

Life-history plasticity and inbreeding depression under mate limitation and predation risk: cumulative lifetime fitness dissected with a life table response experiment

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Abstract Environmental effects on the evolution of mating systems are increasingly discussed, but we lack many examples of how environmental conditions affect the expression and consequences of alternative mating systems. Variation in mate availability sets up a trade-off between reproductive assurance and inbreeding depression, but the consequences of both mate limitation and inbreeding may depend on other environmental conditions. Predation risk is common under natural conditions, and known to affect allocation to reproduction, but we know little about the effects of isolation and inbreeding under predation risk. We reared selfed and outcrossed hermaphroditic freshwater snails (*Physa acuta*) in four environments (predator cues present or absent crossed with mating partners available or not) and quantified life-history traits and cumulative lifetime fitness. Our results confirm that isolation from mates can increase longevity and growth, resulting in higher lifetime fecundity. Thus, we observed no evidence for mate limitation of reproduction. However, reproduction under isolation (i.e., selfing) resulted in inbreeding depression, which should counteract the benefits of selfing. Inbreeding depression in fitness occurred in both predator and no-predator environments, but there was no overall change in inbreeding depression with predator cues. This represents, to our knowledge, the first empirical estimate of the effect of predation risk on inbreeding depression in an animal. Cumulative fitness was most influenced by early survival and especially early fecundity. As predation risk and inbreeding (both ancestral and due to a lack of mates) reduced early fecundity, these effects are predicted to have important contributions to population growth

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under natural conditions. Therefore life-history plasticity (e.g., delayed reproduction) is likely to be very important to overall fitness.

Keywords Age/size at first reproduction · Delayed selfing · Inducible defense · Lifetime fecundity · Longevity · Waiting time

Introduction

There is a great diversity of mating systems among natural populations of plants and animals (Goodwillie et al. 2005; Jarne and Auld 2006). Due to the role that the mating system plays in determining genetic structure and directly affecting fitness (Jarne 1995; Charlesworth 2003), understanding the basis of this variation is central to understanding evolution in natural populations. While we know that traits related to the mating system (e.g., the age at first reproduction, sex allocation) and the effects of various mating systems (e.g., the fitness consequences of inbreeding) often change among environments (e.g., Carr and Eubanks 2002; Stephenson et al. 2004; Armbruster and Reed 2005; Waller et al. 2008; Auld and Relyea 2008), a functional understanding of how these components combine to affect cumulative lifetime fitness is lacking in most systems.

In general, the effects of mating systems on fitness-related traits such as survival and fecundity may differ among environments for several reasons. For example, genetic effects such as inbreeding depression (i.e., the relative fitness decrement suffered by inbred individuals compared to outbred individuals; Charlesworth and Charlesworth 1987) may differ among environments (e.g., Bijlsma et al. 1999; Henry et al. 2003; Waller et al. 2008). Also, plasticity in response to environmental conditions such as the availability of mates can alter patterns of reproduction (Tsitrone et al. 2003a; Kalisz et al. 2004). Mate limitation (e.g., pollen limitation in plants) refers to situation where reproductive fitness is lower than it hypothetically could be with adequate access to mates (Knight et al. 2005). Mate (pollen) limitation is common in plants (Ashman et al. 2004; Knight et al. 2005; but see Knight et al. 2006), but much less is known in animals. Typically, researchers demonstrate mate (pollen) limitation by supplying additional unrelated mates (or pollen) and observing whether reproduction increases. Simultaneously, many studies on inbreeding depression use hermaphrodites that are either forced to self-fertilize or outcross, potentially confounding the amount and type of mate access. Thus, there is an important distinction between the fitness effects of isolation (i.e., the number of available mates) and inbreeding (i.e., the relatedness of available mates). Understanding how these factors combine, potentially interact, and change among environments is thus important for a general perspective on mating system evolution in nature.

The ability to reproduce under isolation (i.e., reproductive assurance; Jain 1976) has obvious benefits that might favor the evolution of selfing, many of which are counterbalanced by inbreeding depression (Jain 1976; Lloyd 1979; Goodwillie et al. 2005). The balance between reproductive assurance and inbreeding depression may change in different environments. For example, stress (e.g., an environmental factor with negative effects on fitness) may exacerbate the effects of inbreeding such that inbreeding depression is greater in stressful environments compared to benign environments (Bijlsma et al. 1999; Armbruster and Reed 2005). Furthermore, stress and other ecological perturbations are predicted to alter patterns of mate limitation (Knight et al. 2005), potentially altering reproductive assurance. Predation risk is a potential stress that may alter the effects of isolation and inbreeding. While the effects of predation risk on isolation have recently been

discussed (Auld and Relyea 2008), the effects of predation risk on inbreeding depression have not received any empirical attention (to our knowledge; Steets et al. 2007).

In simultaneously hermaphroditic animals, the combined effects of isolation and inbreeding have been termed “self-fertilization depression” (Jarne et al. 1991) to reflect the fact that fitness may be affected not only by inbreeding but also by mate availability. For example, in internally fertilizing species that preferentially outcross, inbreeding depression is typically strong and individuals without mates often delay selfing (Tsitrone et al. 2003a; Escobar et al. 2007). Such plasticity in the time to reproduction (i.e., delayed selfing or the “waiting time”) is favored because it facilitates the avoidance of inbreeding depression but still provides reproductive assurance (Lloyd 1979). During this waiting time, individuals should allocate resources to growth and therefore initiate reproduction at a larger size (Tsitrone et al. 2003a). Size-specific predation risk may affect the waiting time because individuals must balance investment in growth, reproduction and defense (Auld and Relyea 2008). Previously, we examined whether predation risk and mate availability interact to affect the waiting time and longevity in a simultaneously hermaphroditic snail (Auld and Relyea 2008). We found that predation risk resulted in delayed reproduction, but no change in the waiting time. However, we found that predation risk reduced the longevity and cumulative lifetime reproduction of virgin (i.e., selfing) snails relative to mated (i.e., outcrossing) snails. Conversely, without predation risk, virgin snails lived longer and laid more eggs than mated snails. Thus the effect of isolation from mates on cumulative lifetime reproduction was negative with predators and positive without predators (Auld and Relyea 2008). As reproduction under isolation is strictly through self-fertilization, these effects are difficult to interpret without simultaneously understanding of the role of inbreeding depression (i.e., the fitness depression that results from inbreeding in the parental generation) and how this is affected by predation risk.

Here, we took this question a step further by incorporating the effects of inbreeding in the parental generation so that we could distinguish the effects of being the offspring of a selfed parent from the effects of selfing due to a lack of mates. Therefore, we could disentangle the effects of parental mating system and current mating system. Using the offspring of selfed or outcrossed freshwater snails that were exposed to a factorial combination of mate access (present or absent) and predation risk (present or absent), we examined treatment effects on several fitness traits including cumulative lifetime reproduction to address the following questions: How do parental inbreeding, isolation from mates and predation risk affect life-history traits separately and in various combinations? How do parental inbreeding and isolation from mates, both separately and together, affect cumulative fitness in the presence and absence of predation risk? We predicted that parental inbreeding would reduce growth, survival and reproduction, while isolation from mates would decrease reproduction but increase growth. Predation risk is predicted to increase growth at the expense of delayed reproduction.

Methods

We examined life history and cumulative lifetime fecundity of *Physa acuta* snails that were the second-generation (i.e., G_2) descendants of wild-caught snails from Geneva pond #3 in northwestern Pennsylvania. *P. acuta* is a self-compatible, but preferentially outcrossing species that copulates with and stores sperm from multiple partners (Wethington and Dillon 1991; Henry et al. 2005). While this species is highly outcrossing in natural populations, spatiotemporal variation in snail density exists such that individuals may

experience periods of isolation from potential mates (Henry et al. 2005). For this reason, we held all G_0 snails together under high density for >5 h after collection to ensure that each snail had the opportunity to copulate. Likewise, variation in predation risk exists in natural populations of *P. acuta*, including the sampled population (J.R. Auld, *pers. obs.*), making this an ideal species to examine the effects of predation risk on life-history traits and the consequences of alternative mating systems.

We set up breeding lines of *P. acuta* to produce selfed and outcrossed G_2 offspring of equal age. These breeding lines were set up by outcrossing or selfing siblings from ten G_1 families. G_1 snails were outcrossed by placing a new potential mate (marked with non-toxic paint; Henry and Jarne 2007) into each focal snail's container every day for 14 days. G_1 snails were selfed by leaving them in isolation until they reproduced. All other experimental conditions were identical to Auld and Relyea (2008) including snail food (*ad libitum* Spirulina three times/week) and weekly water changes.

We reared individual G_2 snails in 1 L of water under a randomized, factorial design of two parental mating systems (G_1 parents were selfed or outcrossed), two mate-availability treatments (G_2 snails were given a mate or not), and two predation-risk treatments (G_2 snails were exposed to predator cues or not) for a total of eight treatment combinations. Thus, selfed or outcrossed snails were reared in four different conditions (Fig. 1). By rearing an individual from each of the 10 outcrossing and 10 selfing lines in each of these four conditions, we had a potential total of 80 experimental units. However, two of the selfed lines went extinct (i.e., all offspring were dead) by the time that we set up the experiment. Therefore, the sample size was $n = 8$ for treatment combinations involving selfed snails (i.e., the G_1 mating system) and the total sample size was 72 experimental units. By simultaneously manipulating the G_1 and G_2 mating systems in one experiment, we can tease apart the effects of inbreeding depression (i.e., the relative fitness of inbred and outbred individuals) and mate limitation (hereafter referred to as "isolation depression", i.e., the relative fitness of selfing and outcrossing individuals). To streamline the terminology, we will hereafter refer to these treatment combinations as "outcrossed-outcrossed" ("XX"), "outcrossed-selfed" ("XS"), "selfed-outcrossed" ("SX") and "selfed-selfed" ("SS") to reflect the G_1 and G_2 mating systems, respectively (see Fig. 1).

Individual juvenile G_2 snails were placed in their containers on 30 May 2006 (age = 29 days; initial mass <1 mg); predator-cue and mate-availability treatments began on 31 May and 7 June, respectively, and were implemented as in Auld and Relyea (2008). In short, we produced predator-conditioned water by feeding a pond-dwelling crayfish (*Procambarus acutus*) 150 mg of *P. acuta* three times/wk. Prior to each feeding, we collected the water in which each crayfish was held, pooled the water from all crayfish ($n = 20$), removed 400 ml of water from each experimental unit assigned the predator treatment and replaced it with 400 ml of predator-cue water. Similarly, we removed 400 ml of water three times/wk from all no-predator containers and added 400 ml of freshwater. Mate availability was manipulated by adding marked mates to the appropriate containers three times/wk for 3 h at a time, a duration that is sufficient for copulation (Tsitrone et al. 2003b). Snails in the no-mate treatment remained in isolation.

The experiment lasted the entire life of the snails (the last snail died at 267 days old) and we measured a set of life-history traits including age/size at first reproduction, longevity and lifetime fecundity. Experimental units were checked daily, egg masses were marked, and the number of oviposited eggs was counted weekly. During egg counting, the number of eggs that failed to hatch was counted to quantify egg-hatching proportion. Snails were blotted dry and weighed when they produced their first egg mass and at death (i.e., size at first reproduction and death).

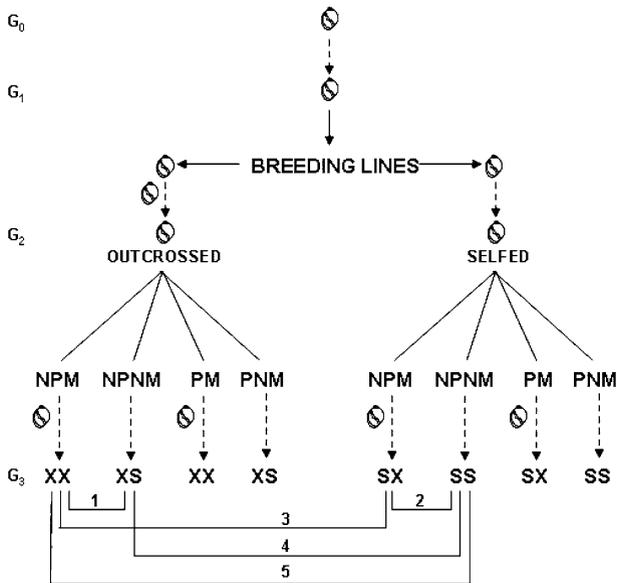


Fig. 1 Experimental design that was followed for each replicate. G_0 snails were collected, breeding lines were established using G_1 snails ($n = 10$), and parallel outcrossing or selfing lines were established using sibs. G_2 snails were reared in one of four treatments: no predator–mate available (NPM), no predator–no mate (NPNM), predator–mate (PM), or predator–no mate (PNM). *Dashed arrows* show reproduction (snails along these *arrows* show outcrossing). Letters at the bottom describe the mating system; the last letter is the mating system of the experimental snails and preceding letters show their parent’s mating system (e.g., SX means that snails produced through self-fertilization were outcrossed). The five possible comparisons used to assess fitness depression are numbered (see text); depressions were calculated for both predator treatments but are only labeled once for clarity

We analyzed the life-history response variables using 3 analyses. All analyses were conducted in R (R Development Core Team 2006). First, we used univariate analysis of variance (ANOVA) to examine treatment effects on age and size at first reproduction and death and the number of reproductive days. Age at first reproduction and age and size at death were *log*-transformed prior to analysis to improve normality. The total number of eggs laid was analyzed using generalized linear models with Poisson error variance. These models included main effects of our treatments and all two- and three-way interactions. The effect of each term was assessed by sequentially deleting terms starting with those of highest order and conducting an analysis of deviance. That is, we compared the deviance ($-2 \times \log$ likelihood) that remains unexplained by the model before and after the deletion of each term. We calculated deviance ratios, which are equivalent to *F*-ratios, and account for overdispersion (Crawley 2005). Post-hoc tests were used to parse treatment effects when specific response variables could be considered to be redundant. We controlled for running multiple ANOVAs by adjusting the significance threshold to control for the false discovery rate (i.e., to balance Type I and II error risk; Verhoeven et al. 2005).

Second, to examine the effects of predation risk, isolation, and inbreeding on total fitness (i.e., survival and fecundity) across the entire lifespan of the snails, we built age-structured transition matrices for each treatment combination and compared their dominant eigenvalues. These matrices were constructed with seven stages: eggs, hatchling to 49, 50–99, 100–149, 150–199, 200–249, and >250 days (no snails survived beyond 267 days).

We used the egg-hatching rate as the probability of transitioning from egg to hatchling and calculated the other survival probabilities as the probability of surviving from one age class to the next. Fecundities were estimated as an average number of eggs laid per day. Note that the late-life transition rates may be biased relative to those early in ontogeny due to both the decrease in sample size through time as well as differential survival of the most vigorous individuals. Our cumulative measure of fitness, the dominant eigenvalue, was extracted for each transition matrix. We estimated 95% confidence intervals on these eigenvalues by bootstrapping each matrix element 1000 times across replicates. This was done in the same way for all treatments regardless of sample size. Subsequently, we combined each bootstrapped element into a matrix with other bootstrapped elements, and estimated the eigenvalue for each bootstrapped matrix (for a total of 8000 matrices).

We used prospective perturbation analyses to reveal the relative contribution of age-specific fecundities and survival probabilities to the dominant eigenvalue (λ) of each matrix and retrospective perturbation analysis to decompose treatment effects on λ (Caswell 1989, 2000, 2001). In other words, we calculated the sensitivities and elasticities of each matrix element to λ and fit linear models to decompose treatment effects on λ . The basic model was:

$$\lambda^{(rst)} = \lambda^{(\dots)} + \alpha^{(r)} + \beta^{(s)} + \gamma^{(t)} + (\alpha\beta)^{(rs)} + (\alpha\gamma)^{(rt)} + (\beta\gamma)^{(st)} + (\alpha\beta\gamma)^{(rst)},$$

where (...) represents an average, $\alpha^{(r)}$, $\beta^{(s)}$ and $\gamma^{(t)}$ are treatment effects (r = no predator or predator, s = mate or no mate and t = selfed or outcrossed), and the other terms are interactions. Each term was calculated based on deviation from a reference matrix ($A^{(\dots)}$). For example,

$$\hat{\alpha}^{(r)} = \lambda^{(r..)} - \lambda^{(\dots)} \quad \text{and} \quad (\hat{\alpha}\hat{\beta})^{(rs)} = \lambda^{(rs..)} - \alpha^{(r..)} - \beta^{(s..)} - \lambda^{(\dots)}$$

where each term can then be decomposed to obtain the contribution of each matrix element to λ :

$$\hat{\alpha}^{(r)} = \sum_{a_{ij}} \left(a_{ij}^{(r..)} - a_{ij}^{(\dots)} \right) \frac{\partial \lambda}{\partial a_{ij}} \Big|_{\frac{1}{2}(A^{(r..)} + A^{(\dots)})}$$

By examining the first term in the summation (i.e., the difference between a_{ij} in the treatment-mean and reference matrices) we see the effect of a treatment on each matrix element with respect to the reference matrix. The product of this difference and the sensitivity reveals the contribution of each matrix element to the dominant eigenvalue (Caswell 1989, 2001). The original data and elasticity matrices are provided as Appendix Tables 1–8 (See Supplementary material) and the age-specific survival probabilities and fecundities are plotted in Appendix Fig. 1 (See Supplementary material).

In the third step of our analysis, we compared the relative fitness (i.e., the relative magnitude of λ) among our mating treatments both with and without predation risk. We estimated fitness depression using the formula $\delta = 1 - w_s/w_o$, where w is the fitness of selfed (w_s) or outcrossed (w_o) snails (Johnston and Schoen 1994). Note that this is the same as what is classically called “inbreeding depression”; we refer to it as “fitness depression” because we are using the concept to compare the fitness of individuals based on both the parental and current mating systems. We made the following five comparisons to distinguish the effects of inbreeding (i.e., G_1 mating system) and isolation (i.e., G_2 mating system) in both predator treatments (see Fig. 1): (1) *Outcrossed isolation depression*: the fitness depression of outcrossed-selfed snails relative to outcrossed-outcrossed snails, (2)

Selfed isolation depression: the fitness depression of selfed-selfed snails relative to selfed-outcrossed snails, (3) *Inbreeding depression with mates*: the fitness depression of selfed-outcrossed snails relative to outcrossed-outcrossed snails, (4) *Inbreeding depression without mates*: the fitness depression of selfed-selfed snails relative to outcrossed-selfed snails, and (5) *Total self-fertilization depression*: the fitness depression of selfed-selfed snails relative to outcrossed-outcrossed snails. We predict that #5 should be greater than all the other depressions, but note that #5 must equal the sum of #1 + #4 as well as #2 + #3 because both of these pairs are different ways of partitioning #5. In this way, we can determine whether isolation or inbreeding represents a larger fraction of total self-fertilization depression (i.e., is $\#4/\#5 > \#1/\#5$ and is $\#3/\#5 > \#2/\#5$?). We also test the hypothesis that depression should be stronger in more “stressful” environments (i.e., stronger in the predator treatment).

Results

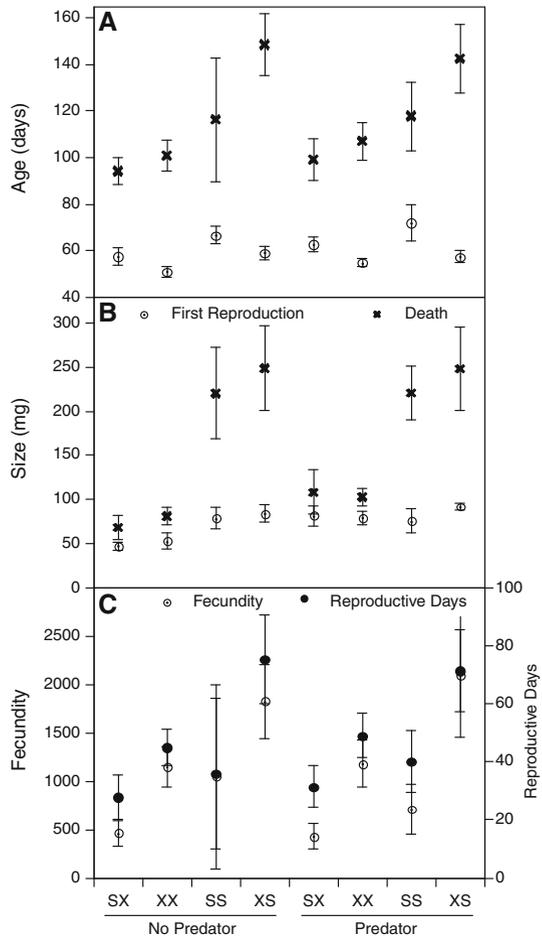
The parental mating system (i.e., selfed or outcrossed G_1 snails) had a significant effect on age at first reproduction, number of reproductive days, and number of eggs laid by G_2 snails (Table 1). Inbred snails (i.e., the offspring of G_1 selfed snails) experienced a 17% delay in the time to reproduction (i.e., 9 days) and tended to die earlier than outbred snails (Fig. 2a), which resulted in a 44% decrease in the number of reproductive days and a 58% decrease in fecundity (Fig. 2c). Predator cues caused a 25% increase in the size at first reproduction (Fig. 2b). Mate availability (i.e., G_2 treatment) affected age and size at first reproduction and death as well as total fecundity. Snails with mates reproduced and died earlier and at a smaller size than isolated snails (Fig. 2a, b) resulting in a 43% reduction in fecundity. The negative effect of mate availability on size at first reproduction was larger in the absence of predator cues (a 6 and 38% reduction with mates, with and without predator cues respectively), an effect represented by a mate-availability-by-predator interaction that was marginally non-significant ($F_{1,57} = 3.74$, $P = 0.058$). None of the other two- or three-way interactions were statistically significant.

Table 1 Results of univariate analyses on eight traits showing the effects of parental mating system (selfing or outcrossing), predation risk (present or absent), and mate availability (present or absent)

Trait	G ₁ mating system		Predation risk		Mate availability	
	<i>F</i> _{df}	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Age first reprod.	13.56 _{1,57}	<0.001	1.39	0.24	7.46	0.008
Size first reprod.	0.76 _{1,57}	0.39	6.25	0.015	7.51	0.008
Age at death	4.40 _{1,59}	0.040	0.04	0.85	7.93	0.007
Size at death	2.11 _{1,54}	0.15	1.79	0.19	38.78	<0.001
Reproductive days	8.77 _{1,63}	0.004	0.04	0.84	4.36	0.041
Total fecundity	11.45 _{1,64}	0.001	0	1	4.79	0.032

Age and size at first reproduction, age and size at death, and the number of reproductive days were analyzed using ANOVAs. Age and first reproduction and the age and size at death were log-transformed to improve normality. The total number of eggs (i.e., total fecundity) was analyzed using a generalized linear model with Poisson error variance. All models included the three two-way interactions and the three-way interaction, but only one of these interactions (out of the total 24) had a *P*-value <0.1; it is discussed in the text. *Boldface* denotes statistical significance while controlling for multiple testing using the method of Verhoveven et al. (2005)

Fig. 2 The effects of parental mating system, predation risk and mate availability on age at first reproduction and death (a), size at first reproduction and death (b), and total fecundity and the number of reproductive days (c). Along the x-axis, S and X represent the mating system (S for selfing, X for outcrossing); the first letter represents the ancestral (G_1) mating system and the second letter represents the mate-availability treatment manipulated in the experiment (i.e., G_2 mating system). These abbreviations follow Fig. 1 and are described in the text. No Predator and Predator treatments are labeled. Data are means \pm 1 S.E



Given the greater mass of snails when isolated, we were interested in whether this change resulted from an increase in growth rate or simply a longer time over which growth could occur. To address this, we fit models of size at death including either age at death or a measure of growth rate as a covariate and examined whether the mate effect was still significant. We estimated individual growth rate in two ways, either as a total linear growth rate (size at death divided by age at death) or an “adult growth rate” (the difference between size at death and size at first reproduction divided by the difference between age at death and age at first reproduction). An analysis of both estimates of growth rate revealed negative effects of mate availability (total: $F_{1,52} = 20.09$, $P < 0.0001$; adult: $F_{1,54} = 29.01$, $P < 0.0001$). Models of size at death including either growth rate or the age at death as a covariate revealed significant effects of the covariates and mate availability was still significant. Therefore, we conclude that snails in isolation attain a larger size due to both longer life and a faster growth rate.

Similarly, given the increased fecundity of snails when isolated, we were interested in whether fecundity differed due to increased egg-production rate or simply a longer time over which eggs could be laid. To address this, we fit models of total fecundity including

either the total number of reproductive days or a measure of egg-production rate (total fecundity divided by the number of reproductive days) as a covariate and examined whether the mate effect was still significant. Egg-production rate was lower for selfed snails (G_1 mating-system effect: $F_{1,56} = 12.70$, $P = 0.0008$), but not affected by any other treatment. When we included egg-production rate as a covariate, there were no longer any significant treatment effects. When we included the total number of reproductive days as a covariate, the model contained significant effects of the covariate and G_1 mating system, but no effect of mate availability. Therefore, we conclude that mate availability did not affect egg-production rate; isolated individuals laid more eggs because they lived longer and therefore had a greater number of reproductive days.

Our analyses of cumulative lifetime fitness (i.e., the dominant eigenvalue of each transition matrix; λ) revealed treatment effects that varied throughout the lifespan of the snails. Under similar environmental conditions, inbred snails (i.e., G_1 mating system) had significantly lower estimates of cumulative fitness than outbred snails (Table 2A). Elasticity analyses revealed that, in each treatment combination, fitness was disproportionately influenced by egg-hatching proportion, survival to 50 days, and fecundity during 50–99 days (Appendix Tables 1B–8B in Supplementary material). Under certain environmental conditions (e.g., no predator cues, mates available; Appendix Table 5 in Supplementary material), early fecundities (hatchling to 49 days) were also influential to λ .

Table 2 Cumulative fitness (A) and fitness depression (B) with and without predation risk

A. Results of transition matrix analysis to determine eigenvalues (i.e., cumulative fitness)

Mating system of parents	Predator presence	Mate availability	Dominant eigenvalue	Bootstrapped 95% C.I.
Outcrossed	Absent	Mate	3.12	3.00–4.19
		No mate	2.85	2.41–2.99
	Present	Mate	2.67	2.66–3.21
		No mate	2.78	2.27–2.87
Selfed	Absent	Mate	1.84	1.41–2.23
		No mate	1.69	1.28–2.15
	Present	Mate	1.85	1.29–1.96
		No mate	1.72	1.39–2.04

B. Estimates of cumulative fitness depression due to isolation, inbreeding, and both (see text)

Comparison	Predator	Depression	Bootstrapped 95% C.I.
1. Outcrossed isolation depression	No predator	0.08	–0.07 to 0.21
	Predator	–0.04	–0.24 to 0.12
2. Selfed isolation depression	No predator	0.08	–0.29 to 0.45
	Predator	0.07	–0.25 to 0.33
3. Inbreeding depression with mates	No predator	0.41	0.27 to 0.55
	Predator	0.31	0.15 to 0.47
4. Inbreeding depression without mates	No predator	0.41	0.24 to 0.65
	Predator	0.38	0.22 to 0.55
5. Total self-fertilization depression	No predator	0.46	0.29 to 0.68
	Predator	0.36	0.22 to 0.55

The treatment effects on survival and fecundity and their influences on λ are elucidated by examining the results of the life-table-response-experiment calculations. In general, these models fit the data quite well with only a 6.6% difference on average between the actual and estimated eigenvalues. Predator cues caused a small decrease in fecundity between 0 and 50 days (Fig. 3a), but this resulted in a substantial negative contribution to λ (Fig. 3d). Mate availability caused an increase in fecundity during 0–100 days and a decrease in fecundity later in life, but the increase in early fecundity had a larger contribution to λ than the decrease late in life (Fig. 3b and e). Selfed snails experienced decreased fecundity through 200 days of age and the contribution of this depression to λ was strongest during 51–100 days (Fig. 3c and f). Predator cues caused a relatively small decrease and large increase in survival probability during 51–100 and 101–150 days,

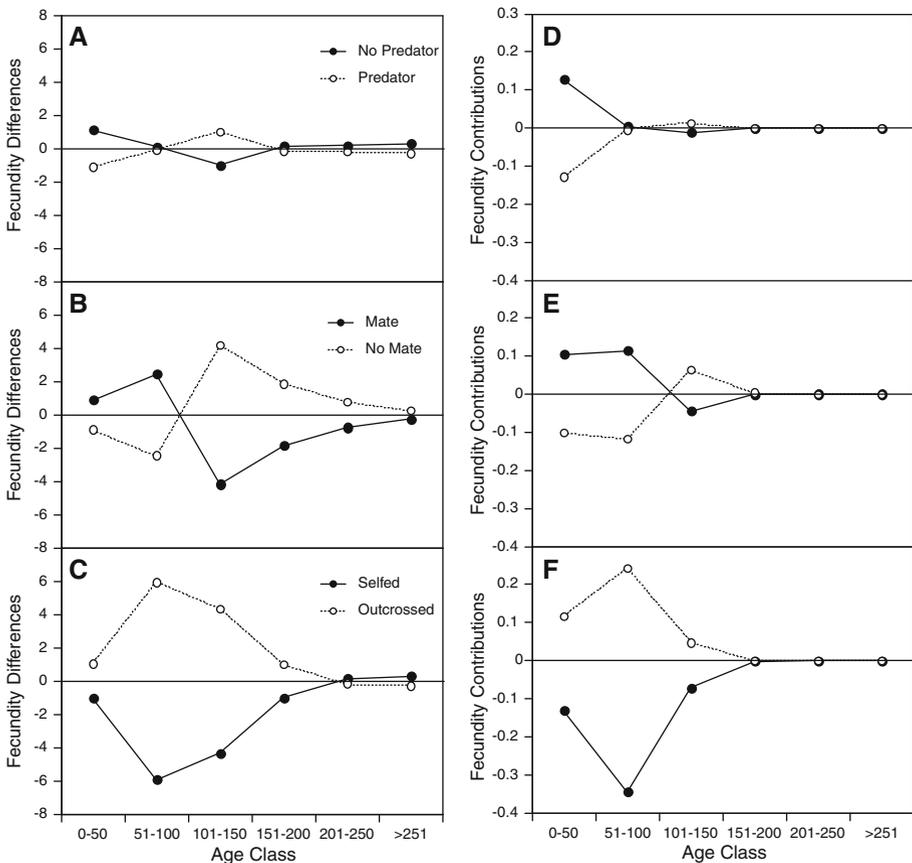


Fig. 3 Decomposition of the effects of predator treatment (a, d), mate availability (b, e), and parental mating system (c, f) on age-specific fecundities. The *left-hand panels* (a–c) show the difference between the reference (i.e., average) matrix and the treatment matrix while the *right-hand panels* (d–f) show the contributions of each fitness component to the treatment effect on the dominant eigenvalue. Symbols are the same for the *left* and *right panels*, but note the difference in scale. Note that while the differences are necessarily symmetrical around zero, the contributions are not necessarily so as the contributions are the product of the difference (*left panel*) and the sensitivity of the reference matrix element (see text for further details)

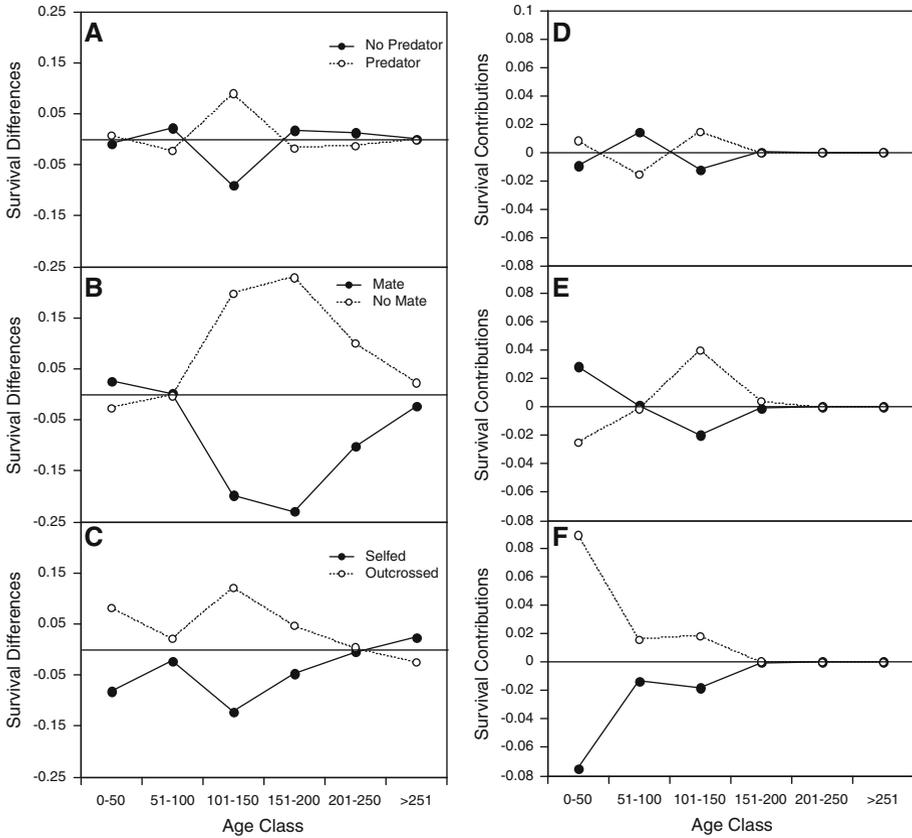


Fig. 4 Decomposition of the effects of predator treatment (**a, d**), mate availability (**b, e**), and parental mating system (**c, f**) on age-specific survival probabilities. As in Fig. 3, the *left-hand panels* show the difference between the reference and treatment matrix while the *right-hand panels* show the contributions of each fitness component to the treatment effect on the dominant eigenvalue. See Fig. 3 legend and text for further information

respectively, but these effects had similar contributions to λ (Fig. 4a and d). Mate availability caused a relatively small increase in early survival and large decrease in late survival, but these changes had similar contributions to λ (Fig. 4b and e). Inbred (i.e., G_1 mating system) snails experienced reduced survival through 200 days, but the contributions to λ were the most important during 0–50 days (Fig. 4c and f). Interaction effects (two-way and three-way) are plotted in Appendix Figs. 3–6 (See Supplementary material). Similar to the main effects, the contributions of fecundities to λ are of greater magnitude than the contributions of survival. The magnitude of the contribution of these interaction effects sometimes exceeds the main effects. Overall, the treatment effects on fecundity, especially the effects of previous mating history, had the most influence on our measure of cumulative fitness.

The age-structured transition matrices revealed that self-fertilization depression stems primarily from inbreeding (not isolation) and varies in magnitude and direction throughout the lifespan (Table 2B, Appendix Fig. 2 in Supplementary material). Based on overlapping confidence intervals, total self-fertilization depression was not significantly larger than

inbreeding depression and we found no difference in overall fitness depression across predator environments. Isolation depression was weak early in life, and negative later in life showing that isolated snails had higher fitness than mated snails late in life (Appendix Fig. 2 in Supplementary material). Inbreeding depression exists throughout the lifespan except for isolated snails reared without predator cues. Fitness depression in fecundity was typically greater than fitness depression in survival.

Discussion

This study reveals ways in which inbreeding in the prior generation combined with mate availability and predator cues in the current generation affect life history, survival and reproduction in a hermaphroditic snail. The effects of isolation and predation risk on snail traits were similar to those previously observed (Auld and Relyea 2008) in that isolation led to larger size (i.e., improved growth) and longer life. In addition, isolation reduced early fecundity but, contrary to the prediction that snails would be mate limited, isolation did not reduce overall fitness. However, one might expect the negative effects of inbreeding to outweigh these benefits of isolation. Indeed, inbreeding negatively affected every variable except size at first reproduction and death, but effects of inbreeding were not exacerbated by predation risk. Our analyses revealed that fecundity, especially early fecundity, was particularly important to overall fitness. Therefore, life-history plasticity (e.g., delayed reproduction) can directly affect fitness, potentially scaling up to population-level effects.

Despite the weak effects of isolation on overall fitness, we did observe a delay in self-fertilization (i.e., a waiting time). This reinforces the perspective that, in this outcrossing species, self-fertilization is avoided due to past selection against individuals that self-fertilized and whose offspring incur inbreeding depression (i.e., selection for delayed selfing when inbreeding depression is strong; Tsitrone et al. 2003a; Escobar et al. 2007). This delay in selfing was slightly reduced in the predator environment (i.e., 2 days) compared to the no-predator environment (i.e., 8 days). In other studies on this species, the fitness effects of isolation were more negative (e.g., decreased fecundity; Jarne et al. 2000; Tsitrone et al. 2003b); total self-fertilization depression was estimated at 90% (in a no-predator environment; Jarne et al. 2000). This discrepancy may be related to a difference in resource quality among studies. While this and previous studies have supplied food *ad libitum*, we used *Spirulina* food while other studies have used boiled lettuce. The former has higher protein content than the latter. Therefore, the effects of mate limitation on reproduction may be ameliorated under higher resources, a hypothesis that extends to a connection between resource availability and the mating system. Future studies are needed to evaluate the effect of additional environmental factors (such as resource quality) on the effects of isolation and parental inbreeding.

Similar to our previous study (Auld and Relyea 2008) and contrary to predictions of mate limitation, isolated snails had higher fecundity than mated snails, a pattern explained by differences in longevity. In both cases, the egg-production rate, a measure of allocation to female reproductive function, was not affected by environmental conditions. As mentioned before (Auld and Relyea 2008), differential allocation to male function under varying conditions of mate availability is a theoretically predicted response (Charnov 1982) that may provide an explanation for these life-history changes. Future work on the reaction norms of sex allocation across mate and resource availability environments should prove to be very illustrative.

While a few plant studies have examined the effects of inbreeding in environments with herbivores and pathogens (e.g., Ouborg et al. 2000; Carr and Eubanks 2002; Ivey et al. 2004; Stephenson et al. 2004), this appears to be the first study to estimate the effect of predation risk on inbreeding depression in an animal. Inbreeding depression did not differ between predator environments, and while there was a negative contribution to early fecundity under predation risk (Fig. 3d), the levels of predation risk used may not have been extremely stressful. Therefore, while predation risk did not magnify inbreeding depression, which is contrary to earlier hypotheses (Bijlsma et al. 1999; Armbruster and Reed 2005), we need further studies using other levels of predation risk as well as other stressors (e.g., food reduction, temperature extremes, etc.) to investigate the effects of stress on inbreeding depression in this system. However, note that other studies using different types of stressful environments have also found evidence that is not consistent with the inbreeding depression-stress hypothesis (e.g., Henry et al. 2003; Coutellec and Lagadic 2006; Waller et al. 2008). Indeed, we lack a general theoretical perspective on why inbreeding depression should increase under stress. The change of inbreeding depression among environments may be related to the novelty of the environment, where environment-specific purging of inbreeding depression might occur most efficiently in common environments compared to rare or novel environments (Bijlsma et al. 1999).

In conclusion, our study demonstrates the effects that life-history plasticity can have on cumulative lifetime fitness and dissects the effects of parental inbreeding, mate availability and predation risk to show that these effects are largest early in the life cycle. Importantly, predation risk in this study was nonlethal—snails could smell the predators, but the predators were not able to kill the snails. Given that predators caused a delay in reproduction and thus a negative contribution to early fitness, this clearly suggests that our estimates of the effects of predation risk on fitness (and therefore inbreeding depression) may underestimate the negative consequences of predation in nature. Future experiments that evaluate these effects under natural conditions will be useful to illustrate how our findings translate to a natural population.

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