



Life-history traits and density dependence in metapopulations of a tropical moss: a monoicous species that is almost dioicous

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Abstract

Life-history traits, such as reproductive allocation, sexual expression, sex ratio, and reproductive success, are central aspects of a species' ecology and evolution. For example, bias in male and female sex expression may play a large role in determining the viability of populations in the face of environmental pressures, such as population fragmentation, climate change and habitat occupancy. Thus, in this study, we investigated reproductive traits in 10 meta-populations of *Fissidens flaccidus* Mitt. From each meta-population, 30 patches were randomly selected, and 1 cm² samples were collected from each patch. A total of 20,173 ramets were analyzed and classified into male, non-sporophytic female, sporophytic female, and non-sex expressing. In addition, population density in each patch was quantified. Our results showed that relative reproductive allocation in perigonia and sporophytes is greater than perichaetia. Trade-off between sexual relative reproductive allocation and asexual gemma production was observed, suggesting an important role of female ramets in asexual reproduction. The number of male ramets does not influence the reproductive success observed in each patch, and ramet density may induce male sex expression. Thus, we concluded that reproductive allocation in male function is efficient, since fewer male ramets can assure a considerable reproductive success. Furthermore, our results suggest that there may be a habitat preference between the sexes, since male ramets are found in patches with high density and mostly below female ramets, suggesting an avoidance of direct sunlight by male ramets.

Keywords Reproductive allocation · Sexual dimorphism · Sex ratio · Sexual expression · Trade-off

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This study has the potential to be a Highlighted Student Paper because most of the ideas, hypotheses, and questions were conceived by me during the period of pandemic restrictions and because the findings are unprecedented considering the data available in the literature for monoicous bryophytes. In this article, we investigated the biology and ecology of a tropical moss species with a monoecious sexual system, *Fissidens flaccidus*, which becomes functionally dioecious due to sex segregation. Several reproductive traits found in *F. flaccidus* such as female-biased sex ratio, low male sexual expression, and higher male reproductive allocation are not common in monoicous species. Our data indicate that *F. flaccidus* behaves like a dioecious species, expanding our view regarding the diversity of reproductive strategies found in bryophytes.

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Introduction

Life history is a schedule of key events in an organism's life cycle, and is usually defined in terms of life-history traits averaged across individuals within a population (Oli and Coulson 2016). This life history theory asserts that vital functions, such as growth, reproduction, maintenance and defense, compete for limited resources obtained by an organism (Delph 1999). It is often observed that the allocation of resources in different traits of the organism's life history induces trade-offs (Stearns 1976; Delph et al. 1996). Such trade-offs represent the costs paid when the available resource is allocated differentially among traits (Stearns 1989; Obeso 2002). For example, in the aquatic monocot *Butomus umbellatus* L., plants that are pollinated show a significant reduction in clonal bulbil (Thompson and Eckert 2004). Thus, trade-offs are commonly observed in reproductive traits, showing a crucial influence in the demography and maintenance of populations (Rydgren and Okland 2003; Horsley et al. 2011).

Reproductive traits, such as sex expression (proportion of individuals that are expressing sex) and sex ratio (proportion of females and male in a population), are important variables influencing the reproductive performance of population (Glime and Bisang 2017b). In this context, many biotic or abiotic factors can affect development and reproductive traits. For instance, some species need high levels of humidity to express sexuality, as during the rainy season, while others do not (Maciel-Silva et al. 2012; Maciel-Silva and De Oliveira 2016). Sexual reproduction plays an important role in maintaining species, and in some cases, population density is a determining factor of reproductive performance. Density dependence has been recurrently reported in species of plants and animals (Hanski 1990; Gunton and Kunin 2009). Furthermore, sexual systems which are defined by Leonard (2018) as “pattern of gender allocation that characterizes a species”, have also been linked to the reproductive success of populations, for example in *Fissidens scarious* Mitt., and *Fissidens submarginatus* Bruch., which have reproductive traits differently associated with sexual systems (Santos et al. 2020).

In plants, the sexual system is classified in relation to the distribution (distance) between reproductive structures (Bergh and Verboom 2011). Species that have male and female reproductive structures on the same plant are classified as monoecious, and dioecious species that have reproductive structures on separate plants. However, in bryophytes, as the reproductive structures are produced in the haploid phase, these systems are called monoicous and dioicous (Glime and Bisang 2017a).

The simple architecture of vegetative and reproductive organs of bryophytes makes them excellent models for ecological studies (Harris et al. 2020). Indeed, bryophytes are considered models to understand the ecology and evolution of sexual systems (Suzuki et al. 2018; Harris et al. 2020), reproductive allocation (Stark and Brinda 2013; Santos et al. 2022), and reproductive cost (Bisang and Ehrlén 2002; Rydgren and Økland 2002). Evidence suggests that reproductive allocation is strongly related to sexual systems in bryophytes. Sexual systems of bryophytes present a gradient of distance between the sexes, and the more distant the sexes, the greater is the relative reproductive allocation (proportion of resource allocated to reproduction) in the male function is expected (Stark and Brinda 2013). Reproductive allocation may also change over different reproductive seasons, mainly on functionally dioecious plants, in which sex expression may change following environmental variation (Glime and Bisang 2017a). Indeed, reproductive allocation may be more responsive to different habitat conditions in functionally dioicous bryophytes (rhizautoicous system which male and female ramet are spatially separated, however attached only by rhizoids) (Stark and Brinda 2013; Santos et al. 2018) than in dioicous species (Alvarenga et al.

2013). Given the importance of dioecious systems in the promotion and maintenance of genetic diversity of plants (Renner and Ricklefs 1995; Barrett 2002), studies on functionally dioecious species may clarify the role of habitat heterogeneity in the sex expression (Stehlik et al. 2008), including reproductive allocation.

In this study, we quantified the reproductive allocation (absolute and relative), and the following reproductive traits: sexual expression, sex ratio, reproductive success, and population density for a monoicous species that has similarities with dioicy. *Fissidens flaccidus* Mitt. is a species of moss with a rhizautoicous sexual system that reproduces sexually and asexually (by clavate gemma in stem tissues). The rhizautoicous system presents individualized male and female ramets that are connected, at least initially, by rhizoids. This sexual system, therefore, functionally resembles the dioicous system, since the ramets presumably do not compete for resources for their development and formation of reproductive structures. In this context, we investigate the following questions: First, is male relative reproductive allocation greater than female? This is the usual pattern found in dioicous mosses for pre-fertilization allocation (Stark and Brinda 2013), and rhizautoicous species have segregation of sexual functions (functionally dioicous). Therefore, we expect the reproductive allocation at the prezygotic level to be greater in male sexual function compared to female sexual function. Second, is there a trade-off between sexual and asexual reproduction? Since, according to life history theory, the resources available to individuals are finite, and these resources are subject to competition among different life history features or phases (Oli and Coulson 2016). Therefore, we expect that ramets that have higher relative reproductive allocation will produce less gemmae. Third, is the number of male ramets a determinant greater reproductive success for females? Since the greater the quantity of male ramets expressing sex, the greater the quantity of male gametes and consequently the chance of fertilization of the female gametes. Thus, we expect that the meta-populations that have the highest number of males will, consequently, have greater reproductive success. Fourth, is meta-population density related to the sexual expression of ramets? As density dependence effects are recurrent in many animals and plants, we expect that meta-population density influences the reproduction of the species.

Materials and methods

Study species, study site, and sampling

Fissidens flaccidus Mitt. is a monoicous acrocarpous moss with rhizautoicous sexual system. The rhizautoicous system has male ramets, those that have perigonia (modified leaves

enclosing antheridia) and female ramets, those that have perichaetia (modified leaves enclosing archegonia) connected by rhizoids. *F. flaccidus* is distributed in the Neotropical region, Africa, New Guinea, and Australia (Pursell 2007). In Brazil, the species has a wide distribution, so that they are found in all Brazilian phytogeographic domains (Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Wetland) (Bordin and Yano 2013; Flora do Brasil 2021). *F. flaccidus* produces sporophytes in the rainy season, in addition to presenting clavate gemmae in ramets (Santos et al. 2018).

Sampling was carried out in July 2021 in the gardens of the Biosciences Center of the Federal University of Pernambuco (Recife, Brazil). According to Alvares et al. (2013), Recife has a tropical, hot, and humid climate according to the Köppen classification, with an average monthly temperature of 23 °C. The rainy season occurs in the autumn–winter period (March–August), with June–July being the rainiest months (Coutinho et al. 1998). The Biosciences Center has isolated gardens, in which meta-populations of *F. flaccidus* are often found. Thus, we selected 10 gardens to collect the material. The size of the gardens where the samples were collected ranged from 144m² (12 × 12 m) to 400m² (20 × 20 m), and each garden presented a minimum distance of 10 m. The sizes of the patches varied considerably, since some patches had few ramets while others had an area larger than 900cm² (30 × 30 cm). In each meta-population, 30 patches were randomly selected. In each selected patch, samples of 1 × 1 cm were collected, totaling 30 cm⁻² per meta-population. The samples were placed in small Petri dishes to preserve the structures in the ramets.

In the laboratory, the samples were analyzed to confirm the species identification. The ramets were then quantified for each sample and classified into the following categories: (1) male—ramets with perigonia; (2) non-sporophytic female—ramets with perichaetia; (3) sporophytic female—ramet with sporophyte, and (4) non-sex expressing ramet—without gametocia or sporophyte.

Reproductive allocation and gemma production

To quantify reproductive allocation and gemma production, 50 ramets were randomly selected from each of the following categories: non-sporophytic female; sporophytic female, and non-sex expressing, and 48 male ramets (male ramet density was lower in meta-populations of *F. flaccidus*) for the entire study. These collections were performed in meta-populations where male ramets had already been found. Since, where male ramets were found, the other categories of ramets were always found. For each ramet, the gemmae were separated of ramets and counted. Posteriorly gametangia were counted without destroying gametocia. *Fissidens* simple structure and distichous leaf arrangement makes it possible to quantify gametangia without destroying

gametocia. After, ramets were washed with distilled water. Then, the perigonia, perichaetia and sporophytes were extracted and stored in small paper envelopes together with its respective ramets.

Envelopes with the ramets and reproductive structures were then covered with aluminum foil and placed to dry in an oven for 72H at 70 °C. Once removed from the oven, the envelopes were placed in a box sealed with silica gel. Then, reproductive structures (perigonia, perichaetia and sporophytes), and ramets were weighed. To carry out the weighing, an ultra-micro-analytical balance model SE2 ultra-micro-balance, of Sartorius, Goettingen, Germany, precision of 0.1 µg was used.

After weighing, the reproductive allocation was quantified as absolute and relative. Absolute reproductive allocation was quantified by the biomass of the reproductive structures (perigonia, perichaetia, and sporophyte). Relative reproductive allocation was quantified as the proportion of biomass allocated to reproduction. We follow the formula: $RA = \frac{RB}{RB+VB}$ where RA is relative reproductive allocation, RB is reproductive biomass, and VB is ramet biomass (McLetchie and Puterbaugh 2000).

Reproductive traits

Sex expression, sex ratio, reproductive success, and meta-population density

Sex expression was calculated as the proportion of ramets that expressed sex for both the sample (1 × 1 cm) and for the entire meta-population. The sex ratio was quantified as the ratio of female to male ramets. Reproductive success was calculated by the proportion of female ramets that formed sporophytes. Finally, the meta-population density was quantified as the number of ramets/cm². All reproductive traits were calculated for the samples and for the entire meta-population.

Statistical analysis

Reproductive allocation and gemma production

After transformation (log), the data were not normally distributed, and non-parametric analyses were used. The data used was without the transformation. The Kruskal–Wallis test was used to compare ramet biomass, absolute reproductive allocation, relative reproductive allocation and gemma production between different sexual conditions. To compare gametangia of male and non-sporophytic female ramets, we applied a Wilcoxon test.

Once it was clear that gemma production varied with sex expression and sex, we tested possible trade-offs between the different functions. To test possible trade-off among reproductive traits (sexual *versus* asexual), we used Generalized Linear Model (GLM). First, we tested if gemma production is related to ramet biomass and absolute reproductive allocation. To answer this question, we created two models, a full model in which the amount of gemmae produced as response variable and the ramet biomass and the absolute reproductive allocation as predictor variables, with a Poisson distribution along with a model null, which tests the randomness of the data. Then a comparison was carried out between the two models with chi-square test. Since there were no differences between the models, no significance was observed. Even so, we checked the full model summary to see the results.

The second trade-off among reproductive traits was also tested with a GLM. We created two models, one being a full, with response variable as gamma production and predictor variables as relative reproductive allocation and ramet biomass and a null model. Since the chi-square test between null and full model was significantly different, we excluded the null model and analyzed the full model. The model summary was then analyzed for data interpretation, and interactions were tested. The distribution applied in the model was Poisson; since under-dispersion was observed, the distribution was changed to quasi-Poisson.

Reproductive traits

First, sexual expression was analyzed. The normality of the variable was tested, and non-parametric tests were applied, given the non-normality of the data. Thus, the expression rate between meta-populations was compared using the Kruskal–Wallis test. The sex ratio was quantified by the ratio of female ramets (sum of sporophytic and non-sporophytic ramets) divided by the number of males. The Chi-square test was applied to confirm the sexual bias of the meta-population. We applied the Kruskal–Wallis test to compare reproductive success between meta-populations. Metapopulation density was reported by density graphs.

Once we observed that the fertilization rate was higher in samples with high density and with greater numbers of male ramets, we applied a GLM to determine if the amount of male ramets and the density of the samples influence reproductive success. We created a full model, where the response variable was the reproductive success, and the predictor variables were the amount of male ramets and the sample density. Thus, full model was compared with null model. For both models, the distribution was binomial and compared via the chi-square test. Since the result was significant, the null model was excluded. The dispersion of the data was checked, and since it was over-dispersion, the model

distribution was changed to quasi-binomial. The model was analyzed using a test of variance via the F test.

The GLM was also applied to determine if meta-population density influences sex expression on male ramets. For that, two models were created, namely: full model, having as response variable the amount of male ramets and as predictor variable the density of samples, and null model. For both models, the distribution was Poisson. The full and null models were compared via chi-square test. Once the test was significant, the null model was excluded, and the full model was used to analyze the data. Data dispersion was checked and neither over-dispersion nor under-dispersion was observed.

The analyses were performed using the RStudio software version 1.4.1717 (RStudio Team 2021). The Multicomp package (Hothorn et al. 2016) was used to perform the post analysis of the GLMs. The Package ‘dunn.test’ (Dinno and Dinno 2017) was used to perform Dunn's test as a post hoc analysis in the Kruskal–Wallis test. The ggplot2 (Wickham et al. 2016) package was used to make the maps.

Results

Reproductive allocation and gemmae production

The male ramets had lower vegetative biomass compared to other ramets category (non-sex expressing, sporophytic and non-sporophytic females) (Table 1), which in turn did not differ from each other ($X^2 = 73.55$, $df = 3$, $P < 0.0001$) (Fig. 1. a). Allocation at the prezygotic level (perigonia and perichaetia) did not differ from each other (Fig. 1b). However, the absolute allocation of the sporophyte was significantly higher ($X^2 = 169.85$, $df = 3$, $P < 0.0001$) (Table 1). Relative reproductive allocation was similar between male plants and female sporophytic plants (Table 1), whereas non-sporophytic females had a significantly lower relative reproductive allocation than male and sporophyte plants ($X^2 = 151.26$, $df = 3$, $P < 0.0001$) (Fig. 1c). Gemma production was significantly lower in male plants ($X^2 = 62.38$, $df = 3$, $P = 0.0001$), whereas the other ramet categories did not differ from one another (Fig. 1d). Male and female ramets produced similar numbers of gametangia ($W = 948.5$, $P = 0.10$) (Table 1).

Regarding the trade-off between gemma production and the ramet and reproductive biomass variables, the full and null GLM models did not show significant differences, suggesting the model's non-significance; the summary of the full model indicated no association between the variables. Thus, ramet biomass and absolute reproductive allocation do not show any association (Table 2). On the other hand, the model that tested whether gemma production is associated to relative reproductive allocation and ramet biomass

Table 1 Mean and standard deviation of ramet mass, absolute reproductive allocation, relative reproductive allocation, and gemma production

Variables	Sexual condition				Kruskal–Wallis		
	Male ($X \pm SD$)	Non-sporophytic female ($X \pm SD$)	Sporophytic female ($X \pm SD$)	Non-expressing sex ($X \pm SD$)	X^2	df	P
Vegetative mass (mg)	0.0139 ± 0.0137	0.0639 ± 0.0315	0.0568 ± 0.0324	0.0655 ± 0.0377	73.55	3	< 0.01
Absolute reproductive allocation (mg)	0.0046 ± 0.0030	0.0070 ± 0.0028	0.0223 ± 0.0085	–	169.76	2	< 0.01
Relative reproductive allocation (%)	30.32 ± 14.22	11.88 ± 7.87	30.49 ± 8.19	–	150.98	2	< 0.001
Gemma amount (n)	2.44 ± 4.71	18.66 ± 14.15	14.84 ± 12.41	12.04 ± 9.67	62.38	3	< 0.001

Kruskal–Wallis parameters are represented in the last columns of the table

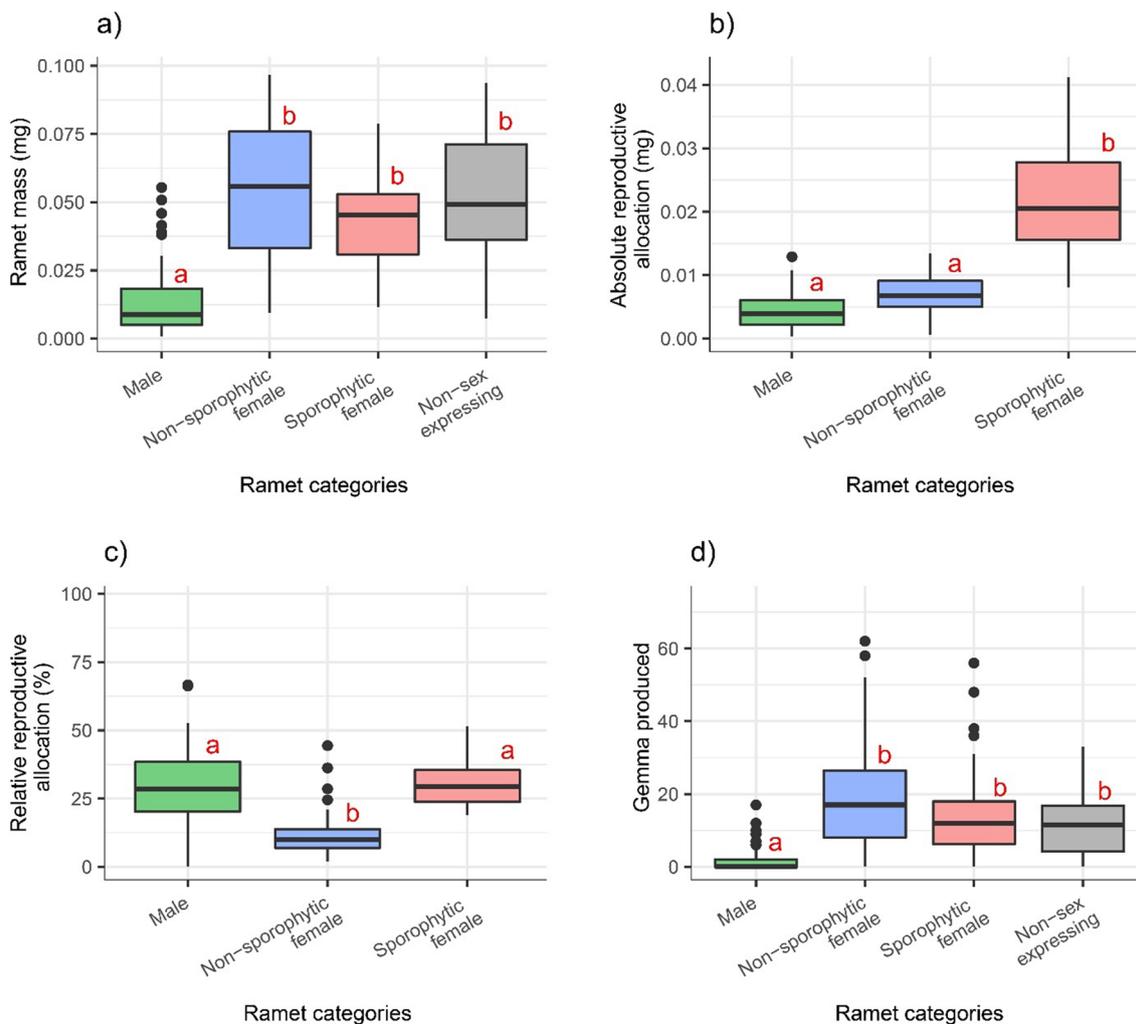


Fig. 1 Graphs reporting **a** ramets biomass, **b** absolute reproductive allocation, **c** relative reproductive allocation and **d** amount of gemma produced. The letters indicate the significance parameters

was significant. According to the results, those plants that allocate a greater amount of resource to relative reproductive allocation, have a lower gemma production (Fig. 2). Furthermore, the model showed significance in the interactions

between relative reproductive allocation and vegetative biomass, suggesting that the smaller the growth and relative reproductive allocation, the greater the gemma production (Table 3).

Table 2 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM)—Gemma production		
	Df	Deviance
Gametangia biomass	1	20.10 ^{n.s}
Ramet biomass	1	2.42 ^{n.s}
Observations		197
Akaike information criterion		NA
Residual deviance		2506.5 (Df= 194)
Null deviance		2529 (Df= 196)

Response variable (gemma production), predictor variables (gametangia biomass, and ramet biomass)

^{n.s.}not significant

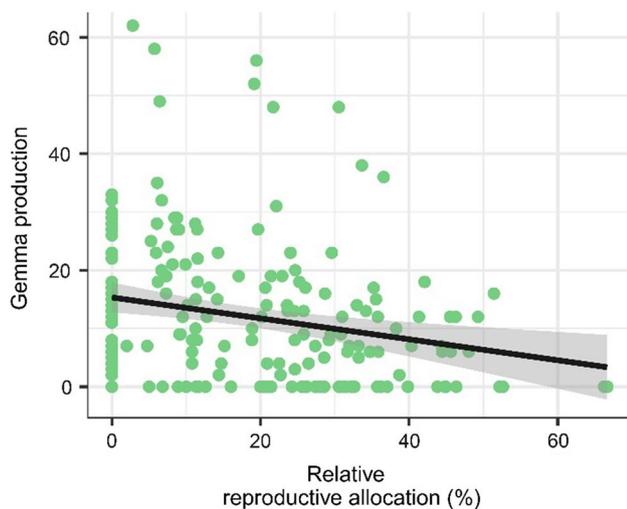


Fig. 2 Scatterplot of trade-off between relative sexual reproductive allocation (% ramet biomass) and gemma production (number ramet/cm²)

Table 3 Results of Generalized Linear Models (GLM). Response variable (gemma production), predictor variables (Relative reproductive allocation, and ramet biomass)

Generalized Linear Models (GLM)—Gemma production		
	Df	Deviance
Relative reproductive allocation	1	20.10 ^{**}
Vegetative biomass	1	117.44 ^{n.s}
Reproductive relative allocation versus vegetative biomass	1	215.24 ^{***}
Observations		197
Akaike information criterion		NA
Residual deviance		2176.2 (Df= 193)
Null deviance		2529.0 (Df= 196)

^{**}*p* < 0.01

^{***}*p* = 0.001

n.s Not significant

Reproductive traits

The results indicated that sexual expression is roughly equivalent among meta-populations ($X^2 = 252.11$, *df* = 218, *P* = 0.05) (Table 4). Metapopulations 2, 3, 7, and 10 had the highest sexual expression proportion (Fig. 3). While meta-population 6, has a no sex expression. Regarding sex ratio, all meta-populations showed a bias toward females (Table 4). However, the exact sex ratio was quantified for meta-populations 1, 3, 5, 7 and 10, as they were the only meta-populations that presented male ramets. Thus, the analyses showed that all meta-populations that expressed sex were biased toward females (Supplementary tab 1).

Reproductive success (proportion of females that formed sporophytes) did not differ between meta-populations that presented sporophytes ($X^2 = 108.46$, *df* = 91, *P* = 0.09) (Fig. 4). The meta-populations that showed greater reproductive success (1, 3, 5 and 7) presented higher meta-population density (Fig. 5). While meta-population 10 showed lower density and a lower tendency in the production of sporophytes. Suggesting that meta-population density can influence the reproductive success of populations.

Regarding the GLM result that we investigated whether reproductive success is higher in samples with high density and with a greater amount of male ramet, it did not show any significant result (Table 5). On the other hand, the model that was applied to investigate if population density entails male sex expression, presented a significance (Table 6). This model showed that population density is an important factor to male ramets express their sex.

Discussion

Sexual systems differ in morphological and reproductive attributes, which confer different mechanisms for each sex. However, the variation in the spatial distance between the sexes can characterize the reproductive allocation and meta-population dynamics of the species. Our main findings indicated that: (1) reproductive allocation is higher in male function; (2) there is a trade-off between sexual and asexual reproduction and (3) that density is a limiting factor in male sexual expression (the greater the population density, the greater the sexual expression of male ramets). These results indicate a similarity in reproductive patterns observed recurrently in dioicous bryophytes species. Thus, we can state that rhizautoicous sexual systems are monoicous sexual systems that behave like a dioicous system. Since in this sexual system, male and female ramets are individualized and connected only by rhizoids, that is, each ramet has a resource intended only for a sexual function, male or female, as in dioicous species. Since the only function of the rhizoids is to make the adhesion of the ramets to the substrate.

Table 4 Reproductive trait data by patches and for the entire meta-population

Meta-population	Ramets				Mean of reproductive traits to samples					Absolute reproductive traits to meta-population				
	Male	Non-sporophytic female	Sporophytic female	Non-expressing sex	Sex expression $\bar{X} \pm SD$	Sexual proportion ♀:♂	Sexual proportion	Reproductive success per sample $\bar{X} \pm SD$	Density per sample $1 \times 1 \text{ cm} \bar{X} \pm SD$	Sex expression	Sexual proportion ♀:♂	Reproductive success	Metapopulation density $30 \times 30 \text{ cm}$	
1	29	304	648	2747	28.25 ± 18.76	32.14:1	69.70 ± 25.25	124.3 ± 30.31	26.31	32.83:1***	68.07	3728		
2	0	530	0	1071	31.91 ± 12.60	–	0	31.91 ± 13.33	33.10	–***	0	1601		
3	83	263	704	1852	37.31 ± 13.14	11.70:1	69.66 ± 18.20	69.66 ± 22.98	36.18	11.65:1***	72.80	2902		
4	0	127	0	767	12.26 ± 13.02	–	0	29.80 ± 8.79	14.21	–	0	894		
5	35	348	481	2810	26.46 ± 16.87	23.69:1	55.05 ± 32.26	122.46 ± 37.08	23.52	23.69:1***	58.02	3674		
6	0	0	0	1182	0	–	0	39.40 ± 9.86	0.00	–***	0	1182		
7	74	269	745	1945	35.60 ± 11.18	13.70:1	69.90 ± 22.01	101.10 ± 26.87	35.87	13.70:1***	73.47	3033		
8	0	250	0	827	21.96 ± 9.94	–	0	35.90 ± 9.65	23.21	–***	0	1077		
9	0	216	0	819	19.82 ± 13.85	–	0	34.5 ± 6.76	20.87	–***	0	1035		
10	14	229	162	642	38.40 ± 27.08	27.93:1	21.74 ± 37.16	35.56 ± 9.13	38.68	27.93:1***	41.43	1047		

The sexual expression, reproductive success, and density traits calculated for patches are being reported with mean and standard deviation. While the data reported for meta-populations is being represented by the raw data

*** $p < 0.001$

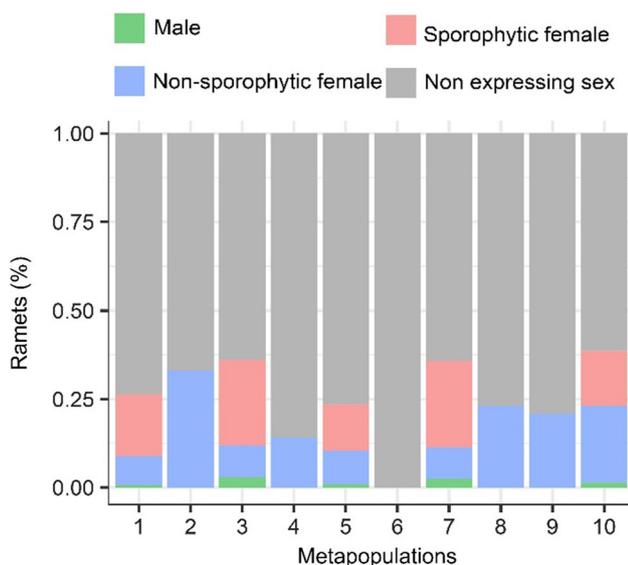


Fig. 3 Sexual expression of rhizautoicous moss *Fissidens flaccidus*. Percentage of ramets male, non-sporophytic female, sporophytic female, and non-sex expressing

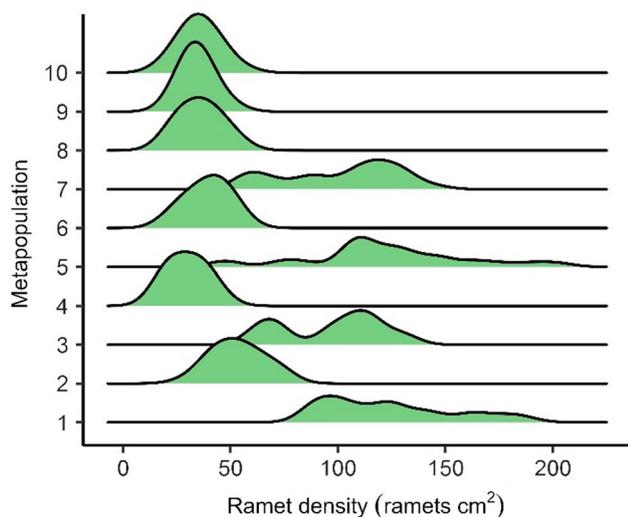


Fig. 4 Density graphics show ramet density per meta-population

The high relative reproductive allocation in the male function reflects a trade-off

Gender differences were not observed in the absolute reproductive allocation in the prezygotic phases (perigonia and perichaetia formation). On the other hand, for relative reproductive allocation, male ramets had a significantly higher mean. This pattern of greater reproductive allocation in male function compared to females is observed in most dioicous species and some rhizautoicous species (Stark et al. 2000; Horsley et al. 2011; Stark and Brinda 2013; Santos et al. 2018). Stark and Brinda (2013) noted that, as the distances

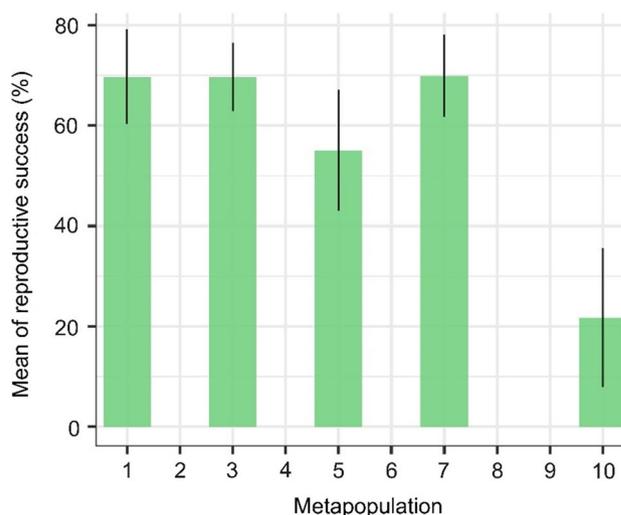


Fig. 5 Bar plot represents reproductive success by meta-population with error bar

Table 5 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM)		
Response variable as reproductive success		
	Df	Deviance
Male ramets	1	0.0026 ^{n.s}
Total ramets	1	11.5135 ^{n.s}
Observations		300
Akaike information criterion		NA
Residual deviance		283.31 (Df=27)
Null deviance		294.82 (Df=29)

Response variable (reproductive success), predictor variables (Male ramets, and total ramets)
^{n.s.} Not significant

Table 6 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM)		
Response variable as male sexual expression		
	Df	Deviance
Populational density	1	298***
Observations		300
Log likelihood		-1,356.703
Akaike information criterion		NA
Residual deviance		644.59 (Df=298)
Null deviance		877.09 (Df=299)

Response variable (male sexual expression), predictor variables (Population density)
 *** $p < 0.0001$

between males and females increase, the greater is the relative reproductive allocation in male function. Thus, in rhizautoicous sexual systems where male and female ramet connections dissolve, plants can be considered functionally dioicous. It is expected that the larger the distance between the male and female ramets, the chances of fertilization decline. Indeed, results reported by several studies confirm this expectation (Glime and Bisang 2017b). Thus, we can infer that this pattern (greater sexual relative reproductive allocation in male function) may be interpreted as an evolutionary strategy that increases the chance of fertilization in sexual systems showing large distances between sexual functions.

Male ramet biomass is lower than other ramet categories (non-sex expressing, sporophytic, and non-sporophytic female). This result has been related to this same species (Santos et al. 2018), and a strong sexual dimorphism was measured. The lower male ramet biomass may be related to reproductive cost using relative reproductive allocation. According to Obeso (2002), there are many definitions of reproductive cost, among them, direct cost is defined as somatic costs of reproduction during the current reproductive season. Therefore, if a direct cost is related to the current reproductive season, a trade-off between growth and reproduction is expected. Indeed, male ramets have a much smaller biomass than females, since the proportion of resources allocated to perigonia is relatively high and these resources are not available for growth. In this context, phenological observations can provide clues to the effect of reproductive allocation on species biology. For instance, Santos et al. (2020) reported on a study where the phenology of two species of *Fissidens* with different sexual systems was observed, namely: *Fissidens scarious* Mitt.—rhizautoicous; and *Fissidens submarginatus* Bruch.—gonioautoicous. The authors observed that in the species with the rhizautoicous sexual system, protandry occurred, as they allocate more resources to reproduction, compared to the gonioautoicous (with male and female sex structures produced distally along single ramets) species. In addition, the sexual system that presented the highest reproductive allocation (rhizautoicous) was more seasonal. While the gonioautoicous system presented antheridia production throughout the year, however, in those ramets that formed sporophytes, a greater amount of aborted antheridia was found, suggesting reproductive cost.

Reproductive allocation competes for resources with asexual reproduction

The results showed that reproductive allocation for sexual reproduction (*i.e.*, resource allocated to the formation of perigonia, perichaetia, and sporophytes) entails fewer resources for gemma production. Trade-offs occur because environments are variable, often seasonal, and the plants are

sessile organisms, they must adjust to these conditions. In this context, trade-offs can be interpreted as adjustments to environmental variations (Liu et al. 2009). A clear example of a trade-off between sexual and asexual reproduction was reported in a liverwort by Laaka-Lindberg (2001) in the species *Lophozia silvicola* H. Buch, where the author found female plants allocating more resources to sexual reproduction compared to male plants. On the other hand, the amount of gemma was higher in plants that did not express sex, and the average increased from males to females. The latter finding makes it clear that sexual and asexual reproduction competes for resources produced by the plant. Nonetheless, this trade-off relationship between sexual and sexual functions has been reported recurrently in bryophytes (Kimmerer 1991; Bisang and Ehrlén 2002; Fuselier and Mcletchie 2002; Rydgren and Okland 2003; McLetchie and Stark 2006; Hedderon and Longton 2008; Stark et al. 2009; Horsley et al. 2011).

Metapopulation density can influence the reproductive fitness of meta-populations

Our results showed that the density of male ramets is not a factor increasing reproductive success in meta-populations of *F. flaccidus*. Thus, our findings indicate that the large reproductive allocation directed at male sexual function is efficient in bringing about the reproductive success of the studied meta-populations. Reese (1984) showed that in dioicous *Syrrhopodon texanus* Sull. male plants are very rare. However, when males are found, there are also females bearing sporophytes, similar to our results. Regarding sex ratio in dioicous bryophytes, commonly populations are found with female bias. For instance, Stark (2002) reports that 2/3rd of the studied dioicous species ($N=30$) have populations with a female bias, 5 with a male bias, and 5 without a sexual bias. In the same sense, Bisang and Hedenäs (2005) show that approximately 80% of analyzed species ($N=103$) presented a female bias. However, although less studied, rhizautoicous species tend to express a female ramet bias, namely: *Atrichum undulatum* (Hedw.) P. Beauv., *Tortula muralis* Hedw. (Longton and Miles 1982), *Weissia controversa* Nees & Hornsch. (Anderson and Lemmon 1972), and *Fissidens scarious* Mitt. (Santos et al. 2020). Deviating from this expected pattern is the moss *Aloina bifrons* (De Not.) Delgad. (Stark and Brinda 2013).

Metapopulation density was associated with male sexual expression induction, that is, the denser the meta-population, the greater the chance of male ramets expressing sex. Male reproductive allocation in dioicous and functional dioicous species, have been related to phenology since it is more costly. According to phenological patterns, usually in those plants where there is greater reproductive allocation in a function, they are developed first. Indeed, protandry

is commonly observed in these plants with greater reproductive allocation in male function. For example, in *Aloina bifrons* (De Not.) Delgad., Stark and Brinda (2013) showed that the development of the male plants preceded the female ramets and, at the end of the experiment, a greater amount of male ramets was observed. Similar to the findings by Santos et al. (2020), however, the number of female ramets was higher in rhizautoicous *Fissidens scarious*. Thus, we can suggest that meta-population density may create alternative microhabitat that favors male ramets' expression, which may take advantage from specific conditions created by higher or lower densities. However, we emphasize that other factors may be influencing the results found. In addition, more studies focusing on understanding the effects of density on sexual expression need to be carried out.

The preference for microhabitat in relation to sex has been reported in some bryophyte studies. For example, Bowker et al. (2000) found habitat preferences by sex in the widely distributed desert dioicous moss *Syntrichia caninervis* Mitt. in populations from the Mojave Desert in Nevada (USA). It was observed that male plants tend to establish and persist in shade, compared to female plants that are found across a span of shaded and open microhabitats. In the same sense, Cameron and Wyatt (1990) reported for the monoicous species *Splachnum ampullaceum* Hedw., *Splachnum sphaericum* Hedw., and dioicous *Splachnum rubrum* Hedw. that low light intensity and other factors favor the formation of male ramets. Thus, an explanation that population density is associated with male sexual expression is suggested, since the greater density of patches leads to a favorable microclimate for the ramets to express the male sex. Furthermore, the male ramets have a reduced size that are mostly protected near the female plants. Thus, more studies need to be carried out with functionally dioicous species to know if male ramets show preference for habitat, as our findings only suggest this association.

Thus, we can conclude that the studied meta-populations present characteristics of dioicous species such as greater reproductive allocation in the male sexual function. In addition, the reproductive allocation in the male function is sufficient to generate greater reproductive success. Since the number of male ramets is not positively correlated with the fertilization rate, meta-populations that have few male ramets have the potential to induce greater reproductive success as well as meta-populations that have many male ramets. Finally, our results suggest a possible preference for habitat in relation to male ramets, since these are always present in meta-populations that present higher density. Thus, we emphasize that the system investigated in this study (rhizautoicous) is strongly plastic, since the same ramet that forms perigonia can form perichaetia when it regenerates. Resembling to the moss *Atrichum undulatum* (Hedw.) P. Beauv., in which the branches have the potential

to produce male and female gametangia, but each one in different seasons. Furthermore, considering that the climate change scenario will certainly impact the reproduction bryophytes (Shortlidge et al. 2017), the rhizautoicous system can be considered an excellent sexual system to investigate the effect of climate change, since it may have identical genotypes for different sexes (Stearns 1989).

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Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

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References

- Alvarenga LDP, Pôrto KC, Zartman CE (2013) Sex ratio, spatial segregation, and fertilization rates of the epiphyllous moss *Crossomitrium patrisiae* (Brid.) Müll. Hal. in the Brazilian Atlantic

- rainforest. *J Bryol* 35:88–95. <https://doi.org/10.1179/174328213X13662092820316>
- Alvares CA, Stape JL, Sentelhas PC et al (2013) Köppen's climate classification map for Brazil. *Meteorol Zeitschrift* 22:711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Anderson LE, Lemmon BE (1972) Cytological studies of natural intergeneric hybrids and their parental species in the moss source. *Ann Missouri Bot Gard* 59:382–416
- Barrett SCH (2002) The evolution of plant sexual diversity. *Nat Rev Genet* 3:274–284. <https://doi.org/10.1038/nrg776>
- Bergh NG, Anthony Verboom G, Verboom GA (2011) Anomalous capitulum structure and monoecy may confer flexibility in sex allocation and life history evolution in the *Iffoga* lineage of paper daisies (Compositae: Gnaphalieae). *Am J Bot* 98:1113–1127. <https://doi.org/10.3732/ajb.1000457>
- Bisang I, Ehrlén J (2002) reproductive effort and cost of sexual reproduction in female *Dicranum polysetum*. *Bryologist* 105:384–397. [https://doi.org/10.1639/0007-2745\(2002\)105\[0384:REACOS\]2.0.CO;2](https://doi.org/10.1639/0007-2745(2002)105[0384:REACOS]2.0.CO;2)
- Bisang I, Hedenäs L (2005) Sex ratio patterns in dioicous bryophytes re-visited. *J Bryol* 27:207–219. <https://doi.org/10.1179/174328205X69959>
- Bordin J, Yano O (2013) Fissidentaceae (Bryophyta) do Brasil. *Inst Botânica São Paulo* 22:351
- Bowker MA, Stark LR, McLetchie DN, Mishler BD (2000) Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). *Am J Bot* 87:517–526. <https://doi.org/10.2307/2656595>
- Cameron RG, Wyatt R (1990) Spatial patterns and sex ratios in dioecious and monoecious mosses of the genus *Splachnum*. *Bryologist* 93:161–166
- Coutinho RQ, Lima-Filho MF, Souza-Neto JB, Silva ED (1998) Características climáticas, geológicas, geomorfológicas e geotécnicas da Reserva Ecológica de Dois Irmãos. In: Machado IC, Lopes AV, Pôrto KC (eds) Reserva ecológica de Dois Irmãos: estudos em um remanescente de mata atlântica em área urbana, 1st edn. Editora Universitária, Recife, pp 21–50
- Delph LF (1999) Sexual Dimorphism in Life History. Gender and sexual dimorphism in flowering plants. Springer, Berlin Heidelberg, pp 149–173
- Delph LF, Galloway LF, Stanton ML (1996) Sexual dimorphism in flower size. *Am Nat* 148:299–320
- Dinno A, Dinno MA (2017) Package 'dunn.test.' CRAN Repos 10:1–7
- Flora do Brasil (2021) Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/>. Accessed 11 Jul 2022
- Fuselier L, McLetchie N (2002) Maintenance of sexually dimorphic preadult traits in *Marchantia inflexa* (Marchantiaceae). *Am J Bot* 89:592–601
- Glime JM, Bisang I (2017a) Sexuality: reproductive barriers and tradeoffs. In: Glime JM (ed) Bryophyte ecology, Chap 3–4, Vol 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 12 April 2021 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>. Accessed 10 Apr 2022
- Glime JM, Bisang I (2017b) Sexuality: sex ratio and sex expression. In: Glime JM (ed) Bryophyte ecology, Chapt 3–2, Vol 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 25 December 2021 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>. Accessed 10 Apr 2022
- Gunton RM, Kunin WE (2009) Density-dependence at multiple scales in experimental and natural plant populations. *J Ecol* 97:567–580. <https://doi.org/10.1111/j.1365-2745.2009.01492.x>
- Hanski I (1990) Density dependence, regulation and variability in animal populations. *Philos Trans R Soc London Ser B Biol Sci* 330:141–150. <https://doi.org/10.1098/rstb.1990.0188>
- Harris BJ, Harrison CJ, Hetherington AM, Williams TA (2020) Phylogenomic evidence for the monophyly of bryophytes and the reductive evolution of stomata. *Curr Biol* 30:2001–2012.e2. <https://doi.org/10.1016/j.cub.2020.03.048>
- Hedderson TA, Longton RE (2008) Local adaptation in moss life histories: population-level variation and a reciprocal transplant experiment. *J Bryol* 30:1–11. <https://doi.org/10.1179/174328208X282175>
- Horsley K, Stark LR, McLetchie DN (2011) Does the silver moss *Bryum argenteum* exhibit sex-specific patterns in vegetative growth rate, asexual fitness or prezygotic reproductive investment? *Ann Bot* 107:897–907. <https://doi.org/10.1093/aob/mcr027>
- Hothorn T, Bretz F, Westfall P et al (2016) Package 'multcomp.' Simultaneous inference Gen Parametr Model, Project for Statistical Computing, Vienna
- Kimmerer RW (1991) Reproductive ecology of *Tetraphis pellucida*. I. Population density and reproductive mode. *Bryologist* 94:255–260
- Laaka-Lindberg S (2001) Biomass allocation to sexual and asexual reproduction in a leafy hepatic *Lophozia silvicola* Buch. *J Bryol* 23:3–8. <https://doi.org/10.1179/jbr.2001.23.1.3>
- Leonard JL (2018) The evolution of sexual systems in animals. In: Leonard JL (ed) Transitions between sexual systems, 1st edn. Springer, pp 1–58
- Liu F, Chen JM, Wang QF (2009) Trade-offs between sexual and asexual reproduction in a monoecious species *Sagittaria pygmaea* (Alismataceae): the effect of different nutrient levels. *Plant Syst Evol* 277:61–65. <https://doi.org/10.1007/s00606-008-0103-2>
- Longton RE, Miles CJ (1982) Studies on the reproductive biology of mosses. *Journ Hattori Bot Lab* 52:219–240
- Maciel-Silva AS, De Oliveira MP (2016) How tropical moss sporophytes respond to seasonality: examples from a semi-deciduous ecosystem in Brazil. *Cryptogam Bryol* 37:227–239. <https://doi.org/10.7872/cryb/v37.iss3.2016.227>
- Maciel-Silva AS, Valio IFM, Rydin H (2012) Altitude affects the reproductive performance in monoicous and dioicous bryophytes: examples from a Brazilian Atlantic rainforest. *AoB Plants* 12:1–14. <https://doi.org/10.1093/aobpla/pls016>
- McLetchie DN, Puterbaugh MN (2000) Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort *Marchantia inflexa*. *Oikos* 90:227–237. <https://doi.org/10.1034/j.1600-0706.2000.900203.x>
- McLetchie DN, Stark LR (2006) Sporophyte and gametophyte generations differ in their thermotolerance response in the moss *Microbryum*. *Ann Bot* 97:505–511. <https://doi.org/10.1093/aob/mcl011>
- Obeso JRR (2002) The costs of reproduction in plants. *New Phytol* 155:321–348. <https://doi.org/10.1046/j.1469-8137.2002.00477.x>
- Oli MK, Coulson T (2016) Life history, what is? Encyclopedia of evolutionary biology. Elsevier, Amsterdam, pp 394–399
- Pursell RA (2007) Fissidentaceae, Flora Neotropica Monograph 101, 1st edn. The New York Botanical Garden, New York
- Reese WD (1984) Reproductivity, fertility and range of *Syrhophodon texanus* Sull. (Musci; Calymperaceae), a North American endemic. *Bryologist* 87:217–222. <https://doi.org/10.2307/3242794>
- Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. *Am J Bot* 82:596. <https://doi.org/10.2307/2445418>
- RStudio Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Rydgren K, Økland RH (2002) Ultimate costs of sporophyte production in the clonal moss *Hylocomium splendens*. *Ecology* 83:1573–1579. [https://doi.org/10.1890/0012-9658\(2002\)083\[1573:UCOSPI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1573:UCOSPI]2.0.CO;2)
- Rydgren K, Okland RH (2003) Short-term costs of sexual reproduction in the clonal moss *Hylocomium Splendens*. *Bryologist*

- 106:212–220. [https://doi.org/10.1639/0007-2745\(2003\)106\[0212:SCOSRI\]2.0.CO;2](https://doi.org/10.1639/0007-2745(2003)106[0212:SCOSRI]2.0.CO;2)
- Santos WL, Pereira Alvarenga LD, Pôrto KC (2018) Sexual dimorphism, vegetative growth and reproductive investment in the rhizautoicous moss *Fissidens flaccidus* (Fissidentaceae, Bryopsida). *Cryptogam Bryol* 39:271–281. <https://doi.org/10.7872/cryb/v39.iss2.2018.271>
- Santos WL, Maciel-Silva AS, Pôrto KC (2020) How do sexual expression, reproductive phenology and reproductive success relate to sexual systems in *Fissidens* Hedw. (Fissidentaceae)? A case study comparing two different sexual systems in mosses. *Plant Biol* 22:573–581. <https://doi.org/10.1111/plb.13122>
- Santos WL, Pôrto KC, Pinheiro F (2022) Sex-specific differences in reproductive life-history traits of the moss *Weissia jamaicensis*. *Am J Bot* 109:645–654. <https://doi.org/10.1002/ajb2.1840>
- Shortlidge EE, Eppley SM, Kohler H et al (2017) Passive warming reduces stress and shifts reproductive effort in the Antarctic moss, *Polytrichastrum alpinum*. *Ann Bot* 119:27–38. <https://doi.org/10.1093/aob/mcw201>
- Stark LR (2002) Phenology and its repercussions on the reproductive ecology of mosses. *Bryologist* 105:204–218. [https://doi.org/10.1639/0007-2745\(2002\)105\[0204:PAIROT\]2.0.CO;2](https://doi.org/10.1639/0007-2745(2002)105[0204:PAIROT]2.0.CO;2)
- Stark LR, Brinda JC (2013) An experimental demonstration of rhizautoicy, self-incompatibility, and reproductive investment in *Aloina bifrons* (Pottiaceae). *Bryologist* 116:43–52. <https://doi.org/10.1639/0007-2745-116.1.043>
- Stark LR, Mishler BD, McLetchie DN (2000) The cost of realized sexual reproduction : and sporophyte abortion in a desert moss. *Am J Bot* 87:1599–1608
- Stark LR, Brinda JC, McLetchie DN (2009) An Experimental demonstration of the cost of sex and a potential resource limitation on reproduction in the moss *Pterygoneurum* (Pottiaceae). *Am J Bot* 96:1712–1721. <https://doi.org/10.3732/ajb.0900084>
- Stearns SC (1976) Life-history tactics: a review of the ideas. *Q Rev Biol* 51:3–47. <https://doi.org/10.1086/409052>
- Stearns SC (1989) Trade-offs in life-history evolution. *Funct Ecol* 3:259. <https://doi.org/10.2307/2389364>
- Stehlik I, Friedman J, Barrett SCH (2008) Environmental influence on primary sex ratio in a dioecious plant. *Proc Natl Acad Sci U S A* 105:10847–10852. <https://doi.org/10.1073/pnas.0801964105>
- Suzuki T, Inoue Y, Tsubota H (2018) Molecular phylogeny of the genus *Fissidens* (Fissidentaceae, Bryophyta) and a refinement of the infrageneric classification. *Mol Phylogenet Evol* 127:190–202. <https://doi.org/10.1016/j.ympev.2018.05.020>
- Thompson FL, Eckert CG (2004) Trade-offs between sexual and clonal reproduction in an aquatic plant: experimental manipulations vs. phenotypic correlations. *J Evol Biol* 17:581–592. <https://doi.org/10.1111/j.1420-9101.2004.00701.x>
- Wickham H, Chang W, Wickham MH (2016) Package ‘ggplot2.’ *Creat elegant data vis using gramm graph version. R Package Version 2.1* 2:1–18
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