Reproductive phenology of *Polygonum hispidum* Kunth and *P. punctatum* Elliott (Polygonaceae), in response to the flooding cycle in the *Pantanal*, Brazil

Fenologia reprodutiva de *Polygonum hispidum* Kunth e *P. punctatum* Elliott (Polygonaceae), em resposta ao ciclo de inundação do Pantanal, Brasil

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Abstract: In flood-driven environments the life cycles of organisms are regulated in function of the water regime. The objective of this work is to analyse the phenophases of flowering and fructification of two species of amphibious aquatic macrophytes, *Polygonum hispidum* Kunth and *P. punctatum* Elliott, with regard to the flooding cycle of the Brazilian *Pantanal*. We collected monthly data in 26 plots of 20 ponds during two years. The flooding phases were classified as drought, rising, flood, and receding. The periods with an abundance of water (rising, flooding, and receding) were the most important for reproduction. Both species bear fruits at flood, when the water is deepest and remains until receding, and most seeds are dispersed. The synchrony between reproductive cycles and these flood phases suggests the importance of seasonality in the reproductive patterns of these species.

Keywords: Plant ecology. Smartweed. Wetlands.

Resumo: Em ambientes inundados, os ciclos de vida dos organismos são regulados em função do regime de água. O objetivo deste trabalho é analisar as fenofases de floração e de frutificação de duas espécies de macrófitas aquáticas anfíbias, *Polygonum hispidum* Kunth e *P. punctatum* Elliott, no que se refere ao ciclo de inundação do Pantanal brasileiro. Coletamos dados mensais em 26 parcelas de 20 lagoas durante dois anos. As fases de inundação foram classificadas como seca, enchente, cheia e vazante. Os períodos com abundância de água (enchente, cheia e vazante) foram os mais importantes para a reprodução. Ambas as espécies produzem frutos na cheia, quando a água é mais profunda, e permanecem até a vazante, na qual a maioria das sementes são dispersas. A sincronia entre os ciclos reprodutivos e essas fases de inundação sugere a importância da sazonalidade nos padrões reprodutivos dessas espécies.

Palavras-chave: Ecologia vegetal. Erva daninha. Planícies de inundação.

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INTRODUCTION

One of the aims of phenology is to reveal patterns of flowering and fruiting in plant communities (Foster, 1982), as an adaptation to cyclic biotic and abiotic factors (Lieth, 1974; Morellato & Leitão-Filho, 1990; Schaik *et al*., 1993), linked to genetic factors (Wilczek *et al*., 2010), and even dispersal of diaspores (Mantovani & Martins, 1988). Such studies are important for understanding the dynamics of ecosystems (Monasterio & Sarmiento, 1976). However, little is known of the phenological relations of the community of aquatic macrophytes and floodplains.

The *Pantanal* wetlands are a plain with regular flood cycles (Junk *et al*., 1989), natural and seasonal events that drive the dynamics in structure and floristic composition (Prado *et al*., 1994; Schessl, 1999), mainly in communities of aquatic macrophytes that are abundant in lakes and ponds throughout the region. In aquatic environments ruled by flooding, biodiversity is organized by function of the water regime (Ortega *et al*., 2015), variations in rainfall, and changes in the dynamics of sediments that are a filter for the establishment and development of species (van der Valk, 1981; Collischonn *et al*., 2001).

Few reports describe the phenology of aquatic macrophytes in the *Pantanal*: for example, on Poaceae *Oryza latifolia* Desv. (Bertazzoni & Damasceno-Júnior, 2011), and on Arecaceae *Attalea phalerata* Mart. ex Spreng. and *Bactris glaucescens* Drude (Fava *et al*., 2011), plus some on shrubs and trees, such as *Combretum lanceolatum* Pohl ex Eichler and *C. laxum* Jacq. (Marestoni, 2011), and *Vochysia divergens* Pohl (Nunes da Cunha & Costa, 2000). Nevertheless, these studies do not address the effects of flooding on the reproductive phenology of the species, regarding the phases when the plain is under flood or in drought, and the fact that aquatic species show adaptation to the annual flood cycle.

Knowledge on the phenological response of plants to the water regime in the production of inflorescences and seeds, for example, is critical to determine the responses of aquatic macrophytes to the flood regime, as well as to predict the species distribution in habitats with moisture gradients. In the *Pantanal*, the flood pulse is an annual cycle that can be of short or long duration and presents a large or small amplitude (Junk *et al*., 1989). Flooding in the southern part of the *Pantanal* is caused mainly by river overflow and not just by local rainfall, and the flood peaks caused by discharge of the Paraguay River in the south occur four months after rainfall peaks on the headwaters (Hamilton *et al*., 1996). In the northern *Pantanal*, floods normally occur between January and April, nearly coinciding with the rainy season (Rebellato & Cunha, 2005), whereas in the south floods are delayed until July and August, but most rainfall in both parts occurs between October and March (Gonçalves *et al*., 2011). Hence, local rainfall is not directly a determinant of flooding of the plain, mainly in the studied subregion of Miranda-Abobral.

Knowledge on flowering and fruiting in these flood cycle phases allows us to predict the periods of reproduction in aquatic macrophytes, their growth cycles, and other characteristics such as adaptation, information of great conservation value for the *Pantanal*. This knowledge has various applications, such as helping to determine strategies to harvest seeds, predict the availability of fruits, seed dispersal (Mariot *et al*., 2003), and in forest management (Fournier, 1974, 1976). The objective of this work is to analyze the phenophases of flowering and fruiting of two species of amphibious aquatic macrophytes with regard to the flood cycle of the *Pantanal*.

MATERIAL AND METHODS

STUDY AREA

The process of seasonal flooding is generally considered simply as flood and drought (Abdon *et al*., 1998), but we divided the cycle into four well-defined phases: drought, rising, flood, and receding (Prado *et al*., 1994). During the drought phase, water of the river stays within its bed while the soil surface of the surrounding open grassland is dry. In the rising phase, water overflows the riverbanks towards the open grassland, generally through seasonal streams on lower ground that spread the water on the plain. In the flood phase, the peak of flooding occurs on grasslands where water depth over the plain becomes level with pond surfaces. In the receding phase, water drains from the grassland back to the rivers through seasonal streams, except from the ponds.

The natural grasslands and flooded grasslands are the vegetation forms covering most of the region (Silva *et al*., 2000), and the proportions between dry and flooded grassland alternate in function of water inflow via intermitent or permanent rivers and seasonal streams (Silva *et al*., 2000). The area of the plain comprehends fresh water lakes and ponds, brackish ponds, paleolevees, seasonal streams and old riverbeds (Almeida & Lima, 1959).

The climate of the region is tropical subhumid, type Aw (Koeppen, 1948), with dry winters and rainy summers, with mean annual rainfall between 1,000 and 1,200 mm, concentrated between November and April (Soriano *et al*., 2001). In the south of the *Pantanal*, flooding occurs from April to June (Hamilton *et al.*, 1996).

In the study period (November 2012 to September 2014), the mean recorded temperature was 26.07 °C, October 2014 being the warmest month (34.73 °C maximum) and August 2013 the coolest (17.1 °C minimum). Accumulated annual rainfall was 2,484 mm, the highest rainfall was in May 2014 (300.4 mm), and the lowest in August 2013 (1.6 mm). In the first sample year, the mean recorded temperature was 25.94 °C, December was the dry phase and the warmest month (34,59 °C) and August, at the begining of the receding phase, was the coolest month (17.1 °C). Accumulated annual rainfall was 1,125.8 mm, the highest rainfall being recorded in March during the rising phase (210,8 mm), and the lowest in August (1.69 mm) at the begining of the receding phase. In the second sampling year, the mean recorded temperature was 26.20 °C in October, at the end of the receding phase, the warmest month (34.73 °C), and July, at the end of the flood phase, the coolest month (18.86 °C). Accumulated annual rainfall was 1,358.2 mm, the highest rainfall being recorded in May (300.4 mm) at the end of the rising phase, and the lowest in June (1.6 mm) at the onset of the rising phase (Figure 1). This information was obtained from the *Centro de Monitoramento de Tempo, do Clima e dos Recursos Hídricos de Mato Grosso do Sul* (CEMTEC-MS).

Flooding in the southern part of the *Pantanal* is caused mainly by river overflow and not just by local rainfall, and the flood peaks caused by discharge of the Paraguay River in the South occur four months after rainfall peaks in the headwaters (Hamilton *et al*., 1996).

SAMPLING

We sampled 20 ponds at monthly intervals along the Park Road located in the *Pantanal* wetlands, between 19° 24' 21.89" S, 57° 01' 44.57" W and 19° 14' 45.14" S, 57° 02' 18.00" W, in the Abobral and southwestern Nhecolândia subregions (Silva & Abdon, 1998), municipality of Corumbá, Mato Grosso do Sul, Brazil.

The phases of the flooding cycle (Prado *et al*., 2002) were checked in our field observations: drought (November/2012 to January/2013 and November/2013 to March/2014); rising (February and March/2013 and April and May/2014); flood (April to July/2013 and June and July/2014); and receding (August to October/2013 and August to October/2014) (Catian, 2015).

PHENOLOGY

We collected phenological data (flowering and fruiting) (Morellato *et al*., 2010) on two amphibious species of Polygonaceae (*Polygonum hispidum* Kunth and *P. punctatum* Elliott) during two years. The ponds were divided into 26 plots, without replicates, utilizing 0.5 x 0.5 m quadrats, placed every two meters from the shore to the middle of the pond. Quantitative (presence or absence) data on flowers and fruits were sampled monthly (Bencke & Morellato, 2002a; D'Eça Neves & Morellato, 2004). The number of quadrats was relative to the distribution of the stand of aquatic macrophytes, at the margin and in the pond. Water depth was measured monthly in each plot.

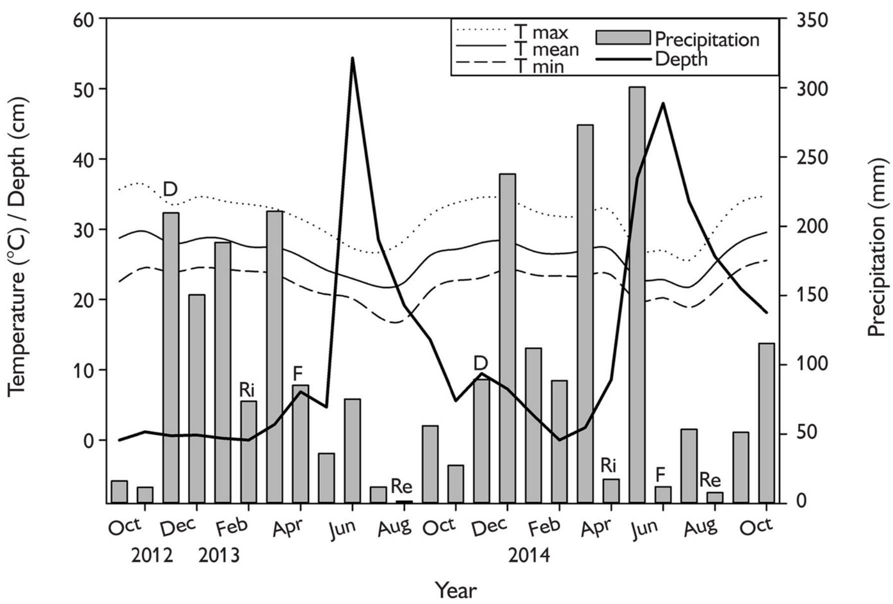

Figure 1. Climatic data for Corumbá, Mato Grosso do Sul, Brazil, for September 2012 to September 2014, and water depth of ponds. Year 1. November 2012-January 2013 (D = drought); February-March 2013 (Ri = rising); April-July 2013 (F = flood); August-November 2013 (Re = receding). Year 2. December 2013-March 2014 (D = drought); April-May 2014 (Ri = rising); June-July (F = flood); August-October 2014 (Re = receding). Source: *Instituto Nacional de Meteorologia* (INMET)/*Secretaria de Estado de Desenvolvimento Agrário, da Produção, da Indústria*, *do Comércio e do Turismo* (SEPROTUR)/*Agência de Desenvolvimento Agrário e Extensão Rural* (AGRAER)/*Centro de Monitoramento de Tempo, do Clima e dos Recursos Hídricos de Mato Grosso do Sul* (CEMTEC-MS).

In this study, we considered two phenophases: flowering (bud/open flower) and fructification (imature/mature fruits), according to Morellato *et al*. (1989). To verify the patterns of flowering/fructification, we utilized the number of flowers and fruits of individuals of each species.

The taxonomic classification of family and genus followed the Angiosperm Phylogeny Group (APG IV, 2016), and the scientific names of the species were checked with the database of *Lista de Espécies da Flora do Brasil* (REFLORA). Life form classification followed Irgang *et al*. (1984). For plant identification, we utilized the *Pantanal* aquatic plant guide book (Pott & Pott, 2000), with abbreviation of authors according to Brummitt & Powell (1992). Collected vouchers of the species were incorporated into the *Herbário de Campo Grande, Mato Grosso do Sul/ Universidade Federal de Mato Grosso do Sul* (CGMS/UFMS) (*P. hispidum* - CGMS 37167; *P. punctatum* - CGMS 37177).

ANALYSES

For the description of the phenology of the species, we utilized the Rayleigh test (Z) for circular distribution (Morellato *et al*., 1989; Zar, 2010), calculating the mean dates for the frequency of species in flowering or fructification, and the concentration of each event around this date (synchrony r) (Morellato *et al*., 2000), Z being the reproductive probability (flower and fruit) uniformly over the year (flood phases). When the mean angle is significant ($p < 0.05$), the pattern is considered seasonal, and this corresponds to the mean date of the year around which the phenological events are concentrated (Morellato *et al*., 2000, 2010). In our work, it indicates the production of flower and fruit in the flooding phases. The distributions of flower and fruit frequencies in each phenophase were ploted in circular histograms, at monthly intervals, with the 365 days of the year corresponding to the 360° of the circunference. The vector length is related to the value of the concentration coefficient (r), varying from 0 to 1, and the angle where this is ploted indicates the mean angle, that corresponds to the mean date of occurrence of the phenophase, *i.e.*, the concentration of flower and fruit production around the mean annual date or degree of seasonality of the phenophase (Morellato *et al.*, 2000, 2010). Calculations were made using the software Oriana 2.0 (Kovach, 2004).

The intensity of synchrony of flowering and fructification was determined following the scale of intensity: values equal to 0 indicate an absence of synchrony; from 0.1 to 0.27 indicate minimum synchrony; from 0.28 to 0.45 indicate low synchrony; from 0.46 to 0.69 indicate median synchrony; from 0.70 to 0.99 indicate high synchrony; and values equal to 1 indicate perfect synchrony (Augspurger, 1983).

To verify if the frequency of flowers and fruits in a certain phenophase responds proportionally to climatic seasonality, the degree of synchrony of each species in the flooding phases was calculated by applying the proportion of flowers and fruits shown in certain phenophases in the four flooding phases, as suggested by Bencke & Morellato (2002a, 2002b): asynchronic (< 20% of the individuals showing the phenophase); little synchronic/low synchrony (20-60%); or high synchrony (> 60%).

To evaluate the correlation between abiotic variables (depth, temperature, and rainfall) and each phenological event, we utilized Pearson's correlation (Zar, 2010).

RESULTS AND DISCUSSION

POLYGONUM HISPIDUM

In the first year of sampling, this species presented a seasonal flowering peak with mean date in February (Figure 2A), during the rainy season but still in the dry phase (Figure 1). Synchrony of flowering was median ($r = 0.48$), with seasonal phenophases ($Z = 48.497$; $p < 0.05$). The fruiting peak was seasonal ($Z = 1052.596$; $p < 0.05$), with mean date in April (Figure 2B), at flood when rainfall decreased (Figure 1), with high synchrony ($r = 0.77$). For synchrony in the flooding phases, flowering presented asynchrony only in the receding phase, with low synchrony during the other phases, yet for fruiting there was a high synchrony at flood phase with deeper waters, and low synchrony in the rising phase, with asynchrony in the other phases (Table 1). However, there was no significant correlation between flowering, fruiting, and the measured parameters.

Table 1. Synchrony of phenophases with flooding phases (drought, rising, flood, and receding), over two years, in percentage of flowers/buds (flowering) and imature/mature fruits (fruiting), for the aquatic macrophytes *Polygonum hispidum* and *P. punctatum* in ponds of the *Pantanal* wetlands, Corumbá, Mato Grosso do Sul. Degree of synchrony: a = asynchronic, b = low synchrony, c = high synchrony (Bencke & Morellato, 2002a). Year 1 (2012-2013) = November 2012-January 2013 (drought); February-March 2013 (rising); April-July 2013 (flood); August-November 2013 (receding); Year 2 (2013-2014) = December 2013-March 2014 (drought); April-May 2014 (rising); June-July (flood); August-October 2014 (receding).

	Flowering				Fructification			
Flood phase	Drought	Rising	Flood	Receding	Drought	Rising	Flood	Receding
P. hispidum								
Year 1	37.38^b	30.1^b	35.52^b	0^a	0^a	32.13^b	67.87^c	0^a
Year 2	0^a	39.13^b	0^a	60.97^c	0^a	41.97^b	0^a	58.03^b
P. punctatum								
Year 1	26.45^b	31.48^b	39.43^b	2.65^a	13.9^a	35.3^b	47.96^b	2.85^a
Year 2	44.79^b	27.88^b	6.95^a	20.38^b	24.01^b	24.46^b	7.98^a	43.55^b

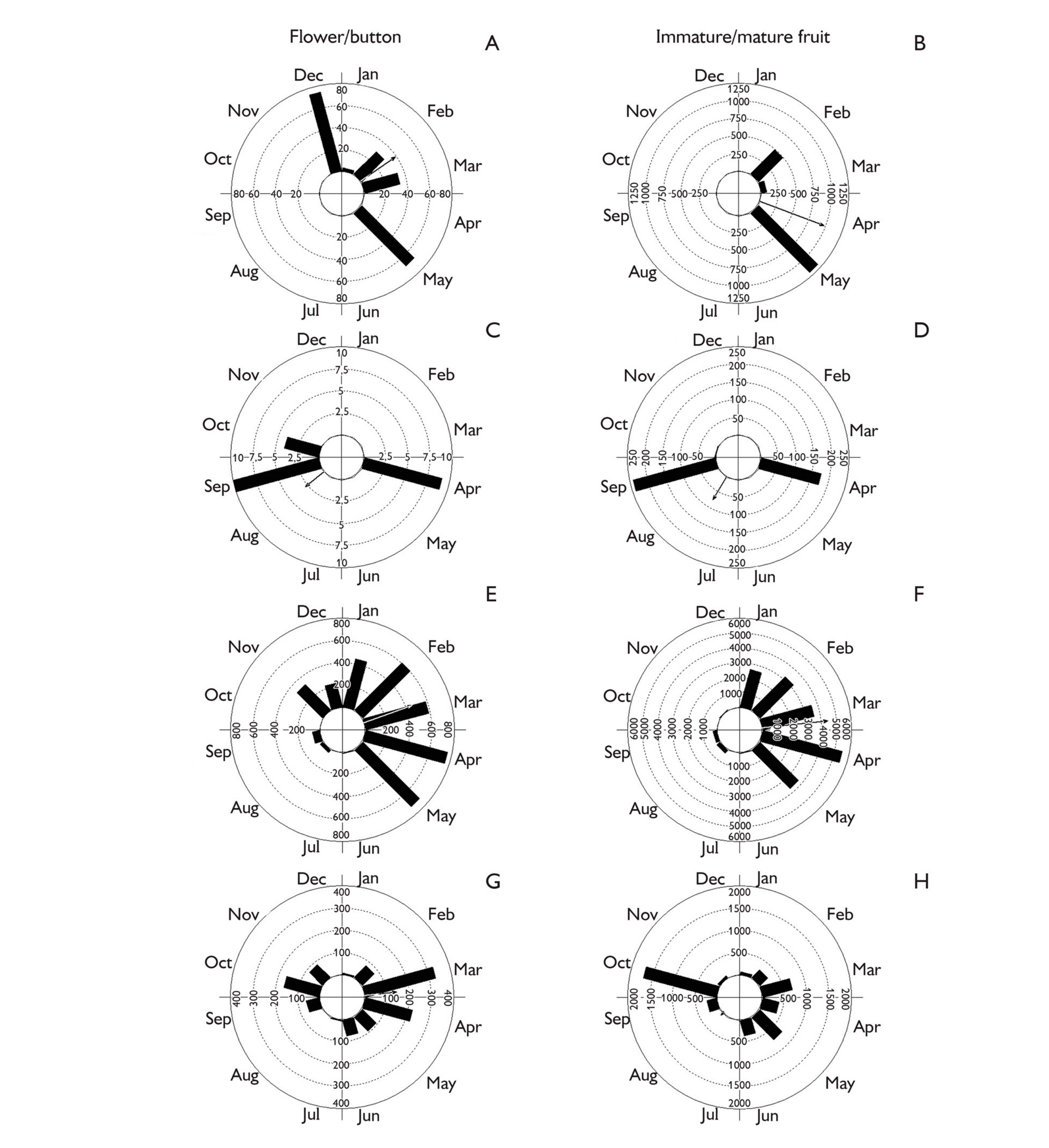

Figure 2. Circular histogram of *Polygonum hispidum* (A, B, C, D) and *P. punctatum* (E, F, G, H) in ponds of the *Pantanal*, with the number of flowers/buds (A, C, E, G) and immature/mature fruits (B, D, F, H), over two annual cycles (2012-2014). A, B, E, F = 2012-2013; C, D, G, H = 2013-2014 (rA = 0.73, $p < 0.001$; rB = 0.68, $p < 0.001$; rC = 0.83, $p < 0.005$; rD = 0.79, $p < 0.001$; rE = 0.73, $p < 0.001$; rF = 0.68, $p < 0.001$; rG = 0.83, $p < 0.005$; rH = 0.79, $p < 0.001$). September 2012-January 2013 (drought); February-March 2013 (rising); April-July 2013 (flood); August-November 2013 (receding); December 2013-March 2014 (drought); April-May 2014 (rising); June-July (flood); August-October 2014 (receding).

In the second year, the community also exhibited a seasonal pattern for phenophases, with the seasonal flowering peak and mean date in August (Figure 2C) at the begining of the rising phase when depth is shallow (Figure 1), and low synchrony ($r = 0.27$) but non seasonal ($Z = 1.708$; $p > 0.05$). The fruiting peak showed the mean date in the same month and phase (Figure 2D), with low synchrony ($r = 0.30$) but significantly seasonal ($Z = 38.312$; $p < 0.05$). Throughout the flooding phases, the synchrony of flowering was high in the receding phase when water was still deep (Figure 1) and low in the rising phase with deep water and low rainfall (Figure 1), and asynchronic in the other phases. For fruiting, the asynchrony was evident in the drought and flood phases but with low synchrony in the rising and receding phases (Table 1). The correlation was only significant between flowering and water depth ($r = 0.60$; $p < 0.05$) but not for fruiting.

Amphibious plants grow on pond margins and can present different propagation strategies, with seeds being stored in the soil seed bank, and vegetative sprouting from the stem nodes (cloning). During the flood cycle, as soon as water enters the plain in the rising phase, the soil becomes wet and new seedlings are recruited from the diaspore bank, as well as new vegetative sprouts, thereby increasing the population density. Both *Polygonum* spp. are commonly found in the soil seed bank (Catian, 2015). In both sampling years, drought was prominent and extrapolated the usual duration. This fact is explained by the annual flood pulse in years following large floods and pronounced droughts, considered as pluriannul periods by Nunes da Cunha & Junk (2001), as the rising phase brought back water, the main resource to stimulate plant reproduction and propagation.

Like other macrophytes, this species propagates itself in phases with greater presence of water, leading plants to fruit at flood when water reaches its greatest depth and remains until receding, at which time most seeds are dispersed. This process enlarges areas of the species occurrence, with higher survival rates of young individuals, allowing genetic fluctuations between populations (Janzen, 1980; van der Pijl, 1982; Howe & Miriti, 2004). Between average floods, soils remain moist and ponds do not dry out, favoring reproduction, what explains the correlation with depth in the second year. The fact that the cycle of the previous year presented higher rainfall favored emergence of seedlings, which reached maturity in the receding phase. Since the species disperses its fruits hydrochorically, plants were apt to develop flowers at rising and to fruit at flood. Yet, we observed in the field that individuals can flower at any time of the year as long the soil is moist, which ends up interfering with the synchrony. According to Pott & Pott (1986), in this genus flowers are produced in various months of the year. However, some degree of fruiting of amphibious species occurs in flooding phases, since many have hydrochoric traits on their diaspores, *e.g.*, the achene of *Polygonum*, with the remaining perianth keeping stored air, facilitates dispersal by water, as observed by Staniforth & Cavers (1976). For colonization of new areas, the transport of diaspores (Hamilton & May, 1977) and the spectrum of seed dispersal are essential (Hughes *et al*., 1994).

POLYGONUM PUNCTATUM

In the first sampling year, this species presented a peak of seasonal flowering with mean date in March, at the end of the rising phase (Figure 2E). Synchrony of flowering was median ($r = 0.56$), with seasonal phenophases ($Z = 1183.214$; $p < 0.05$). The fructification peak was seasonal ($Z = 10231.518$; $p < 0.05$), with mean date in the same month and phase (Figure 2F), with high synchrony ($r = 0.73$). In the flood phases, flowering was asynchronic in the receding phase, with low synchrony in the other phases, while fructification had low synchrony in the rising and receding phases and asynchrony in the other phases (Table 1). There was significant correlation only between fruiting and water depth ($r = 0.66$; $p < 0.05$), but not regarding flowering.

In the second year of sampling, the community also showed a seasonal pattern for the phenophases, with a seasonal peak of significant correlation and mean date and phase similar to the first year (Figure 2G), and median synchrony ($r = 0.36$), significantly seasonal ($Z = 139.822$; $p < 0.05$). However, the fruiting peak presented the mean date in August (Figure 2H), onset of the receding phase, with low synchrony ($r = 0.032$), significantly seasonal ($Z = 4.465$; $p < 0.05$). Between the flooding phases, flowering showed asynchrony at flood and low synchrony in the other phases, the same occurring for fruiting (Table 1). There was no significant correlation between flowering, fruiting, and the measured parameters.

This species occurs in various aquatic environments (Pott & Pott, 2000). We observed various individuals on the margin of ponds at drought and inside the ponds at flood. This occurs due to the morphological adaptations of species of this group, such as aerenchyma. Furthermore, according to Lorenzi (2000), this species is weedy due to a high seed set and its high adaptation capacity. When in water, it flowers and fruits. At the onset of the rising phase, many seedlings are recruited from the seed bank, and at flood these already flower, which explains the median synchrony. At the end of the rising flood phase, there was high rainfall (Figure 1), thus favoring flowering, although probably influenced by other environmental factors and by pollinator activity (Morellato, 1991; Morellato & Leitão-Filho, 1992). Ponds directly favor communities of pollinators due to the water requirement by various species of bees (Abou-Sharaa, 2012; Jönsson *et al*., 2015; Stewart *et al*., 2017), increasing the pollination of the plant species present.

Although the seeds do not have apparent hydrochoric traits, many are released during the aquatic phases, and the persistent perianths trapping air confer buoyancy to the achenes (Staniforth & Cavers, 1976). Despite the lack of correlation between the parameters, a dependence on water for fruit dispersal was observed in the second year. Many species of aquatic macrophytes produce fruits in the receding phase, with a drop of water level, for dispersal. As observed by Catian (2015), receding is the phase when this species invests more in the biomass of flowers, fruits, and vegetative propagation structures. We perceive an adjustament to the optimal period of seed dispersal for amphibious species, such as observed for *cerrado* species by Oliveira (2008). However, this would need further studies.

The flood phases do not reflect the variations in the abiotic factors such as rainfall, which means that in the Miranda-Abobral subregion the flood phase does not indicate high rainfall in spite of increased flood depths that occur in the *Pantanal* from north to south due to the increasing water level of the Paraguay River floodplain. According to Hamilton *et al*. (2002), the flood extends into the dry season. Although the species is amphibious, it produces more fruits in the season when there is more water on the plain and not necessarily with greater rainfall, relating to the way of diaspore dispersal.

CONCLUSIONS

The flooding cycle has a great influence on the phenology of *Polygonum hispidum* and *P. punctatum*, with flooding phases with an abundance of water being the most important for the production of flowers and fruits.

The synchrony between reproductive cycles and the rising, flood, and receding phases suggests a prominent importance of seasonality of the flooding cycle in the phenological patterns of these *Polygonum* species in the *Pantanal*.

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