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An Overview of Reproductive Allocation and Reproductive Costs in Bryophytes: Challenges and Prospects

Wagner Luiz dos Santos[1,2] · **Kátia Cavalcanti Pôrto**[2] · **Fábio Pinheiro**[1]

[1] Department of Plant Biology, Biology Institute, Universidade Estadual de Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil
[2] Biosciences Center, Department of Botany, Universidade Federal de Pernambuco, Moraes Rego Av., S/N, Recife, PE 50670-901, Brazil
[3] Author for Correspondence; e-mail: wag.lsantos1@gmail.com

Abstract

Reproductive allocation and cost play a crucial role in the survival of organisms, but research on these traits in bryophytes has been limited and inconsistent. To address this, we conducted a literature review focusing on bryophyte studies. Our goal was to clarify inconsistencies and explore reproductive allocation and cost concepts, as well as current trends in bryophyte reproduction. We examined different approaches and highlighted advantages and limitations. We emphasized five key topics: the importance of understanding reproductive allocation and reproductive cost in bryophytes; the significance of bryophytes as model organisms; historical research; terminological and methodological inconsistencies; sexual dimorphism and reproductive allocation; and measurement methods. Furthermore, we provided insights into future perspectives. Based on our findings, we advocate for standardized quantification of reproductive allocation. Standardization would enhance comparability and synthesis of results, ultimately advancing our understanding of reproductive allocation and cost of reproduction in bryophytes.

Keywords Biomass Allocation · Liverworts · Moss · Reproductive Effort · Reproductive Investment · Trade-off

Introduction

Life history theory, an approach that investigates the timing of key events in an organism's life cycle, predicts that every resource produced by an organism is allocated to its different vital functions (Oli and Coulson, 2016), namely, maintenance, growth, and reproduction (Morris, 2009). In plants, maintenance includes activities such as water regulation, maintenance of photosynthetic systems, production of secondary metabolites, and production of structures to reduce predation and to provide resistance against competitors (Thornely, 1970; Rungsung et al., 2015). Growth refers to the increase in the biomass of vegetative structures, which, ultimately, will give support to reproductive organs (Agren, 1985; Ingestad and Lund, 1986; Barrett, 2015). The reproductive cycle includes the production of gametangia, gametes, and offspring (Reekie and Bazzaz, 2002; Block et al., 2009).

The amount of an organism's resources that is invested in reproduction is called reproductive allocation (RA) (Reekie and Bazzaz, 2002). This resource can be carbon, energy, and even water (Reekie and Bazzaz, 1987; Delph et al., 1996; Ehrlén et al., 2000). However, the quantification of biomass, measured in absolute (reproductive biomass) or relative terms (proportion of resource allocated to reproduction or RA), is the most frequent method used in studies addressing the theme. When a large part of the resources available for an organism is allocated to reproduction, the other functions (growth and homeostasis) receive a decreased amount of such resources (Stearns, 1976, 1989). However, if the amount of resource varies over time, especially in highly seasonal environments, an adjustment in resource allocation trade-offs may be necessary (Stark, 2002; Maciel-Silva and De Oliveira, 2016). The partition of the acquired resources between multiple traits has been reported since Darwin's time. In his book "The different forms of flowers on plants of the same species", Darwin (1877) described the resource limitation as the *Law of Compensation*. He postulated that when a resource was not allocated to one specific trait, it was naturally reallocated to another. As an example, he used the morphology of monoecious flowers. He reported that plants that had larger (*i.e.*, greater mass) pistils (i.e. greater allocation of resources to this trait) had the smallest stamens. Currently, this condition is referred to as a trade-off (Charlesworth and Morgan, 1991).

Trade-offs can be governed by ecological, environmental, and evolutionary forces like natural selection (Stearns, 1976, 1989, 2000). According to Wilson (1983), natural selection should favor the RA pattern that promotes the highest fitness of individuals. Thus, we should expect a variation in RA related to several factors such as sex, age, and environmental conditions where the organisms are found. However, variations in RA strategies may be due to genetic or/and phenotypic factors (Smith-gill, 1983). For example, greater individuals have higher fitness in RA than smaller ones. Resource levels allocated for basic maintenance can be extremely high (Tuomi et al., 1983). Thus, an organism that allocates large amounts of resources to reproduction and growth (without a trade-off) may be excluded from the population by natural selection unless there are plenty of available resources (Wilson, 1983).

When the reproductive cycle of a plant begins, an important change in the pattern of allocation of resources occurs (Karlsson and Méndez, 2005a, b). Various structures begin to develop and grow, going through different phenophases. Metabolic energy and nutrients are required for the formation of reproductive structures (gametangia, gametes, and offspring) (Thornely, 1970). When there is fertilization, the reproductive cost (RC) tends to be greater for female plants because they need to allocate resources not only to the production of eggs (pre-zygotic investment) but also offspring (post-zygotic investment) (Obeso, 2002; Karlsson and Méndez, 2005a, b).

Reproductive cost is defined by Obeso (2002) as the "losses in the potential future reproductive success caused by current investments in reproduction". In flowering plants, RC has been widely investigated and shown to vary among sexes and across environments and sexual systems (Delph, 1999; Reekie and Bazzaz, 2002; Harris and Pannell, 2008). Sexual systems in plants refer to the organization and distribution of reproductive structures, such as male and female organs, in a plant (Charlesworth 2002), and it is strongly related to RA and RC in plants (Charlesworth 2002, Obeso 2002). The influence of sexual systems on RC is widely observed in plants in the most diverse ways across different levels of disturbance (Obeso, 2002; Karlsson and Méndez, 2005a, b; Rydgren et al., 2010; Santos et al., 2018a, b). For example, variations in RA within a sexual system across different ecosystems or levels of disturbance can indicate how sexual performance responds to environmental changes (Abrahamson and Gadgil, 1973; Hickman, 1977). Further, cases of sexual systems in which there is a pattern of greater RA to the male or female sexual function may suggest that the RC for one of the sexes is greater (Cruden and Lyon, 1985), with consequences at individual and populations levels.

Bryophytes as Model Organisms to Understand RA and RC

Bryophytes (mosses, liverworts, and hornworts) are a monophyletic group of plants comprising the simplest plants on Earth living in terrestrial environments (Harris et al., 2021). Because bryophytes do not have lignin in their cells, they are smaller in size than tracheophytes and cannot control water loss (Shaw and Goffinet, 2000). Unlike vascular plants, bryophytes have an alternation of generations in which the sporophyte (diploid phase) is supported and nourished by the gametophyte (haploid phase) (Haig, 2016). Gametophytes in bryophytes can be thallose or leafy (Shaw and Goffinet, 2000). All mosses are leafy, liverworts can be thallose or leafy, and hornworts are exclusively thallose (Glime, 2017a). Bryophyte reproduction depends on the formation of male gametangia (antheridia) that produce male gametes (antherozoids) and female gametangia (archegonia) that produce female gametes (eggs) (Haig, 2016). Gametangia are formed in structures called gametoecia (modified leaves that protect the gametangia). Gametoecia producing antheridia and archegonia are called perigonia and perichaetia, respectively (Shaw and Goffinet, 2000). When antherozoids are viable, they swim in the water film and fertilize the eggs, forming sporophytes (Haig, 2016) (Fig. 1).

As in angiosperms, bryophyte species may produce male and female reproductive organs on the same individual or on separate individuals. These species are respectively known as monoecious and dioecious in angiosperms, but in bryophytes they are called monoicous and dioicous because the reproductive structures are produced in the haploid phase (Wyatt, 1985; Maciel-Silva and Pôrto, 2014) in contrast to vascular plants. The monoicous and dioicous sexual systems of bryophytes present subdivisions according to variations in the position of the gametoecia in the gametophyte (Fig. 2) (Wyatt, 1985; Maciel-Silva and Pôrto, 2014). This diversity of sexual systems provides an opportunity to experimentally investigate the effect of the distance between the sexes on reproduction.

Bryophytes have proven to be powerful models for understanding complex processes in plants (Wyatt, 1985; Wood et al., 2000; Müller et al., 2016). Some aspects that make bryophytes excellent model organisms for the study of RA and RC are:

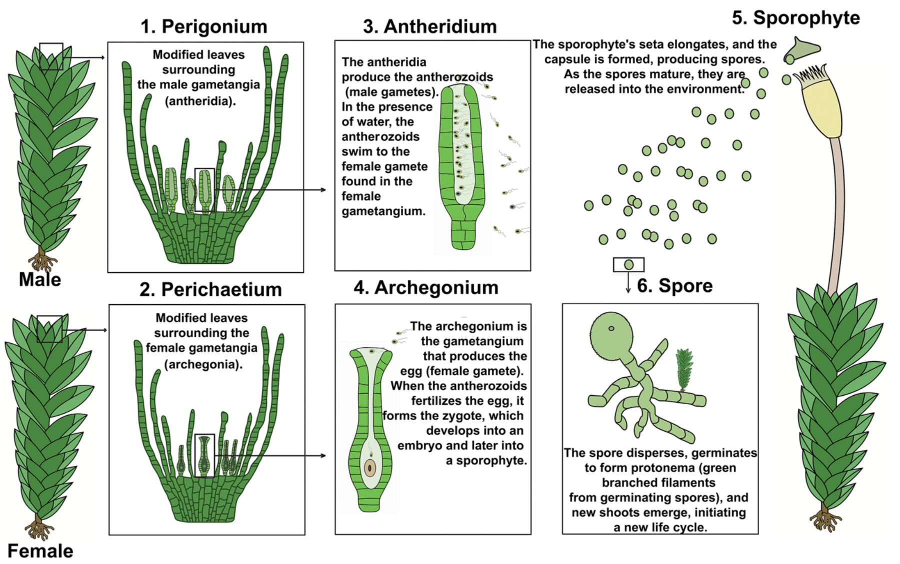

Fig. 1 Scheme of the reproductive cycle of a dioicous moss. The male gametoecium (perigonium) is the structure that produces and protects the male gametangia (antheridia). Antheridia produce male gametes (antherozoids), which are flagellated and swim to the female gametoecia (perichaetia) to fertilize the female gamete (egg) found within the female gametangia (archegonia). With the fertilization of the egg, cell divisions occur to form the sporophyte, which produces the spores. In the environment, under favorable conditions, the spores germinate and form new plants, starting a new life cycle

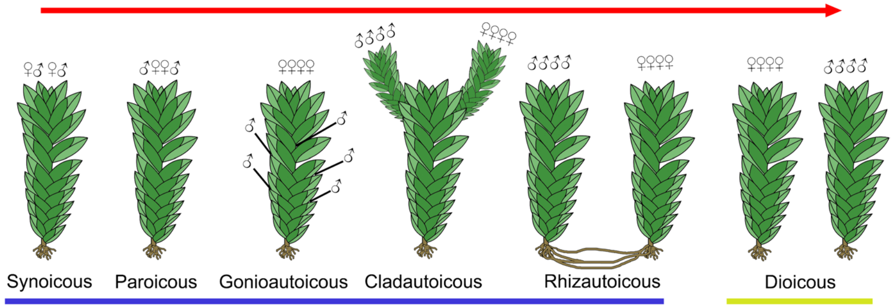

Fig. 2 Sexual systems of bryophytes. The distance between sexes increases from left to right. Synoicous – male and female gametangia mixed in the same gametoecia; Paroicous – male and female gametangia in the same gametoecia, but male gametangia are clustered and adjacent to female gametangia; Gonioautoicous – female gametoecia in the apex of the ramet and male gametoecia in the axil of the ramet; Cladautoicous – male and female gametoecia on separate branches of a principal ramet; Rhizautoicous – male and female gametoecia on separate ramets, however, the ramets are attached by rhizoids; Dioicous – male and female gametoecia in different plants

[1] the high potential for clonal propagation (Rydgren and Økland, 2003; Maciel-Silva, 2017) that facilitates the quantification of parameters such as RA and RC, because fitness is quantified as the growth rate of a clonal population (Stearns, 2000); [2] the small size of the plants and their reproductive structures that allows

the quantification of the biomass of entire vegetative and reproductive parts, which is virtually unfeasible for trees, for example; [3] the small size of the plants associated with high cloning and regeneration rates that facilitates experimental studies in which the plant and its development can be manipulated; and [4] the great morphological diversity associated with sexual and asexual reproduction (Frey and Kürschner, 2011; Maciel-Silva and Pôrto, 2014). Thus, bryophytes encompass several characteristics that facilitate the experimental analysis and interpretation of data related to RA and RC in plants. Notwithstanding the usefulness of these plants, bryophytes have only used to investigate the ecology and evolution of RA and the trade-offs involved in this process.

Bryophytes have a much faster life cycle compared to angiosperms (Oliveira and Porto, 2001; Maciel-Silva and Marques Válio, 2011; dos Santos et al., 2020). For example, in many species of flowering plants, it takes several years for the first reproduction to occur (Munguía-Rosas et al., 2011; Grogan and Schulze, 2012). In contrast, many species of bryophytes and some of flowering plants complete their reproductive cycle within a few months, especially tropical species (Glime, 2017b). Additionally, the small size of bryophytes and the diversity of substrates they colonize allow their manipulation in the field and in the laboratory, which is impossible for many angiosperms (Stark et al., 2009; Stark and Brinda, 2013; Santos et al., 2022).

History of the Study of RA and RC in Bryophytes

The first studies that quantified RA in bryophytes were carried out with moss species from the polar region (Convey and Smith, 1993; Convey, 1994a, b). They used the terminology “reproductive investment” and “reproductive effort” to refer to post-zygotic RA. Convey and Smith (1993) quantified the RA in several ways, including the measurement of the biomass of the reproductive and vegetative structures. The same was done by Convey (1994a) to compare the RA of eight mosses species in different ecosystems in arctic regions. He observed that the pattern of RA was different among species, highlighting the diversity of reproductive strategies found in bryophytes. Finally, Convey (1994b) associated physiological aspects to RA to verify whether environmental variations could cause changes in RA in moss species from a maritime Antarctic locality. He reported a weak correlation between net photosynthesis and RA, suggesting that these species had a high and regular investment in sporophyte production. However, the quantification of RA in these studies is somewhat misleading, since RA was calculated as a ratio of reproductive to vegetative structures and not as the proportional biomass allocated to reproductive structures. Thus, these studies do not portray the true RA of the studied species.

Kimmerer (1994) quantified the RA for sporophytes in two bryophyte species, *Dicranum flagellare* Hedw. and *Tetraphis pellucida* Hedw., in a forest. Similar to the studies mentioned above, RA was quantified as the ratio of reproductive to vegetative biomass. In this study, the author also investigated the allocation to asexual reproduction. The species differed in their reproductive characteristics: both had asexual reproduction, but *D. flagellare* showed a predominance of asexual reproduction, while *T. pellucida* produced asexual propagules at a lower intensity. *Dicranum flagellare* invested significantly more biomass in asexual reproduction, while the overall RA to sexual reproduction of *T. pellucida* was greater than that of *D. flagellare*. Furthermore, branches of *D. flagellare* germinated significantly faster than gemmae of *T. pellucida*. Thus, this was the first study to show that RA to sexual reproduction limits the resources available for vegetative reproduction. These results suggested that reproduction could entail a RC, although the way in which RA was quantified does not inform the real RA of the species studied.

González-Mancebo and During (1997) investigated the RA in nine species of mosses from the perspective of their life forms. The authors applied two different ways to quantify the RA and compared them between acrocarpous and pleurocarpous life forms, which are, respectively, forms in which the sporophytes are produced at the apex of the stem/main branch of upright shoots and forms in which sporophytes are produced on short, specialized lateral branches or buds of typically prostrate shoots, forming freely branched mats (Glime and Chavoutier, 2017). Reproductive allocation, which they referred to as reproductive effort, was calculated per area and per individual. In this study, the true RA was quantified for the first time for bryophytes. The authors investigated questions exploring the interplay between RA and life history traits such as: (1) Do short-lived acrocarpous mosses have greater reproductive effort than perennials? and (2) Is shoot size correlated with sporophyte weight, or is sporophyte weight relatively flexible, with reproductive mass independent of gametophyte size? The results showed that the RA per area did not differ significantly between the two groups. In the six acrocarpous species, the RA to sporophytes per sporophyte-bearing shoot was generally quite low compared to the pleurocarpous species. The two measures of RA (area and individual) produced different results and apparently reflect different trade-offs. The lower RA in the acrocarpous species conveys the idea that the production of several branches in pleurocarpous mosses results in a greater amount of resource for the formation of sporophytes because a greater amount of resources is produced and can be allocated to sexual reproduction (sporophyte formation). Furthermore, it was observed that the biomass of the sporophytes was positively correlated with branch size in some species and negatively correlated in others. This finding may give an idea of the earliest steps of plant reproduction on terrestrial ecosystems.

The studies until then had focused only on the quantification of RA. Then, for the first time, Ehrlén et al. (2000) quantified the RC in the dioicous moss *Dicranum polysetum* Sw. The authors tested the hypothesis that RA to sporophyte production may imply costs, such as reduced performance of gametophytes in the later steps of the life cycle. Sporophyte production was investigated in three different ways: (1) by examining the variation in sporophyte production in unmanipulated sporophytic shoots; (2) by comparing sporophytic and non-sporophytic shoots (not carrying perichaetia); and (3) by comparing control sporophytic shoots with shoots whose reproductive sinks were experimentally removed. They estimated that 74.8% of the total carbon investment during the growth interval went to sexual structures in sporophyte-producing shoots. Branches that aborted all sporophytes had significantly higher growth rates than those that formed sporophytes. The difference in apical vegetative growth between control branches and branches in which sexual reproduction was manipulated was mainly due to different length increments, because biomass did not differ significantly. Thus, post-zygotic RC was quantified for the first time in a bryophyte and it was demonstrated that sporophyte maturation competes for resources, affecting the future development of new branches in *D. polysetum.* The RC in *D. polysetum* was further investigated by Bisang and Ehrlén (2002) using a slightly different approach. In this case, the authors proposed in a new experiment a way to quantify RA that considers only the photosynthetic portions of the plants. Making use of precise innate markers for growth intervals, the position of gynoecia in *D. polysetum*, the authors dissected the individuals of this moss according to growth intervals, quantifying the sporophytes and perichaetia from previous years. They removed the brown portions of the gametophytes, leaving only the photosynthetically active parts to be weighed. This is because the brown parts can be dead, and even if they are alive (when they can regenerate), they do not influence the production of resources for reproduction. They found that the RA in plants that formed sporophytes was 16% while in those that did not form sporophytes was 1.3%. The authors also found several results that suggest the existence of RC: (1) the reproductive investment was inversely proportional to annual segment biomass prior to sporophyte initiation; (2) sporophyte formation was negatively related to annual sprouting segment and innovation size; (3) sporophyte formation reduced the probability of new perichaetia initiation; (4) investments in innovations and reproductive structures were negatively correlated; and (5) the mean mass per sporophyte + perichaetium decreased with the increase in the number of mature sporophytes per perichaetium. Thus, this study deepened the knowledge about the RC in the studied species. Furthermore, they showed that forming sporophytes entails a greater RC than forming perichaetia.

Using a population approach to investigate the relationship between sex ratio and RA, Stark et al. (2000) studied the moss *Syntrichia caninervis* Mitt., a species that has strongly female biased populations (Bowker et al., 2000). They investigated the ‘cost of sex hypothesis’, which predicts that “the sex that is more expensive (the one that requires more energy) is the rarest in populations”. Thus, they investigated the assumptions that male sexual expression is more expensive than female sexual expression and that sexual reproduction resource is limited. This study was innovative because it was the first to quantify RA at the pre-zygotic level in both sexes (male and female) in addition RA to sporophytes at a population level in bryophytes. Reproductive biomass was quantified by removing perichaetial and perginonial leaves and weighing sets of gametangia. The authors reported that the reproductive costs in the studied populations were lower to the female than to the male function. Furthermore, they discussed resource limitation and observed a relationship between sporophyte production and population density. This study was very relevant for a better understanding of sex ratios in bryophytes. However, in the same vein of Stark et al. (2000), Bisang et al. (2006) investigated the possible association of RC with sex ratios in *Pseudocalliergon trifarium* Loeske. This species, like *S. caninervis*, has populations strongly biased towards the female sex and low fertilization rates. However, the findings contradicted the proposals of Stark et al. (2000), since the RA in *P. trifarium* was higher to the female function than to the male function. The RC did not influence the sex ratios in *P. trifarium*. Thus, with this result, it became clear that the two species behave differently in terms of RC and reproductive traits.

Until this point, RA had been quantified in relation to sexual reproduction (perigonia, perichaetia, and sporophytes). McLetchie and Puterbaugh (2000) quantified, for the first time, the RA to asexual reproduction and also for the first time in a liverwort. The authors estimated the biomass of the gemmae produced using the formula: $= \frac{\text{Reproductivemass}}{\text{Reproductivemass+Vegetativemass}}$. Male plants allocated proportionally more biomass to gemmae than females. In turn, females grew faster and produced more meristematic tips, which, in a way, influenced the population parameters. Another species of liverwort whose RA has been investigated is *Lophozia silvicola* Buch. Laaka-Lindberg (2001) investigated possible relationships between RA to sexual and asexual structures and RC in *L. silvicola*. Higher RA to sexual structures was inversely proportional to the number of gemmae and associated with lower the investment in growth. Female plants had higher RA to sexual structures, but these results must be analyzed with caution because RA was estimated by weighing female plants with pre- and post-zygotic structures altogether. Thus, it is not possible to establish whether vegetative growth and gemmae production are negatively related with pre- or with post-zygotic RA.

To investigate long-term reproductive costs, Rydgren and Økland (2002) followed a population of the dioicous moss species *Hylocomium splendens* (Hedw.) Schimp. for five years. They divided the population into two subcategories: female plants with and without sporophytes. Their findings showed that population growth was more influenced by female plants that did not form sporophytes than by those which did form sporophytes. The sub-population that did not form sporophytes had higher branching rates, greater survival, and greater size development. In another study the authors Rydgren and Økland (2003) investigated the short-term RC in the species, demonstrating that sporophyte formation induced significant costs in terms of less favorable size development of new segments, lower branching frequency, and greater risk of not producing new annual segments. Furthermore, they claimed that the costliest phase of sporophyte development was the last one (when the capsule expands and matures and spores are produced), because a large amount of resources is consumed in this phase. The RC was also reported in the tropical species *Crossomitrium patrisiae* (Brid.) Müll. Hal. (Alvarenga et al., 2016). In this study, the researchers observed lower growth rates of new branches in the colonies where sporophytes were formed. Thus, in the latter three studies mentioned, the costs brought about by RA to sporophytes was detected using different types of measurements, although RA was not measured in any of them.

In the attempt to understand why the distribution of *Pogonatum dentatum* (Brid.) Brid. had expanded (spreading from the mountains down to the lowlands) in Fennoscandia, Hassel and Söderström (2003) quantified several reproductive traits of the species. The results of the studies showed that male plants were smaller than the female ones and matured in the second year of life and that female, mountain plants produced sporophytes only in the third year of life, while lowland plants formed sporophytes in the second year. Despite this difference in the formation of sporophytes in female plants, the RA did not differ between the two areas. The authors also mentioned that the establishment of populations by spores was more common in lowland areas, because in the mountain region, there were few spores available in the soil. The associations between life history traits and RA (referred to as reproductive effort) in the species *Pogonatum aloides* (Hedw.) P. Beauv., *Polytrichum commune* Hedw., and *Polytrichum juniperinum* Hedw. were explored by Hedderson and Longton (2008). The authors transplanted plants in their respective environments between upland and lowland localities. They observed that there were differences in life history traits and that some traits were genetically determined, although the environment and phenotypic plasticity were also significant contributors to the observed variation. In addition, the transplants indicated divergences in the plasticity regarding male reproductive effort and investment in vegetative shoots by female plants among populations. The RA to female and male sexual functions was respectively 35% and 50% in *P. aloides* (35% and 50%), 18% and 6.7% in *P. commune* (18% and 6.7%), and 18% and 35% in *P. juniperinum*. (18% and 35%).

Several studies have investigated the reproductive traits and costs in various mosses species to understand their ecological strategies (Glime 2017b). The RC in the widespread moss *Pterygoneurum ovatum* (Hedw.) Dixon was investigated by Stark et al. (2009) to test the hypothesis that a trade-off exists between current sexual reproduction and subsequent clonal regeneration and that reproduction and regeneration are resource limited. After the experimental removal of leaves and sporophytes in early stages of development (embryo) from gametophytes, the following findings of the study indicated the existence of RC in the species: [1] shoots whose sporophytes were removed presented a higher protonemal growth rate; [2] natural abortion of sporophytes presented a positive correlation with time to protonemal emergence; [3] shoots whose leaves were removed showed lower probability of sporophyte maturation in relation to control shoots with intact leaves. In the same vein, evidence of RC was found in the moss *Weissia jamaicensis* (Mitt.). The relative and absolute biomass of reproductive structures of *Weissia jamaicensis* were quantified, among other reproductive traits, by Santos et al. (2022). Relative biomass was higher in sporophytic shoots, followed by male and female non-sporophytic shoots. Absolute biomass also varied among the three categories of shoots. Reproductive cost was indicated by a negative correlation between regeneration rate and RA: plants that allocated more resources to reproduction had lower regeneration rates. These results suggest that RA influences reproductive traits. Since sexual reproduction is more expensive, the more expensive sex tends to have lower regeneration rates, what may consequently lead to biases in population sex ratios.

As mentioned in the studies by Stark et al. (2009) and Santos et al. (2022), some species may show patterns that contradict the expectations. For example, Horsley et al. (2011) investigated the reproductive traits the dioicous moss *Bryum argenteum* Hedw. in cultivation, specifically sex-specific patterns in parameters related to the life-history traits of the species. Among other objectives, they investigated whether there were sex-specific patterns of pre-zygotic RI (evaluating the production and biomass of gametangial structures). They found that male and female plants showed different development patterns. Male plants showed higher rates of sexual expression than female plants, while female plants were greater in size. Male plants showed a significantly higher RA than female plants. This result was contrary to what was expected according to the life history theory, because although male plants had a RA that was approximately 24 times higher than that of female plants, they presented faster sex expression and higher rates of sexual expression.

Brzyski et al. (2014) quantified the RA and its impact on the population genetic diversity of the liverwort *Marchantia inflexa* Nees & Mont. in natural (rivers) and disturbed (roadsides) habitats. Reproductive allocation was quantified based on the number of gemmae-producing cups. This was, thus, an indirect measure of RA. The source environment had a considerable impact on the responses of male and female plants. Roadside-collected female plants had higher growth and asexual reproduction than river-collected female plants, while males showed the reversed pattern, although these differences were not significant. An indirect method of quantification of RA was also used by Pereira et al. (2016). They assessed the RA in six sympatric species of Calymperaceae by counting the number of gametangia. They observed that plants that reproduced asexually (by gemmae) had a lower number of gametangia. Shortlidge et al. (2017) investigated the effects of global warming on the moss *Polytrichastrum alpinum* (Hedw.) G.L. Sm. using open-top chambers to follow the development of the species. They demonstrated that passive warming reduces the stress and shifts the reproductive effort (measured as the number of sporophytes produced) of the species. Although passive warming did not increase sporophyte production in *P. alpinum*, it increased gametangia production in both males and females, suggesting that warming may alleviate the environmental constraints on reproductive effort. Although Brzyski et al. (2014), Pereira et al. (2016), and Shortlidge et al. (2017) claimed to have quantified the RA, they actually quantified the (sexual or asexual) reproductive structures.

Stark and Brinda (2013) studied the reproductive parameters, including the pre-zygotic RA for the first time in a monoicous moss, the rhizautoicous moss *Aloina bifrons* (De Not.) Delgad. They observed that the cultured plants were protandrous in gametangial development and in time to maturation, but mature gametes were present at the same time. Male plants were smaller in size and presented greater RA (which was calculated by area) than female plants. In addition, the authors created ideal conditions for fertilization, but fertilized females aborted the sporophytes, suggesting self-incompatibility. In this study, the authors hypothesized that RA to the male function increases as the distance between sexes increases, and this is expected to be seen across the range of sexual systems existing in bryophytes (See Fig. 2). The first tropical moss to have the RA quantified was the rhizautoicous moss *Fissidens flaccidus* Mitt. Santos et al. (2018a, b) quantified the RA in terms of absolute and relative biomass allocation and investigated the existence of sexual dimorphism in the species. They showed that male ramets had a higher RA, even exceeding the allocation to sporophytes. Furthermore, the analyses showed that there was a trade-off between relative RA and vegetative growth. Thus, these results suggest that there is a RC in the male function because ramets that presented greater allocation to the sexual reproduction grew less. Furthering the study of the relationship between RA and the population ecology of *F. flaccidus*, Santos et al. (2023) observed that the formation of male plants was dense density dependent. The number of male plants did not influence the fertilization rates of the populations, since the large RA to the male function ensured fertilization even when male individuals were fewer in the populations. Thus, the compiled data on RA in this rhizautoicous species suggest the functionality of this system as a dioicous system, as proposed by Stark and Brinda (2013). Finally, more recently, in the attempt to test the pattern of RA across the sexual systems of bryophytes proposed by Stark and Brinda (2013), Santos et al. (2023) compared the RA of rhizautoicous, cladautoicous, and gonioautoicous species of the genus *Fissidens*. Among the main findings, the rhizautoicous species behaved like a dioicous species (greater allocation to the male function), while the other systems showed no sex-specific differences in RA.

Reproductive allocation and RC have been studied in different families (Fig. 3A) and genera (Fig. 3B). However, as presented in this section, RA has been quantified in various ways (Table 1), leading to significant methodological inconsistencies among the studies. Thus, in the next section of this article, we will discuss the methodological and terminological issues associated with the study of RA and RC in bryophytes.

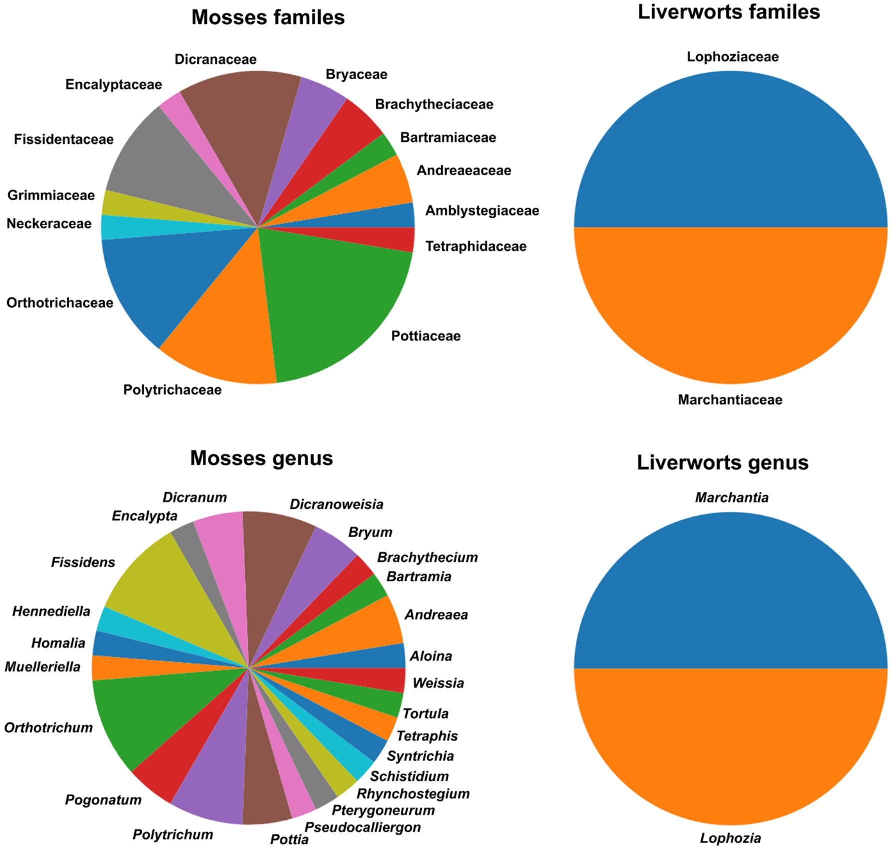

Fig. 3 Number of liverwort and moss species per family (**A**) and genus (**B**) whose reproductive allocation or reproductive cost has been investigated

Table 1 Compilation of results from studies that quantified reproductive allocation in bryophytes. Absolute reproductive allocation is the biomass of reproductive structures. Relative reproductive allocation is the proportion of resources allocated to reproduction. Mean values per species and sex are presented. ♂ = male sexual structures (perigonia or antheridia), ♀ = female pre-zygotic structures (perichaetia or archegonia), and ⚥ = post-zygotic reproductive structures (sporophytes). Note that: ^{a} = reproductive allocation was quantified by area in mm^{2}, and ^{b} = pre- and post-zygotic structures were weighed together

Species	Absolute Reproductive Allocation (Biomass)			Relative Reproductive Allocation (%)			Reference
Mosses	♂	♀	⚥	♂	♀	⚥	
Aloina bifrons (De Not.) Delgad	204 mg mm^{-2}	24 mg mm^{-2}	–	–	–	–	Stark and Brinda (2013)
Andreaea gainii Cardot	–	–	26.4 µg	–	–	0.24	Convey and Smith (1993)
Andreaea regularis Müll. Hal	–	–	27.3 µg	–	–	0.31	Convey and Smith (1993)
Andreaea regularis Müll. Hal	–	–	27.3 µg	–	–	0.31	Convey (1994b)
Andreaea regularis Müll. Hal	–	–	26.8 µg	–	–	0.31	Convey (1994b)
Andreaea regularis Müll. Hal	–	–	54.3 µg	–	–	0.37	Convey (1994b)
Bartramia patens Brid	–	–	439.9 µg	–	–	0.56	Convey and Smith (1993)
Bartramia patens Brid	–	–	439.9 µg	–	–	0.61	Convey (1994b)
Bartramia patens Brid	–	–	1338 µg	–	–	0.49	Convey (1994b)
Bartramia patens Brid	–	–	694.3 µg	–	–	0.52	Convey (1994b)
Brachythecium rutabulum (Hedw.) Schimp	–	–	286 mg cm^{-2}	–	–	0.056a	González-Mancebo and During (1997)
Bryum argenteum Hedw	26.5 mg mm-^{2}	11.36 mg mm-^{2}	–	–	–		Horsley et al. (2011)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb	–	–	637 µg	–	–	2.65	Convey and Smith (1993)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb	–	–	673 µg	–	–	2.73	Convey (1994b)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb	–	–	201.4 µg	–	–	0.99	Convey (1994b)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb	–	–	1541 µg	–	–	2.73	Convey (1994b)
Dicranoweisia antarctica (Müll. Hal.) Kindb	–	–	130 µg	–	–	0.69	Convey and Smith (1993)
Dicranoweisia antarctica (Müll. Hal.) Kindb	–	–	130 µg	–	–	0.74	Convey (1994b)

Table 1 (continued)

Species	Absolute Reproductive Allocation (Biomass)			Relative Reproductive Allocation (%)			Reference
Dicranoweisia antarctica (Müll. Hal.) Kindb	_	_	159.9 µg	_	_	0.61	Convey (1994b)
Dicranoweisia cirrata (Hedw.) Lindb. ex Milde	_	_	106 mg cm^{-2}	_	_	0.021a	González-Mancebo and During (1997)
Dicranoweisia cirrata (Hedw.) Lindb. ex Milde	_	_	0.034 mg	_	_	0.034	González-Mancebo and During (1997)
Dicranoweisia grimmiacea (Müll. Hal.) Broth	_	_	147.6 µg	_	_	0.48	Convey and Smith (1993)
Dicranoweisia grimmiacea (Müll. Hal.) Broth	_	_	147.6 µg	_	_	0.5	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth	_	_	206.8 µg	_	_	0.29	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth	_	_	94.4 µg	_	_	0.36	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth	_	_	206.4 µg	_	_	0.43	Convey (1994b)
Dicranum flagellare Hedw	_	_	12.2 µg	_	_	_	Kimmerer (1994)
Dicranum polysetum Sw	_	_	_	_	_	74.8	Ehrlén et al. (2000)
Dicranum polysetum Sw	_	_	_	_	1.3	16	Bisang and Ehrlén (2002)
Encalypta patagonica Broth	_	_	265.7 µg	_	_	1.24	Convey and Smith (1993)
Fissidens flaccidus Mitt	7.8 µg	11.69 µg	29.9 µg	35.15	12.61	25.4	Santos et al. (2018a, b)
Fissidens flaccidus Mitt	0.0046 mg	0.0070 mg	0.0223 mg	30.32	11.88	30.49	Santos et al. (2023)
Fissidens pseudoplurisetus Bordin, Pursell & O. Yano	0.0090 µg	0.0087 µg	_	1.60	7.42	_	Santos et al. (2023)
Fissidens scariosus Mitt	0.0024 µg	0.0123 µg	_	27.36	16.84	_	Santos et al. (2023)
Fissidens weirii Mitt	0.0050 µg	0.0081 µg	_	4.41	6.93	_	Santos et al. (2023)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	127.8 µg	_	_	1.47	Convey (1994b)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	329.8 µg	_	_	1.32	Convey (1994b)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	207.3 µg	_	_	1.48	Convey (1994b)
Homalia trichomanoides (Hedw.) Brid	_	_	292 mg cm^{-2}	_	_	0.04a	González-Mancebo and During (1997)
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	300.4 µg	_	_	0.92	Convey and Smith (1993)

Table 1 (continued)

Species	Absolute Reproductive Allocation (Biomass)			Relative Reproductive Allocation (%)			Reference
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	300.4 µg	_	_	0.98	Convey (1994b)
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	326.8 µg	_	_	0.92	Convey (1994b)
Orthotrichum affine Brid	_	_	188 mg cm^{-2}	_	_	0.122a	González-Mancebo and During (1997)
Orthotrichum affine Brid	_	_	0.123 mg	_	_	0.15	González-Mancebo and During (1997)
Orthotrichum diaphanum Schrad. ex Brid	_	_	117 mg cm^{-2}	_	_	0.087a	González-Mancebo and During (1997)
Orthotrichum diaphanum Schrad. ex Brid	_	_	0.036 mg	_	_	0.16	González-Mancebo and During (1997)
Orthotrichum pulchellum Brunt	_	_	273 mg cm^{-2}	_	_	0.089a	González-Mancebo and During (1997)
Orthotrichum pulchellum Brunt	_	_	0.042 mg	_	_	0.12	González-Mancebo and During (1997)
Orthotrichum striatum Hedw	_	_	250 mg cm^{-2}	_	_	0.026a	González-Mancebo and During (1997)
Orthotrichum striatum Hedw	_	_	0.059 mg	_	_	0.04	González-Mancebo and During (1997)
Pogonatum aloides (Hedw.) P. Beauv	_	_	_	50.17	35.13	_	Hedderson and Longton (2008)
Pogonatum aloides (Hedw.) P. Beauv	_	_	_	48.77	34.34	_	Hedderson and Longton (2008)
Pogonatum aloides (Hedw.) P. Beauv	_	_	_	53.46	36.13	_	Hedderson and Longton (2008)
Pogonatum dentatum (Brid.) Brid	_	_	_	_	_	0.34	Hassel and Söderström (2003)
Polytrichum alpinum Hedw	_	_	2636 µg	_	_	0.32	Convey (1994b)
Polytrichum commune Hedw	_	_	_	6.6	18.36	_	Hedderson and Longton (2008)
Polytrichum commune Hedw	_	_	_	6.7	12.33	_	Hedderson and Longton (2008)
Polytrichum commune Hedw	_	_	_	6.7	21.41	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw	_	_	_	36.19	21.45	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw	_	_	_	34.73	22.8	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw	_	_	_	23.69	14.03	_	Hedderson and Longton (2008)
Pottia austrogeorgica Cardot	_	_	127.8 µg	_	_	1.38	Convey and Smith (1993)
Pottia heimii (Hedw.) Hampe	_	_	280.6 µg	_	_	1.99	Convey and Smith (1993)

Table 1 (continued)

Species	Absolute Reproductive Allocation (Biomass)				Relative Reproductive Allocation (%)			Reference
Pseudocalliergon trifarium (F. Weber & D. Mohr) Loeske	121.15 µg	174.24 µg	–		8.60	11.20	–	Bisang et. al. (2006)
Pterygoneurum ovatum (Hedw.) Dixon	–	–	124.4 µg		–	–	2.09	Convey and Smith (1993)
Rhynchostegium confertum (Dicks.) Schimp	–	–	129 mg cm^{-2}		–	–	0.069a	González-Mancebo and During (1997)
Schistidium antarctici (Cardot) L.I. Savicz & Smirnova	–	–	90.6 µg		–	–	0.67	Convey and Smith (1993)
Syntrichia caninervis Mitt	17.1 µg	2.7 µg	–		–	–	–	Stark et al. (2000)
Tetraphis pellucida Hedw	–	–	54.2 µg		–	–	–	Kimmerer (1994)
Tortula saxicola Cardot	–	–	168.2 µg		–	–	1.48	Convey and Smith (1993)
Weissia jamaicensis (Mitt.) Grout	0.0629 mg	0.0479 mg	0.148 mg		15.28	6.74	21.56	Santos et al. (2022)
Liverworts	♀	♂	⚥		♀	♂	⚥	
*Lophozia silvicola*Buch	4.8 µg	83.3 $µg^{b}$	–	–	–	–	–	Laaka-Lindberg (2001)
Marchantia inflexa Nees et Mont	0.0292 mg	0.0092 mg	–	–	–	–	–	McLetchie and Puterbaugh (2000)

Terminological Inconsistencies and Methodological Problems

Studies carried out with higher plants in general present several terminologies related to RA and RC (Table 2). It is not different in studies with bryophytes. As shown in the previous section, many terminologies have been used, and sometimes incorrectly. For example, some authors have used the terms reproductive effort, reproductive investment, and biomass production to refer to RA (Table 3). This imprecise application of different terms for the same concept contributes to confuse and mislead the conclusions in the studies. The term reproductive investment has been the most used, followed by RA, reproductive effort, and biomass allocation (Table 3).

In a review of RA, Karlsson and Méndez (2005a, b) mentioned that the most adequate term is RA (proportion of resources destined to reproduction). Terminological inconsistencies regarding RC, in turn, are discussed by Obeso (2002). Another problem found in the investigation of RA, is the way it has been quantified. In the previous section, we showed that RA has been measured in different ways, either counting or weighing reproductive structures. Of the 25 studies that investigated RA in bryophytes, 50% correctly quantified this parameter (Table 3). Among the other studies, most quantified RA by calculating the ratio of reproductive to vegetative biomass, and the others counted the reproductive structures (Table 3). Not only standardizing the terminology but also the methodology for quantification is a necessity in the field of investigation of RA. Terminological and methodological variations preclude the correct understanding of the works, the comparisons of the results of multiple studies, and consequently the understanding of ecological processes and mechanisms of the species investigated.

As for the measurement of RC, its quantification cannot be standardized, since this variable can be reflected in several ways and at several stages of the life of the organisms. For example, Bisang and Ehrlén (2002) found RC in terms of reduced growth of new branches in *D. polysetum* while Stark et al. (2009) found a cost in terms of lower regeneration of asexual propagules in *Pterygoneurum ovatum* (Hedw.) Dix. Therefore, there is no single correct way of quantifying RC.

Relationship Between Sexual Dimorphism and RA

Sexual dimorphism is defined as any physiological or morphological differences related to sex (Delph, 1999). These differences are often related to RA. In morphological terms, female plants are often larger than male plants (Delph, 1999) because they need to support the offspring (Lewin, 1988). Thus, a large vegetative biomass is necessary for the development and maintenance of the offspring.

In bryophytes, sexual dimorphism has been reported in mosses and liverworts, and in some cases, this morphological variation has been attributed to reproduction. Some species have larger male plants (Laaka-Lindberg, 2001; Rydgren and Økland, 2002), others have larger female plants (Shaw et al., 1993; McLetchie

Table 2 Terminology used in studies to refer to reproductive allocation and reproductive cost in plants along with the definitions used by the authors

Terminology	Theme	Summary	References
Direct costs	Reproductive cost	Costs of reproduction during the same breeding season	(Obeso, 2002)
Short-term costs	Reproductive cost	Reduction of growth or reproduction in the next breeding season	(Rydgren and Okland, 2003)
Long-term costs	Reproductive cost	Have short-term implications in the production process, that is, used in a short production interval	(Nicotra, 1999)
Physiological costs	Reproductive cost	Physiological trade-offs derived from reproductive allocation	(Karlsson, 1994)
Relative somatic costs	Reproductive cost	Differences in vegetative growth rate associated with differences in reproductive allocation	(Tuomi et al., 1983)
Absolute reproductive allocation	Reproductive allocation	Biomass allocated in reproduction in terms absolute or relative reproductive allocation	(Harris and Pannell, 2008)
Relative reproductive allocation	Reproductive allocation	Proportion of resource allocated to reproduction	(Karlsson and Méndez, 2005a, b)
Reproductive investment	Reproductive allocation	Resource allocated to reproduction (quantified by area or by shoot)	(Stark and Brinda, 2013; Santos et al., 2018a, b)
Reproductive effort	Reproductive allocation	Resource allocated to reproduction	(Reekie and Bazzaz, 2002)
Sex allocation	Reproductive allocation	Biomass allocated to reproduction	(Laaka-Lindberg, 2001)

Table 3 Compilation of studies that quantified reproductive allocation (RA) and/or reproductive cost (RC) in bryophytes and respective terminologies adopted in the study, variable measured for estimation of RA, form of reported results, bryophyte group to which the species belongs, and life phase (pre- or post-zygotic) in which the reproductive parameters were quantified

Paper	RA terminology	RC terminology	RA measurement	Bryophyte group	Phase
(Alvarenga et al., 2016)	-	Reproductive cost	-	Moss	Post
(Bisang and Ehrlén, 2002)	Reproductive effort	Reproductive cost	Biomass (%)	Moss	Pre and Post
(Bisang et al. 2006)	Reproductive effort	Reproductive cost	Biomass (%)	Moss	Pre
(Brzyski et al., 2014)	Reproductive allocation	-	Count of gemmae-producing cups	Liverwort	Asexual
(Convey and Smith, 1993)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Convey, 1994a)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Convey, 1994b)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Ehrlén et al., 2000)	Reproductive investment	Reproductive cost	Biomass (%)	Moss	Post
(González-Mancebo and During, 1997)	Reproductive effort	-	Biomass (%)	Moss	Post
(Hassel and Söderström, 2003)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Hedderson and Longton, 2008)	Reproductive effort	-	Biomass (%)	Moss	Pre and Post
(Horsley et al., 2011)	Reproductive investment	-	Biomass (gametangia)	Moss	Pre
(Kimmerer, 1994)	Biomass allocation	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Laaka-Lindberg, 2001)	Biomass allocation	-	Biomass (%)	Liverwort	Pre and Post
(McLetchie and Puterbaugh, 2000)	Biomass allocation	-	Biomass (%)	Liverwort	Asexual
(Pereira et al., 2016)	Reproductive allocation	-	Count of gametangia	Moss	Pre
(Rydgren and Økland, 2002)	-	Ultimate cost	-	Moss	Post
(Rydgren and Økland, 2003)	-	Short-term costs	-	Moss	Post

Table 3 (continued)

Paper	RA terminology	RC terminology	RA measurement	Bryophyte group	Phase
(Santos et al., 2018a, b)	Reproductive investment	-	Biomass (%)	Moss	Pre and Post
Santos et al. (2022)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre and Post
Santos et al. (2023)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre and Post
Santos et al. (2023)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre
(Shortlidge et al., 2017)	Reproductive effort	-	Count of sporophytes	Moss	Post
(Stark and Brinda, 2013)	Reproductive investment	-	Biomass per area	Moss	Pre
(Stark et al., 2000)	Reproductive allocation	-	Biomass per area	Moss	Pre and Post

and Puterbaugh, 2000; Laaka-Lindberg, 2001, 2005; Stark et al., 2001; McDaniel, 2005; Horsley et al., 2011; Stark and Brinda, 2013; Santos et al., 2018a, b), and others show little sexual dimorphism in size (Shaw et al., 1999; Stark et al., 2001; Bisang et al., 2006; Santos et al., 2022). Furthermore, sexual dimorphism has been observed at various ontogenetic stages, such as in cases of anisospory (sexual dimorphism in the spore size) (Sérgio et al., 2020).

Morphological sexual dimorphism is associated with RA because allocation of resources to reproduction limits the resources available for growth. Thus, when RA is consistently larger in one of the two sex functions in a given species, the lower amount of resources left for vegetative growth results in smaller sizes in the plants of the sex with greater RA. For example, in the liverwort *Lophozia. silvicola*, Laaka-Lindberg (2001) reported that RA led to sexual dimorphism in the species: the sex that allocated more resources to reproduction had smaller vegetative development. The influence of RA on sex-specific morphological traits has been demonstrated in many other studies (Table 4).

Methods Used to Measure Reproductive Allocation in Bryophytes

The quantification of RA is not a trivial process. It requires attention from the selection of ramets to the weighing of branches and reproductive structures. Since inconsistencies in the weighting of bryophytes for the quantification of RA are observed, we propose eight steps for standardization of the method, namely: (1) ramet selection; (2) cleaning; (3) removal of brown parts; (4) detachment of reproductive structures; (5) storage of vegetative and reproductive structures; (6) drying; (7) weighing, and (8) calculation of RA.

1 – Ramet Selection To quantify RA, it is necessary to sort the material and select the ramets to be weighed. The selected sex-expressing ramets need to be viable (current growth cycle).The color and appearance are key characteristics to ensure that the plant analyzed corresponds to an actively growing ramet in the current growth cycle. Ramets of the current growth cycle are normally green and do not look brittle. Ramets are classified into two groups according to the phase of reproduction: (1) pre-zygotic (not fertilized), that is, ramets with antheridia and/or archegonia; and (2) post-zygotic (fertilized, that is, with sporophytes). The selection of sporophytic ramets must meet some requirements: (1) the ideal phenophase must be 'intact operculum' (Stark, 1985, 2002; Santos et al., 2020); (2) the operculum, capsule and seta must be intact; and (3) aborted sporophytes (brown and withered) should not be selected.

2 – Ramet Cleaning Each ramet must be cleaned separately and any residual substrate must be removed from the plant. Otherwise, tiny residues, such as soil, leaf remnants, insect exoskeletons and so on will affect the estimate reproductive or vegetative biomass, leading to misinterpretations of the data (Fig. 4A and B).

Table 4 Trade-offs: Type 1 = Sexual reproduction vs. vegetative growth; Type 2 = Asexual reproduction vs. vegetative growth; Type 3 = Sexual reproduction vs. asexual reproduction; Not observed (no conflicting demand detected)

Species	Sexual system	RA	Sexual dimorphism	Trade-off	Reference
Aloina bifrons (De Not.) Delgad	Rhizautoicous	F < M	F > M	Type 2	Stark and Brinda (2013)
Anastrophyllum hellerianum (Nees ex Lindenb.) R.M.Schust	Dioicous	-	F > M	Type 2	Pohjamo and Laaka-Lindberg (2004)
Bryum agenteum Hedw	Dioicous	F < M	F > M	Type 1	Horsley et al. (2011)
Ceratodon purpureos (Hedw.) Brid	Dioicous	F < M	F < M	Type 1	Shaw et al. (1999)
Crossomitrium patrisiae (Brid.) Müll. Hal	Dioicous	-	F = M*	Type 1	Alvarenga et al. (2013, 2016)
Dicranum polysetum Sw	Dioicous	F < E	F > M	Type 1	Bisang and Ehrlén (2002)
Fissidens flaccidus Mitt	Rhizautoicous	F < M	F > M	Type 1, 2 and 3	Santos et al. (2018a, b, 2023)
Fissidens scariosus Mitt	Rhizautoicous	F < M	F > M	Type 2	Santos et al. (2023)
Hylocomium splendens (Hedw.) Schimp	Dioicous	-	F > M	Type 1	Rydgren and Økland (2002)
Lophozia silvicola H.Buch	Dioicous	F > M	F < M	Type 1 and 3	Laaka-Lindberg (2001)
Marchantia inflexa Nees & Mont	Dioicous	F > MG	F < M	Type 4	McLetchie and Puterbaugh (2000)
Pseudocalliergon trifarium (F. Weber & D. Mohr) Loeske	Dioicous	F > M	F = M*	Type 1	Bisang et al. (2006)
Sphaerocarpus texanus Austin	Dioicous	-	F > M	Type 2	McLetchie (2001)
Syntrichia carninervis Mitt	Dioicous	F = M	F > M	-	Stark et al. (2001)
Weissia jamaisencis	Dioicous	F < M	F > M	Type 2	Santos et al. (2022)

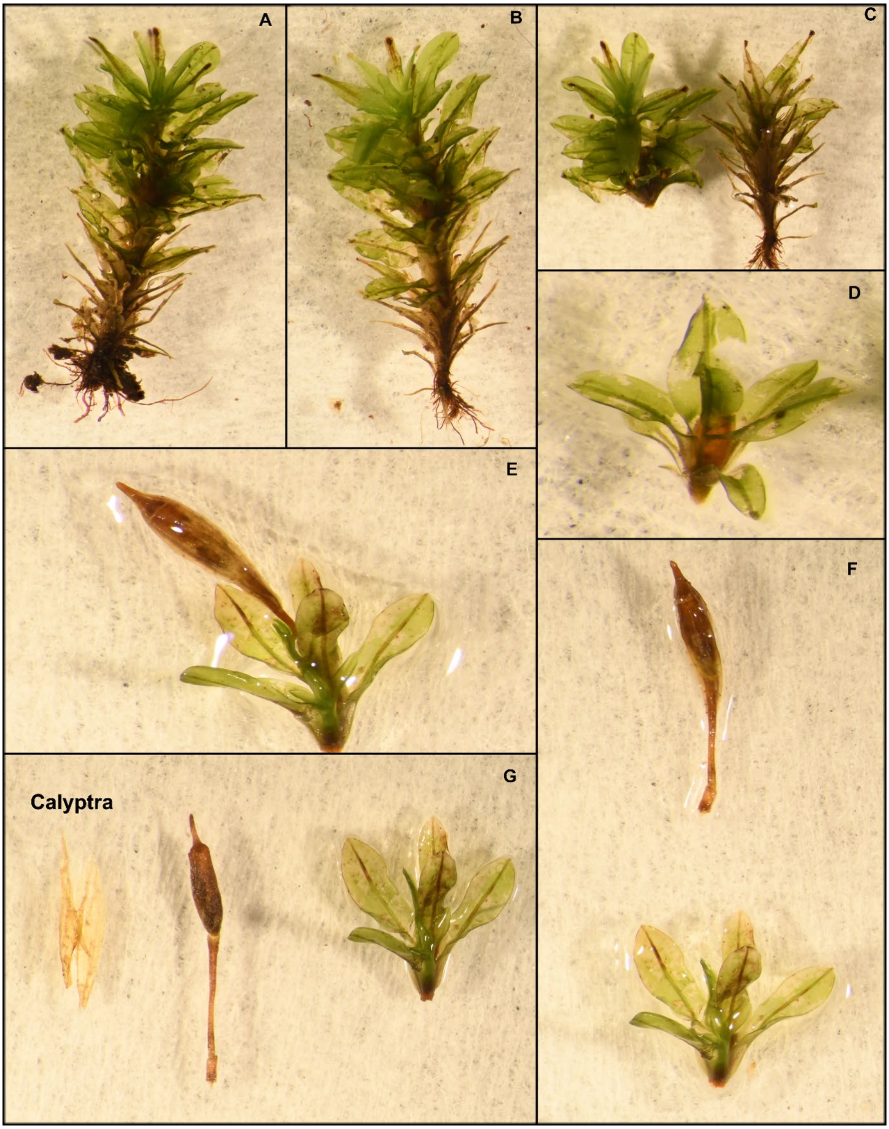

Fig. 4 *Calymperes palisotii* Schwaegr. individuals used as a model for the schematic representation of the steps for quantification of reproductive allocation in bryophytes. **A**—dirty ramets before cleaning; **B** – ramet after being cleaned with distilled water; **C** – separation of the brown portion (bottom part of the ramet) from the green, photosynthetic -portion (upper part of the ramet); **D**—selection of a gametoecium (a "perigonium" is being used here as an example); **E**—sporophytic female ramet with attached sporophyte; **F**—sporophytic female ramet with detached sporophyte; **G**—perigonium with detached antheridia; H—sporophyte with closed operculum and detached calyptra

3 – Removal of Brown Parts Once the ramets are clean, it is necessary to remove the brown parts (bottom of the gametophyte) (Fig. 4C). As Bisang et al. (2006) remark, although the lower brown portion of the gametophytes may be alive (when they can regenerate), they do not influence the RA because they are not photosynthetically active.

4 – Detachment of Reproductive Structures After the ramets have been selected and cleaned and the lower brown portion has been removed, the process of detaching the reproductive structures begins. Here we will discuss how to detach pre- and post-zygotic reproductive structures. In pre-zygotic ramets, only gametoecial leaves and the male and female gametangia, that is, the gametoecia, must be detached from the plant (Fig. 4D). This step must be done with great care because when the reproductive structures are detached, gametangia may come off and be lost. The manipulation of post-zygotic ramets and sporophytes, in turn, is easier. To detach the sporophyte, the gametophyte must be held on one side and the sporophyte must be gently plucked at the base using tweezers (Fig. 4E, F). For further analysis, the calyptra should be removed from the sporophyte, since this structure is a remnant of female gametangium (Fig. 4G).

5 – Storage of Vegetative and Reproductive Structures The vegetative (gametophytes) and reproductive (gametoecia and sporophytes) structures must be packed in small paper envelopes to be dried. When placing the structures in small paper envelopes, it is advisable to protect the envelopes with aluminum foil so as to avoid the risk of them to open and release the structures. In this step, it is important not to press the envelopes while the structures are being placed in them because the reproductive structures are small and may be squashed and lost. At this stage, it is important to label the envelopes describing the vegetative structures and the respective reproductive structures stored in each one of them.

6 – Drying Drying the plants is an important step because this process will eliminate all the water from the plant tissue and allow the measurement of the real biomass of the structures. Thus, each envelope must be placed in an oven for 72 h at 70 °C. This temperature is efficient for mosses, but a longer drying time may be necessary for other groups, as for example for complex thalloid liverworts such as Marchantiaceae and Ricciaceae, due to the complexity of their tissues. Structures can also be dried in desiccation chambers, but in this case, more time will be required for proper drying.

7 – Weighing Once the structures are dry, they must be weighed. For this, a microanalytical or preferably an ultramicroanalytical balance should be used. the high sensitivity of ultramicroanalytical balances (0.1 μg) make them ideal for weighing gametangia, providing greater accuracy in the quantification of biomass. As mentioned earlier, some studies quantified RA as the biomass of gametangia by weighing pre-zygotic structures. However, Stark et al. (2009) argue that the biomass of the gametoecia, and not only of the gametangia, need to be weighted because the gametoecial leaves protect the gametangia and help in the dispersion and or capture of the gametes. Therefore, the biomass of gametoecia must be weighed for the quantification of RA. Sporophytes and ramets must be weighed on a microbalance or ultramicrobalance.

8 – Calculation of RA After weighing the reproductive and vegetative structures, the RA can be calculated in two ways: (1) Absolute RA – absolute biomass of reproductive structures; (2) relative RA – proportion of the total biomass of the ramet (vegetative plus reproductive parts) that corresponds to reproductive structures.

By following the steps proposed here, it is possible to know the real amount of resources allocated to reproduction in absolute and relative terms, and this standardization will allow comparisons among different studies.

Perspectives in the Studies of RA and RC in Bryophytes

The synthesis of concepts in RA and RC of bryophytes presented in previous sections provides a base for new and experienced scientists interested in the field. In the next section, we will point out some topics that still need to be addressed in order to deepen the knowledge of RA and RC in bryophytes.

The simple morphological and functional characteristics of bryophytes, which were practically the first plants to successfully colonize terrestrial environments, are favorable for the study of RA and RC, allowing the implementation of experiments and the measurement of the total pool of resources.

As stressed earlier in this study, the varied forms of estimation of RA and RC in bryophytes complicate the comparisons among the studies published so far. The most appropriate ways to quantify RA and RC in bryophytes were presented. We will conclude this article by sigesting some promising areas for future research on RA and RC in bryophytes.

1 - Sexual Systems The variation of RA and RC across sexual systems is a topic that still deserves to be explored in bryophytes. Most moss and liverwort species studied so far have a dioicous sexual system. And as far as the monoicous system is concerned, only the rhizautoicous system (which is considered functionally dioicous) has been investigated. The investigation of RA in cosexual monoicous systems is fundamental to understand possible patterns and implications for the reproduction of these systems. Stark and Brinda (2013) predicted that larger distance between the sexes should increase the relative RA to the male function. If this prediction is confirmed in future studies, this could potentially explain several patterns related to the reproductive biology of bryophytes, such as, for example, the absence of sporophytes in many dioicous mosses. The only study carried out with cosexual monoicous species was the one performed with species of the genus *Fissidens*. New data from other families may shed further light on the patterns of RA in bryophyte species.

2 - Sexual Lability Sexual lability has been reported in some species of bryophytes (Korpelainen, 1998). Thus, understanding the pattern of lability of the species is important to understand their reproduction and population dynamics. Therefore, we emphasize the importance of investigating the relationship between labile sex expression and RA, because lability can be a strategy to ensure reproduction in bryophyte populations. An important question to be explored is whether the RA of genotypes exhibiting sexual lability follows the same pattern of RA as genotypes that do not exhibit liability. When genotypes that change sex present greater RA to the new sex, this may indicate that enhancing that sexual function is important for maintaining the populations of this species.

3 – Genomics No molecular biology studies related to sexual systems in bryophytes have been conducted so far. Thus, we highlight the importance of looking into this theme. We suggest investigating the transcriptome (complete set of transcripts of a given organism) to find out which genes are expressed for sexual expression. Also, it is important to investigate whether the intensity of sexual expression affects the lability or the pattern of RA in the species, since several physiological aspects controlled by gene expression can influence the biology of organisms.

4 – Reproductive Cost As defined earlier, RC is the effect of current reproductive effort on future reproductive success. However, most studies that investigated RC did not quantify RA. In some cases, RA was estimated in order to provide information on RC. Thus, the correct estimation of RA is essential to measure the RC. Further, the RC could be investigated in the context of other parameters besides future reproductive success, as for example, in terms of tolerance to abiotic factors, photosynthetic rates, and even morphological differences.

5 – Ecosystems Terrestrial ecosystems have a great diversity of climatic characteristics, such as temperature, precipitation, solar radiation, and so forth. It is very important to know how certain species behave in these ecosystems. Some bryophyte species are cosmopolitan, occurring in all continents. One example is *B. argenteum* (Longton, 1981; Castetter et al., 2019). This species is found naturally occurring in the most diverse terrestrial ecosystems, from the polar region to deserts, tropical forests, savannas, and temperate forests. It has been proven that the environment can influence the reproduction of mosses, as reported by Bisang et al. (2020) in the moss *Drepanocladus lycopodioides* (Brid.) Warnst., whose sexual expression varies according to region and environment in wetlands. Thus, studies investigating how resources are allocated in different environments are essential to understand the reproductive biology of the species.

Concluding Remarks and Perspectives

It is clear that RA and RC in bryophytes are not trivial topics and require further research for the clarification of ecological patterns and processes. To date, studies have been conducted in temperate forests in Europe and the US, deserts in the US, tropical forests in South America, and the Arctic region (Fig. 5). Thus, there is a gap in studies in different ecosystems such as tropical wetlands, savannas, and dry

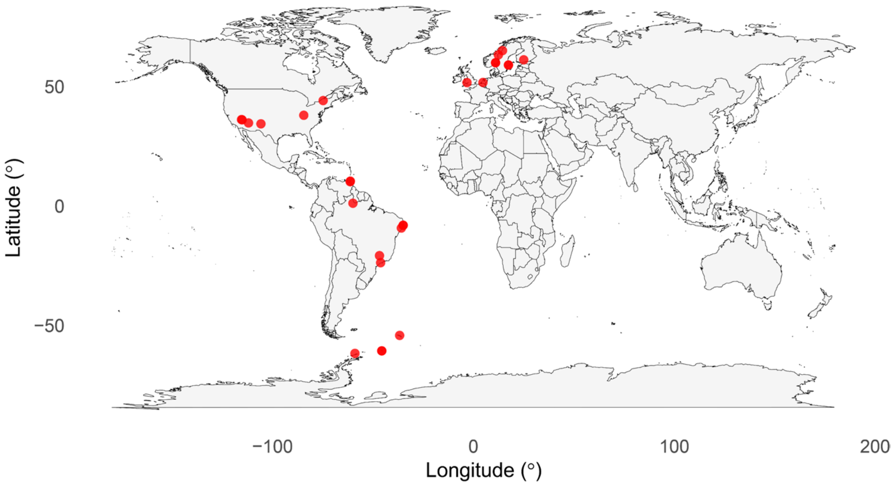

Fig. 5 Map showing localities in the world where studies on reproductive allocation and reproductive cost of bryophytes have been conducted

forests. Furthermore, measuring the RA following the steps proposed in the topic "Methods used to measure reproductive allocation in bryophytes" will provide absolute and relative RA data and even RA per square area that can be straightforwardly compared among multiple studies.

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Declarations

Research involving Human Participants Was not applicable.

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