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Biomass production of the aquatic macrophyte *Ceratopteris pteridoides* (Hook.) Hieron (Pteridaceae) in nutrient addition treatments

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ABSTRACT

Aquatic macrophytes are a key group in flooded areas due to their high primary productivity, and several species present in the Amazonian floodplains have potential for human food use. This study evaluated biomass production and nutrient levels in fronds of *Ceratopteris pteridoides* (Pteridaceae), under the following nutritional treatments: (T1) artesian well water, (T2) natural lake water, and (T3) artesian well water with a nutritive solution. Each replicate had 25 plants of 25 g total fresh biomass each. The experiment lasted for 35 days and total fresh biomass weight, root system length, leaf dry biomass weight, root dry biomass weight and sprout number were assessed. The plants from T3 had the greatest increase in total fresh biomass with an average of 430.02 g, showing that the nutritive solution used in the experiment provided the best conditions for plant growth. The daily ingestion of *C. pteridoides* could contribute to mineral supplementation, in addition to diversifying existing crops and contributing to sustainable agriculture.

Keywords: amazonian floodplain, mineral nutrition, potential food, unconventional food plants (UFPs), wild vegetables.

Introduction

Studies show that for over 10,000 years the domestication, cultivation, and management of plant species have been practiced by farmers, indigenous peoples and traditional communities around the world and this knowledge of the free use of biodiversity is one of the main bases of agriculture (Packer 2012). However, the modern, globalized food system has led to a simplified diet and increased dependence on some basic crops for most nutritional needs (Johns & Eyzaguirre 2006). A survey of the world's vegetables indicates that only 402 species are grown worldwide (Kays & Dias 1995). Young leaves or buds are the most consumed (53 % of the total) and, as most are perishable, sale should occur soon after harvest, usually in local markets (Dias 2012).

As a general trend, the daily consumption of vegetables in the diet is closely associated with good health and the reduction of the risk of various diseases, as vegetables are important sources of vitamins, minerals, dietary fibers and phytochemicals for humans. Consumers are increasingly becoming more interested in healthy vegetable products due to their functionalities and food benefits (Dias 2015). Nutrition is a matter of quantity (biomass production) and quality (nutritional content), and vegetables ensure adequate intake of most compounds (Kays & Dias 1995). Although they still require studies related to propagation and consumption, several wild vegetables are gaining more interest, especially in regions with a rich diversity of plant

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species such as the Amazon (Brasil 2010; Kinupp & Lorenzi 2014).

The Neotropical region has the highest diversity of macrophyte species on the planet, with about 1,566 species (Chambers *et al.* 2007). There are a variety of fruits and seeds of aquatic plants that could be used as food for human, since many are rich in oils, starch, or proteins. Numerous rhizomes and tubers are rich in carbohydrates, especially starch, sugar, and mucilage. The foliage of many may also be used as salad ingredients. The rhizome of many aquatic macrophytes is also used as flour to produce cookies and cakes, among others. In Brazil, one of the most used macrophytes with nutritional value for humans is watercress (*Nasturtium* sp.) (Thomaz *et al.* 2009).

Aquatic macrophytes are a key group in freshwater ecosystems due to their high primary productivity, and several species growing in wetlands are potential sources of food. One example is *Victoria amazonica*, whose seeds and rhizomes are used in the diet of the riverine populations of the Brazilian Amazon (Piedade *et al*. 1991; 2010). Some frequent species, such as *Neptunia oleraceae*, *Ipomoea aquatica* and the pteridophytic *Ceratopteris pteridoides*, are among the species commonly commercialized and consumed in Asia as vegetables; however, in the Brazilian Amazon, they are used only to feed domestic animals (Piedade & Junk 2000). The population in the Amazon region does not consume some of the native plants often because of their inability to identify the species, lack of information regarding their forms of use or their edible parts, and also due the loss of traditional knowledge (Kinupp & Lorenzi 2014). However, some aquatic native species have potential for human consumption (Kinupp & Barros 2008), provided that their biomass production, nutritional value and yield is known.

Ensuring access to nutritious food for the world's population is one of the main challenges of the 21st century (ONU 2015). In order to produce sufficient, high-quality biomass that can be converted into leafy vegetables, it is necessary to identify the production strategies for traditional species (Mafeo & Mashela 2009). The demand for a greater variety of plant species can be satisfied by assessing the suitability of less well-known edible species, including *C. pteridoides*.

In Brazil, *C. pteridoides* grows in the Atlantic Forest, the Pantanal, the Cerrado and the Amazon biomes (Hirai & Prado 2020), especially in the nutrient-rich floodplain ecosystem found along the Amazon River (Piedade & Junk 2000). The fronds of the species are consumed in oriental countries in salads, often together with fruit, and mainly in rural areas are also frequently eaten cooked (Liu *et al*. 2012; Panda 2015). There is currently no information on the cultivation of macrophytes, *i.e.*, *C. pteridoides,* for human consumption, especially in hydroponic systems. Hydroponics is an efficient technique that allows the modulation of the nutrient solution based on the exclusive needs of each cultivated species (Furlani *et al.* 2009). It also provides greater yield with fewer fertilizer inputs and without compromising the quality and safety of the vegetables (Rodrigues 2002). Therefore, it is of extreme importance to develop methodologies for adapting promising species from the Amazon for large-scale production using hydroponics. Through greenhouse experiments, this study analyzed the growth and the incorporation of biomass of *C. pteridoides* that were collected in the natural environment and cultivated in water with different mineral contents in order to evaluate the species potential for human consumption.

Materials and methods

Ceratopteris pteridoides (Hook.) Hieron. is a fern of the Pteridaceae family. It is an aquatic plant that is free-floating or rooted in mud and occurs in environments with a high nutritional level such as the Amazonian floodplain lakes (Guterres *et al*. 2008; Piedade *et al*. 2019). This species has dimorphic fronds composed of a petiole with 4–many vascular bundles and sterile lamina that are simple or deeply-lobed to pinnatifid or 1–3-pinnate, glabrous, succulent to herbaceous (Hirai & Prado 2020). It also has vegetative reproduction through the formation of sprouts (Piedade *et al*. 2019).

Specimens of *C. pteridoides* with only vegetative fronds were collected in December 2014, in the Catalão Lake (3º10'04" S; 59º54'45" W), which is located in the Central Amazon. The lake is periodically influenced by the acidic and nutrient poor waters of Negro River and by the Solimões River, which is rich in nutrients and sediments (Brito *et al*. 2014). White-water rivers, like the Solimões, present fertile waters, with a pH close to neutral and high electrical conductivity due to the high concentration of dissolved ions (Sioli 1968). Despite receiving the waters of the Negro River, throughout the year, the physical and chemical properties of the Solimões River predominate in the Catalão Lake, which is why it is classified as a fertile floodplain lake (Brito *et al*. 2014).

The plants collected for the experiment were allocated in pots containing water from the same collection site and brought to the Instituto Nacional de Pesquisas da Amazônia (INPA), where they were washed in a sieve with running water and then placed on absorbent paper for 5 minutes to remove excess water. Every individual plant was weighed using a precision scale 0.1000g (Gehaka, AG 200, Brazil). The initial fresh weight of the plants varied from 0.60 to 1.30g. The individuals were taken to the greenhouse of the MAUA Group – INPA/MAUA Project, where the experiment was conducted. The experimental design consisted of 3 treatments with five repetitions. Each repetition was composed of 5 plants with a total of 5 grams of fresh weight in each pot. The experiment comprised a total of 25 plants and 25 grams per treatment, as follows: T1 – water from the artesian well at INPA (37 µmol.L^{-1} Na^+, 24 µmol.L^{-1} Cl^-, 8 µmol.L^{-1} K^{+8}, 8 µmol.L^{-1} Ca^{2+}, 3 µmol.L^{-1} Mg^{2+}; Souza-Bastos *et al*. 2017); T2 – water from the Catalão Lake (208.0936 µmol.L^{-1} Na^+, 131.1856 µmol.L^{-1} Cl^-, 87.6928 µmol.L^{-1} K^{+8}, 964.7092 µmol.L^{-1} Ca^{2+}, 118.8096 µmol.L^{-1} Mg^{2+}; Souto *et al*. 2015); T3 – water from the artesian well of INPA + nutritive solution (See the composition below). The plants were distributed in a completely randomized design with five replicates per treatment. The same procedure was carried out for the distribution of the experimental units on the benches of the greenhouse.

For the climate of the northern region of Brazil, nutritional solution should be diluted until reaching an electrical conductivity in the range of 1.0 to 1.5 µS.cm^{-1} (Abou-Hadid *et al*. 1995). This is because the high temperature increases the physiological activity, and plants absorb more water than nutrients, thus, the ideal pH for cultivation is between 5.5 and 6.5 (Furlani *et al.* 2009). The commercial nutrient solution kit for hydroponics Hortibras® ($ 4.00 for 1,000 L) was used to simulate hydroponic conditions that could easily be performed at low cost by farmers. To establish the proper dosage for the growth of the species, 40 concentrations ranging from 0.5 mL.L^{-1} up to 10 mL.L^{-1} of water were previously tested, according to the manufacturer's recommendations, and the dosage 0.5 mL.L^{-1} resulted in plants that appeared visibly healthy. The T3 treatments followed the chemical adjustment method of the nutrient solution proposed by Furlani *et al.* (2009), which is based on the adjustment of electrical conductivity and pH, containing 1,200 g KNO_3 10 L^{-1}; 200 g $NH_4H_2PO_4$ 10 L^{-1}; 240 g $MgSO_4.7H_2O$ 10 L^{-1}; 600g $Ca(NO_3)_2.4H_2O$ 10 L^{-1}; 300g NH_4NO_3 10 L^{-1}; 10 g $MnSO_4.4H_2O$ L^{-1}; 5 g H3BO_3 L^{-1}; 2 g $ZnSO_4.5H_2O$ L^{-1}; 1 g $CuSO_4.5H_2O$ L^{-1}; 1 g $Na_2MoO_4.2H_2O$ L^{-1} and 200 mL of Fe-EDTA. The pH of the solution was kept in the range of 5.5 to 6.5 in both T1 and T3. The electrical conductivity recorded was 1.3 µS.cm^{-1} and 1.2 µS.cm^{-1}, which is within the appropriate recommended range.

The plants were allocated in 15 polyethylene containers, with 39 cm in diameter at the base, 50 cm at the opening and 30 cm high with a capacity of 40 liters each and allocated in a bench with dimensions of 3 m × 2 m and 1.5 m high. The greenhouse was covered with black screens providing 25 % shade. In all treatments, the water was changed weekly, and the pH and conductivity were monitored daily.

The fresh weight, root length and the number of sprouts were assessed every 10 days and the plants were returned to the experiment. At 35 days, the fronds of T3 start to produce fertile leaves, and were therefore considered to be in the harvesting phase. The parameters analyzed were the weight of dry leaf biomass (DLB), and the weight of dry root biomass (DRB). The length of the roots was measured with a ruler graduated in millimeters. The DLB and DRB were obtained by separating the leaf and root compartments, with subsequent drying of the materials in a forced ventilation oven at 45 °C, until they reached a constant weight.

The increment of fresh biomass, root length and number of sprouts over time was analyzed using ANOVA of the repeated measurements. The data of the dry biomass at the end of the experiment were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test at a 5 % probability level. The statistical analyses were performed using the statistical package Systat version 12.0.

Results

The water pH varied between 3.9 and 6.3, and conductivity varied between 47.1 and 73.9 µS/cm. No plant mortality was observed in any of the three treatments. However, in T1 (well water) growth was inhibited early in the experiment (Fig. 1A). In T2 (Catalão Lake water) significant biomass accumulation was expected due to the use of the same water from the plant collection site, however, from the 20th day onwards, there was a reduction in the gain of fresh biomass (Fig. 1A); the leaves in T2 were losing their color and, at the end of the experiment, they presented generalized chlorosis both in the old fronds and in the sprouts (see Tabs. S1, S2 and Fig. S1 in supplementary material). An intense growth of algae was also observed in this treatment. In T3 (well water + mineral supplement), fresh biomass has increased considerably throughout the treatment (Fig. 1A). Thus, total fresh biomass was influenced by treatments ($F=179.657$, $p<0.0001$), time ($F=156.709$, $p<0.0001$) and interaction between time and treatment ($F=82.296$, $p<0.0001$) (Fig. 1A).

Root length was also influenced by treatments ($F=78.539$, $p<0.0001$), time (F=80,319, $p<0.0001$), and interaction time and treatment ($F=23.725$, $p<0.0001$). Only the T3 plants showed root growth (Fig. 1B) and the production of new roots. The number of sprouts was influenced by treatments ($F=70.247$, $p<0.0001$), time ($F=62.898$, $p<0.0001$) and interaction time and treatment ($F=29.641$, $p<0.0001$). Treatment 3 showed the greatest increase in the number of sprouts. In treatments 1 and 2, the plants formed sprouts, but the increment was not significant (Fig. 1C).

In treatment 3 there was a greater increase in dry leaf biomass than T1 and T2 ($F=83.467$, $p<0.0001$; Fig. 2); greater dry root biomass ($F=30.468$, $p<0000.1$; Fig. 2) and total dry biomass ($F=78.028$, $p<0000$; Fig. 2).

Discussion

Although no plant mortality was observed in all three treatments, this study revealed that the cultivation of the species *C. pteridoides* as a crop in the artesian well water with a nutritive solution to be the most promising cultivation practice. The water of the artesian well alone (T1 treatment) may not have provided the necessary amount of nutrients, since *C. pteridoides* prefers nutrient-rich environments

(Piedade *et al*. 2019). On the other hand, we expected a higher growth and biomass accumulation in T2 in which the water of the natural environment was used, due to the high amount of nutrients dissolved in the water (Furch 1997). This may be explained by the process of decantation that occurs after some time, which favors the penetration of light, leading to the development of algae (König 2000), as observed in this study.

Free-floating plants affect phytoplankton biomass through competition for solar radiation, nutrients and secretion of allelopathically active compounds (Dent *et al*. 2002; Van Donk & Van de Bund 2002; Gross 2003; Dong *et al*. 2018). In a field mesocosm experiment in a temperate lake, there was decrease in phytoplankton biomass in the presence of aquatic macrophytes (O'Farrell *et al*. 2009). In warm, nutrient-rich wetlands, dominant floating plant stands also

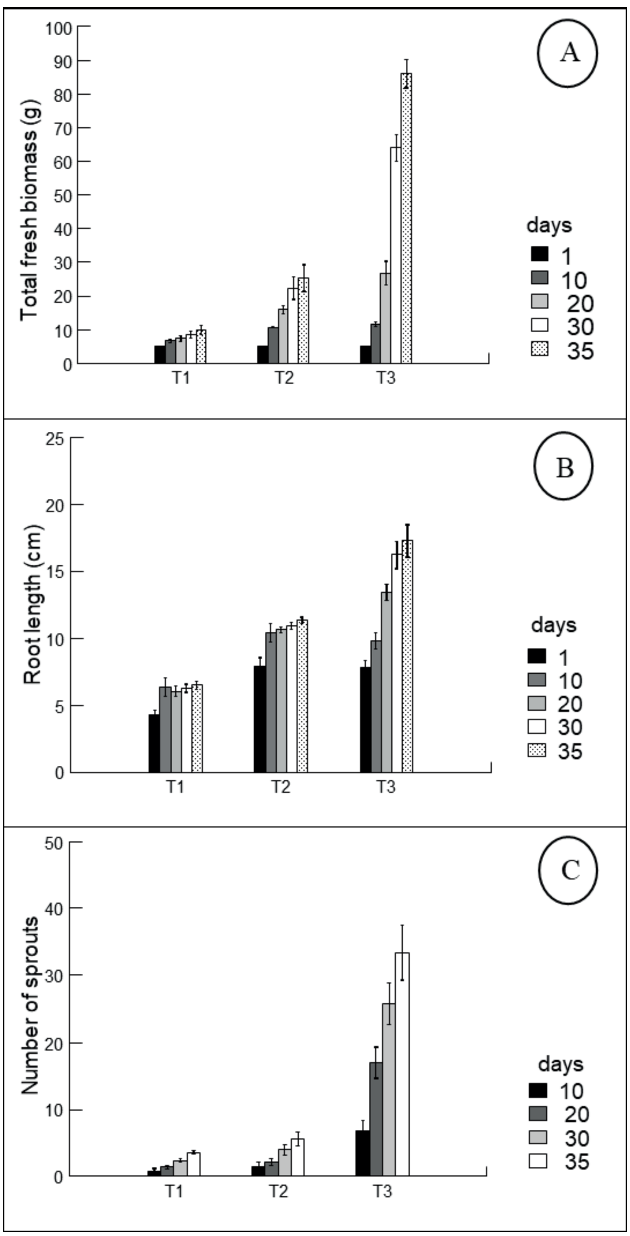

Figure 1. Effect of nutrient level on *C. pteridoides*: **A**) Total fresh biomass (g); **B**) Root length (cm); **C**) Number of sprouts. Where: T1 = Water from the Instituto Nacional de Pesquisas da Amazônia (INPA) artesian well (control); T2 = Water from the Catalão Lake; T3 = Water from the INPA artesian well + nutritive solution. Error bars denote the standard deviation. Different letters indicate significant differences according to Tukey test ($p < 0.05$).

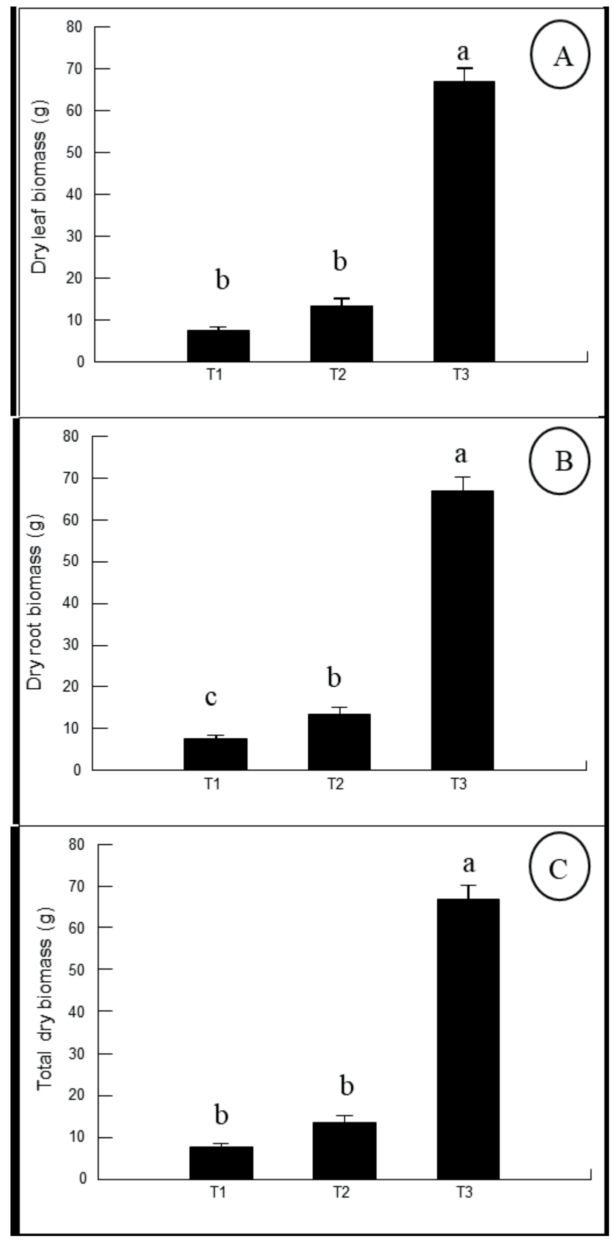

Figure 2. Effect of nutrient level on dry biomass of frond and root of *C. pteridoides*. Where: T1 = Water from the Instituto Nacional de Pesquisas da Amazônia (INPA) artesian well (control); T2 = Water from the Catalão Lake; T3 = Water from the INPA artesian well + nutritive solution. Error bars denote the standard deviation. Different letters indicate significant differences according to Tukey test ($p < 0.05$).

result in low phytoplankton biomass (Bicudo *et al.* 2007; Tezanos Pinto *et al.* 2007). However, information about competition between algae and floating plants is scarce, and the observed patterns are not consistent (Meerhoff *et al.* 2003). Thus, on its own, the growth of algae in the T2 treatment may not explain the unexpected low performance in growth in comparison to the T3 treatment, especially since the water of the tray was changed weekly using fresh water from the Catalão Lake. Therefore, studies regarding algal competition and floating aquatic macrophytes need to be performed in order to understand the real influence of algae on hydroponic production systems.

The fresh and dry total biomass obtained in T3 allowed us to infer that the nutrient solution promoted better development of the root system and the aerial part of *C. pteridoides*, which shows that the nutrient concentration of the mineral nutrient solution selected is adequate for the cultivation of this species. The correct dosage of the nutrient solution is fundamental for the development, the increase in productivity, and the quality of the vegetable to be produced. Several hydroponic crops have been shown to be unsuccessful when provided with incorrect nutritional management (Furlani *et al.* 2009). In T3, the pH of the water varied between 3.9 and 6.3, an important indicative of the adequacy of the solution. The nitric and ammoniacal forms in the composition of the nutrient solution provide greater pH stability (Andriolo 1999). Values below 4.0 affect the integrity and permeability of cell membranes, which can lead to loss of nutrients already absorbed by the plant and delayed root growth; values greater than 6.5 can lead to the precipitation of elements such as calcium, phosphorus, iron and manganese, making them unavailable to plants (Martinez 2002).

When compared to other aquatic macrophyte species, the total biomass production of *C. pteridoides* over 35 days at T3 treatment can be considered remarkably high, since it increased by 1,720.0 %. *Eichhornia crassipes* increased 177.6 % and *Egeria densa* Planch. 2 % in their production of fresh biomass in 40 days when cultivated with organic aquaculture effluents (Henry-Silva *et al.* 2008). In comparison to other vegetables that are hydroponically cultivated, *C. pteridoides* also performed very well. For example, arugula (*Eruca sativa*) increased its fresh aerial biomass by 29.0 % in 30 days (Genuncio *et al.* 2011), while unconventional food plants (UFPs), such as Florida spinach (*Talinum triangulare*), which was grown using hydroponics, increased its fresh aerial biomass by 27.0 % in 21 days (Araújo *et al.* 2018).

Considering the biomass production, the mineral content (Tab. S1 in supplementary material) and the protein content (11.3 %) (Albuquerque 1981) of *C. pteridoides,* the species has proved to be a very promising vegetable for human consumption using a low-cost mineral nutrition kit. In fact, the congeneric species, *Ceratopteris thalictroides,* is regularly consumed raw or cooked in several Asian countries (Edwards 1980), or even cultivated as a spring vegetable in Japan (Cook *et al.* 1974). The practice of cultivating floating crops (Radulovich *et al.* 2015) is scarce, if not absent, in the floodplains of the Brazilian Amazon, since the flood-pulse impedes plantations during the period of high water levels (Junk *et al.* 2000). The introduction of such a practice using *C. pteridoides* may enhance the economy and food security especially of the Amazonian riverine populations.

Conclusion

This study has confirmed that *C. pteridoides* has great potential for cultivation. The nutritive solution caused a high accumulation of biomass and, thus, it can be cultivated all year round. Since the species is particularly rich in K, Ca, Fe, and Zn, which are essential dietary minerals, we may conclude that its ingestion could contribute to a large proportion of the body's mineral requirement if consumed on a regular basis. We also suggest palatability tests to promote consumption by the local population. Further experiments in the field, where water current and a continuous natural nutrient supply may allow a better growth than that achieved in our greenhouse experiments, are also recommended.

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