Senescence in duckweed: age-related declines in survival, reproduction, and offspring quality

Authors and affiliations

Patrick M. Barks and Robert A. Laird

Department of Biological Sciences

University of Lethbridge

Lethbridge, AB, T1K 3M4

Canada

Corresponding author

Patrick Barks

Department of Biological Sciences

University of Lethbridge

Lethbridge, AB, T1K 3M4

Canada

Email: patrick.barks@gmail.com

Running title

Senescence in duckweed

Summary

1. As they grow old, most organisms experience progressive physiological deterioration resulting in declining rates of survival and reproduction – a seemingly maladaptive phenomenon known as senescence.

2. Although senescence is usually defined with respect only to survival and reproduction, a third component of fitness, offspring quality, may also decline with age. Few studies, however, have assessed age-related changes in offspring quality using measures that truly reflect fitness.

3. In a controlled environment, we tested for age-related declines in three demographic components of fitness (survival, reproduction, and offspring quality) in *Lemna minor*, a small aquatic plant in the subfamily Lemnoideae (the duckweeds) with a short lifespan and rapid rate of asexual reproduction. Our primary measure of offspring quality, the intrinsic rate of increase, more closely approximates fitness than measures used in previous studies such as size, lifespan, and total reproductive output.

4. We observed strong age-related declines in all three components of fitness: old plants had lower rates of survival and reproduction, and produced lower-quality offspring than younger plants.

5. Theoretical and empirical research on the evolutionary biology of senescence should devote more attention to offspring quality. This often unrecognized component of fitness may change with age – as we have shown in *L. minor* – and may be shaped by, and feed back into, the same evolutionary forces that give rise to senescence.

Key-words: ageing, Lansing effect, *Lemna minor*, life history, parental age effects

Introduction

Senescence is characterized by progressive physiological deterioration and age-related declines in survival and reproduction (reviewed in Kirkwood & Austad 2000; Hughes & Reynolds 2005; Williams *et al*. 2006; Sherratt & Wilkinson 2009). Such declines are seemingly deleterious from the perspective of an ageing individual, and yet senescence occurs in many taxa (Jones *et al*. 2014). Explaining the evolution and maintenance of senescence has therefore been an important challenge in evolutionary biology.

In the most general sense, the evolutionary paradox of senescence concerns age-related declines in the expectation of future genetic representation (i.e. fitness). All else being equal, a lineage that is not subject to age-related declines in fitness should have greater future representation than one that is. Although many authors define senescence with respect only to survival and reproduction, there is increasing evidence that another component of fitness, offspring quality, may also decline with age (Kern *et al.* 2001). For example, a decline in offspring lifespan with increasing parental age (known as the Lansing effect) has been observed in a variety of taxa including rotifers (Lansing 1947, 1948), ladybird beetles (Singh & Omkar 2009), duckweeds (Ashby & Wangermann 1949), and humans (Bell 1918; Gavrilov & Gavrilova 1997) (additional examples are cited in Priest, Mackowiak & Promislow 2002). Similarly, advanced parental age has been shown to negatively affect offspring fecundity schedules in great tits (Bouwhuis *et al*. 2010) and pre-industrial humans (Gillespie, Russell & Lummaa 2013a).

Age-related declines in offspring quality are paradoxical in much the same way as age-related declines in survival and reproduction. All else being equal, lineages not subject to age-related declines in offspring quality should have greater future representation than

70 those that are. Of course, this argument is only valid insofar as offspring 'quality' reflects
71 biological fitness. Lifespan is generally a poor measure of fitness (e.g. Jenkins, McColl &
72 Lithgow 2004), so despite the apparent prevalence of age-related declines in offspring
73 lifespan, the extent to which offspring fitness declines with parental age remains unclear.
74 Resolving this gap in our understanding is important because established theories of life
75 history evolution and senescence implicitly assume that offspring fitness is constant with
76 parental age (e.g. Williams 1957; Hamilton 1966; Kirkwood & Rose 1991). If this is not the
77 case, then the force of selection cannot be understood simply in terms of age-specific
78 survival and fecundity, but may also depend on age-specific patterns of change in offspring
79 fitness (e.g. Pavard, Koons & Heyer 2007). As Caswell (2001, p. 280) points out: "The
80 paradoxes of life history theory mean that selection must be studied in terms of the entire
81 life cycle. The alternative – analysis in terms of a subset of vital rates, or what are called
82 *components of fitness* – risks getting answers that are qualitatively wrong." Thus, if
83 offspring fitness does indeed change with parental age, evolutionary analyses that ignore
84 such changes may lead us astray.
85 Here we test for age-related declines in three major demographic components of
86 fitness (survival, reproduction, and offspring fitness) in *Lemna minor* L., a small and short-
87 lived aquatic plant (Landolt 1986). Our primary interest is to understand whether offspring
88 fitness declines with increasing parental age. *Lemna minor* is an excellent species in which
89 to address this question for two reasons. First, reproduction in *L. minor* is almost
90 exclusively asexual, which simplifies the analysis of parental age effects (there is only one
91 parent to account for and it is easy to identify). Second, previous research suggests *L. minor*
92 may be subject to parental-age-related declines in various offspring traits potentially

relating to fitness, including offspring size, lifespan, and lifetime reproductive output (Wangermann & Ashby 1950, 1951; but see Claus 1972). Because there is a premium on early reproduction, lifespan and lifetime reproductive output may be poor measures of overall fitness (Stearns 1992; Partridge & Barton 1996). Thus, to understand whether *L. minor* is subject to age-related declines in offspring fitness (in addition to age-related declines in survival and reproduction), we employ a demographic measure that better approximates realized fitness – the intrinsic rate of increase (*r*) measured at the level of individual offspring.

Materials and methods

STUDY SPECIES

Lemna minor is a small aquatic plant belonging to Lemnoideae (the duckweeds), a subfamily comprising "the simplest and smallest of flowering plants" (Hillman 1961, p. 222). It occurs in slow-moving freshwater bodies on every continent except Antarctica (Landolt 1986), and is tolerant to a wide range of environmental conditions (Wang 1990; Mkandawire & Dudel 2000). Individual plants are about 3–5 mm long and consist of a free-floating frond (also called a thallus; a combination of leaf and stem) and a single root that emanates from the frond's lower surface (Lemon & Posluszny 2000). Proliferation of *L. minor* is dominated by vegetative reproduction – offspring (often referred to as daughter fronds) develop asexually in alternating succession from one of two meristematic pockets within the parent (Landolt 1986). Under optimal laboratory conditions, each plant will produce about 15 offspring within a lifespan of approximately 30 days (Lemon, Posluszny

& Husband 2001). We note that, unlike most vascular plants, duckweeds have a unitary growth form and determinate growth potential – maximum frond size is usually achieved prior to a frond detaching from its parent (Hillman 1961).

OVERVIEW

We tested for age-related declines in components of *L. minor* fitness in two phases.

Phase One: Survival and Reproduction

First, to measure the influence of age on rates of survival and reproduction, we isolated 216 fronds individually in Petri dishes containing a liquid growth medium, and observed the fronds daily for the duration of their lives. The first day of life was defined as the day that a frond detached from its parent, and death was defined as the day that a frond's final daughter detached (there are no obvious physiological definitions of death in *L. minor*, as the progression of cell death during frond senescence generally spans 10 or more days). Every day during a frond's lifetime, we observed whether or not the frond reproduced – i.e. whether any of its daughters detached since the previous day's observation. Detached daughters were aseptically removed from the Petri dish and discarded.

Phase Two: Offspring Quality

The second phase of our study examined changes in offspring quality (measures included the intrinsic rate of increase, total reproductive output, latency to reproduce, lifespan, and frond size) as a function of parental age. We isolated 41 'parental' fronds

individually in Petri dishes, and observed them daily for the duration of their lives as described above. This time, however, instead of being discarded, the daughters (the 'focal' generation, N = 542) of the 41 parental fronds were transferred to their own Petri dish upon detaching from the parent, randomly assigned to one of three growth chambers, and observed for reproduction daily for the duration of their lives. Four of the 542 focal fronds (all of which were the final daughters produced by their respective parents) remained attached to their parent for a prolonged period of time – well into their reproductive lifespan. We defined the first day of life for these four individuals as the day that their first daughter detached.

PLANTS AND GROWTH CONDITIONS

The plants used in this study were derived from a clonal lineage that we obtained from the Canadian Phycological Culture Centre (CPCC 492 *Lemna minor*; originally collected from Elk Lake, British Columbia, Canada; 48° 31' 30" N, 123° 23' 18" W). We studied a genetically homogeneous sample because heterogeneity (both genetic and environmental) can sometimes mask true patterns of senescence (Zens & Peart 2003). Due to the possibility of parental-age effects in *L. minor* (e.g. Wangermann & Ashby 1950, 1951), we also strove for 'genealogical' homogeneity among our focal plants. Specifically, the 216 focal fronds in *Phase one* and 41 parental fronds in *Phase two* were each first daughters of first daughters (etc.) going back at least five generations.

Plants were aseptically cultured in 60 × 10 mm Petri dishes containing 10 ml of Modified Hoagland's E+ growth medium (Environment Canada 2007), and kept inside growth chambers set to 25°C with a 12:12 photoperiod and a photosynthetic photon flux

density at plant height of approximately 500 μmol m^{-2} sec^{-1}. To ensure environmental constancy (e.g. to account for evaporation, nutrient depletion, etc.), we aseptically transferred each plant into a new Petri dish with 10 ml of fresh growth medium every four days. Two of the 216 fronds from *Phase one* developed bacterial contamination and so were discarded and not included in the analyses below. There was no bacterial contamination during *Phase two*. Low rates of fungal contamination occurred in both phases of our study, always taking the form of an isolated clump of stringy white fungus within the growth medium. When such contamination was detected, the corresponding frond was aseptically transferred to a new Petri dish with fresh growth medium. This intervention was successful given that no plant was ever subject to more than a single instance of fungal contamination.

FITNESS MEASURES

Phase One: Survival and Reproduction

Our measures of fitness in *Phase one* were daily rate of survival and daily rate of reproduction conditional on survival. Although fronds occasionally released two daughters on the same day (this occurred in 8.6% of the reproductive events that we observed), we chose to analyze reproduction as a binary event (0 = did not reproduce, 1 = released one or two daughters). Treating reproduction as binary instead of ordinal made it easier (statistically) to account for non-independence due to repeated observations on the same individuals.

Phase two: Offspring Quality

Our primary measure of offspring fitness was the intrinsic rate of increase (*r*) measured at the level of individual fronds, as described in McGraw and Caswell (1996). Intrinsic rate of increase is an appropriate measure of fitness for stable populations under constant environmental conditions (Metcalf & Pavard 2007), and can be calculated as the natural logarithm of the dominant eigenvalue of a Leslie matrix. To construct a Leslie matrix for single individuals, the age-specific survival rate was set to 1 for each age at which an individual survived, and 0 for every other age (McGraw & Caswell 1996). Measuring fitness in this way – at the level of the individual – is sometimes problematic due to a lack of replication (Link, Cooch & Cam 2002). However, our use of a single clone negates this problem. The realized fitness of replicate fronds of a given parental age should reflect the same underlying fitness propensity (or 'latent fitness'), and thus, our approach entails appropriate replication.

In addition to our primary measure of offspring fitness (the intrinsic rate of increase), we examined four secondary measures of offspring quality (not necessarily directly related to fitness): total number of offspring produced, latency to first reproduction (days between detachment from parent and first daughter detaching; inversely related to fitness), lifespan (days between detachment from parent and last daughter detaching), and frond surface area. Frond surface area was measured in ImageJ v. 1.43u (Rasband 2012) using images captured with a microscope-mounted digital camera. Images used for surface-area measurement were captured late in a frond's life when it had no attached daughters. Occasionally, fronds produced late in their parent's life were 'curled' (see Figure S1 under Supporting Information), which complicated the measurement of surface area. For the 42 focal fronds in *Phase two* that were curled, we estimated surface

area based on the length of each frond's longitudinal axis (Fig. S1). These 'corrected' estimates were interpolated from a linear regression of surface area on length for the 500 non-curled fronds (Fig. S2).

DATA ANALYSIS

All analyses were conducted in R v. 3.0.2 (R Core Team 2013).

Phase One: Survival and Reproduction

To understand how daily rates of survival varied with age, we fit and compared four candidate survival models (described in Pletcher, Khazaeli & Curtsinger 2000; Sherratt *et al.* 2010): exponential, Weibull, Gompertz, and logistic. The exponential model serves as a null hypothesis of no senescence because it assumes a constant rate of survival with age, whereas survival may decline with age in the other models. All survival models were fit by maximizing log-likelihood functions using the *optim* function in R, and strength of evidence was assessed using the Akaike information criterion corrected for small sample sizes, AIC_C (Burnham & Anderson 2002).

To test for age-related declines in the daily rate of reproduction, we used generalized estimating equations (GEE) with a binomial error structure and logit link, fit with the *geeglm* function in the R package *geepack* (Halekoh, Højsgaard & Yan 2006). The GEE approach was ideal for our analysis given the possibility of within-individual negative temporal autocorrelation in reproduction (i.e. an individual that reproduces on a given day is somewhat less likely to reproduce the very next day). Due to this possibility, we favoured (based on biological relevance) a first-order autoregressive (AR-1) correlation structure,

which assumes that the correlation between repeated observations on the same subject is inversely related to the distance (or time) between those observations. Other common correlation structures include 'exchangeable' (constant within-subject correlation; similar to a mixed-effects model with subject-level random intercepts) and 'independence' (no within-subject correlation; equivalent to a generalized linear model) (Zuur *et al.* 2009). We used the Rotnitzky–Jewell (RJ) criteria (Rotnitzky & Jewell 1990) and the rule-out criterion proposed by Shults *et al.* (2009) to compare the three correlation structures described above, and a Wald test to assess the effect of age on probability of reproduction. The RJ criteria include three metrics by which to compare robust (empirical) estimates of a covariance matrix to naïve (model-based) covariance estimates. The model in which the working correlation structure best approximates the 'true' correlation structure is the model for which empirical and model-based covariance estimates are most similar (Wang & Carey 2004; Shults *et al.* 2009). The rule-out criterion rejects correlation structures yielding estimated covariance matrices that are not positive definite – indicative of a misspecified correlation structure (Crowder 1995; Schults *et al.* 2009). Note that, in the analyses of reproduction described above, we excluded data for the first day of each frond's life because none of the 216 focal fronds in *Phase one* reproduced on day one.

Phase Two: Offspring Quality

To understand whether offspring quality declined with parental age, we modeled our primary measure of offspring fitness (intrinsic rate of increase) and secondary measures of offspring quality (total offspring, latency to first reproduction, lifespan, and surface area) as functions of the age of the parent when the focal frond (i.e. offspring)

254 detached, while controlling for the growth chamber that the focal frond was assigned to. All
255 of the relationships between offspring quality and parental age were nonlinear and could
256 not be transformed to linearity, so in all cases we examined polynomials of parental age up
257 to a degree of three.

258 The modeling approach described hereafter follows Zuur *et al.* (2009). To account
259 for potential non-independence of offspring derived from the same parent, we initially fit
260 linear mixed models describing a given measure of offspring quality as a function (either
261 linear, quadratic, or cubic) of parental age and linear function of growth chamber, with one
262 of three random effect structures: (i) random intercept and slope terms for parent identity,
263 (ii) random intercept term for parent identity, or (iii) no random effects. These models
264 were fit via restricted maximum likelihood (REML) using the *lme* or *gls* functions (*gls* was
265 used for models without random effects) in the package *nlme* (Pinheiro *et al*. 2010). To
266 identify the best random effect structure (separately for each measure of offspring quality),
267 we compared the nine models (3 random effect structures × 3 polynomials of parental age)
268 using AIC_C. We did not encounter any instances in which the 'best' random effect structure
269 differed between the three polynomials of parental age for a given measure of offspring
270 quality (i.e. selection of the best random effect structure was always unanimous).

271 Once the best random effect structure was established, we moved on to the fixed
272 effects (parental age and growth chamber). In this portion of the analysis, models were fit
273 via maximum likelihood (ML), again using either the *lme* or *gls* functions. Our approach
274 here was to construct 'full' models describing each of the five measures of quality as a cubic
275 function of parental age and linear function of growth chamber (with the appropriate
276 random effect structure, as described above). We then compared all fixed-effect subsets of

each full model using the *dredge* function in the package *MuMIn* (Bartoń 2013) and AIC_C values. Our all-subsets approach yielded eight models for each measure of offspring quality: three polynomials of parental age (either with or without a term for growth chamber), a growth chamber only model, and a null model with only an intercept.

We visually assessed model assumptions (independent, normally-distributed error with homogeneous variance) for each measure of offspring quality using standard diagnostic plots including quantile-quantile plots, histograms of model residuals, scatterplots of residuals versus fitted values, and scatterplots or histograms of residuals versus independent variables (including the random effect term for parent identity). Diagnostic plots suggested that parametric assumptions were violated for the best model of intrinsic rate of increase (residuals were positively skewed). We therefore repeated the above-described protocol on log-transformed intrinsic rates of increase, which resulted in a best model that was more closely in line with parametric assumptions.

Results

PHASE ONE: SURVIVAL AND REPRODUCTION

We observed a significant decline in daily rates of survival with increasing frond age (Fig. 1a). In particular, of the four candidate survival models that we examined, the three models in which survival rates declined with age received greater statistical support (i.e. had much lower AIC_C values) than the exponential model which assumes a constant survival rate (Table 1). We also observed significant age-related declines in the daily probability of reproduction (Wald test, $\chi^2 = 652.3$, df = 1, $P < 0.001$; Fig. 1b). Predicted daily probability of

reproduction from the fitted GEE declined from 0.65 at day one to 0.28 at day thirty. The Wald test and predicted probabilities of reproduction described above were based on a GEE with autoregressive (AR-1) correlation, which was selected as a more appropriate working correlation structure than 'independence' based on the RJ criteria (Table 2). The 'exchangeable' correlation structure was ruled out because it yielded an estimated covariance matrix that was not positive definite, potentially indicating a misspecified correlation structure (Crowder 1995; Schults *et al.* 2009). The estimate for the correlation parameter of the AR-1 model was -0.28 (± 0.02, SE), indicating moderate within-subject negative temporal autocorrelation in reproduction.

PHASE TWO: OFFSPRING QUALITY

There was a strong decline in our primary measure of offspring fitness, the intrinsic rate of increase, with increasing parental age (Fig. 1c). We also observed parental-age-related declines in three of our four secondary measures of offspring quality: total offspring produced, latency to first reproduction (this inverse measure of quality technically increased with parental age), and frond surface area (Fig. 2a,b,d). Lifespan, conversely, did not decline with increasing parental age (Fig. 2c).

The models of offspring quality selected as best (lowest AIC_C) were in all cases non-linear with respect to parental age. Specifically, best models always described offspring quality as either a quadratic or cubic function of parental age (Table 3). Except for frond surface area, best models (or a close second-best model in the case of latency to reproduction, $\Delta AIC_C = 0.1$) always included a term for growth chamber, suggesting that measures of offspring quality consistently differed among the three growth chambers that

we used (Table 3). Excepting latency to reproduction and lifespan, best models also always included random intercept and slope terms for parent identity, suggesting non-independence of offspring derived from the same parent (Table 3).

Discussion

We observed strong age-related declines in three demographic components of fitness in *L. minor*. Old plants had lower rates of survival and reproduction, and produced offspring of lower fitness than younger plants. While many species are known to experience age-related declines in at least one component of fitness, our study is to our knowledge the first to demonstrate simultaneous age-related declines in these three major demographic components of fitness, and also one of few studies to demonstrate age-related declines in a measure of offspring quality that closely approximates fitness (see also Gillespie *et al*. 2013a). Of course, these results were obtained in a controlled, laboratory setting, so we encourage further research examining how the demographic patterns we identified manifest in the wild.

OFFSPRING QUALITY AND THE EVOLUTION OF SENESCENCE

Classic theories for the evolution of senescence implicitly assume that all offspring are of equal fitness, so that the action of natural selection depends only on age-specific rates of survival and reproduction (e.g. Williams 1957; Hamilton 1966; Kirkwood & Rose 1991). Our results suggest that this assumption does not always hold, in which case selection may

346 depend additionally on age-specific trajectories of offspring fitness. Why would this
347 matter? There are few theoretical results to guide us here, but a recent analysis by Gillespie
348 *et al*. (2013b) suggests that birth-order-related declines in offspring fitness (similar in
349 principle to parental-age-related declines) lead to steeper declines in the force of selection
350 compared to what would be expected under classical models of senescence. In other words,
351 not accounting for declining offspring fitness, where it occurs, may lead us to
352 underestimate age-related declines in the force of selection. As many authors have argued,
353 senescence, or more generally the action of selection, cannot be understood in terms of a
354 single 'vital rate' or component of fitness (Partridge & Barton 1996; Caswell 2001; Nussey
355 *et al*. 2008). We suggest, following Kern *et al*. (2001), that research on the evolutionary
356 biology of senescence should devote attention to one extra vital rate – offspring quality.
357 This often unrecognized component of fitness can clearly change with age, as we have
358 shown in *L. minor*, and may be just as important in shaping overall fitness as survival and
359 fecundity.
360
361 SENSCENCE IN PLANTS
362 Evolutionary theories of senescence suggest that age-related declines in fitness evolve
363 because, for populations subject to nonzero mortality, the force of natural selection
364 declines with age (Medawar 1952; Williams 1957; Hamilton 1966). Simply put, natural
365 selection discounts old age-classes because relatively few individuals survive into old age,
366 even in the absence of senescence. However, a number of authors have suggested that
367 senescence should be relatively rare among vascular plants (Vaupel *et al.* 2004; Peñueles &
368 Munné-Bosch 2010) or even that plants are predisposed to immortality (Silvertown,

Franco & Perez-Ishiwara 2001). Such views are based on unique aspects of the plant form and life history. For example, unlike other organisms that exhibit determinate growth, many vascular plants exhibit continual growth and regeneration via totipotent apical meristems (Roach 2001). This indeterminate growth pattern potentially allows for a continual increase in reproductive potential with age, which may translate into an increase in the force of natural selection with age (Vaupel *et al*. 2004).

Although some iteroparous plants (e.g. Herrera & Jovani 2010; Shefferson & Roach 2013) and all semelparous plant species exhibit senescence, comparative studies to date have largely confirmed the predicted rarity of senescence among iteroparous vascular plants (Silvertown *et al*. 2001; Baudisch *et al*. 2013). Furthermore, a recent analysis by Caswell and Salguero-Gómez (2013) found that the force of selection does in fact increase with age for many iteroparous plants, especially within later stages of the plant life cycle. Why then is the iteroparous *L. minor* subject to senescent decline when its relatives within Plantae seem mostly immune? Unlike most vascular plants, *L. minor* has a unitary growth form and exhibits determinate growth at the level of individual fronds, which usually reach their full growth potential prior to detaching from their parent (Hillman 1961). This determinate growth pattern, combined with potentially high rates of extrinsic mortality due to herbivory and disease (Landolt 1986), likely leads to a decline in the force of natural selection with age, making *L. minor*'s age-related declines in fitness consistent with evolutionary theory.

PROXIMATE EXPLANATIONS FOR DECLINING OFFSPRING QUALITY

Age-related declines in fitness generally coincide with various forms of physiological deterioration or damage (Munné-Bosch 2007; Lindner *et al.* 2008; Monaghan 2010). Although our study did not specifically examine proximate explanations for senescence, we briefly touch on a potential explanation for age-related declines in offspring quality in *L. minor*. In general, age-related declines in offspring quality (including the Lansing effect) are thought to relate either to declines in parental care or provisioning of offspring (Fox 1993), the accumulation of mutations in parental reproductive tissue (Crow 1997), or the accumulation and transfer of deleterious compounds from parent to offspring (Ashby & Wangermann 1951). Of the three explanations above, mutation accumulation seems the least likely in this case given *L. minor*'s almost-exclusive asexual reproduction, which would render it subject to Muller's ratchet and mutation meltdown (Lynch *et al*. 1993). While we are not able to rule out the other two explanations in the case of *L. minor*, we suggest a another, non-exclusive possibility – that declining offspring quality in *L. minor* is caused by age-related structural changes in the environment in which fronds develop. In particular, Lemon and Posluszny (2000) found that when a daughter frond detaches from its parent, a small amount of connective tissue (deriving from a structure called the stipe) is left behind in the parent's meristematic pocket. They report, "after several daughter fronds have been produced, a large amount of stipe tissue will have accumulated in the pockets" (p. 743). We hypothesize that the accumulation of stipe tissue in the meristematic pockets of *L. minor* fronds progressively constricts or otherwise modifies the growth environment experienced by successive daughters, which may play a role in the age-related declines in offspring size and fitness observed in our study. This hypothesis yields a potentially testable prediction:

413 the artificial removal of accumulated stipe tissue should delay age-related declines in
414 offspring size and/or fitness.
415
416 SENESCENCE IN LEMNA
417 Wangermann and Ashby (1950, 1951) documented parental-age-related declines in
418 offspring size, lifespan, and lifetime reproductive output in *L. minor*, whereas Claus (1972)
419 observed a slight increase in offspring lifespan and no change in lifetime reproductive
420 output with increasing birth order (similar in principle to parental age). In Claus's study,
421 birth order was confounded with other aspects of genealogy and there were very few
422 plants representing the highest birth orders (i.e. greatest parental ages), so his results are
423 difficult to interpret and we do not consider them further. Similar to Wangermann and
424 Ashby, our results demonstrate age-related declines in offspring size and lifetime
425 reproductive output, and we extend the results of Wangermann and Ashby in a manner
426 relevant to evolutionary theories of senescence by specifically demonstrating age-related
427 declines in offspring fitness (i.e. intrinsic rate of increase). We did not, however, observe
428 declines in offspring lifespan with increasing parental age. One possible explanation for the
429 conflicting results relates to how we defined death (i.e. the day that a frond's final daughter
430 detached). It is not clear to us exactly how Wangermann and Ashby defined death, but they
431 seem to have assessed death visually based on a loss of pigment. The difference between
432 these two definitions of death might be considered the post-reproductive lifespan (i.e. the
433 time between a final reproduction and the complete loss of pigment). If post-reproductive
434 lifespans (but not reproductive lifespans) tend to decline with increasing parental age in *L.*

435 *minor*, we would expected to see age-related declines in offspring lifespan under
436 Wangermann and Ashby's (presumed) definition of death, but not under our own.
437
438 CONCLUSIONS
439 We found that, in a controlled laboratory environment, *L. minor* fronds exhibited age-
440 related declines in three major demographic components of fitness – survival, reproduction,
441 and offspring fitness. Following Kern *et al.* (2001), we suggest that both theoretical and
442 empirical research on the evolutionary biology of senescence should devote more attention
443 to age-related changes in offspring quality. This often unrecognized component of fitness
444 can clearly change with age, as we have shown in *L. minor*, and may be just as important in
445 shaping overall fitness as survival and fecundity. Incorporating offspring quality into
446 demographic and evolutionary analyses will no doubt be challenging. Indeed, determining
447 the appropriate measure of fitness is difficult even when only the traditional fitness
448 components – survival and fecundity – are considered (Link *et al.* 2002; Metcalf & Pavard
449 2007). Nonetheless, we suggest that treating offspring quality as a component of fitness
450 that may covary or trade-off with other fitness components, and be shaped by age-specific
451 changes in the force of natural selection alongside other fitness components, may provide
452 important insight into the evolutionary biology of senescence.
453
454
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463

464 **Data accessibility**

465 Data deposited in the Dryad repository: http://doi.org/10.5061/dryad.t938n (Barks &
466 Laird 2014)

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Images of non-curled and curled fronds.

Figure S2. Estimating the surface area of curled fronds.

Table 1. Comparison of models describing age-specific rates of frond survival. The best model is in bold.

Model	Parameters	Deviance	AIC_C	ΔAIC_C	AIC_C weight
Logistic	**3**	**1195.9**	**1202.0**	**0.0**	**0.99**
Weibull	2	1222.1	1226.2	24.2	<0.001
Gompertz	2	1258.5	1262.5	60.5	<0.001
Exponential (no senescence)	1	1808.1	1810.1	608.1	<0.001

Table 2. Comparison of working correlation structures for GEE models describing age-specific rates of reproduction. The 'best' working correlation structure (in bold) is the one that yields values of RJ1 and RJ2 closest to 1, and a value of RJ3 closest to 0. Working correlation structures that fail to yield a positive definite covariation matrix are ruled out.

Working correlation structure	Positive definite covariation matrix?	RJ1 ($\bar{c}_1$)	RJ2 ($\bar{c}_2$)	RJ3 ($\bar{d}$)
Independence	yes	0.25	0.07	0.58
Autoregressive (AR-1)	**yes**	**0.42**	**0.19**	**0.36**
Exchangeable	no	–	–	–

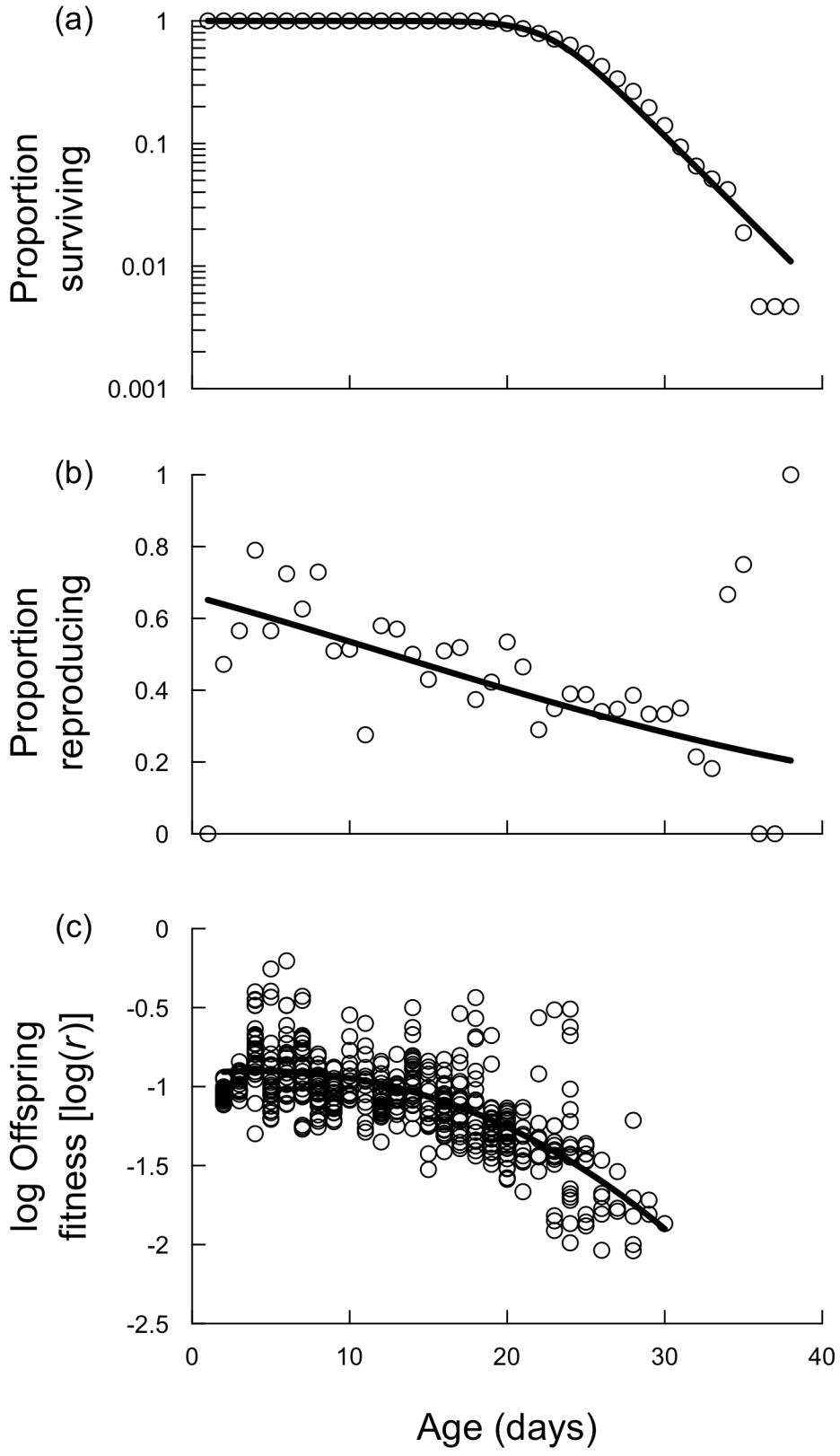
688 **Table 3.** Comparison of models describing measures of offspring quality as functions of
689 parental age (p.age) and growth chamber (chamb). For each measure of quality, the best
690 model is in bold. Only the five best models are displayed for each measure of offspring
691 quality.
692

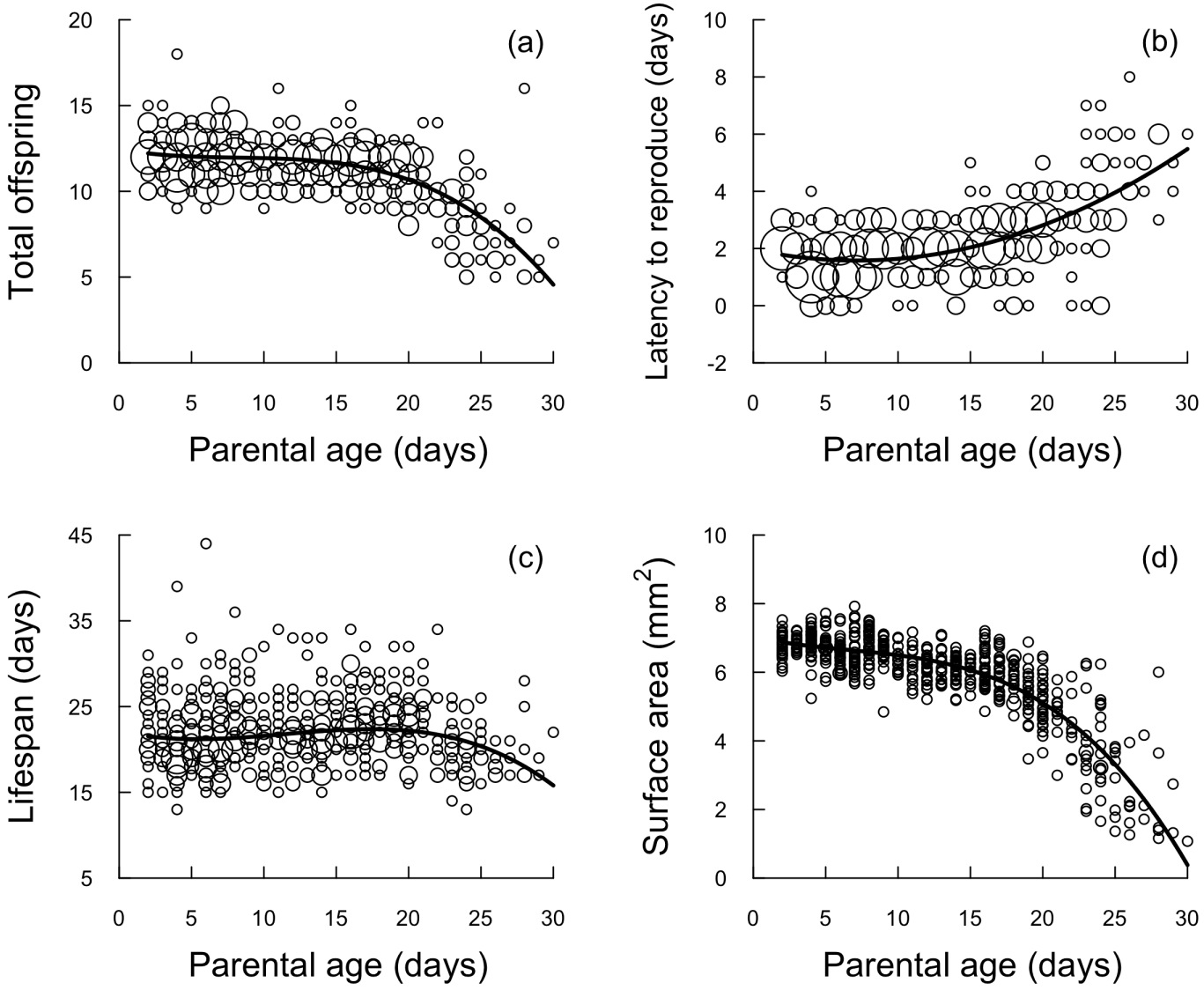
Measure of offspring quality	Model[a]	df	Deviance	AIC_C	ΔAIC_C	AIC_C weight
log (Intrinsic rate of increase)[c]	**$p.age^2$ + chamb**	**9**	**-192.8**	**-174.4**	**0**	**0.44**
	$p.age^2$	7	-187.8	-173.6	0.9	0.29
	$p.age^3$ + chamb	10	-192.9	-172.5	2.0	0.17
	$p.age^3$	8	-187.9	-171.7	2.8	0.11
	$p.age^1$	6	-127.4	-115.2	59.2	<0.001
Total offspring[c]	**$p.age^3$ + chamb**	**10**	**1854.1**	**1874.5**	**0**	**0.83**
	$p.age^3$	8	1861.4	1877.6	3.1	0.17
	$p.age^2$ + chamb	9	1871.0	1889.3	14.9	<0.001
	$p.age^2$	7	1877.0	1891.2	16.7	<0.001
	$p.age^1$ + chamb	8	1963.0	1979.3	104.8	<0.001
Latency to reproduce[b]	**$p.age^2$**	**4**	**1502.4**	**1510.5**	**0**	**0.30**
	$p.age^2$ + chamb	6	1498.4	1510.6	0.1	0.28
	$p.age^3$	5	1501.0	1511.1	0.6	0.22
	$p.age^3$ + chamb	7	1497.1	1511.3	0.8	0.20
	$p.age^1$	3	1571.7	1577.8	67.3	<0.001
Lifespan[b]	**$p.age^3$ + chamb**	**7**	**3005.0**	**3019.2**	**0**	**0.95**
	$p.age^2$ + chamb	6	3013.0	3025.2	6.0	0.05
	chamb	4	3025.2	3033.3	14.1	0.001
	$p.age^1$ + chamb	5	3025.2	3035.3	16.1	<0.001
	$p.age^3$	5	3033.2	3043.3	24.1	<0.001
Frond surface area[c]	**$p.age^3$**	**8**	**970.2**	**986.5**	**0**	**0.67**
	$p.age^3$ + chamb	10	967.5	987.9	1.4	0.34
	$p.age^2$	7	1002.0	1016.2	30.0	<0.001
	$p.age^2$ + chamb	9	998.4	1016.7	30.2	<0.001
	$p.age^1$	6	1236.4	1248.5	262.0	<0.001

693 a. Numeric superscripts beside the parental age term (p.age) indicate polynomial degree. For example, $p.age^3$
694 indicates that the measure of offspring quality was modeled as a cubic function of parental age.
695 b. Models do not include random effects
696 c. Models include random intercept and slope terms for parent identity
697

Figure 1. Age-related changes in rates of survival (a), rates of reproduction (b), and offspring fitness (c) in *L. minor*. Offspring fitness is measured as the log-transformed intrinsic rate of increase (*r*), which has units of day^{-1}. Best-fit models are described in the text and Tables 1-3. In semi-log survival plots such as in panel a, a population with constant survival rates (i.e. with no senescence) would appear as a straight line.

Figure 2. Parental-age-related changes in secondary measures of offspring quality including total offspring produced (a), latency to first reproduction (inversely related to fitness; b), lifespan (c), and frond surface area (d). Point area is proportional to the number of observations at a given set of coordinates. Best-fit models are described in the text and Table 3.


(a)
Proportion surviving
1
0.1
0.01
0.001
(b)
Proportion reproducing
1
0.8
0.6
0.4
0.2
0
(c)
log Offspring fitness [log(r)]
0
-0.5
-1
-1.5
-2
-2.5
0
10
20
30
40
Age (days)


(a)
Total offspring
Parental age (days)
(b)
Latency to reproduce (days)
Parental age (days)
(c)
Lifespan (days)
Parental age (days)
(d)
Surface area (mm2)
Parental age (days)

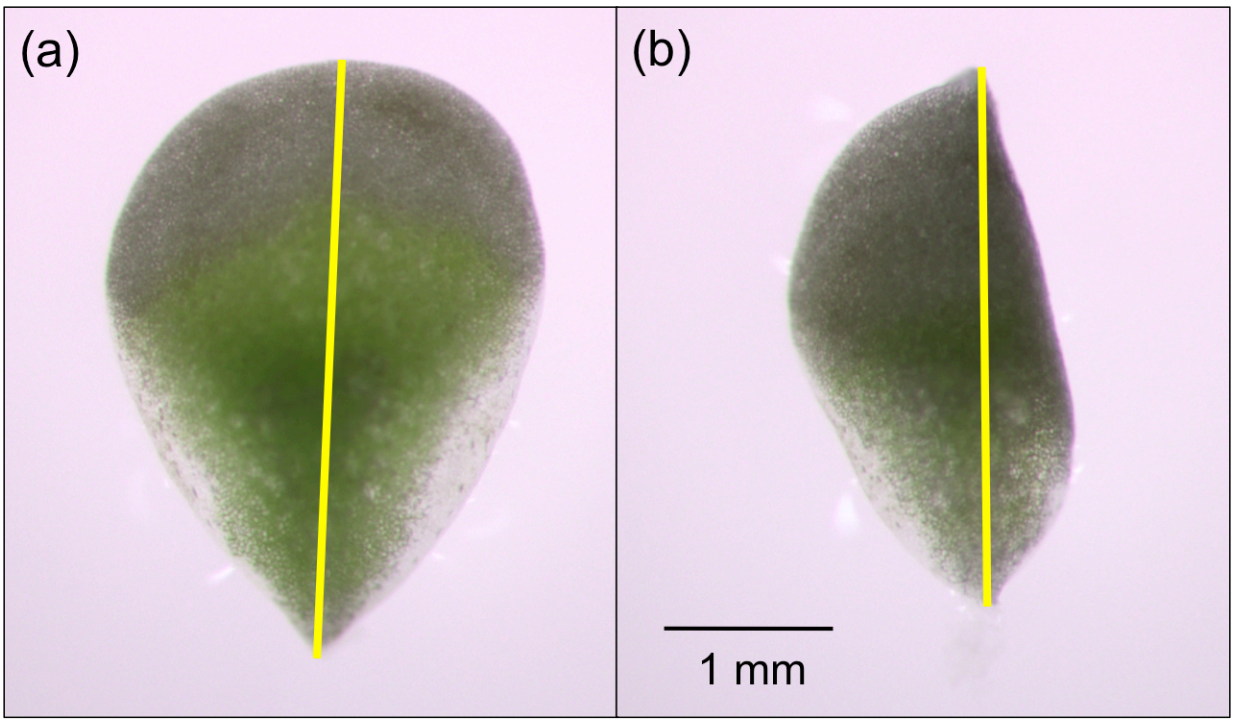


Figure S1. Comparison of non-curled (a) and curled (b) fronds of *Lemna minor*. Yellow lines correspond to each frond's longitudinal axis. We used the strong correlation between surface area and length of the longitudinal axis to estimate the surface area of the 42 (out of 542) fronds in *Phase two* of our study that were curled (see also Fig. S2). Note that the 1 mm scale bar applies to both panels.

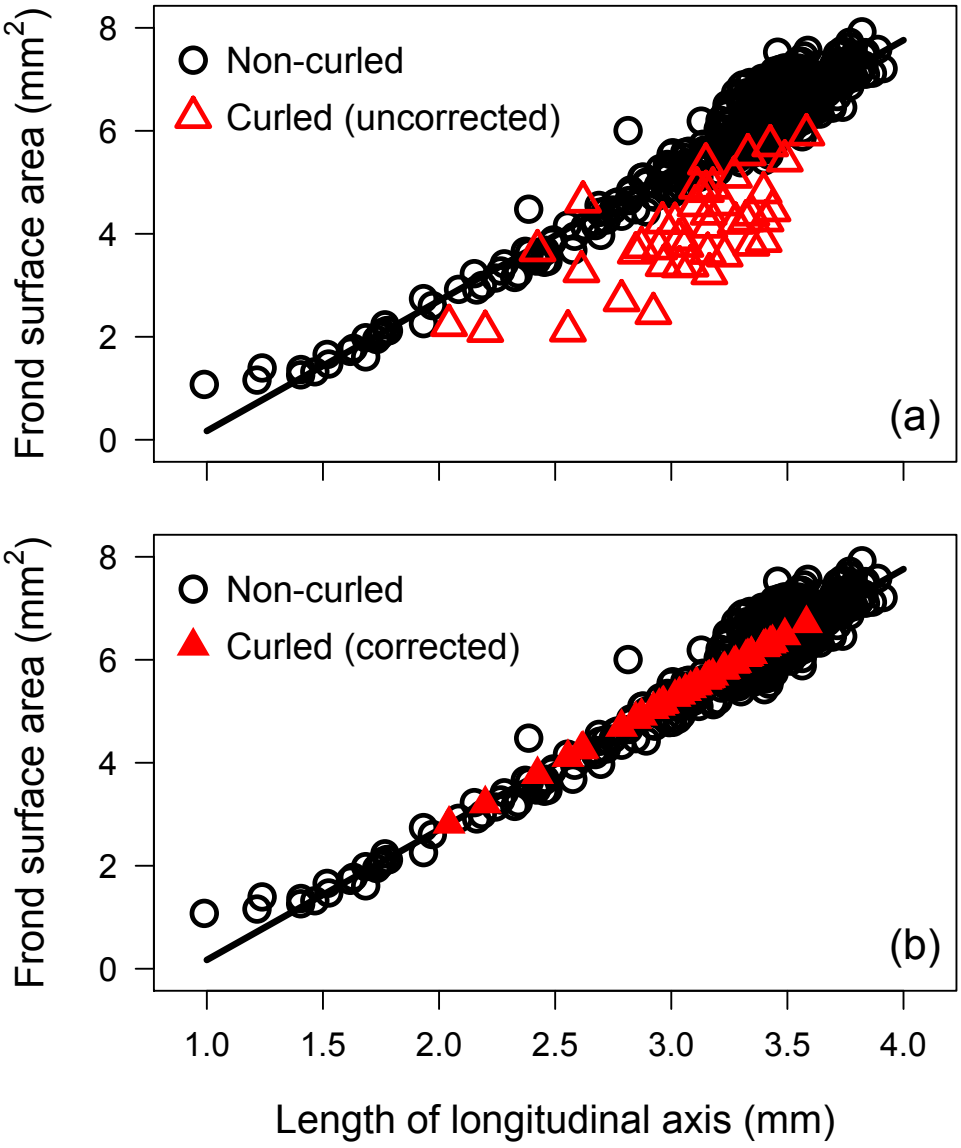


Figure S2. Linear regression of frond surface area (mm^2) versus length of the longitudinal axis (mm) for the 500 non-curled fronds (open black circles) in *Phase two* of our study. Uncorrected (open red triangles, panel a) and corrected (filled red triangles, panel b) surface areas of curled fronds are depicted for comparison. We 'corrected' estimates of surface area for curled fronds by interpolating from the regression line (which was fit using data from the 500 non-curled fronds).