Reproductive phenology and germination of *Eleocharis laeviglumis* R. Trevis. & Boldrini (Cyperaceae)

Camila Luisa Bernhardt Demeda¹*, Guilherme Dubal dos Santos Seger², Neusa Steiner¹ and Rafael Trevisan¹

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ABSTRACT

Aquatic macrophytes are important components of freshwater ecosystems, of which Cyperaceae is the most diverse family. Many species of Cyperaceae form dense populations along lake margins of the southern coast of Brazil, but little is known about their sexual reproductive strategies. We characterized the reproductive life cycle of *Eleocharis laeviglumis*, an abundant emergent macrophyte of coastal wetlands in southern Brazil, by assessing its reproductive phenophases and estimating the number of its flowers and potentially viable fruits per inflorescence in a natural population. We also tested seed germinability and vigor for a period of four months during a single reproductive season. The species possesses dichogamous and protogynous spikelets with an average duration of 34 days (pre-anthesis, 1.7 d; anthesis, 6.9 d; fruit maturation, 22.3 d; fruit dispersion, 3.2 d). More than half of the flowers (62.2 %) developed into fruits, while only 5.5 % of the seeds germinated. Germinability and vigor decreased during the reproductive season. Some culms probably originate from asexual reproduction or sexual reproduction of clones. For restoration purposes, we suggest that *E. laeviglumis* should be propagated by sowing seeds collected at the beginning of the reproductive season, along with the transplantation of rhizomes.

Keywords: aquatic macrophyte, coastal lake, Cyperaceae, *Eleocharis*, sexual reproduction, spikelet, germinability, reproductive phenology, seed vigor

Introduction

Cyperaceae is the third largest monocot family (Govaerts et al. 2007), and is widely dispersed around the globe but concentrated in the tropics (Goetghebeur 1998). The family comprises graminoid herbaceous species of varied sizes. In general, species possess leaves with closed sheaths, tiny and discrete wind-pollinated flowers with various arrangements on spikelets, and abundant and regular diaspore production (Goetghebeur 1998; Leck & Schütz 2005). Each diaspore is generally composed of an achene and reduced perianth parts, usually bristles (Goetghebeur 1998).

Several genera of the family are associated with humid or truly aquatic environments (Cook et al. 1974), composing the most diverse aquatic macrophyte family in freshwater ecosystems (Chambers et al. 2008). The majority of macrophyte species of Cyperaceae are emergent (Cook et al. 1974), with the genus *Eleocharis* being one of the most diverse genera in Brazil (Alves et al. 2009). *Eleocharis* is distinguished from other Cyperaceae genera by possessing culms with a single terminal spikelet, leaves reduced to

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¹ Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil
² Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil

* Corresponding author: camila_demeda@hotmail.com
tubular sheaths in the culm base, and a thick and persistent stylodium on the fruit (Goetghebeur 1998; Trevisan & Boldrini 2008).

Eleocharis laeviglumis is a perennial and emergent macrophyte endemic to southern Brazil that it is well represented in coastal wetlands (Trevisan & Boldrini 2006), where it is usually found grouped in monospecific clusters. As for most perennial macrophytes, E. laeviglumis has a mixed reproductive strategy, involving both sexual and asexual reproduction, which can benefit its propagation (Eckert et al. 2016). Its flowers are hermaphroditic and its achenes hold bristles adapted to water dispersion (Trevisan & Boldrini 2006). In addition to achene production, the species possesses a complex underground stem system that favors the growth of dense populations (Trevisan & Boldrini 2006).

Freshwater ecosystems, especially Neotropical coastal lakes, are among the most vulnerable habitats in the world (Esteves et al. 2008) and frequently need to be restored. Macrophytes can be useful for the recolonization of such disturbed habitats (Brown & Bedford 1997; Nishihiro & Washitani 2007). Moreover, as with other macrophytes (Dhote & Dixit 2009; Sakakibara et al. 2011), E. laeviglumis can accumulate heavy metals in its underground tissues, showing potential for phytoextraction of contaminated environments, especially those at mining sites (Silva 2015). However, the flower dynamics, diaspora production, and seed germinability of E. laeviglumis, as well as its reproductive phenology, remain unknown. Knowledge of the species’ reproductive phenology and seed germinability and vigor are of extreme importance to understanding its reproductive success. Therefore, our study aimed to describe part of the reproductive cycle of E. laeviglumis, including spikelet phenology, fruit set, and seed germinability and vigor.

Materials and methods

Species and study area

Eleocharis laeviglumis R. Trevis. & Boldrini is a perennial herb that produces culms of up to 65 cm in height, which are unbranched and with leaves reduced to tubular sheaths. Clonal expansion occurs via rhizomes and can form large clonal clusters. Vegetative culms have an aborted inflorescence at the apex while fertile culms have one terminal spikelet. Flowers within a spikelet are hermaphroditic with three stamina, a pistil with three stigmatic branches and a single ovule. Diaspores, or dispersion units, are composed of an achene with a reticulated surface, a persistent stylodium and 6-7 bristles adapted to flotation (Goetghebeur 1998; Trevisan & Boldrini 2006).

The species is endemic to southern coastal wetlands of Brazil (Trevisan & Boldrini 2006) and is one of the dominant emergent macrophytes in Lagoa Pequena (LP), a freshwater coastal lake 600m from the ocean on southern Florianópolis island, Santa Catarina, Brazil (27°39’17.9"S; 48°28’36.2”W). The ecosystem supports a rich macrophytic flora, with Cyperaceae being the richest taxon (Ferreira et al. 2017). Although LP is located in an urban area and suffers from human impacts, it is partially surrounded by restinga forests, dunes and wetlands (Falkenberg 1999).

Reproductive phenology

A brief description of spikelet phenophases and their respective developmental time in days was accomplished by marking and following fertile culms daily in LP. A minimum of 20 and a maximum of 26 spikelets per phenophase were followed during November 2016 to January 2017, for a total of 33 observed culms. The mean and standard deviation were calculated for the length of each phenophase.

To estimate flower and seed set per spikelet, fertile culms were collected from LP during the beginning of the 2016-2017 reproductive season (December and January). We collected 20 culms for each of the phenophases of anthesis and fruit maturation, and counted the number of flowers and potentially viable fruits, respectively. Diaspores with a filled achene were considered potentially viable fruits due to their clear differentiation from empty (i.e., not developed) diaspores. The mean and standard deviation were calculated for the number of flowers and seed set per spikelet.

Germinability and seed vigor

Diaspores, here also referred as seeds, were collected four times between December 2016 and March 2017. Seeds were processed in the laboratory by selecting those that were considered potentially viable (those that were filled), randomly mixing them (within each temporal sample separately) and separating them into working samples for germination testing. For testing, seeds were immersed in 70% alcohol for 30 seconds followed by 40 seconds in a 2% sodium hypochlorite solution and then washed three times with distilled water, following the standard and accepted protocol for seed germination (adapted from Baskin & Baskin 2014).

For each of the four temporal samples, seven replicates of 15 seeds were placed in plastic Petri dishes with moistened filter papers and then incubated under 25 ± 2 °C and a 12 h photoperiod (142 μmol m-2s-1) in a germination chamber. Temperature and photoperiod simulated local averages during the months of dispersion (spring-summer) in the study area. Seeds were watered and checked daily for germination, with coleoptile protrusion indicating the occurrence of germination (Walters 1950; Bewley et al. 2013). The test lasted 39 days, ending when no more seeds germinated for seven consecutive days.

Seed vigor for each temporal sample was determined and compared using the Germination Speed Index (GSI)
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proposed by Maguire (1962). The index follows the formula: 
\[ \text{GSI} = \sum (\text{NGS} / \text{DAS}) \], where NGS corresponds to the non-cumulative number of germinated seeds and DAS to the number of days after the test started. For tetrazolium test we submitted part of the non-germinated seeds to a 2,3,5-triphenyl tetrazolium chloride solution (1 %) for 24 hours at 35 °C in the dark (Baskin & Baskin 2014). One third of the seeds of each temporal sample were tested (35/105). Seeds were considered to react positively to tetrazolium when embryo tissues stained pink.

**Data analysis**

Data were analyzed by nonparametric statistics because they were not normally distributed and not homoscedastic. Germination percentages, as well as GSI’s, among replicates and samples were analyzed separately using the Kruskal-Wallis test. When significant differences were found, a Dunn’s post-hoc test was performed. Differences were considered significant at the 95 % confidence interval for both tests. All statistical analyses were performed using R Statistical Environment v. 3.3.2 (R Core Team 2016).

**Results**

**Reproductive phenology**

The sampled population of *Eleocharis laeviglumis* is relatively small and forms a dense and reasonably isolated monospecific cluster along the north margin of LP. The species reproduces during spring and summer (November to March) with a flowering peak in November (personal observation), according to the number of culms observed. Each spikelet took on average 34.1 days to complete its developmental cycle (Tab. 1), with relatively synchronous flowering. Although fertile culms were not quantified, a decrease in the amount was observed over the course of the reproductive season. We observed the following reproductive phenophases: pre-anthesis, gynoecium anthesis (♀), concomitant gynoecium and androecium anthesis (♀/♂), androecium anthesis (♂), fruit maturation, and fruit dispersion (Tab. 1 and Fig. 1); fruit dispersion was considered the detachment of the diaspore from the spikelet.

Spikelets developed completely underwater, taking 1.7 ± 0.6 (min. of 1 and max. of 3) days from emersion until...
anthesis. Flowers within a spikelet were hermaphroditic and dichogamous, with androecium and gynoecium anthesis segregated over time. Gynoecium anthesis preceded androecium anthesis, characterizing a protogynous inflorescence. Gynoecium anthesis occurred in the first 3.6 ± 1.1 (min. of 2 and max. of 6) days, and was followed by concomitant exposure of the stigma and stamens for 0.5 ± 0.5 (min. of 0 and max. of 1) days in 53.8% of the spikelets observed. Both stigma and stamens protruded from the inflorescence. Stamen exposure lasted 2.8 ± 1.2 (min. of 1 and max. of 5) days, for a total of 6.9 days for a spikelet to fully complete anthesis.

Spikelets in anthesis produced 57.2 ± 6.1 flowers (min. of 46 and max. of 67) (Fig. 1). The next and longest phenophase observed was fruit maturation, with a duration of 22.3 ± 4.1 (min. of 17 and max. of 31) days. During this phenophase, each spikelet produced 35.6 ± 11.5 fruits (min. of 13 and max. of 50), with 62.2% of the flowers developing into potentially viable fruits. Fruit dispersion took 3.2 ± 1.1 (min. of 1 and max. of 6) days with each fruit being dispersed with its respective glume. Diaspores located on the bottom of spikelets were rarely dispersed, and remained attached to the inflorescence rachilla.

Germinability and seed vigor

The seeds of *Eleocharis laeviglumis* began to germinate after 14 days during the test, with only 5.5% germinating (23/420), considering the four temporal samples. Within temporal samples, 15 seeds germinated (14.3%) in the first sample, six (5.7%) in the second, and only one seed (0.9%) in the third and fourth samples each (Fig. 2A). Significant differences in germination were not observed among replicates ($\chi^2 = 3.19; P = 0.78$) but were observed among samples ($\chi^2 = 13.05; P = 0.004$). The first sample, from the beginning of the 2016-2017 reproductive season, differed from the last two (both with $Z = 3.09$ and $P = 0.001$), which corresponded to the end of the season. Although the number of seeds per sample was not quantified, seed availability for collection was observed to be scarcer for the last two samples.

According to GSI, replicates did not differ significantly in seed vigor ($\chi^2 = 3.78; P = 0.7$), but significant differences were found among samples ($\chi^2 = 12.1; P = 0.007$). The calculated GSI for samples decreased temporally (first=0.08; second=0.03; third=0.01; fourth=0) (Fig. 2B). Similar to the germination results, the GSI of the first temporal sample differed from that of the last two (Dec-Feb with $Z = 2.92$; Dec-Mar with $Z = 3.01$; and both with $P = 0.001$). Only 22.5% (32/140) of all the seeds tested with tetrazolium reacted positively. Seed vigor, according to tetrazolium reaction, also decreased as the reproductive season progressed. Whereas 48% of seeds reacted positively to tetrazolium in the first sample, just 23% showed some reaction in the second. This rate was even lower in the third and fourth samples, with positive reactions in 11% and 8% of the seeds, respectively (Fig. 2C).

Discussion

The spikelet of *Eleocharis laeviglumis* is dichogamous and protogynous, and takes around 34 days to develop and disperse seeds, which have low germinability and vigor. Dichogamy, the temporal segregation of androecium and gynoecium anthesis, is a strategy that promotes outcrossing (Lloyd & Webb 1986) and is common among wind-pollinated species (Friedman & Barrett 2009). Protogyny has been previously attributed to Cyperaceae (Goetghbeur 1998), as
well as concomitant exposure of stigma and stamens (Snyder & Richards 2005; Costa & Machado 2012). This concomitant exposure of female and male whorls characterizes incomplete dichogamy and can open a temporal gate for self-pollination (Lloyd & Webb 1986). Although little is known about breeding systems in Cyperaceae, both auto-compatibility (Cladium jamaicense, Snyder & Richards 2005) and auto-incompatibility (Scirpus maritimus, Charpentier et al. 2000; Rhynchospora ciliata, Costa & Machado 2012) have been previously described for the family.

We found that each E. laeviglumis spikelet produced on average 57.2 flowers, which differs from the 20-50 flowers per spikelet reported by Trevisan & Boldrini (2006). Wiens (1984) states that number of flowers can be determined by environment factors and resource availability via selection of particular genotypes, and that there can be variation in locations and time scales. The seed set of 62.2% found for E. laeviglumis in the present study is consistent with the 57.2% estimate of seed-ovule ratio for herbaceous perennials of the North American flora (Wiens 1984). This is because each flower of E. laeviglumis has one ovary, which develops into an only-seeded fruit, thus the percentage of ovules developing into seeds (seed-ovule ratio) is equivalent to flowers developing into fruits. Although seed set was regular, seeds presented low germinability (5.5%). Low germinability rates were also described for other perennial Eleocharis species, such as E. acuta (0.9 %), E. dietrichiana (3.4 %) and E. pusilla (2.6 %) from Australia. Even with low germinability, most seeds of these species maintained vigor after a 33-month burial (E. acuta 91.4 %, E. dietrichiana 83.1 % and E. pusilla 90.4 %), which indicates persistence in seed banks (Bell & Clarke 2004). However, this does not seem to be the case for E. laeviglumis, since seeds showed relatively low vigor (22.5 %).

Seeds collected in December 2016 resulted from flowers fertilized during the flowering peak in November/2016, which could explain the differences in both germinability and seed vigor found between this sample and the last two. The greater quantity of fertile culms observed at the beginning of the reproductive season could have affected the amount of pollen available for fertilization, and thus increasing the probability of cross-fertilization. The relatively small size of the species throughout its development, the fewer fertile culms at the end of the season, and the consequent greater distance between culms could have contributed to reduced pollen dispersion and fertilization (Friedman & Barrett 2009). In this way, the higher seed germinability and vigor observed early in the reproductive season could be a result of cross-fertilization between flowers of the previous month.

Vegetative reproduction of E. laeviglumis can also be understood as a factor that influences the effectiveness of sexual reproduction. Perennial aquatic plants are known to use both sexual and asexual reproduction (Eckert et al. 2016), which when combined represents a strategy that can maximize fitness (Fenner & Thompson 2005). However, E. laeviglumis can form extensive clonal clusters, which can result in fertilization among clones, a phenomenon previously observed in species of Carex (Friedman & Barrett 2009) and C. jamaicense (Ivey & Richards 2001), both of which are wind-pollinated species of Cyperaceae with unisexual flowers. Geitonogamy, or fertilization among clones, can reduce fitness through inbreeding depression and stigmatic clogging in both self-compatible and self-incompatible plants, respectively (Eckert et al. 2016), being that inbreeding depression was already reported for some Carex species (Ohkawa et al. 2000, M’Baye et al. 2013). We thus suggest that pollination among clones can occur in the population sampled and may be causing the observed decrease in seed vigor and germinability.

The dynamics of sexual reproduction of E. laeviglumis is consistent with that previously observed for wind-pollinated species. We observed temporal gaps in flowering that can enable self-pollination within the same spikelet. There are no data on the compatibility of flowers of E. laeviglumis, which we believe it is a subject worth exploring. Nevertheless, our results suggest that geitonogamy is a plausible explanation for the low germinability and vigor of seeds in the studied population. However, this suggestion can only be confirmed by studying the genetic composition of the population.

Considering that fertile culms of E. laeviglumis develop reasonably fast and produce a fair amount of fruit, we suggest that the species could be a good candidate for wetland restoration. Therefore, we conclude that for restoration purposes the mixed reproductive strategy of the species should be taken into consideration; that is, the propagation of E. laeviglumis would be most effective by sowing seeds along with transplanting rhizomes. We also recommend that seeds be collected at the beginning of the reproductive season. Such a propagation strategy could help to guarantee genetic variability for the established population.

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