)
- Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, Netherlands) for nutrients analysis
- Air Pump to enhances water mixing (Aeration was provided through the laboratory's centralized piped air system, delivering a constant airflow directly to each mesocosm. This setup replaced the need for individual air pumps and ensured uniform water mixing and oxygenation across all experimental units).

Material

- 20 Polypropylene 30 L containers (size: 400 × 300 × 243 mm).
- Graph paper to take a picture and measure plants structures.
- Syringes and Glass filter (GF 0.45 μm) for solution nutrients analysis
- Volumetric glassware for solution preparation (beakers and volumetric flasks)

Reagents

Tables 2 and 3.

Experimental procedures

Cleaning the plants

In the laboratory, the stolons should be identified on the plants and cut between the two nodes, leaving roots, without leaves, and allowing new leaves to grow (Fig. 1). As stolons may become detached from the parent plant due to mechanical forces resulting from disturbances in the aquatic environment [20], the potential for producing new plants from these detached stolons was investigated. For whole plant propagules, a plant containing only one rosette (a group of floating leaves with a crown-like stem, and submerged roots) were used.

Before placing them in the solution, they should be washed with tap water to remove residues and undesirable organisms. To ensure consistency in the experiment, it is important to standardize the size and weight of the stolons and plants.

The plants (N=20 units) used in the experiment were selected to ensure morphological and physiological uniformity. They had four leaves, with and a total biomass per plant of 36.± 13.23 g.Their stolon biomass (13.± 7.52 g) and length (14.33 ± 5.05 cm) were also within these ranges. Prior to placement in the mesocosms, the roots were carefully washed to eliminate any organisms or debris adhering to them that could potentially affect the ecological balance of the system.

Preparing the solution

The growth medium was based on Hoagland's nutrient solution [26] and was modified for use in aquatic mesocosms. The experimental setup consisted of 20 opaque polypropylene boxes (internal dimensions: 400 × 300 × 243 mm; total volume: 30 litres), each of which was filled with 15 litres of nutrient solution. A central air system provided continuous aeration, with tubing supplying air to each box via individual valves that allowed independent control of airflow. The preparation involved the following:

First, six stock solutions were prepared using distilled water and stored in the refrigerator at 4 °C. These stock solutions were prepared as indicated in Table 3 and kept in the refrigerator for up to two weeks. These stock solutions formed the basis for preparing the Hoagland nutrient solution. Each week, 150 litres of Hoagland solution were prepared by diluting the required volumes of each stock solution with purified tap water from a V2Pure 360 RO system (TMC, Hertfordshire, UK). The pH and salinity were tested prior to

Table 2
Reagents for Hoagland nutrient solution.

Reagents	Chemical Formula	Chemical Abstracts Service (CAS)
Ammonium dihydrogen orthophosphate	(NH4) H2PO4	7722-76-1
Potassium nitrate	KNO_3	7757-79-1
Calcium nitrate tetrahydrate	Ca(NO3)2 4 H2O	13477-34-4
Magnesium sulfate heptahydrate	MgSO4 · 7H2O	10034-99-8
Boric acid	H_3BO_3	10043-35-3
Copper (II) sulfate pentahydrate	$CuSO_4$ * 5 H_2O	231-847-6
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	7446-20-0
Manganese (II) chloride tetrahydrate	$MnCl_2$	13446-34-9
Molybdic acid	H2MoO4	7782-91-4
Eethylenedinitrilotetraacetic acid disodium salt dihydrate (EDTA)	$C_{10}H_{14}N_2O_8$*2Na*$2H_2O$	6381-92-6
Potassium hydroxide	KOH	1310-58-3
Ferrous Sulphate	$FeSO_4 \cdot . 7H_2O$	7782-63-0

Table 3
Adapted Hoagland Solution nutrients components.

Hoaglands Stock Solutions (SS)	Stock Solution Concentration	Volume SS in Final Hoagland Solution
Macro nutrients SS		
1	1.00 M $NH_4H_2PO_4$	1ml/L
2	1.00 M KNO_3	6ml/L
3	1.00 M $Ca(NO_3)_2$ $4H_2O$	4ml/L
4	1.00 M $MgSO_4 \cdot 7H_2O$	2ml/L
Micro nutrient SS		
5	2.86 g H_3BO_3 /1L 0.08 g $CuSO_4$.5 H_2O /1L 0.22 g $ZnSO_4 \cdot 7H_2O$ /1L 1.81 g $MnCl_2$ /1L 0.02 g H_2MoO_4 /1L	1ml/L
Iron stock		
6	26.1 g EDTA /1L	0.25ml/L

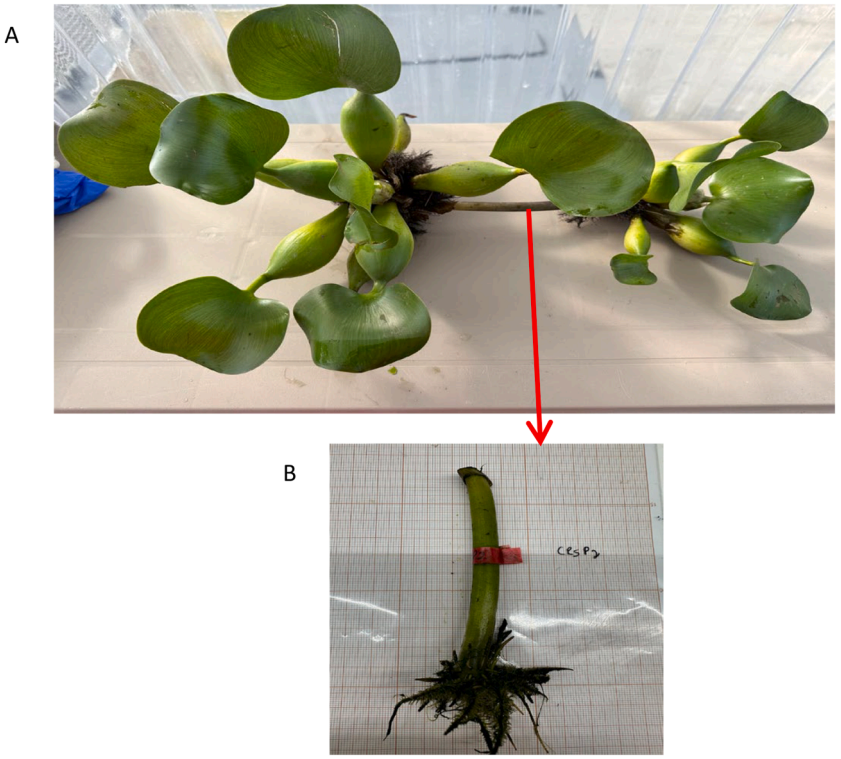


Fig. 1. *Pontederia crassipes* in detail of the vegetative structure of stolon joining the two plants (A) and only stolon (B) used in by experiment.

use: salinity was <1.5 and the pH was around 6.0. To ensure the nutrient solution was completely homogenized, continuous water movement was maintained using the laboratory's centralized piped-air system instead of a mechanical pump. Airflow was delivered to each mesocosm via identical valves, creating a gentle, constant bubbling action to keep the solution mixed. While the exact airflow rate was not quantified, equivalent aeration was provided to all units, ensuring uniform circulation.

Acclimatation time

To avoid death, resulting stolons and plants were placed in an opaque polypropylene box (internal volume:400 × 300 × 243 mm) with 15 L of Hoagland's solution before starting the experiments for one week. Continuous aeration was utilized to enhance oxygen and water flow and reduce the boundary layer around the plants.

Daily monitoring water parameters

To ensure stable conditions and prevent anomalous responses in plant performance, water quality parameters were measured daily throughout the experiment (Table 4). Temperature, salinity, pH, dissolved oxygen, and electrical conductivity were recorded between 10:00 and 12:00 h to minimize diel variation.

This continuous monitoring allowed us to verify water quality, detect potential fluctuations, and better interpret the interactions between *Pontederia crassipes* growth and environmental conditions.

Nutrient analyses

Water samples were filtered using a syringe and glass fiber (GF, 0.45 μm) filters before and after solution renewal. The Hoagland solution was partially renewed while the plants remained under acclimatisation conditions. Each week (Acclimatisation week, Week 1, Week 2, and Week 3), half of the solution (7.5 L out of 15 L) was removed and replaced with an equal volume of fresh Hoagland solution. This procedure is necessary to restore the nutrients that were taken up by macrophytes. Distilled water was also added as needed to compensate for natural evaporation. Concentrations of dissolved nutrients were determined using a Skalar San++ Continuous Flow Analyzoer (Skalar Analytical, Breda, Netherlands), following standard automated colorimetric methods:

- Ammonium (NH_4^+-N): Modified Berthelot reaction
- Orthophosphate (PO_4^{3-}-P): Total UV-digestible phosphate
- Nitrogen oxides (NO_x-N): Total UV-digestible nitrate + nitrite

Table 4
Water parameters during the whole experiment.

Structure	Box Identific	Oxygen (mg/	Temperature	Conductivity	Salinity	pH
	Acclimation					
	Replicate 1	10.95 ± 0.69	21.23 ± 2.97	557.38 ± 35.97	0.2 ± 0	6.35 ± 0.33
	Replicate 2	10.53 ± 0.55	21.36 ± 2.8	2330 ± 165.36	1.25 ± 0.05	5.56 ± 0.11
	Replicate 3	10.55 ± 0.54	21.52 ± 2.59	2370 ± 145.9	1.3 ± 0	5.56 ± 0.14
	Replicate 4	10.59 ± 0.64	22 ± 2.57	1660.12 ± 118.	0.85 ± 0.05	5.69 ± 0.06
	Replicate 5	10.6 ± 0.53	21.62 ± 2.63	1425.75 ± 152.	0.74 ± 0.05	5.76 ± 0.07
	Replicate 1	9.7 ± 0.56	17.7 ± 2.06	812 ± 37.81	0.4 ± 0	5.11 ± 0.39
	Replicate 2	9.35 ± 0.38	18.14 ± 1.84	1568.38 ± 56.4	0.9 ± 0	4.91 ± 0.42
	Replicate 3	9.47 ± 0.4	17.89 ± 1.94	1752.25 ± 73.4	1 ± 0	4.64 ± 0.73
	Replicate 4	9.28 ± 0.47	18.8 ± 1.87	2065.75 ± 79.1	1.2 ± 0	4.58 ± 0.56
	Replicate 5	9.47 ± 0.4	18.25 ± 1.81	973 ± 39.9	0.5 ± 0	4.53 ± 0.78
	Expriemental week					
	Replicate 1	11.73 ± 0.64	18.64 ± 2.51	817.1 ± 325.65	0.4 ± 0.22	6.19 ± 0.43
	Replicate 2	11.78 ± 0.64	18.93 ± 2.62	1858.19 ± 214.	1.03 ± 0.09	4.67 ± 0.89
	Replicate 3	11.81 ± 0.6	18.88 ± 2.41	1562.19 ± 215.	0.87 ± 0.1	4.89 ± 0.81
	Replicate 4	11.65 ± 0.56	19.93 ± 2.42	1784.1 ± 168.2	0.97 ± 0.06	5.24 ± 0.52
	Replicate 5	11.64 ± 0.62	19.47 ± 2.44	1382.86 ± 239.	0.73 ± 0.13	4.85 ± 0.73
	Replicate 1	9.7 ± 0.7	15.23 ± 2.61	1480.76 ± 270.	0.89 ± 0.18	3.84 ± 0.19
	Replicate 2	9.72 ± 0.61	15.96 ± 2.39	2024.43 ± 103	1.25 ± 0.86	3.92 ± 0.2
	Replicate 3	9.72 ± 0.65	15.56 ± 2.53	1767.33 ± 116.	1.06 ± 0.05	3.9 ± 0.51
	Replicate 4	9.57 ± 0.58	16.94 ± 2.13	1880.52 ± 96.8	1.09 ± 0.03	3.54 ± 0.15
	Replicate 5	9.64 ± 0.64	16.26 ± 2.36	1636.48 ± 207.	0.97 ± 0.13	3.85 ± 0.2

The following calibration curves were established for each nutrient: 0–25 μmol L^{-1} for ammonium; 0–10 μmol L^{-1} for orthophosphate; and 0–50 μmol L^{-1} for nitrogen oxides. As the nutrient concentrations in the mesocosms were much higher than those typically found in natural ecosystems and exceeded the analytical system's linear range (Skalar Analytical, Breda, Netherlands), the samples required dilution factors of 167 for ammonium and orthophosphate, and 501 for nitrogen oxides, to ensure the final concentrations fell within the validated calibration ranges.

The calibration was based on a six-point linear curve, with correlation coefficients consistently above 0.999 for all nutrients. Concentrations were expressed using the limits of quantification (LOQ): 252 μmol N L^{-1} for ammonium, 117 μmol P L^{-1} for orthophosphate and 651 μmol N L^{-1} for nitrogen oxides. A certified reference material (Seawater QC3179) was analysed to ensure analytical accuracy, yielding recoveries within the acceptable range. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+-N and NOx-N form. Nutrient concentrations in the water were calculated using the following equation:

Concentration of nutrient in water = (Nutrient initial concentration in water – Nutrient final concentration) * *Volume* (Hoagland Solution volume total).

Biomass measurements

The experiment lasted four weeks. Week 1 corresponded to the acclimatisation period, after which there were three experimental weeks (weeks 2–4). All biomass measurements and relative growth rate (RGR) calculations were performed at the end of weeks 1, 2 and 4.

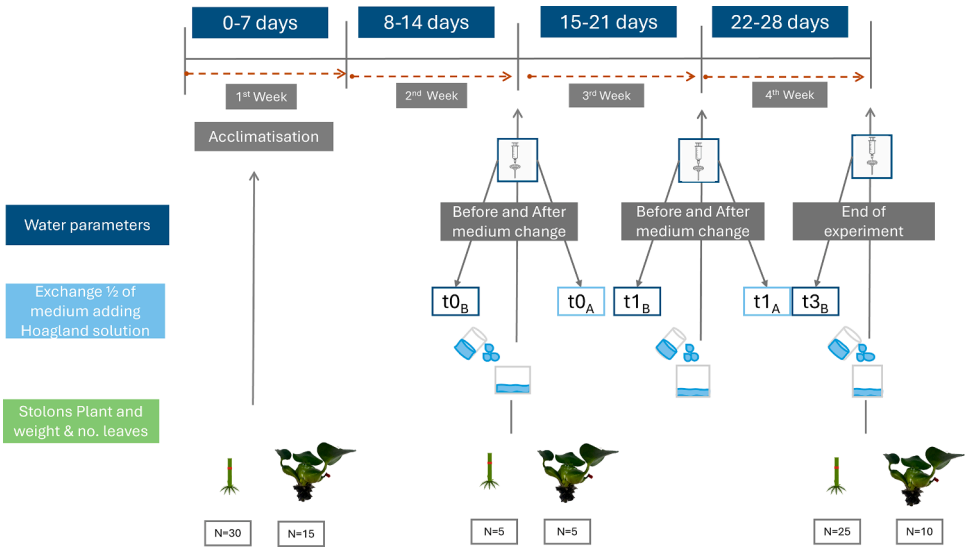


Fig. 2. Schematic representation of the experimental protocol, summarizing water parameter monitoring, nutrient analyses, and biomass measurements.

Total biomass

Fresh biomass was assessed three times throughout the four-week experiment. During week 1 (acclimatisation), the fresh biomass of all the plants and stolons was recorded at the start and end of the week. At the end of week 2, one plant and one stolon were removed from each experimental unit, weighed to determine their fresh biomass, dried and not returned to the system. On the week 4 all the remaining plants and stolons were collected and weighed to determine the final fresh biomass.

Relative growth rate

Relative growth rate (RGR) is a widely used parameter to describe plant growth dynamics, as it normalises biomass accumulation relative to initial mass. In this study, RGR was calculated using the same weekly intervals applied to biomass measurements. Growth measurements were obtained for weeks 1, 2, and 4, and RGR was calculated for the intervals between weeks 1–2 and weeks 2–4, based on total dry biomass.A moisture content of 95% was assumed to convert biomass from wet weight (WWt) to dry weight (DWt), in accordance with Penfound and Earle [2].

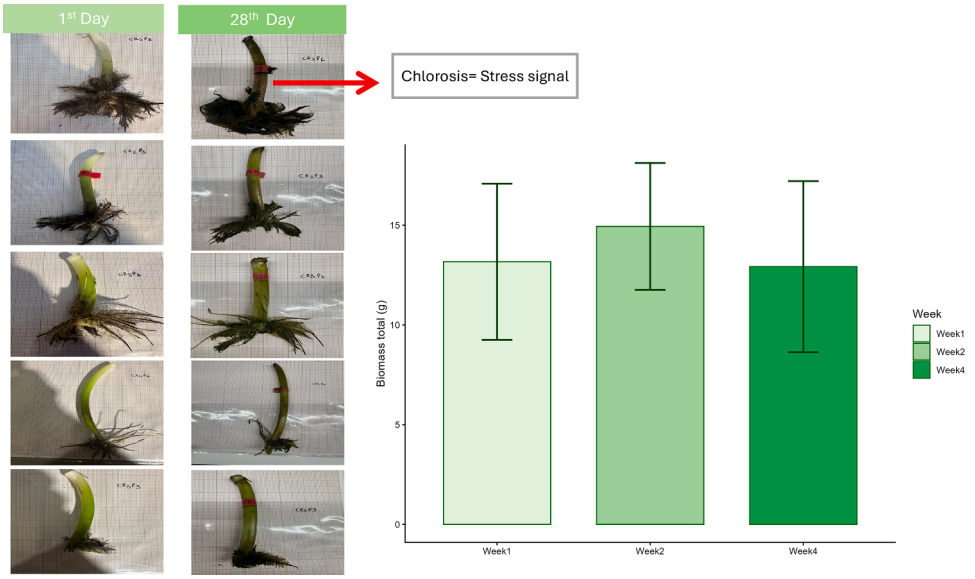


Fig. 3. Evolution of the physical condition of *Pontederia crassipes* stolon as registered by visual signs of chlorosis between the beginning and end of the experiment (left) and by total biomass variation (right) after acclimatisation (week 1), during (week 2) and at the end of the experiment (week 4).

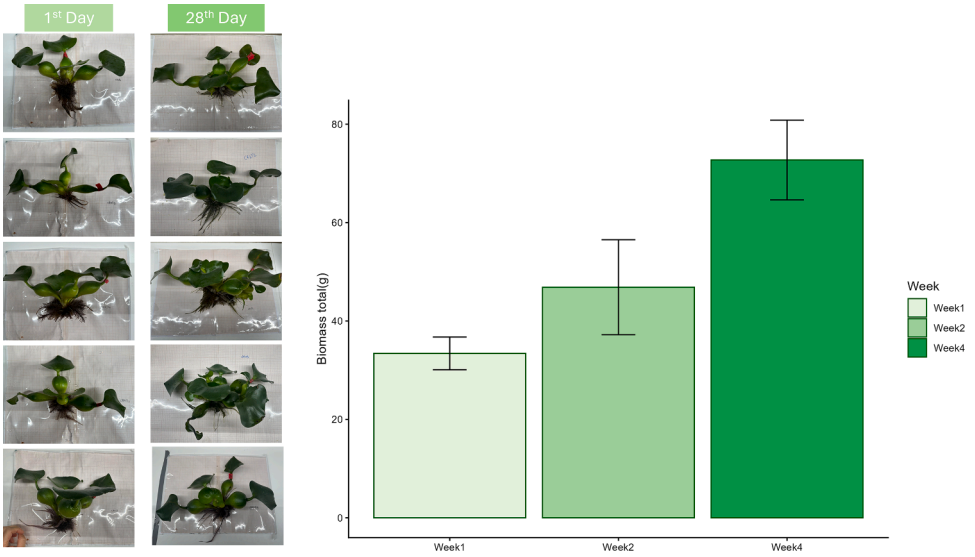


Fig. 4. Evolution of the physical condition of *Pontederia crassipes* whole plant as registered by visual signs of chlorosis between the beginning and end of the experiment (left) and by total biomass variation (right) after acclimatisation (week 1), during (week 2) and at the end of the experiment (week 4).

Table 5
Total Biomass and relative growth rate (RGR) of *Pontederia crassipes* stolon and whole plant in mesocosm experiments (mean ± sd), including *F*-Test results and *p* values.

Plant part	Week	n	Biomass total mean ± sd (g)	*F*-value	*p*-value	RGR mean ± sd (g dw d^{-1})	*F*-value	*p*-value
Stolon								
	Week 1	30	13.167 ± 3.914			0.006± 0.014		
	Week 2	5	14.940 ± 3.180	0.5235	0.5954	0.015 ± 0.023	6.487	0.002956
	Week 4	25	12.921 ± 4.289			−0.003 ± 0.004		
Whole plant								
	Week 1	15	33.415 ± 12.879			0.020 ± 0.036		
	Week 2	5	46.866 ± 21.569	12.436	0.0001	0.016 ± 0.089	0.13509	0.874225
	Week 4	10	72.717 ± 25.606			0.027 ± 0.017		

At the end of week 2, one plant and one stolon were removed from each experimental unit, weighed for fresh biomass, and not returned to the system. Each experimental unit (box) was treated as an independent replicate, with stolons considered sub-replicates and plants considered sub-replicates.

The same procedure described for determining fresh biomass was applied here. The relative growth rate (RGR, day^{-1}) of *P. crassipes* can then be estimated using the following equation [27] for total plant dry mass:

$$RGR = (lnM_n - lnM_n - 1) * t^{-1}$$

M_n= total dry mass in the present week,
M_n-1=total dry mass in the previous week, and
t=time in days

The following scheme (Fig. 2) provides a brief description of water parameters, nutrient analyses and biomass measurements. The scheme demonstrates that the mesocosm was divided into two phases: acclimatisation (days 0–7) and the experimental weeks (days 8–28), totaling 28 days.

Protocol validation

The present study examined the growth of *Pontederia crassipes*, revealing contrasting physiological responses between stolons and whole plants. During the acclimatisation phase, 72.5 % of the stolons (biomass total mean 13.17 ± 3.91 g, and size=13.71 ± 3.95 cm) produced new leaves. However, after 14 days (week 2), stolons exhibited visible signs of stress, such as discoloration and tissue degradation (see Fig. 3). No significant changes in total biomass were observed over the experiment for the stolons, suggesting that stolons did not regenerate new plants during the studied period. Furthermore, green coloration in the water of all experimental tanks indicated cyanobacterial proliferation, which may have negatively affected plant growth through cyanotoxins, since these compounds

impact macrophytes [28].

Growth of the plant and stolon could also be limited by pH. For both experiments, this parameter ranged from 3.5 to 5.1 for the whole plant and from 4.7 to 6.3 for the stolon. The mean reported in the literature ranges from 4 to 8 [29] the decrease of the pH associated with the phytoremediation process, through deprotonation of functional groups in the roots of water hyacinth during the adsorption of metal ions [30].

Conversely, experiments conducted on entire plants demonstrate alterations in net biomass overtime (Table 5). The initial phase of growth occurs during the first week and conitnues until week 4. Evidence of these values was also manifested in the production of new leaves (Fig. 4). The RGR remained relatively constant (Table 5). The values obtained from this study did not demonstrate any significant differences.

Monitoring water parameters in mesocosm experiments is crucial because stolons (Fig. 5) and plants (Fig. 6) can significantly alter

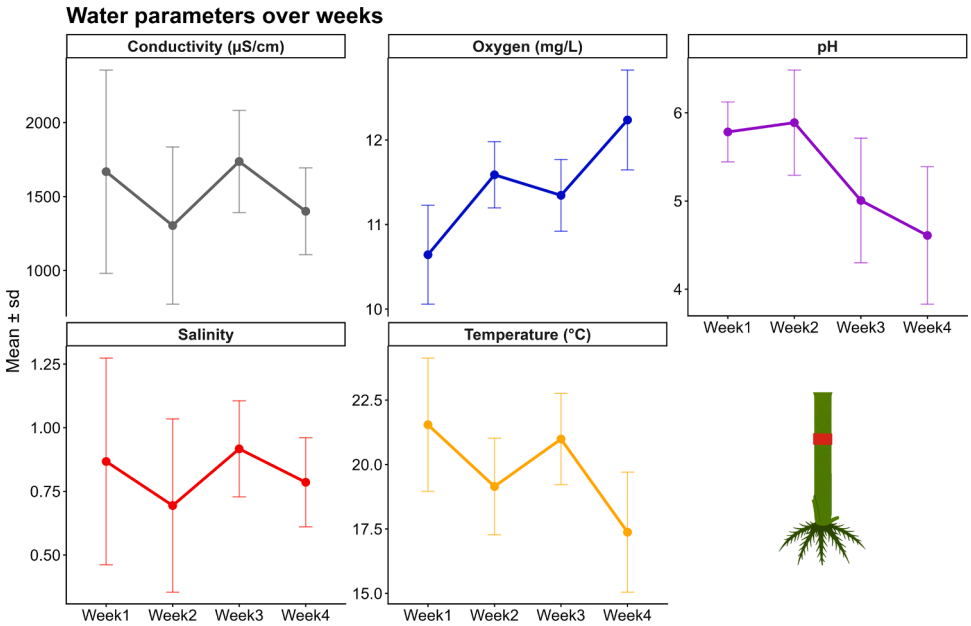


Fig. 5. Variation the of water parameters throughout the *Pontederia crassipes stolon* mesocosm experiment.

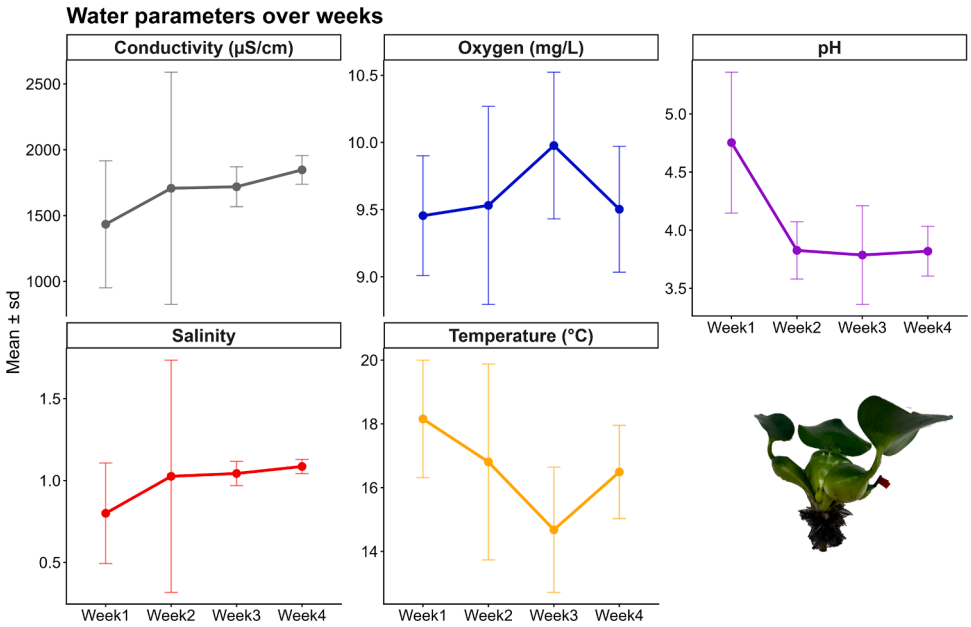


Fig. 6. Variation of water parameters throughout of *Pontederia crassipes* whole plant mesocosm experiment.

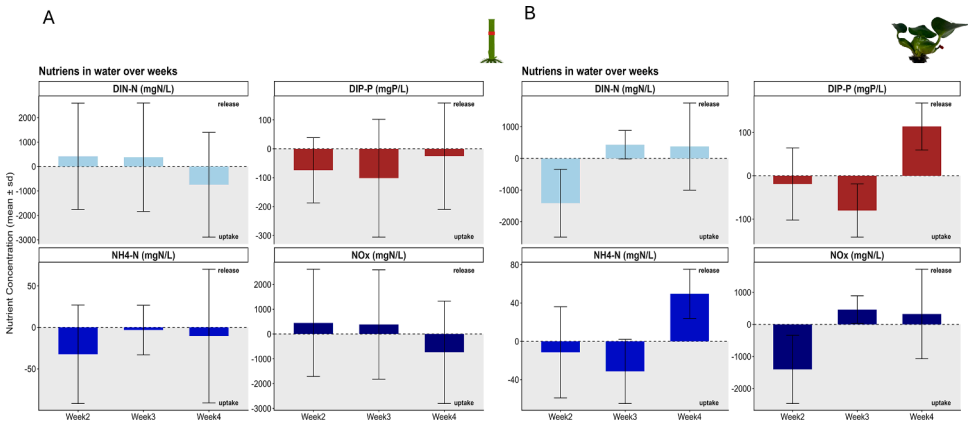


Fig. 7. Results of nutrient changes calculated as the difference between consecutive sampling times (every 7 days from day 7 to day 28), used as an indicator of plant uptake in stolon (A) and (B) in whole plant of *Pontederia crassipes* during the mesocosm experiment.

Table 6
Comparative data from stolons and whole plants in mesocosms experiments.

Repetition	Plant Part	Water volume (L)	Box Size (mm)	Days	Replicates	Acclimatisation time (days)	Initial Weight (g)	Final Weight (g)
1	Stolon	10	270 × 170 × 170	20	12	22	10	*
2	Stolon	10	270 × 170 × 170	22	6	7	13.64	14.61
3	Stolon	20	400 × 300 × 243	32	6	7	13.64	13.96
4	Whole plant	20	400 × 300 × 243	32	3	7	36.64	73.74

* All stolon died.

physical and chemical conditions, including oxygen levels, pH levels, conductivity and salinity. Changes to these parameters can directly influence ecological processes, the interpretation of treatment effects, and the reproducibility of the experiment.

The concentration of nutrients in the water is a key factor in the growth and development of plant structures (Fig. 7). Monitoring these parameters indicates the availability of resources and provides insight into how well the plants can take up and release nutrients. This directly influences mesocosm dynamics and overall water quality.

This protocol does not intend to represent a unique standardized way for *P crassipes* growth experiments, but to showcase what are the main points that should be reported for setting up a mesocosm that ensures comparability of results. Authors should clearly detail all the steps of the experimental set-up, namely the physical and chemical conditions, plus the aim of the study, i.e., if study targets the whole aquatic plant or different plant organs (leaves, stem, root, stolon), including a detailed description of the initial plant/organs characteristics and the metrics used (e.g., biomass, length, number of new leaves). Safety procedures and waste handling practices must also be in place and duly reported to prevent the accidental release of plant material into the environment.

Limitations

The limitations of this experiment consist of the stolon starting to decompose after 10 days. This may have occurred due to the cutting off of the stolon node, which could have impacted vegetative growth and impeded its development. It is important to note that the root is responsible for absorbing nutrients, but it was not large enough for this structure (Table 6).

Related research article

None.

CRediT authorship contribution statement

Letícia da Silva Brito: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Sidinei Magela Thomaz:** Validation, Writing – review & editing. **Heliana Teixeira:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing. **Ana I. Lillebø:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

LB was funded by FCT under the PhD Grant 2021.07101.BD. HT thanks FCT Foundation for Science and Technology, I.P. support through reference https://doi.org/10.54499/2022.08095. CEECIND/CP1720/CT0017. The research was conducted under the RESTORE4Cs project, funded by the European Commission (Grant agreement ID: 101056782; 10.3030/101056782) in collaboration with BESIDE project funded by the ERA Chair BESIDE project (DOI: 10.3030/951389) financed by the European Union's Horizon 2020 (Grant Agreement ID: 951389). We thank Foundation for Science and Technology (FCT), I.P. for funding CESAM FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020).

We are grateful to Sara Ribeiro, the technician, for preparing the Hoagland stock solution and analyzing the nutrient water.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2026.103800.

Data availability

Data will be made available on request.

References

[1] T. Wang, J. Hu, C. Liu, D. Yu, Soil type can determine invasion success of Eichhornia crassipes, Hydrobiologia 788 (2016) 281–291, https://doi.org/10.1007/s10750-016-3003-4.
[2] Wm.T. Penfound, T.T. Earle, The biology of the water hyacinth, Ecol. Monogr. 18 (1948) 448–473, https://doi.org/10.2307/1948585.
[3] Lowe S., Browne M., Boudjelas S., De Poorter M. (2000) 100 of the World's worst Invasive Alien Species A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. First published as special lift-out in Aliens 12, December 2000. Updated and reprinted version: November 2004.
[4] IPBES, 2023. Thematic Assessment Report On Invasive Alien Species and their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Roy, H. E., Pauchard, A., Stoett, P., and Renard Truong, T. (eds.). IPBES Secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.7430682.
[5] A. Monroy-Licht, D. Méndez-Cuadro, J. Olivero-Verbel, Elemental mercury accumulation in Eichhornia crassipes (Mart.) Solms-Laubach, Environ. Sci. Pollut. Res. 30 (2023) 9898–9913, https://doi.org/10.1007/s11356-022-22521-y.
[6] A.B.D. Nandiyanto, R. Ragadhita, S.N. Hofifah, et al., Progress in the utilization of water hyacinth as effective biomass material, Environ. Dev. Sustain. 26 (2024) 24521–24568, https://doi.org/10.1007/s10668-023-03655-6.
[7] M.D. Wilkinson, M. Dumontier, I.J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L. Bonino da Silva Santos, P.E. Bourne, J. Bouwman, A.J. Brookes, T. Clark, M. Crosas, I. Dillo, O. Dumon, S. Edmunds, C.T. Evelo, R. Finkers, A. Gonzalez-Beltran, A.J.G. Gray, P. Groth, C. Goble, J.S. Grethe, B. Mons, The FAIR Guiding Principles for scientific data management and stewardship, Sci. Data 3 (2016) 160018, https://doi.org/10.1038/sdata.2016.18.
[8] K.R. Reddy, J.C. Tucker, Productivity and nutrient uptake of water hyacinth, *Eichhornia crassipes*I. Effect of nitrogen source, Econ. Bot. 37 (1983) 237–247, https://doi.org/10.1007/BF02858790.
[9] K.R. Reddy, Water hyacinth (Eichhornia crassipes) biomass production in Florida*, Biomass 6 (1984) 167–181, https://doi.org/10.1016/0144-4565(84)90019-2.
[10] K.R. Reddy, W.F. DeBusk, Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: I. Water hyacinth, water lettuce, and pennywort, Econ. Bot. 38 (2) (1983) 229–239, https://doi.org/10.1007/BF02858838.
[11] K.R. Reddy, M. Agami, J.C Tuccker, Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (Eichhornia crassipes) plants, Aquat. Bot. 36 (1989) 33–43, https://doi.org/10.1016/0304-3770(89)90089-2Get, rights and content.
[12] K.R. Reddy, M. Agami, J.C Tuccker, Influence of phosphorus on growth and nutrient storage by water hyacinth (Eichhornia crassipes) plants, Aquat. Bot. 37 (1990) 355–365, https://doi.org/10.1016/0304-3770(90)90021-C.
[13] Y.B. Ho, W. Wong, Growth and macronutrient removal of water hyacinth in a small secondary sewage treatment plant, Resour. Conserv. Recycl. 11 (1994) 161–178, https://doi.org/10.1016/0921-3449(94)90087-6.
[14] Y.;. Xie, M.;. Wen, D.;. Yu, Y. Li, Growth and resource allocation of water hyacinth as affected by gradually increasing nutrient concentrations, Aquat. Bot. 79 (2004) 257–266, https://doi.org/10.1016/j.aquabot.2004.04.002.
[15] M.W. Jayaweera, J.:.C. Kasturiarachchi, R.K.A. Kularante, L.J Wijeyeknoon, Contribution of water hyacinth (Eichhornia crassipes (Mart.) solms) grown under different conditions to Fe-removal mechanisms in constructed wetlands, J. Enviorn. Manag. 87 (2008) 450–460, https://doi.org/10.1016/j.jenvman.2007.01.013.
[16] W. You, D. Yu, D. Xie, L. Yu, W. Xiong, C. Han, Responses of the invasive aquatic plant *water hyacinth* to altered nutrient levels under experimental warming in China, Aquat. Bot. 119 (2014) 51–56, https://doi.org/10.1016/j.aquabot.2014.06.009.
[17] M.N. Kumwimba, B. Zhu, D.K. Muyembe, M. Dzakpasu, Growth characteristics and nutrient removal capability of eco-ditch plants in mesocosm sediment receiving primary domestic wastewater, Environ. Sci. Pollut. Resour. 24 (2017) 23926–23938, https://doi.org/10.1007/s11356-017-9992-3.
[18] S.C.H. Barrett, Sexual reproduction in Eichhornia crassipes (water hyacinth). I. Fertility of clones from diverse geographical origins, J. Appl. Ecol. 17 (1) (1980) 101–112, https://doi.org/10.2307/2402968.
[19] Jane H. Bock, Productivity of the water hyacinth eichhornia crassipes (Mart.) solms, Ecology 50 (1969) 460–464, https://doi.org/10.2307/1933898.
[20] S.M. Thomaz, Asexual reproduction of aquatic macrophytes via stem fragments: a review on determinants of plant fragmentation and propagule dispersal, Hydrobiologia (2025), https://doi.org/10.1007/s10750-025-05942-x.
[21] A.M. Villamagna, B.R. Murphy, Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review, Freshw. Biol. 55 (2010) 282–298, https://doi.org/10.1111/j.1365-2427.2009.02294.x.

[22] D. Sasaqi, Pranoto, P. Setyono, Estimation of water losses through evapotranspiration of water hyacinth (Eichhornia crassipes), Caraka Tani 34 (1) (2019) 86–100, https://doi.org/10.20961/carakatani.v34i1.28214.
[23] H. Wu, J. Ding, Abiotic and biotic determinants of plant diversity in aquatic communities invaded by water hyacinth (Eichhornia crassipes (Mart.) solms), Front. Plant Sci. 11 (2020) 1306, https://doi.org/10.3389/fpls.2020.01306.
[24] I. Harun, H. Pushiri, A.J. Amirul-Aiman, Z. Zulkeflee, Invasive water hyacinth: ecology, impacts and prospects for the rural economy, Plants 10 (8) (2021) 1613, https://doi.org/10.3390/plants10081613.
[25] M.B. Djihouessi, Olokotum, L., .F Chabi, F. Mouftaou, M.P Aina, Paradigm shifts for sustainable management of water hyacinth in tropical ecosystems: a review and overview of current challenges, Environ. Chall. 11 (2023), https://doi.org/10.1016/j.envc.2023.100705.
[26] D. Hoagland. The water culture method for growing plants without soil. California Agriculture Experimental Station Circular 347, College of Agriculture, University of California, Davis, California, USA, 1950.
[27] W.A Hoffmann, H. Poorter, Avoiding bias in calculations of relative growth rate, Annals of Botanica 90 (1) (2002) 37–42, https://doi.org/10.1093/aob/mcf140. PMID: 12125771; PMCID: PMC4233846.
[28] Y. Zhang, S. Vo Duy, G. Munoz, S. Sauvé, Phytotoxic effects of microcystins, anatoxin-and cylindrospermopsin to aquatic plants: a meta-analysis, Sci. Total Environ. 810 (2022) 152104, https://doi.org/10.1016/j.scitotenv.2021.152104.
[29] A.S. El-Gendy, N. Biswas, J.K. Bewtra, Growth of water hyacinth in municipal landfill leachate with different pH, Environ. Technol. 25 (7) (2004) 833–840, https://doi.org/10.1080/09593330.2004.9619375.
[30] M.E. Soltan, M.N. Rashed, Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations, Adv. Environ. Res. 7 (2) (2003) 321–334, https://doi.org/10.1016/S1093-0191(02)00002-3.