

**Article information****Article title**

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

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**Keywords**

*Eichhornia crassipes*; mesocosm; experimental reproducibility; growth rate: invasive alien species

**Related research article**

None.

**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, pH and water transparency influence its performance. 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 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*.

**Graphical abstract****Specifications table**

Subject area	Environmental Science
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<b>More specific subject area</b>	Aquatic Ecology / Applied Botany
<b>Name of your protocol</b>	Growth of <i>Pontederia crassipes</i> under mesocosm controlled experiment.
<b>Reagents/tools</b>	<p><b>Reagents:</b>            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 (<math>\geq 85\%</math> MoO<sub>3</sub>)            Titriplex® III (ethylenedinitrilotetraacetic acid disodium salt dihydrate) EDTA            Potassium hydroxide            Ferrous Sulphate</p> <p><b>Tools:</b>            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 o enhances water mixing</p>
<b>Experimental design</b>	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 sediment source to minimize variation due to substrate.
<b>Trial registration</b>	Not applicable.
<b>Ethics</b>	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).
<b>Value of the Protocol</b>	Standardizes the mesocosm experiment for assessing <i>Pontederia crassipes</i> 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.

## 1 Background

2 *Pontederia crassipes* (Mart.), formerly *Eichhornia crassipes* (Mart.) Solms and commonly known as water  
 3 hyacinth, is a free-floating aquatic plant widely recognized for its rapid growth and high invasive potential (Wang et  
 4 al., 2016). Native to South America, it was first recorded outside its natural range in New Orleans in 1884 (Penfound  
 5 & Earle, 1948). Since then, it has been reported as an invasive species on five continents, excluding Antarctica (EPPO,  
 6 2008). It is considered among the 100 world's worst invasive alien species by IUCN (Lowe et al., 2000) and in 2023, it

1 was identified as one of the major threats to global biodiversity (IPBES, 2023). Conversely, it has also been used for  
2 phytoremediation purposes, particularly to treat industrial effluents under ex-situ conditions (Monroy-Licht et al.,  
3 2024). Moreover, its biomass has been utilized for a variety of purposes (Nandiyanto et al., 2024). Although both sexual  
4 and asexual reproduction have been described in the literature, to the best of our knowledge, the enabling conditions  
5 for reproduction of this species under ex-situ settings are not sufficiently documented (Table 1). Addressing this  
6 knowledge gap is essential to ensure experimental reproducibility and thereby advance scientific research following  
7 the FAIR (Findable, Accessible, Interoperable, and Reusable) principles of Open Science (Wilkinson et al., 2016). To this  
8 end, the first step was to analyze the available literature on mesocosm experiments targeting biomass production,  
9 including studies on constructed wetlands and phytoremediation. Table 1 summarizes the findings of ten mesocosm  
10 studies, which ranged in duration from 22 to 150 days. The table shows the use of different container volumes (100–  
11 1000 L) and temperature ranges (5–35 °C). In terms of hydrological conditions, the growth medium included artificial  
12 nutrient water, well water, pond water, lake water and wastewater, with a retention time mostly ranging from one to  
13 six weeks. Nutrient concentrations ranged from 0.05 to 28 mg/L for nitrogen and from 0.05 to 8.86 mg/L for  
14 phosphorus. In addition to variability in experimental setup, initial biomass varied from 15 to 680 g dry weight per m<sup>2</sup>,  
15 while in one experiment, the authors used plant length (cm) rather than biomass. While most authors report on plant  
16 biomass, the description of the included plant organs is not sufficiently clear, with some studies referring to shoots  
17 and others only to plants, without providing detailed measurement criteria. Overall, the literature reveals a  
18 heterogeneity of protocols, as well as short-term and low representativeness and incomplete reporting of conditions.  
19 The lack of standardized reporting with sufficient detail to enable the validation and reproducibility of experimental  
20 conditions must be addressed to foster progress in this field of research. The main objective of this protocol is to share  
21 a set of *ex situ* growth experiments with *Pontederia crassipes* and clearly demonstrate the most and least effective  
22 methods to support progress in this field.  
23

1 **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 (days)	Hydrological conditions				Nutrient source	Nutritional conditions			Growth rate			Reference
			Volume (L)	Temperature (°C)	Retention time (weeks)	Water circulation		Nitrogen (mg L <sup>-1</sup> )	Phosphorus (mg L <sup>-1</sup> )	micronutrients (mg L <sup>-1</sup> )	T0 (g DW m <sup>-2</sup> )	Tf (g DW m <sup>-2</sup> )	Rate (g DW m <sup>-2</sup> day <sup>-1</sup> )	
<b>Whole plant (Petiolo+lamina+stem+root)</b>														
Net productivity (dry weight)	1	84	300	26°C-28°C	1	daily by submersible pump	constructed wetlands	20	5	NA	445	2303	0.0193	Reddy & Tucker 1982
	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 1984
	3	80	1000	NA	1	12h 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 & Debusk, 1984
	4	83	1000	15°C-33°C	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., 1989
	5	96	1000	5°C-35°C	1	daily by submersible pump	well water	20	0.06	K: 25	225	830	0.0147	Reddy et al., 1990
	6	22 *in two seasons	500-1000	17.8°C-24.8°C	48 h and 60 h	NA	wastewater from residencials home that has passed through a primary sedimentation	20	7.5	K: 16.5	48	225	0.0112	Ho & Wong , 1994
	7	56	200	15°C -25°C	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., 2004
	8	105	300	NA	6	NA	constructed wetlands	28	7.7	NA	plant with 20 cm NA	NA	NA	Jayaweera et al., 2008
	9	42	100	23.5°C-28.8°C	1	NA	lake water	0.6	0.05	NA	15	235	0.042	You et al., 2014
	10	150	104	NA	1		pond water	0.58	8.86	NA	260	405.83	0.0112	Kumwimba et al., 2017

1 What is well known: Sexual reproduction in this species occurs through seeds (Barrett, 1980), while asexual  
2 reproduction occurs via vegetative propagation (Bock, 1969; Thomaz 2025). A small portion of plant's horizontal  
3 stem, known as a stolon, may break due to physical or biological agents and act as a propagule capable of producing  
4 new individuals. Under favorable conditions, such as optimal temperature and nutrient availability, the plant can  
5 cover large areas of the water surface within just a few weeks (Wang et al., 2016). This rapid proliferation has  
6 negative ecological impacts, including oxygen depletion and consequent fish mortality (Villamagna & Murphy, 2010),  
7 increased water evaporation (Sasaqi et al., 2019), and reduced biodiversity due to sunlight blockage and decreased  
8 net productivity (Wu & Ding, 2020). In economic terms, it results in high costs associated with control and  
9 management strategies (Harun et al., 2021). Socially, it affects local communities that depend on aquatic ecosystems  
10 (Harun et al., 2021). The remaining knowledge gap lies in determining the optimal conditions for propagation to both  
11 manage the spread of *P. crassipes* and explore potential sustainable applications, as well as in developing effective  
12 control strategies (Djihouessi et al., 2023). For instance, none of the above-mentioned studies addressed the role of  
13 stolons or reported the challenges of maintaining this species under controlled mesocosm conditions.

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

## 18 Description of protocol

### 19 1 Site collection

#### 20 Collecting the material

21 *Pontederia crassipes* was collected from the Pateira de Fermentelos freshwater lagoon in Aveiro, Portugal  
22 (40°34'48"N, 8°31'12"W) in September and October 2024. Two independent experiments were conducted using  
23 different *Pontederia crassipes* propagules: stolon and whole plant. Both experiments were initiated separately in late  
24 autumn 2024. Separate collections were made for each propagation type. Knowing the plant's life cycle in the  
25 collection area is important for planning the collection according to its availability in the ecosystem.

26 Experimental units were standardized by selecting plants with comparable leaf numbers and sizes. They were  
27 then conditioned in plastic bags and transported to the laboratory. The material should be collected when the species  
28 reaches its peak biomass.

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

### 31 2 Material and Equipment

#### 32 Sample

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

#### 36 Equipment

- 37 • Analytical Balance.
- 38 • Conductivity, Temperature, and Salinity portable meters: ProfiLine Cond 3320 (WTW, Weilheim, Germany)
- 39 • pH portable meter: ProfiLine pH 3310 (WTW, Germany)
- 40 • Dissolved Oxygen portable meter: ProfiLine Oxi 3310 (WTW, Germany)
- 41 • Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, Netherlands) for nutrients analysis
- 42 • Air Pump o enhances water mixing.

#### 44 Material

- 45 • 20 Polypropylene 30L containers (size : 400 x 300 x 243mm).

- 1 • Graph paper to take a picture and measure plants structures.
- 2 • Syringes and Glass filter (GF 0.45  $\mu\text{m}$ ) for solution nutrients analysis
- 3 • Volumetric glassware for solution preparation (beakers and volumetric flasks)
- 4

## 5 Reagents

**Table 2:** Reagents for Hoagland nutrient solution

Reagente	Chemical Formula	Chemical Abstracts Service (CAS)
Ammonium dihydrogen orthophosphate	$(\text{NH}_4) \text{H}_2\text{PO}_4$	7722-76-1
Potassium nitrate	$\text{KNO}_3$	7757-79-1
Calcium nitrate tetrahydrate	$\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$	13477-34-4
Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10034-99-8
Boric acid	$\text{H}_3\text{BO}_3$	10043-35-3
Copper (II) sulfate pentahydrate	$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	231-847-6
Zinc sulfate heptahydrate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	7446-20-0
Manganese (II) chloride tetrahydrate	$\text{MnCl}_2$	13446-34-9
Molybdic acid	$\text{H}_2\text{MoO}_4$	7782-91-4
Ethylenedinitrilotetraacetic acid disodium salt dihydrate (EDTA)	$\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8 \cdot 2\text{Na} \cdot 2\text{H}_2\text{O}$	6381-92-6
Potassium hydroxide	$\text{KOH}$	1310-58-3
Ferrous Sulphate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	7782-63-0

## 6 **3. Experimental procedures**

### 7 **3.1 Cleaning the plants**

8 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 (Figure 1). As stolons may become detached from the parent plant due to mechanical forces resulting from disturbances in the aquatic environment (Thomaz, 2025), 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.

9 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.

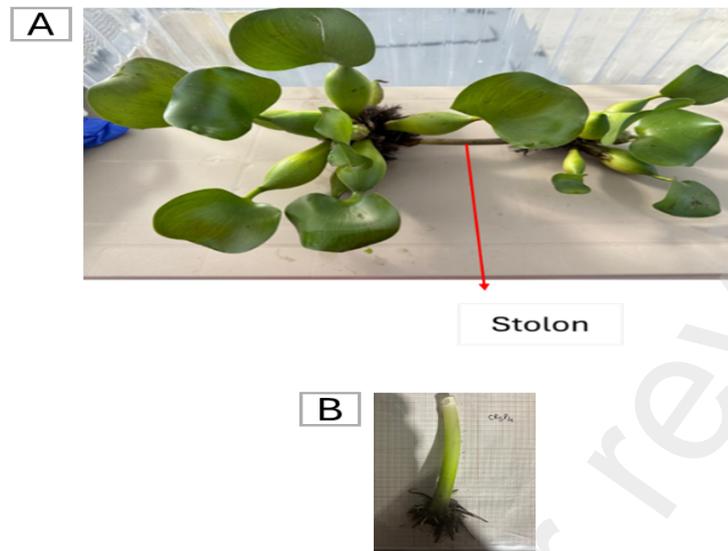


Figure 1: *Pontederia crassipes* in detail of the vegetative structure of stolon joining the two plants (A) and only stolon (B) used in this experiment.

The plant units used in the experiment were selected to ensure morphological and physiological uniformity. They had an average size of  $450.86 \pm 172.22$  cm<sup>2</sup> (length  $\times$  height), four leaves and a total biomass per plant of  $36.34 \pm 13.23$  g, and their stolon biomass ( $13.64 \pm 7.52$  g) and length ( $14.33 \pm 5.05$  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.

### 3.2 Preparing the solution

The growth medium was based on Hoagland's nutrient solution (Hoagland 1950), modified for use in aquatic mesocosms. The preparation involved the following:

**Table 3:** Adapted Hoagland Solution nutrients components

Hoaglands Stock Solutions (SS)	Stock Solution Concentration	Volume SS in Final Hoagland Solution
<b>Macro nutrients SS</b>		
1	1.00M NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	1ml/L
2	1.00M KNO <sub>3</sub>	6ml/L
3	1.00M Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	4ml/L
4	1.00M MgSO <sub>4</sub> · 7H <sub>2</sub> O	2ml/L
<b>Micro nutrient SS</b>		
5	2.86g H <sub>3</sub> BO <sub>3</sub> /1L 0.08 g CuSO <sub>4</sub> ·5 H <sub>2</sub> O /1L 0.22g ZnSO <sub>4</sub> ·7H <sub>2</sub> O /1L 1.81g MnCl <sub>2</sub> /1L 0.02g H <sub>2</sub> MoO <sub>4</sub> /1L	1ml/L
<b>Iron stock</b>		
6	26.1g EDTA /1L	0.25ml/L

1 First, the six different stock solutions were prepared using distilled water and stored in a refrigerator at 4 °C. These  
2 stock solutions formed the basis for preparing the Hoagland nutrient solution. Subsequently, 200 litres of Hoagland  
3 solution were prepared by diluting the appropriate volumes of each stock solution with purified tap water from a  
4 V2Pure 360 RO System (TMC, Hertfordshire, UK). To ensure complete homogenization of the solution components, it  
5 is important to maintain continuous water circulation using a pump.

### 7 3.3 Acclimatation time

8 To avoid death resulting, stolon and plants were placed in an opaque polypropylene box (internal volume:400 x  
9 300 x 243mm) with 15 L of Hoagland's solution before starting the experiments for one week. Continuous aeration  
10 was utilized to enhance oxygen and water flow and reduce the boundary layer around the plants.

### 12 3.4 Daily monitoring

13 To avoid anomalous results, water quality parameters were measured daily throughout the experiment at the  
14 experiment, between 10:00 and 12:00h.

### 16 3.5 Nutrient analyses

17 Water samples were filtered using a syringe and glass fiber (GF, 0.45 µm) filters before and after solution renewal.  
18 The Hoagland solution was partially renewed while the plants remained under acclimatisation conditions. Each week  
19 (Acclimatisation, Week 1, Week 2, and Week 4), half of the solution (7.5 L out of 15 L) was removed and replaced with  
20 an equal volume of fresh Hoagland solution. This procedure is necessary to restore the nutrients that were taken up  
21 by macrophytes. Distilled water was also added as needed to compensate for natural evaporation. Concentrations of  
22 dissolved nutrients were determined using a Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda,  
23 Netherlands), following standard automated colorimetric methods:

- 24 • Ammonium (NH<sub>4</sub><sup>+</sup>-N): Modified Berthelot reaction
- 25 • Orthophosphate (PO<sub>4</sub><sup>3-</sup>-P): Total UV-digestible phosphate
- 26 • Nitrogen oxides (NO<sub>x</sub>-N): Total UV-digestible nitrate + nitrite

27 Calibration curves were established for each nutrient as follows: 0–25 µmol/L for ammonium; 0–10 µmol/L for  
28 orthophosphate; and 0–50 µmol/L for nitrogen oxides. To ensure concentrations fell within the calibration ranges,  
29 samples were diluted by factors of 167 for ammonium and orthophosphate and 501 for nitrogen oxides.  
30 Concentrations were expressed as limits of quantification (LOQ): 252 µmol N/L for ammonium, 117 µmol P/L for  
31 orthophosphate and 651 µmol N/L for nitrogen oxides. A certified reference material (Seawater QC3179) was analyzed  
32 to ensure analytical accuracy, with acceptable recovery ranges.

33 Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>x</sub>-N. The calculation for each  
34 nutrient in the water was done using the formula:

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

### 38 3.6 Biomass measurements

#### 39 **Total Biomass**

40 Fresh biomass was assessed at three time points during the experiment. Initially (day 1, acclimatization  
41 period), all plants and stolons were weighed. After one week (week 1), one stolon and one plant per experimental unit  
42 were removed, weighed for fresh biomass determination, and dried; these samples were not returned to the system.

At the end of the experiment (week 3), all remaining plants and stolons were harvested and weighed to determine their fresh biomass."

### Relative growth rate

The relative growth rate (RGR) is a widely used parameter for describing population dynamics and seasonal responses in ecosystems. It is particularly important because it normalizes plant growth according to initial biomass, enabling reliable comparisons; the initial biomass has a strong effect on plant growth performance. Growth measurements were conducted at different times: during the acclimatization week, the first week and the third week. After one week, one stolon and one plant for each mesocosm were removed from each experimental unit, dried and weight. The same procedure described for fresh biomass determination was applied here. The relative growth rate (RGR,  $\text{day}^{-1}$ ) of *P. crassipes* can then be estimated is the following equation (Hoffmann et al., 2002) for total plant dry mass:

$$\text{RGR} = (\ln M_n - \ln M_{n-1}) * t^{-1}$$

$M_n$  = total dry mass in the present week,

$M_{n-1}$  = total dry mass in the previous week, and

$t$  = time in days

A brief description of water parameters, nutrient analyses and biomass measurements is presented in the following scheme (Figure 2).

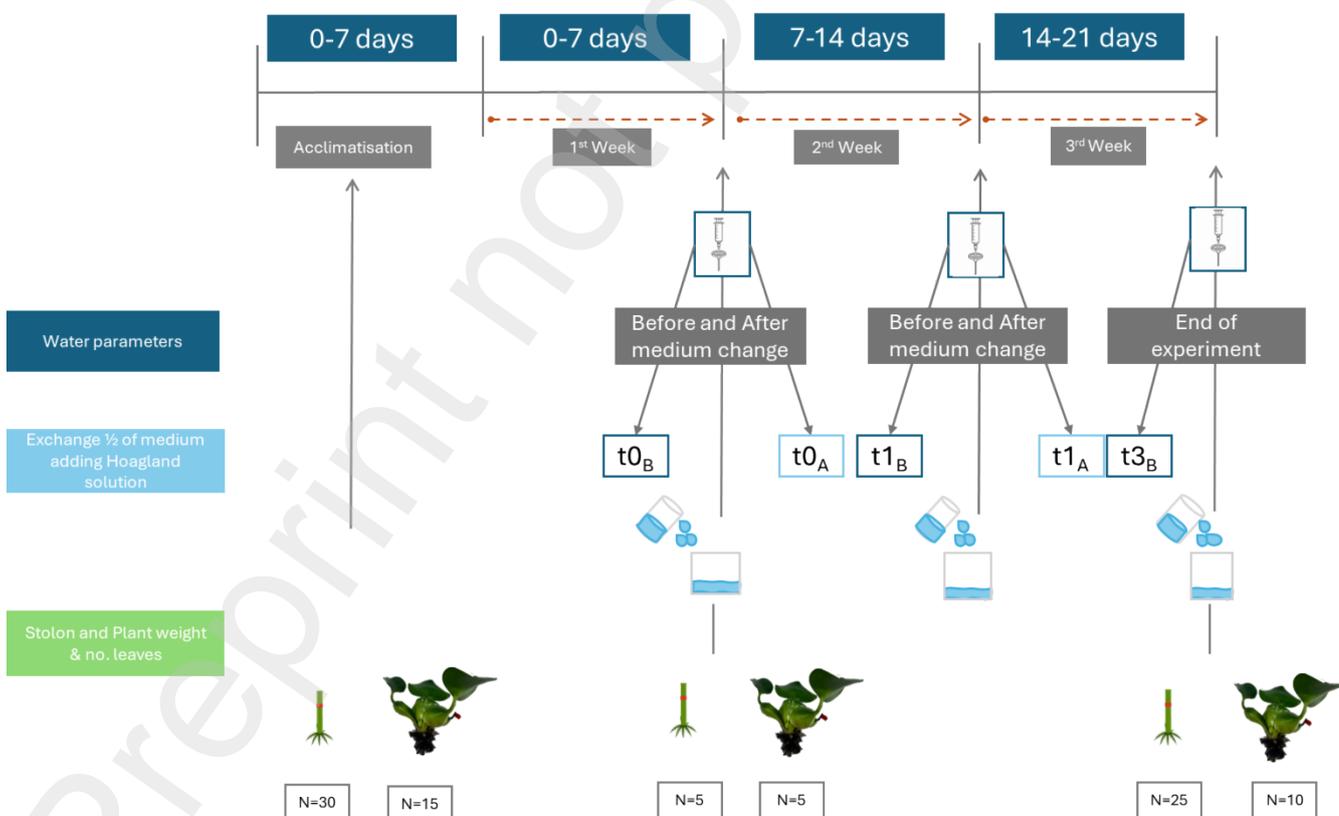
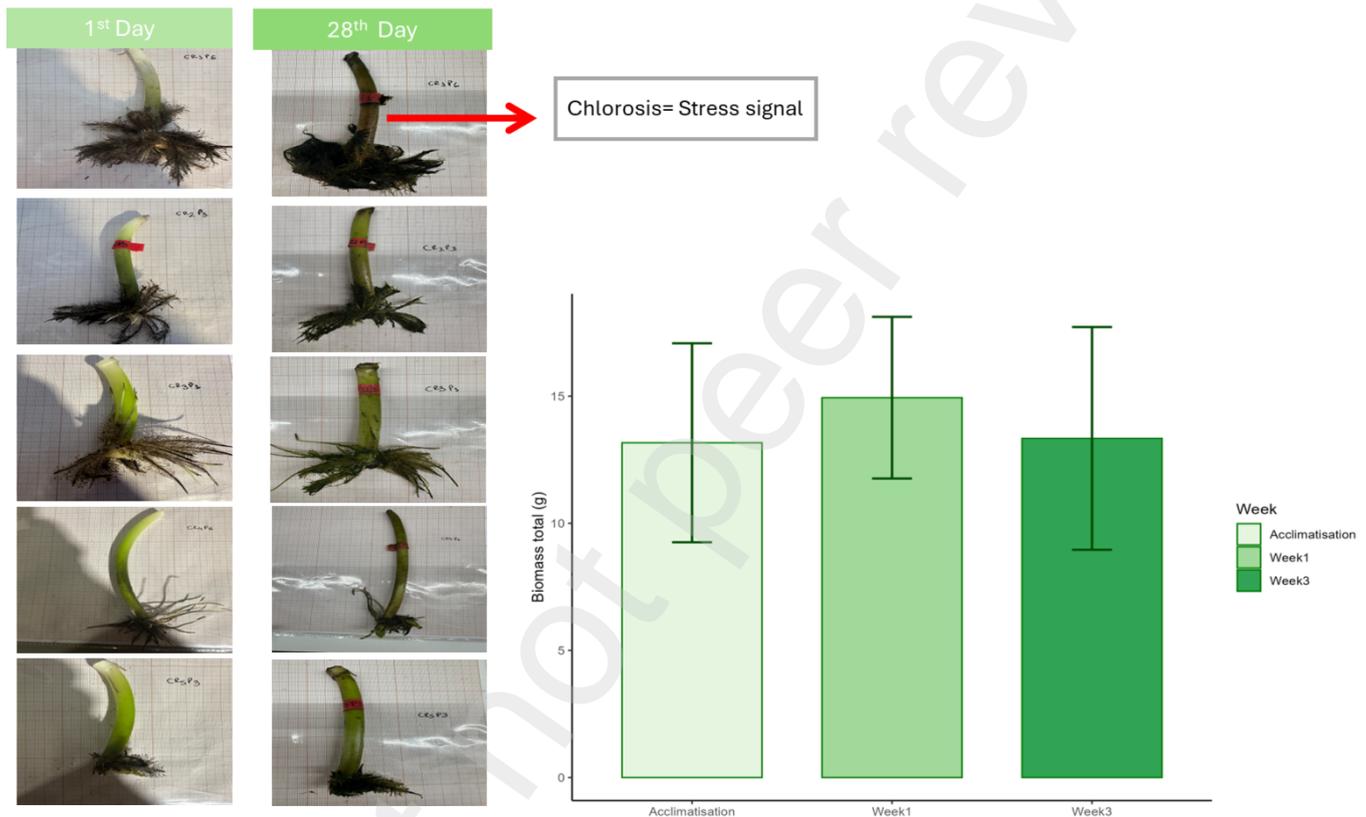


Figure 2: Schematic representation of the experimental protocol, summarizing water parameter monitoring, nutrient analyses, and biomass measurements.

## 1 Protocol validation

2 The present study examined the growth of *Pontederia crassipes*, revealing contrasting physiological responses  
 3 between stolons and whole plants. During the acclimatisation phase, 72.5% of the stolons (biomass total mean  
 4  $13.17 \pm 3.91$  g, and size =  $13.71 \pm 3.95$  cm) produced new leaves. However, over a 14-day period, the sample exhibited  
 5 visible signs of stress, such as discoloration and tissue degradation (see Figure 3). No significant changes in total  
 6 biomass were observed over the experiment, suggesting that stolons did not regenerate new plants during the period  
 7 studied. Furthermore, green coloration in the water of all experimental tanks indicated cyanobacterial proliferation,  
 8 which may have negatively affected plant growth through cyanotoxins, since these compounds impact macrophytes  
 9 (Zhang et al., 2022)



24 Figure 3: Presentation of *Pontederia crassipes* stolon on the first and last day of experiment and biomass total (g) weeks under  
 25 mesocosm experiment.

26 Conversely, experiments conducted on entire plants demonstrate alterations in net biomass overtime. The  
 27 initial phase of growth occurs during the first week, followed by a decline in the third week. Evidence of these values  
 28 was manifest in the production of new leaves. The RGR has been found to remain relatively constant (Figure 4). The  
 29 values obtained from this study did not demonstrate any significant differences.

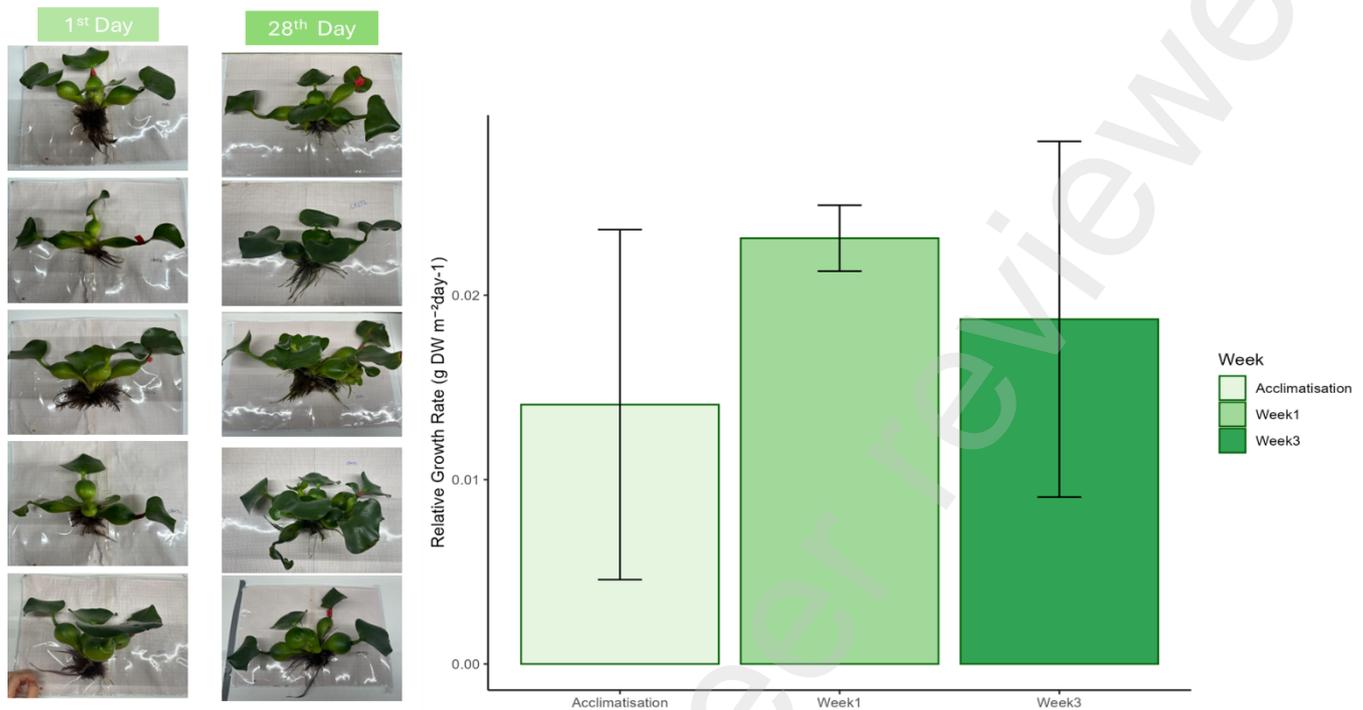


Figure 4: Presentation of *Pontederia crassipes* whole plant before and after exposure under mesocosm experiment and Relative Growth rate (g dw d<sup>-1</sup>) during mesocosm.

**Table 4.** Biomass Total and relative growth rate (RGR) of *Pontederia crassipes* and stolon and whole plant in mesocosm experiments (mean  $\pm$  SD), including *F* and *p* values

Plant part	Week	n	Biomass total			RGR		
			mean $\pm$ sd (g)	<i>F</i> -value	<i>p</i> -value	mean $\pm$ sd (g dw d <sup>-1</sup> )	<i>F</i> -value	<i>p</i> -value
<u>Stolon</u>								
	Acclimatisation	30	13.167 $\pm$ 3.914			0.006 $\pm$ 0.014		
	1 <sup>st</sup> Week	5	14.940 $\pm$ 3.180	0.5235	0.5954	0.015 $\pm$ 0.023	6.487	0.002956
	3 <sup>rd</sup> Week	25	12.921 $\pm$ 4.289			-0.003 $\pm$ 0.004		
<u>Whole plant</u>								
	Acclimatisation	15	33.415 $\pm$ 12.879			0.020 $\pm$ 0.036		
	1 <sup>st</sup> Week	5	46.866 $\pm$ 21.569	12.436	0.0001	0.016 $\pm$ 0.089	0.13509	0.874225
	3 <sup>rd</sup> Week	10	72.717 $\pm$ 25.606			0.027 $\pm$ 0.017		

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

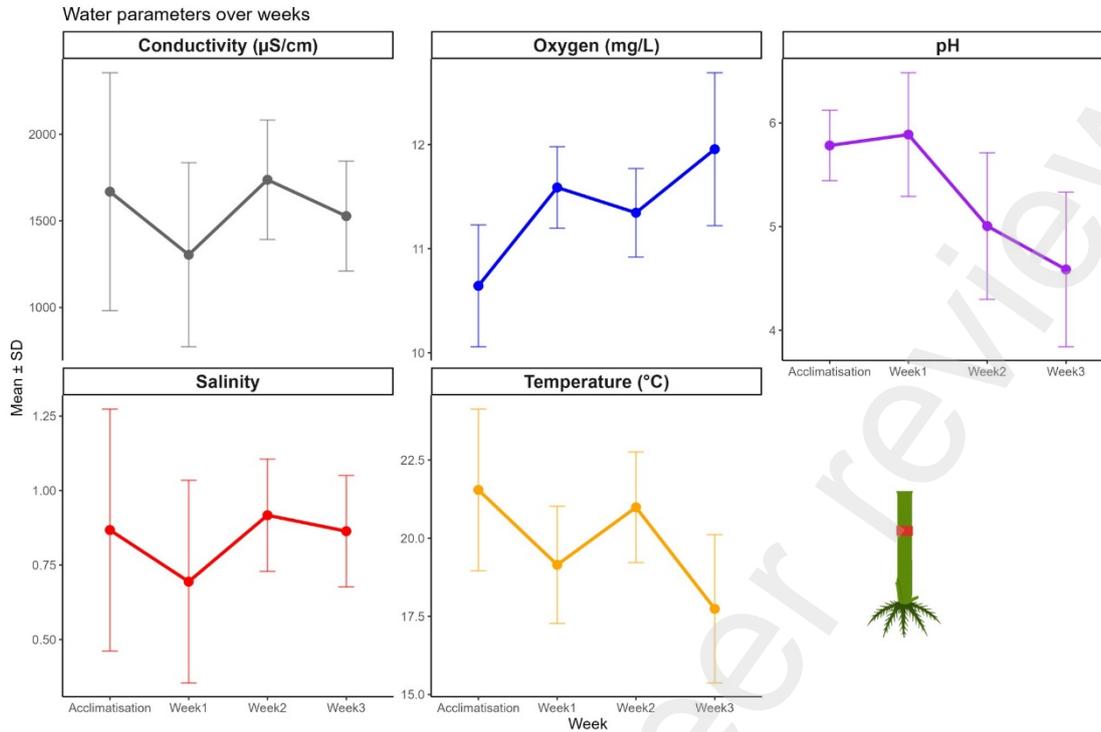


Figure 5: Parameters of water in stolon of *Pontederia crassipes* over weeks during mesocosm experiment.

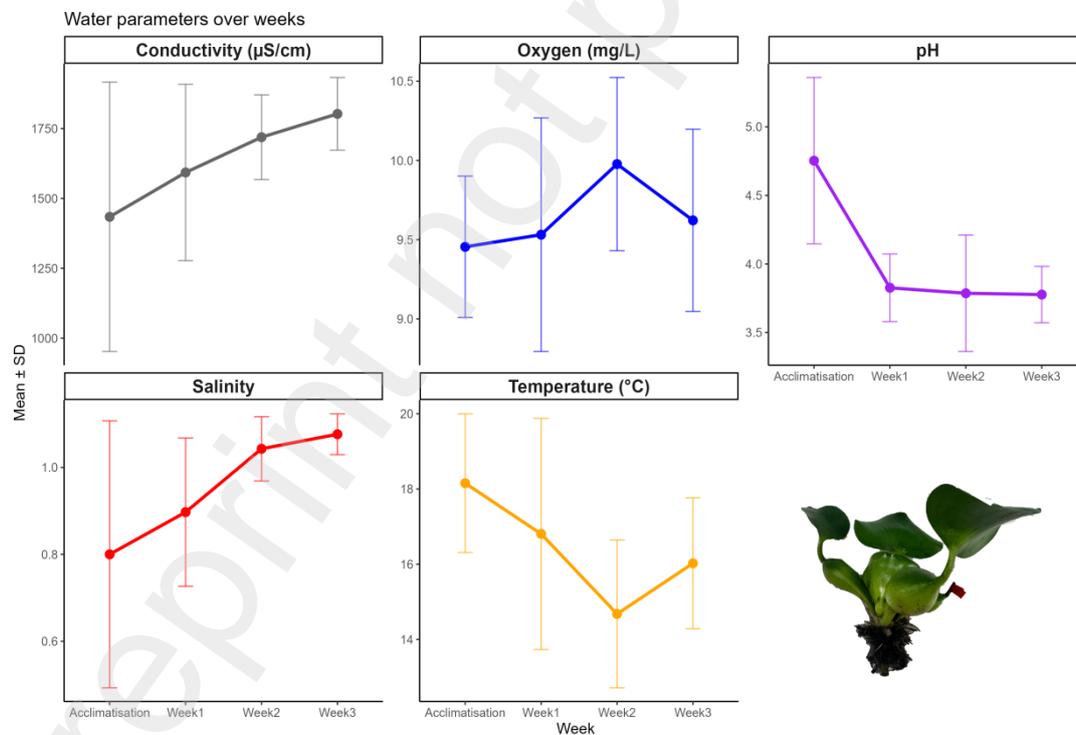


Figure 6: Parameters of water in whole plant of *Pontederia crassipes* over weeks during mesocosm experiment.

The concentration of nutrients in the water is a key factor in the growth and development of plant structures (Figure 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.

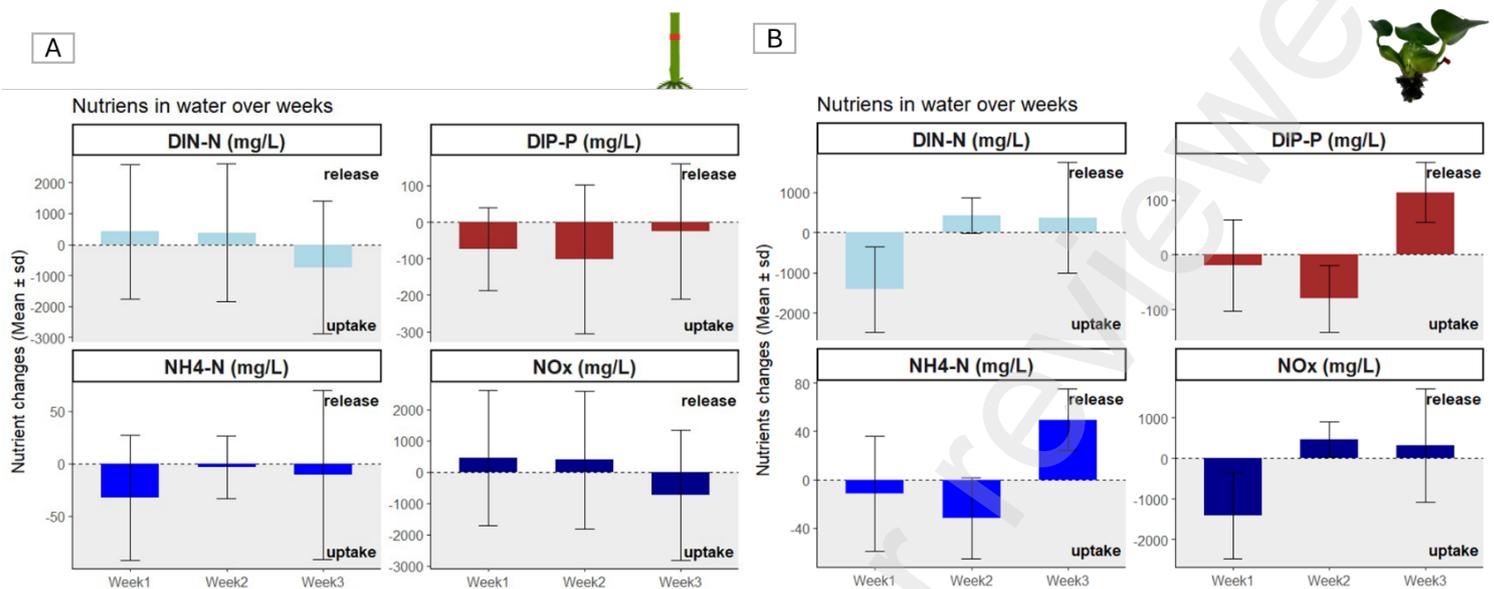


Figure 7: Results of nutrient changes calculated as the difference between consecutive sampling times (every 7 days from day 7 to day 28), were used as an indicator of plant uptake in stolon (A) and (B) in whole plant of *Pontederia crassipes*.

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 the setting up a mesocosm to ensure 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 detail description of the initial plant/organs characteristics and the metrics (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 this 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.

## CRedit author statement

Leticia da Silva Brito was responsible for the methodology of preparing the material, collecting the plants, analyzing the plant measurements, and writing and correcting the first draft. Ana Isabel Lillebø and Heliana Teixeira were guided through the conceptualization and methodology research processes, as well as the validation and editing stages. Sidinei Magela Thomaz was responsible for validation and editing.

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## 1 Declaration of interests

2  
3  The authors declare that they have no known competing financial interests or personal relationships that could  
4 have appeared to influence the work reported in this paper.

5  
6  The authors declare the following financial interests/personal relationships which may be considered as potential  
7 competing interests:

## 9 Supplementary material *and/or* additional information [OPTIONAL]

10 A summary of the experiment with the stolons, which were repeated three times, is provided below.  
11 Adjustments such as the acclimatization period and aeration were made in the last experiment. A summary of the  
12 standardization of each experiment is included in the table below.

14 **Table 5:** Comparative data from stolon and whole plants in mesocosms experiments under salinities levels.

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 x 300 x 243	32	6	7	13.64	13.96
4	Whole plant	20	400 x 300 x 243	32	3	7	36.64	73.74

\*All stolon died

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**Title:**Ex-situ growth protocol for the invasive macrophyte *Pontederia crassipes*  
**Authors:** Brito, Leticia; Thomaz, Sidinei M; Teixeira, Heliana; Lillebø Ana

**Table 1.** Comparative summary of mesocosm experiments with *Pontederia crassipes* from the liter

Growth indicator	Mesocosm experiment	Duration (days)	Hydrological conditions		
			Volume (L)	Temperature (°C)	Retention time (weeks)
<b>Whole plant (Petiolo+lamina+steam+root)</b>					
Net productivity (dry weight)	1	84	300	26°C-28°C	1
	2	100	1000	NA	1
	3	80	1000	NA	1
	4	83	1000	15°C-33°C	1
	5	96	1000	5°C-35°C	1
	6	22 *in two seasons	500-1000	17.8°C-24.8°C	48 h and 60 h
	7	56	200	15°C -25°C	1
	8	105	300	NA	6
	9	42	100	23.5°C-28.8°C	1
	10	150	104	NA	1

perature

Water circulation	Nutrient source	Nutritional conditions		
		Nitrogen (mg L <sup>-1</sup> )	Phosphorus (mg L <sup>-1</sup> )	micronutrients (mg L <sup>-1</sup> )
daily by submersible pump	constructed wetlands	20	5	NA
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
12h 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:
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
daily by submersible pump	well water	20	0,06	K: 25
NA	wastewater from residencials home that has passed through a primary sedimentation	20	7.5	K: 16.5
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
NA	constructed wetlands	28	7,7	NA
NA	lake water	0,6	0,05	NA
	pond water	0,58	8,86	NA

Growth rate			Reference
T0 (g DW m <sup>-2</sup> )	Tf (g DW m <sup>-2</sup> )	Rate (g DW m <sup>-2</sup> day <sup>-1</sup> )	
445	2303	0,0193	Reddy & Tucker 1982
680	3300	14,25	Reddy 1984
500	2000	0,0173	Reddy & Debusk, 1984
225	2000	0,0263	Reddy et al., 1989
225	830	0.0147	Reddy et al., 1990
48	225	0,0112	Ho & Wong , 1994
30	120	0,0248	Xie et al., 2004
plant with 20 cm	NA	NA	Jayaweera et al., 2008
15	235	0,042	You et al., 2014
260	405,83	0,0112	Kumwimba et al., 2017