Age matters: Demographic senescence in the moss *Polytrichastrum formosum*

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**Abstract**
1. It is known that many animal species senesce demographically, showing a decrease in survival and/or fertility with age. Though there is mounting evidence for and against senescence in various flowering plant species, the question of whether senescence also occurs in other plant taxa, such as mosses and other bryophytes, remains unanswered.

2. We used GAMMs (generalized additive mixed models) and GLMMs (generalized linear mixed models) to assess effects of age on survival, size and the monocarpic female ramets’ sexual reproduction in *Polytrichastrum formosum* using demographic, ramet-level data collected over 25 years in Solhomfjell nature reserve, Norway.

3. Survival was found to be negatively correlated with ramet age, with one-year survival decreasing from 83% in 1-year-old to 43% in ten-year-old plants. Stem growth was positively correlated with survival but accounting for it in our models did not change the relationship between age and survival qualitatively. Annual growth decreased during the first years of the ramets’ life but then stabilised. Sexual reproduction in the monocarpic females peaked at ramet age 2–4 years but was observed at ages up to 13 years. The longest-living (non-reproducing) ramet died at age 14.

4. **Synthesis.** Our findings indicate that *Polytrichastrum formosum* ramets experience constant actuarial senescence, at least in terms of survival. This finding improves our understanding of age-dependent demography in mosses, as well as in plants in general. This new knowledge about moss demography may also aid in management decisions for threatened species, as it can improve the precision of predictions of population longevity.

**Keywords:** ageing; bryophyte; growth; monocarpy; mortality; ramet; survival; vital rates

**INTRODUCTION**

Demographic senescence, a decrease in survival and/or fertility with age, has been shown to occur in all investigated mammals and birds, which are the most well-studied taxa in this regard (Jones *et al.* 2014). Historically, the main focus of plant demography has been on size and/or life-cycle stage, as these are considered to be accurate predictors of vital rates in many or most plants (Caswell 2001). However, recent research indicates that age also matters and that there is substantial variation in how age affects vital rates such as survival, reproduction and growth (Edelfeldt, Bengtsson & Dahlgren 2019; Roach & Smith 2020). In some species, survival shows no significant relationship with age or may increase towards higher ages (Hutchings 2010; Garcia, Dahlgren & Ehrlén 2011; Edelfeldt, Lindell & Dahlgren 2019), while other species show a significant decrease in survival with age, i.e. actuarial senescence (Picó & Retana 2008; Roach, Ridley & Dudycha 2009; Dahlgren *et al.* 2016). Similarly diverse relationships have been documented.
between reproduction and age in polycarpic species (i.e. plants that are capable of sexual reproduction multiple times in their life cycle): negative (Tuomi et al. 2013; Barks & Laird 2015), negligible (Rose, Clarke & Chapman 1998; Müller et al. 2014), and positive (Garcia, Dahlgren & Ehrlén 2011; Tuomi et al. 2013). The relationship between reproduction and age is not fully understood in monocarpic species (i.e. plants that reproduce sexually once and then subsequently terminate growth). In general, there seems to exist a size-threshold for reproduction in monocarpic species (Metcalf, Rose & Rees 2003), but some studies indicate that older plants may reproduce at smaller sizes than expected due to an increased tendency of flowering with advancing age (Klinkhamer, de Jong & Meelis 1987; Childs et al. 2003). Also, growth rates of plant individuals have been shown to be affected by age in various ways (e.g. Baden et al. (2020)). However, studies on age-based demography have predominantly been made on vascular plants (Roach & Smith 2020), while very little is known about the effects of age on vital rates of bryophytes (Salguero-Gómez, Shefferson & Hutchings 2013).

Besides ageing, bryophyte mortality is brought about by a large number of factors (Økland 1995), exemplified by various disturbance agents such as burial in leaf litter (Halvorsen 2012), desiccation (Watson 1975; During 1979; Pedersen, Hanslin & Bakken 2001) and amensalism, i.e. deprivation of light by burial in carpets of larger plants (Økland 1995; Halvorsen 2012). This implies that size may be an important driver of bryophyte survival, which indeed has been demonstrated for some species. For example, ramet size was found to be positively correlated with survival in *Hylocomium splendens* (Økland 1995). However, a few studies also suggest that age plays a significant part in determining survival of bryophytes. Thus, in a study of six closely related moss species of the family Polytrichaceae, Watson (1979) found that adult ramet survival decreased with age until levelling off in the oldest age classes. Pohjamo and Laaka-Lindberg (2004) attributed low survival of tall stems of a liverwort to senescence. Observations suggesting adult survival that is independent of age has also been reported for some bryophyte species (During & ter Horst 1985). It is worth noting, however, that none of these studies in which age-related relationships have been inferred, are based on long-term data. The longest-lasting of these observational field studies lasted for less than three years (During & ter Horst 1985) and age distributions were inferred from indirect measures, such as seasonally induced variation in leaf morphology (Watson 1979). Long-term studies of bryophyte survival in which individuals are followed throughout their life spans are so far lacking, and we are not aware of any studies assessing effects of age on the reproduction of bryophytes. In general, the effect of age on bryophyte growth is also poorly understood, although decreasing annual segment lengths with age has been reported in a study of *Polytrichum alpestre* (Longton 1979).
Current evolutionary theories of demographic senescence predict that early-life separation between somatic and germ cell lines in an organism will be associated with an age-related increase in mortality (and possibly decreased fertility) after reaching sexual maturity (Hamilton 1966; Kirkwood 2017). Plants do have this separation between cell lines, but it is currently unclear exactly when the separation takes place. However, research on angiosperms has shown that early-life separation is possible (Lanfear 2018). In angiosperms (and most other vascular plants), the diploid sporophyte is the longer- and free-living generation, while it is the haploid gametophyte that has this role in bryophytes. It is currently unknown whether this difference may influence eventual age-related senescence in this group.

The main aim of this study is to assess to what extent the demography of *Polytrichastrum formosum* ramets is age dependent. More specifically, we assessed the question of whether *P. formosum* ramets experience demographic senescence in terms of negative correlations between age and the vital rates survival, reproduction, and growth. We present analyses of data on ramets of *P. formosum* collected over a twenty-five-year period between 1994 and 2019 in fifteen permanent plots in Solhomfjell nature reserve, Norway. As the length of the study period exceeds the maximum recorded lifespan for the ramets followed during the study by a factor of two, these data are well suited for the purpose. Three hypotheses are evaluated: 1) the effect of age on survival and stem growth of ramets is positive early in life and negative later in life; 2) the effect of stem growth rate of ramets on its survival is positive, which may mask some of the effects of age; and 3) in this monocarpic species, sexual reproduction of female ramets peaks in specific age interval.

**METHODS**

**STUDY SPECIES**

*Polytrichastrum formosum* (Hedw.) G.L.Sm. is a dioecious, acrocarpous moss of the family Polytrichaceae which forms extensive, often 50–200 mm tall, dense turfs (Andersen et al. 1976). Females are monocarpic and terminate after producing a sporophyte, while males are polycarpic (Touw & Rubers 1989). The species is common in the temperate zones of the Northern Hemisphere, on moist, mildly acidic, humus-rich soils (Touw & Rubers 1989). Accordingly, *P. formosum* is commonly found in forests on boulders or on the ground, and it also occurs on well-drained moorland and lowland heath (Touw & Rubers 1989).

Gametophytes of *Polytrichastrum formosum* grow throughout their life span, and older parts form underground rhizomes. They can reproduce asexually by regeneration from fragments or by a branching and eventual separation (During 1990). These clonal processes can result in moss cushions of genetically identical ramets that are either independent, or sometimes connected via rhizomes (van Groenendael & de
Kroon 1990). Other moss cushions can be genetically diverse and consist of multiple genets (Van der Velde et al. 2001). Typically, regeneration from fragments has been found to be of negligible importance for the survival of local populations of this species, whereas branching of the subterranean rhizome is the main process by which new ramets arise (Van der Velde et al. 2001). Sexual reproduction serves long-distance dispersal but is thought to have limited impact on local population dynamics (Van der Velde, Van de Zande & Bijlsma 2001).

STUDY SITE

The study was carried out in Solhomfjell nature reserve, Norway (8°48–51’E, 58°57’N; 350–480 m a.s.l.). The study site is a reference area for monitoring boreal forests and consists of unmanaged Norway spruce (Picea abies) forests with field-layer vegetation dominated by European bilberry (Vaccinium myrtillus). A detailed description of the study site is given by Økland and Eilertsen (1993).

A total of 200 permanent vegetation plots, 1 m² each, were placed using a restricted random procedure along eight transect lines in 1988 (Økland & Eilertsen 1993). The distance between adjacent transects is 2 km or less, and no between-plot distance is less than 3 m. For this study, 14 subplots, 25 × 25 cm each, were censused over the years 1994–2019. A minimum of eight P. formosum ramets had to be observed at census in a specific year for the subplot to be included or retained in the study. If more than 50 ramets were observed in a subplot, the subplot’s “active area” was reduced by a standardised procedure until the number of ramets no longer exceeded 25.

MONITORING

All P. formosum ramets that were observed in the active area of each subplot in any year 1994–2019 were tagged using PVC rings created from HAMA plastic beads (Malte Haaning Plastics Co., Nykøbing Mors, Denmark) with an outer diameter of 4.5 mm and an inner diameter of 2.5 mm. Each ring was sliced into ca. 1 mm tall sections and a slit was provided to allow the rings to be applied to moss stems using two pincers. Ramets were tagged every year by applying a new ring a few millimetres below the apex. This tagging method has been used for studies of Hylocomium splendens over thirty years (Økland 1995). In contrast to other tagging methods (e.g. pins inserted into the substrate next to shoots (Hobbs & Pritchard 1987) and strings tied around the stem at a fixed distance from the apex (Watson 1975)), comparative observations of tagged shoots inside and untagged shoots outside the subplot border over the 25-year study period show no indications of effects of this tagging method on P. formosum ramets, e.g. by attracting herbivores or directly affecting growth and/or survival.

A total of 2,434 ramets were followed during the study period, of which the year of appearance was known for 1,343 ramets. These were used in our analyses. Out of these, 107 female ramets reproduced sexually.
and subsequently terminated, while 885 ramets terminated for other reasons. At each census, the ramets’ stem growth since the previous census was measured. A ramet was considered as terminated (dead) if it was observed with negligible stem growth (< 1 mm) since the previous census and little or no green parts were left on the stem. Fifteen ramets recorded as terminated at one census were found to have regenerated (resumed stem growth) at the next census and thus re-entered the census. The yearly stem growth, hereafter referred to just as “stem growth”, was used as a proxy for size in analyses of survival; the full length of the entire green part of stems was not measured. Female ramets were identified by their sporophytes and ramets with male structures were rare, so the sex of most ramets was therefore unknown.

**ANALYSES AND MODELLING**

Stem growth and survival were modelled using thin-plate spline regressions and the package ‘gamm4’ (Wood & Scheipl 2017) in R (version 3.6.1). This type of generalized additive mixed model (GAMM) enabled identification of possible nonmonotonic age trajectories of both stem growth and survival without assuming specific functional relationships. In GAMMs describing survival, a logit link function and a binomial error distribution were used. When modelling stem growth as a function of age, an identity link function and a Gaussian error distribution were used. Average survival differed among plots (Fig. S1). To account for this variation, and for the repeated measurements of ramets, random intercepts for years, plots and individual ramets were included. Average survival was also found to decrease over the study period, but accounting for this decrease did not affect the estimated age trajectory (Table S1). To limit model complexity, the number of basis functions were limited to 4–6 depending on the model in question.

All models were fitted both excluding and including sexually reproducing females, as well as to sexually reproducing females only. Unless stated otherwise only models excluding sexually reproducing female ramets are reported in the results while all other models are presented in the supplementary material.

Age-dependent survival GAMMs showed linear tendencies regardless of whether stem growth was included or not (this did not, however, hold true for models for sexually reproducing females). Accordingly, we used generalized linear mixed models (GLMMs) for the age-dependent survival models excluding sexually reproducing females. Models of survival were fitted both including and excluding stem growth as a covariate to assess if observed effects of age were affected by a potential correlation between age and stem growth.
RESULTS

Survival probability of *Polytrichastrum formosum* ramets over one-year periods as a function of age, with (slope = -0.15, \(z = -7.02, p < 0.001, n = 3872\)) and without (slope = -0.21, \(z = -10.23, p < 0.001, n = 3872\)) stem growth since the previous census as a covariate in the model. For the model including stem growth, the presented relationship applies to ramets with stem growth equal to the overall mean (15.1 \(\text{mm} \cdot \text{yr}^{-1}\)). Sexually reproducing females are not included in the models. The shaded bands indicate 95% confidence intervals. \(n\) is the number of observations.

Survival decreased almost linearly with age (Fig. 1, Table S1). One-year-old ramets had ca. 83% chance of surviving to the next year in both models, while 10-year-old ramets had a survival probability of 44% when stem growth was not accounted for and 54% when it was. The mean age at termination was 3.4 years (median = 3 years) and the longest recorded lifespan was 14 years.
Survival was positively correlated with stem growth, with ramets growing 5 mm and 20 mm having 53% and 87% chance of survival to the next year, respectively (Fig. 2). Survival levelled off towards high stem growth rates, thus a ramet growing 40 mm in one year had a 90% survival probability. A ramet with a yearly stem growth equal to the average across all ages (15.1 mm) had an 83% chance of survival to the next census.
Fig. 3 Stem growth since previous census of *Polytrichastrum formosum* ramets as a function of ramet age, modelled using a generalized additive mixed model (estimated degrees of freedom = 4.73, $F = 150.7$, $p < 0.001$, $n = 3905$). Sexually reproducing females are not included in the model. The shaded band indicates the 95% confidence interval. Points indicate individual measurements and are jittered horizontally to limit overlap.

Average annual stem growth decreased over the first four years from ca. 19 mm to 12 mm and then levelled off (Fig. 3). The tendency to increased stem growth observed for ramets 10 years or older was based upon very few observations. Stem growth of sexually reproducing females followed largely the same pattern (Fig. S2).
Fig. 4 Left panel: The number of sporophyte-bearing ramets observed during the study period (1995–2019) as a function of ramet age. Right panel: Probability of producing a sporophyte as a function of age (estimated degrees of freedom = 2.89, χ² = 92.23, p < 0.001, n = 483) for female Polytrichastrum formosum ramets that were observed to reproduce during the study. The shaded band indicates a 95% confidence interval. n is the number of observations.

Sporophytes were predominantly found on three to five year-old female ramets (accounting for 84% of all sporophyte-bearing ramets); 36% were four years old (Fig. 4 Left panel). No sporophytes were observed on ramets aged two years or younger (Fig. 4 Left & Right panel). The number of observed sporophytes per year ranged from 0 to 12 (mean = 5.35 for each year in which sporophytes were observed). No sporophyte was observed in the years 1995–97, 2007 and 2019.

**DISCUSSION**

In this uniquely long-term ramet-based study of a monocarpic moss we found a clear negative correlation between age and survival, suggesting actuarial senescence. While stem growth also affected survival, its inclusion in the survival model did not significantly influence the age trajectory. Annual stem growth was negatively correlated to age for young ramets. As expected, sexually reproducing females had a different survival age trajectory compared to other ramets, with the occurrence of sexual reproduction (and termination) in females being centred around ages 3–5 years. These patterns of survival, growth and reproduction suggest that age may matter and has to be taken into account for a full understanding of the demography and life history of at least some bryophytes.

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The negative correlation between survival and age indicates actuarial senescence in *P. formosum*, corroborating findings by Watson (1979), who found similar relationships in a few *Polytrichum* and *Polytrichastrum* species. Similar age-related decreases in survival have been observed in several species of flowering plants, including grasses (Canfield 1957) as well as non-grass monocot (Dahlgren *et al.* 2016) and dicot (Roach, Ridley & Dudycha 2009) herbs. However, it also seems that many flowering plant species do not experience actuarial senescence (Dahlgren & Roach 2017) – their survival and reproduction show no significant negative correlation with age – which may indicate that more studies are needed to sort out general patterns of age trajectories of survival also in bryophytes. Knowing these patterns also for bryophytes will give us a better understanding of senescence in plants in general and may be important for proper management of threatened species, as it has been found that not accounting for age can cause imprecise population projections for plants (Chu & Adler 2014; Edelfeldt, Bengtsson & Dahlgren 2019).

The fact that we find indications of actuarial senescence in ramets does not mean that the same is necessarily the case for genets; that is, the genetic individual potentially consisting of multiple ramets. Theoretical models indicate that both genets (Gardner & Mangel 1997) and ramets (Orive 1995) of plants can escape senescence under some circumstances, due to the modular nature and/or decoupling of size and age generally found in clonal species (Kroon & Groenendael 1997). Due to long life spans of genets and unclear relatedness of ramets, demographic studies of genets of clonal plants are essentially lacking (Dahlgren & Roach 2017). However, molecular techniques, such as those applied by Ally, Ritland and Otto (2010), offer promising opportunities for studies of genets, which may grant us a better understanding of how clonality is related to senescence.

Despite not notably affecting the relationship between age and survival, stem growth appeared to have a positive effect on survival, in accordance with our expectation. Although survival in some species of moss has been found to be mostly independent of size (During & ter Horst 1985), the long-term study of *Hylocomium splendens* by Økland (1995) indicated a strong size-dependent effect on almost all vital rates, including branching and survival. Size is typically also positively correlated to high vital rates in herbaceous vascular plants (e.g. Solbrig (1981), Aarssen and Jordan (2001)). However, this correlation may be brought about by other factors than size as such. A high stem growth rate may simply be indicative of a ramet’s vitality and a sign of the amounts of resources available to it; potentially making stem growth an indicator of higher survival probabilities rather than the cause of it. Regardless of which is the cause, and which is the effect, stem growth was an important predictor of survival of this moss. This strongly motivates taking measures of size, together with age, into account in future demographic research on bryophytes.
The decrease of stem growth rate in the first few years of a ramet’s life could indicate senescence. However, this decrease is followed by a plateau and field observations (D.W. Tholstrup & R. Halvorsen, personal observation) indicated that most ramets stayed connected to the parental plant for the first couple of years. These observations could indicate rapid initial growth being facilitated by the connection to other ramets, which has been observed to occur in other species (e.g. *Populus grandidentata* (Zahner & DeByle 1965)). Thus, age itself is unlikely to drive this pattern in stem growth.

Sporophyte production peaked at ramet ages of 3–5 years. Since sexual reproduction takes place the year before sporophytes mature, the reproductive events peaked at 2–4 years of age. Such relatively “early” sexual reproduction in relation to the possible life span of ramets is in agreement with findings by Metcalf, Rose and Rees (2003), showing that monocarpic plants follow a simple ‘law of diminishing returns’ according to which reproduction takes place once the pay-off from reproduction exceeds the gain from continued survival. Thus, reproduction should take place as soon as enough resources are available for supporting, in the case of mosses, a sporophyte.

We conclude that *Polytrichastrum formosum* ramets appear to experience demographic senescence. However, more studies are necessary to understand how ageing generally affects mosses and other bryophytes, as well as the causes that drive these effects. Long-term individual-based studies are of particular value in cases like this, when age cannot be inferred precisely from morphology. In addition, future studies that identify genets will contribute greatly to a better understanding of ageing, not only in *P. formosum* but in plants in general. Based on our results, we recommend age to be considered in future demographic studies of mosses, as well as in other instances when demographic models are used for population projections.

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**DATA AVAILABILITY STATEMENT**

Data available from the Dryad Digital Repository:

https://datadryad.org/stash/share/r3OHJ2p920TdVVaAj2jy95WeEBZs7DlkwMBs5XXe8 (Tholstrup, Halvorsen & Dahlgren 2021).

**AUTHOR’S CONTRIBUTION**

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RH designed and conducted the fieldwork; DWT, JPD and RH conceived the ideas; DWT analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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