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Phenometric predictors of *Pontederia crassipes* biomass under natural conditions in the Paraná River

Preditores fenométricos de biomassa de *Pontederia crassipe*s em ambientes naturais no rio Paraná

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Abstract: The water hyacinth, *Pontederia crassipes*, is a free-floating aquatic plant native to South America, which has colonized tropical rivers in several continents and has become dominant in floodplains. **Aim:** This study aimed to evaluate the relationship between leaf length and leaf biomass (as an indirect phenometric estimation) and to compare the accuracy of the indirect phenometric estimation (which is a non-destructive method) with that of the direct estimation of aboveground biomass (which is destructive). **Methods:** *Pontederia crassipes* green leaves of all sizes were collected from a floodplain lake of the Paraná River (Argentina). The leaves were oven-dried in the laboratory to obtain the constant dry weight. To determine the accuracy the indirect phenometric estimation was compared with the direct estimation of aboveground biomass. The relationship between leaf weight and leaf length was evaluated by regression analysis. The length of the 279 green leaves collected ranged between 7 and 115 cm. **Results:** The non-destructive method was a good predictor of leaf biomass ($R^2 = 0.87$ $p < 0.0001$). No significant differences were found between the dry weight obtained directly and that estimated by the indirect method. **Conclusions:** Our results suggest that leaf length is a good attribute to estimate the aboveground biomass of *P. crassipes*. This method can contribute to diminish the impact of the direct method of harvest of *P. crassipes* and can be applied in experimental studies aimed to determine the leaf life span and primary productivity of *P. crassipes* clones.

Keywords: wetlands; water hyacinth; large rivers; South America.

Resumo: *Pontederia crassipes* é uma planta aquática flutuante livre nativa da América do Sul, que colonizou rios tropicais em vários continentes, tornando-se dominante nas planícies aluviais. **Objetivo:** Neste estudo testamos a relação entre o comprimento das folhas e a biomassa foliar, e comparamos a precisão da estimativa fenométrica indireta com a biomassa direta acima do solo. **Métodos:** Recolhemos folhas verdes de aguapé com diferentes tamanhos num lago de planície de inundação do rio Paraná (Argentina). As folhas foram secas em estufa de laboratório para obter um peso seco constante. Para determinar a acuracidade da estimativa fenométrica indireta foi comparada com a biomassa direta para a parte aérea. A análise de regressão foi utilizada para testar a relação entre o peso e o comprimento das folhas. O comprimento de 279 folhas verdes variou entre 7 e 115 cm. **Resultados:** O método não destrutivo foi um bom preditor da biomassa foliar (R^2 = 0,87 p < 0,0001). Não foram encontradas diferenças significativas entre o peso seco obtido diretamente e o peso estimado através do método indireto. **Conclusões:** Os nossos resultados sugerem que o comprimento da folha é um bom atributo para estimar a biomassa aérea de *P. crassipes.* Este método é útil para reduzir o impacto do método direto de colheita de aguapé e necessário para aplicação em estudos experimentais que determinam a duração da vida foliar e a produtividade primária em clones.

Palavras-chave: zonas úmidas; aguapé; grandes rios; América do Sul.

1. Introduction

The water hyacinth, *Pontederia crassipes* (Mart.) Solms. (Pellegrini et al., 2018)-Pontederiaceae-, also known as *Eichhornia crassipes*, is one of the most frequent aquatic macrophytes in the floodplains of large South American Rivers (Puhakka & Kalliola, 1993; Colonnello Bertoli, 1996; Navarro & Maldonado, 2002; Neiff et al., 2014) and has invaded warm waters in over 50 countries on five continents (Villamagna & Murphy, 2010). This species is a key element of tropical and subtropical water bodies because it provides habitat and food to other organisms (Blanco Belmonte et al., 1998; Rocha-Ramírez et al., 2007; Poi de Neiff et al., 2020). As other aquatic macrophytes (Wetzel, 1983; Batzer et al., 1999), when the plant dies, it contributes to food webs, and is consumed mainly by detritivores living below the water line. In the Paraná River floodplain, most fish production is supported by trophic webs based on macrophyte detritus (Bonetto, 1986) and the macrophyte biomass above the waterline is consumed by herbivores (Franceschini et al., 2010).

Downstream of the confluence of the Paraná River with the Paraguay River, *P. crassipes* covers 30-100% of the surface of the Paraná River floodplain lakes during prolonged (6-18 months) low water conditions. High of *P. crassipes* biomass can be sustained for long periods without external nutrient inputs from the river (Carignan & Neiff, 1992; Carignan et al., 1994). During extraordinary floods, the height of the green leaves increases, reaching over 90 cm, whereas, during the prolonged isolation phase, they are less likely to exceed 90 cm (Neiff et al., 2007). In other floodplains such as that of the Pilcomayo River (Argentina), they can reach 115 cm during the isolation phase (Poi de Neiff et al., 2020).

Techniques to determine the primary productivity of aquatic plants in the field include direct biomass sampling and indirect phenometric estimation (Thomaz & Esteves, 2011). Destructive methods for direct biomass estimation involve cutting and collecting plants, which generate difficulties in experimental studies that use enclosures to determine the response to nutrient inputs or herbivory damage. In addition, the remarkable amount of plant biomass necessary to accurately estimate productivity is difficult to harvest and transport to the laboratory to determine dry weight. For these reasons, several authors have used the leaf length, leaf width, leaf area, and plant height to evaluate the relationship between these morphological variables and biomass in different macrophyte species of South American Rivers (Gonçalves et al., 2010; Silva et al., 2010; Correia Nunes & Camargo, 2017).

Based on the above, the aims of this research were: 1) to test the relationship between leaf length and aboveground biomass of *P. crassipes* by using a non-destructive method, and 2) to compare the accuracy of the indirect phenometric estimation with that of the direct estimation of aboveground biomass in four different periods (regrowth period, maximum growth period, the beginning of the senescence period and the end of the senescence period) in a floodplain lake of the Paraná River (Argentina).

2. Material and Methods

2.1. Study site

The study area has humid subtropical climate (Bruniard, 1999), with hot summers and mild winters. There is low occurrence of frost, with three frosts per year, over a period of 31 years (Shindoi et al., 2003). The maximum air temperature can reach 41.6 °C in January, whereas the minimum can reach -3.3 °C in June (Neiff et al., 2007). The mean monthly maximum temperature is 32 °C (January) and the mean monthly minimum is 9.2 °C (July). The average annual rainfall is 1598 mm (Poi de Neiff et al., 2006).

For the present study, we selected a small (200 × 3 000 m) and shallow (0.90 - 2.5 m deep) floodplain lake located within the Chaco Wetlands RAMSAR Site (Argentina) and connected with the Paraná River when the water level at the Corrientes datum exceeds 4.20 m (27°26’20”S; 58°51’13”W, Figure 1). This lake, called El Puente,

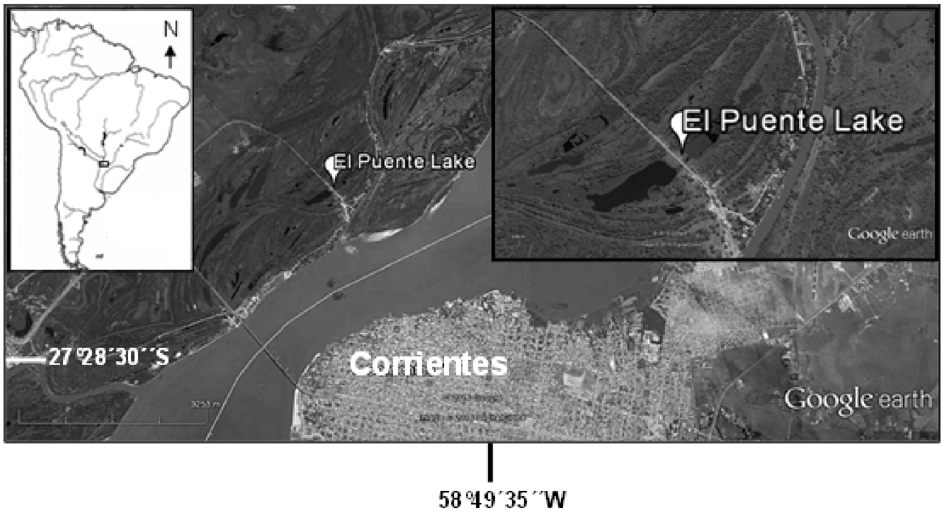

Figure 1. Location of the sampling site on the Paraná River floodplain.

belongs to a complex of several similar oxbow lakes (Figure 1) located on the west riverbank of the Paraná River, and was selected based on the expertise accredited by previous research in the study area (Neiff et al., 2001). The waters have conductivity between 54 and 340 µS.cm^{-1}, slightly acid pH (between 6 and 7.5) and variable nutrient content (between 0.1 µg.L^{-1} and 100 µg.L^{-1} of total nitrogen concentration and between 25 and 150 µg.L^{-1} of total phosphorus concentration). For further characteristics of the lake, see Carignan & Neiff (1992) and Casco et al. (2014). *Pontederia crassipes* forms monospecific stands that cover until 60-80% of the water surface with elongate leaves (large biotype).

2.2. Sampling design

During April 2018, we collected 47 plants with green leaves of all sizes present in six replicate samples of a 0.30 m^2 circular plot. In the laboratory, the green, standing dead, and decomposing leaves were counted and separated. Green leaves were washed and their length (from the base of the petiole up to the end of the blade) was measured with measuring tape. Then, the leaves were oven-dried for 48 hours at 105 °C and weighed to obtain the constant dry weight. Leaf length and leaf dry weight were correlated to obtain a predictive equation.

To determine the accuracy of the phenometric method as a predictor of biomass, we used and re-elaborated data set measurements previously collected at El Puente Lake (Neiff et al., 2007). The dry weight of the green leaves of three replicate samples (sampling unit of 0.30 m^2) grouped in four different periods (regrowth period-August-, maximum growth period-December-, beginning of the senescence period-March- and end of the senescence period -July) was used to determine the direct aboveground biomass. For the indirect phenometric estimation of biomass, we used a predictive equation to determined leaf dry weight and the prior data of leaf densities (mean of 416, 431, 194 and 231 for each period) and leaf length (Neiff et al., 2007).

2.3. Data analysis

For leaf dry weight, normality assumptions were checked using Shapiro tests. A nonparametric Kruskal–Wallis test (and its post-hoc contrasts) was used to test for significant differences in leaf dry weight between the indirect and direct methods used to estimate the aboveground biomass. We considered differences to be significant at an $\alpha = 0.05$ level, using the software InfoStat version 2015 (Di Rienzo et al., 2020).

Regression analysis was used to test the relationship between leaf weight and leaf length.

3. Results

In April 2018, the mean green leaf density per m^2 was 310.16 ± 134.49, whereas green leaf length ranged between 7 and 115 cm (n = 279). The number of standing dead leaves (24.12 ± 16.07 leaves per m^2) and decomposing leaves (19.56 ± 15.17) was smaller than that of green leaves.

The relationship between the dry weight and the length of the green leaves of *P. crassipes* (Figure 2) was significant ($R^2 = 0.87$, $p < 0.0001$). The predictive equation fitted an exponential model with the following Formula 1:

$$W = 0.32.e^{0.03.L} \quad (1)$$

where: W is the weight estimated by the indirect phenometric method; *e* is a constant: 2.71828; and *L* is the measure of the leaf length.

Aboveground biomass determined by both methods was high during the four periods evaluated (Figure 3). The dry weight mean maximum value obtained with both methods ranged between 739.23 $g.m^{-2}$ ± 186.52 (by the indirect method) and 817.01 $g.m^{-2}$ ± 110.38 (by the direct method). No significant differences were found between the dry weight obtained directly and that the estimated by the indirect method (H = 3.22, p = 0.3589).

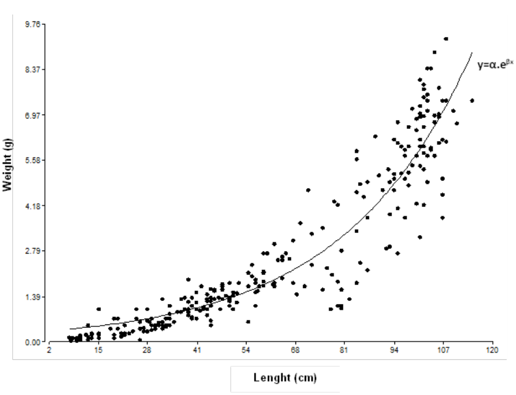

Figure 2. Exponential regression curve obtained from the green leaves length and the dry weight of *Pontederia crassipes*.

4. Discussion

The non-destructive method developed in this study showed a significant correlation, indicating that the morphological variables chosen are good to estimate the aboveground biomass of *P. crassipes*. The accuracy was indicated by the agreement between the dry weight calculated by the indirect phenometric estimation method and the direct estimation of aboveground biomass. Phenometric methods have been extensively evaluated for other macrophytes in floodplains of other South American rivers (Silva et al., 2010), for emergent macrophyte species in the Everglades (Daoust & Childers, 1998), and in estuaries in southeastern Brazil (Correia Nunes & Camargo, 2017). In our study, which was based on a high number of leaves (279) and a wide length range (7 and 115 cm), the non-destructive method showed that leaf length data are useful to calculate biomass in wetlands where *P. crassipes* has leaves with a wide range of sizes. For *P. crassipes*, Gonçalves et al. (2010) also found high efficiency of a non-destructive method using petiole length and limbo width of leaves in a combined model in plants with low height range. In the floating meadows of the Paraná River floodplain, a high proportion (more than 81%) of insect-attacked leaves, which have smaller length, seems to be common during the regrowth period. Nutrient input during floods determines a high frequency of leaves longer than 90 cm (Neiff et al., 2007).

The values of leaf density recorded during this study are in the range of those previously obtained by Neiff et al. (2007) during an annual cycle in

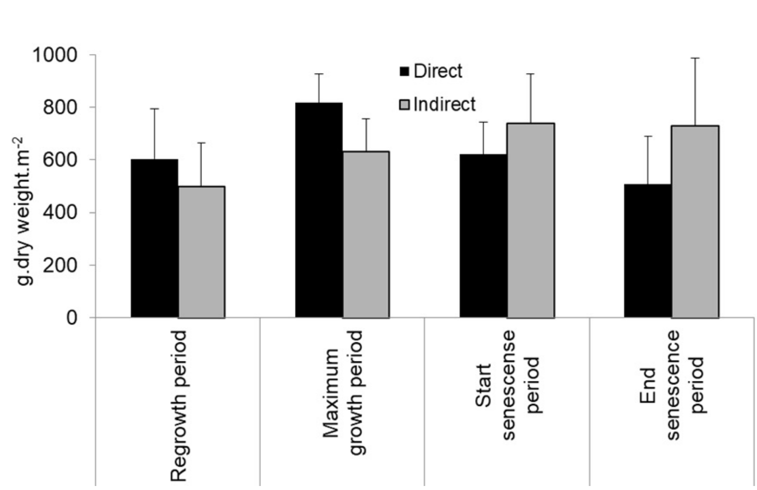

Figure 3. Average aboveground biomass of water hyacinth determined by direct and indirect methods in four different period (regrowth period, maximum growth period, beginning of the senescence period and end of the senescence period).

the same lake. The biomass of green leaves was high during the four periods studied, whereas, in other floodplain lakes, the biomass of green leaves decreases in winter because of thermal stress from low temperatures (Neiff & Poi de Neiff, 1984; Silva & Esteves, 1993).

The aboveground biomass of *P. crassipes* can be determined by the sum of the weight estimated by the indirect method from the length of each leaf in a plot. This method could contribute to diminish the impact of the direct method of harvest of *P. crassipes* and could be useful in experimental studies to determine the leaf life span (Klok & van der Velde, 2017) or the net aboveground primary production of clones (Santos & Esteves, 2002), which is, in turn, necessary to estimate *P. crassipes* production.

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