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Growth, reproduction and longevity in the hermaphroditic freshwater snail *Helisoma trivolvis*

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ABSTRACT

Life-history evolution often results in trade-offs between reproduction, growth and longevity. We investigated the relationships among these traits in a hermaphroditic freshwater snail, *Helisoma trivolvis*, by manipulating opportunities for reproduction and measuring body size and egg production throughout the lifespan. Snails were placed in one of four treatment groups: snails isolated for their entire lives, snails housed with a partner for 1 week at 18 weeks, snails housed with a partner for 1 week at 26 weeks and snails afforded 1 week of mating opportunities six times between 18 and 97 weeks. We monitored egg production and shell diameter regularly throughout the experiment until all snails had died. Isolated snails laid almost no eggs, confirming a low level of self-fertilization in this species. For both groups of snails with only one mating opportunity, the average duration of egg production was 16.8 weeks. Snails in the multiply-mated group continued to lay about 1 egg mass per day with an average of 19–25 eggs per mass for 48 weeks and then egg production decreased. Although all of the snails grew consistently throughout the experiment, when snails were actively laying eggs their growth slowed relative to those no longer producing eggs. There were no significant differences in longevity among snails that were isolated and never mated, those that mated once (either early or later in life) and those that had multiple mating opportunities and continued to lay eggs throughout their lifetimes. These overall patterns of growth demonstrate that costs of reproduction may result in trade-offs in the short term, but not in the long term.

INTRODUCTION

One of the principle goals in the study of life-history evolution is to determine the nature of trade-offs, compromises among reproductive parameters that maximize overall fitness (Reznick, 1985; Roff, 2002; Flatt & Heyland, 2011). The concept of trade-offs is based on the idea that organisms with finite resources must allocate them among the many physiological functions that demand energy. Perhaps the most important trade-off is that between reproduction and other energetically-costly activities such as growth, maintenance and food acquisition. This loss of potential is referred to as the 'cost of reproduction' (Reznick, 1985; Zera & Harshman, 2001; Edward & Chapman, 2011) and includes presumed decreases in future reproductive output and survival resulting from current reproductive investment. Such trade-offs should result in negative correlations between energetically costly life-history parameters, and this relationship has been documented in numerous organisms ranging from the nematode *Caenorhabditis elegans* to *Drosophila* to red deer (see reviews by Bell & Koufopanou, 1986; Roff, 1992; Stearns, 1992).

While there have been many direct tests of reproductive trade-offs, few studies have addressed both the short and long-term consequences of different reproductive strategies. Freshwater snails provide an excellent system in which to study life-history parameters throughout the life span. They are found in a wide range of environments (Dillon, 2000), exhibit a variety of life-history tactics (Brown, 1983; Rollo & Hawryluk, 1988), have easily quantifiable reproductive output, and can be manipulated and observed in the laboratory and in the field. Many species of freshwater snail are also simultaneous hermaphrodites (Dillon, 2000), capable of producing sperm and eggs and mating as male, female or both.

Hermaphrodites have often been the subject of studies on reproductive allocation, but the focus has typically been on allocation of resources to male or female function (see for example De Visser, Ter Maat & Zonneveld, 1994). Here we address whether trade-offs exist between reproductive output and somatic growth in *Helisoma trivolvis* (Hygrophila: Planorbidae), a hermaphroditic freshwater snail, and whether any immediate responses have longer-term consequences. These snails are easily and inexpensively maintained, can be measured reliably even from early ages and produce large numbers of eggs in masses that are easy to observe and count. *Helisoma trivolvis* is a preferentially-outcrossing species with almost negligible self-fertilization (Escobar *et al.*, 2011). Individuals may mate as male, delivering sperm to a partner, or as female, producing eggs, and often mate reciprocally, acting simultaneously as male and female (Abdel-Malek, 1952). Although egg production is related to body size in this species, it is presumed to be influenced by trade-offs with other metabolically-costly functions such as somatic growth. Our previous work has demonstrated a negative correlation between growth and reproduction in a laboratory population—snails that produce large numbers of eggs typically grow less than those that produce fewer eggs (Norton & Bronson, 2006). This observation led us to investigate the relationship between body size, growth and reproduction in snails derived from a natural population of this species throughout their lifespan, in order to document life history trade-offs in both the short and long term.

Although costs of reproduction have been measured in a variety of ways, including estimating phenotypic or genetic correlations between measures of reproductive effort and costs, or assessing correlated responses to selection on life-history characters (Reznick, 1985), we investigated the relationship between reproduction and growth by manipulating opportunities for reproduction (Reznick's method 2) and measuring the response in terms of growth and egg production. We measured the body size of snails (beginning several weeks after hatching) and then compared growth in isolated and nonisolated individuals. Based on general life-history theory and our previous research (Norton & Bronson, 2006), we predicted that mated snails (those producing eggs and that had also donated sperm to a partner) would grow more slowly than their unmated counterparts. We also predicted that snails mated later in life would produce more eggs than those that were mated earlier and at a smaller size, since the delay in reproduction would have allowed them to accumulate resources and grow to a larger size (Ghiselin, 1969; Norton & Bronson, 2006). Finally, since survival is considered an important cost of reproduction (Stearns, 1976), we predicted that snails investing in offspring would have reduced longevity—specifically individuals laying eggs almost continuously for over a year should have reduced survivorship relative to those that produce fewer or no eggs in their lifetimes. The answers to these questions provide a basis for further investigation of the trade-offs among these variables.

In addition to answering questions about the relationship between reproductive output and growth, our experimental design enabled us to answer several additional questions about reproduction in these snails. First, by monitoring egg production in isolated individuals throughout their lives, we could confirm the near absence of self-fertilization in this species. Second, we estimated how long sperm may be stored by monitoring egg production after finite periods of mating followed by isolation. Finally, we determined how long snails can produce eggs when sperm are available, by monitoring egg production in snails repeatedly inseminated at regular intervals.

MATERIAL AND METHODS

Study population

We received fifty adult *Helisoma trivolvis* collected from a pond at the entrance to Charles Towne Landing State Park (Charleston, NC 32.8068°N, 79.9902°W) and placed them in individual plastic cups with tap water for a week. Thirty-eight of the 50 snails laid two or more egg masses and were transferred to new cups. When the snails from these egg masses hatched, *c.* 1 week later, we maintained them in these original cups, changing water and feeding them ad libitum boiled organic romaine lettuce once per week. Approximately 5 weeks after hatching, we transferred the snails to a 7.6-l aquarium, feeding them ad libitum and changing water twice per week. Ten weeks after hatching (before reproductive maturity, measured at 104.3 ± 9.7 d = 14.9 weeks by Escobar *et al.*, 2011), we transferred 104 snails to individual (104 ml) plastic cups. We fed them approximately equal amounts of boiled lettuce (*c.* 2 × 2 cm, an amount typically eaten in 3–4 d by adult snails housed alone in the laboratory) and changed the water twice per week. Trays of snails were kept in the laboratory at room temperature (*c.* 22 °C) and with ambient light on a rolling cart with multiple shelves. We rotated tray placement on the shelves every 1–2 weeks to control for shelf effects.

Experimental treatments

At 18 weeks, we randomly placed snails in one of two treatment groups (Fig. 1). Snails in one group (Unmated; $n = 52$) remained isolated in 296-ml plastic cups and individuals in the other group (Mated; $n = 52$) were paired randomly with another snail and placed together as pairs in cups. Snails were marked with nail varnish to allow individual identification. Snails were not observed during this time, so the number of times each pair mated was not established; however, we do know that each snail mated at least once as a female and once as a male, since both snails from each pair produced eggs. One week later, we separated the paired snails, placed individuals in clean cups and assessed egg production in all 104 snails for the following 3 weeks (see below for details).

At 26 weeks, we subdivided each treatment group. Of the 52 Unmated snails, we continued to isolate 26; this group of snails (Unmat) allowed us to assess the effect of long-term isolation without mating on growth, as well as the extent of self-fertilization in this species. We monitored egg production in these snails on a regular basis for 1 year and, after that time, counted egg masses only when other treatment groups were monitored. The remaining 26 snails were randomly paired for 1 week, as in the previous period. This group (MatLate) allowed us to assess the effect of delayed mating on both growth and reproductive output. Of the 52 snails previously mated at 18 weeks, 26 were isolated for the remainder of the experiment. This group (MatEarly) allowed us to assess the effect of a short period of egg production on overall growth, as well as the duration of sperm storage after one bout of mating. Snails in all three of these treatment groups (Unmat, MatEarly and MatLate) remained isolated until their death. The last 26 snails were paired again for a week, this time with a different randomly-assigned partner. Snails in this group (MatMulti) were paired randomly with new partners a total of six times: first at 18 weeks, second at 26 weeks, then at *c.* 16-week intervals (42, 58, 74 and 97 weeks). The experiment ended at 165 weeks when the last snail died.

Measurements of body size

Beginning 10 weeks after hatching (when snails could be easily handled without crushing), we measured the body size of all snails every week. We placed snails under a dissecting microscope fitted with a camera and used Image-Pro Plus Analysis software to determine maximum shell diameter across the umbilicus, a common measure of snail size (Russell Hunter, 1961). By 21 weeks, snails were sturdy enough to be handled, so shell diameters were measured using handheld digital callipers, a highly reliable measurement (Norton, Johnson & Mueller, 2008). We measured snails weekly until 26 weeks, every 2 weeks until 62 weeks and then every 3 weeks until most snails had died (126 weeks).

Egg production

At 8-week intervals, we transferred snails to clean cups and measured egg production over 3 weeks for all snails in all treatment groups. Using a dissecting microscope, we examined each cup and counted the number of egg masses produced as well as the

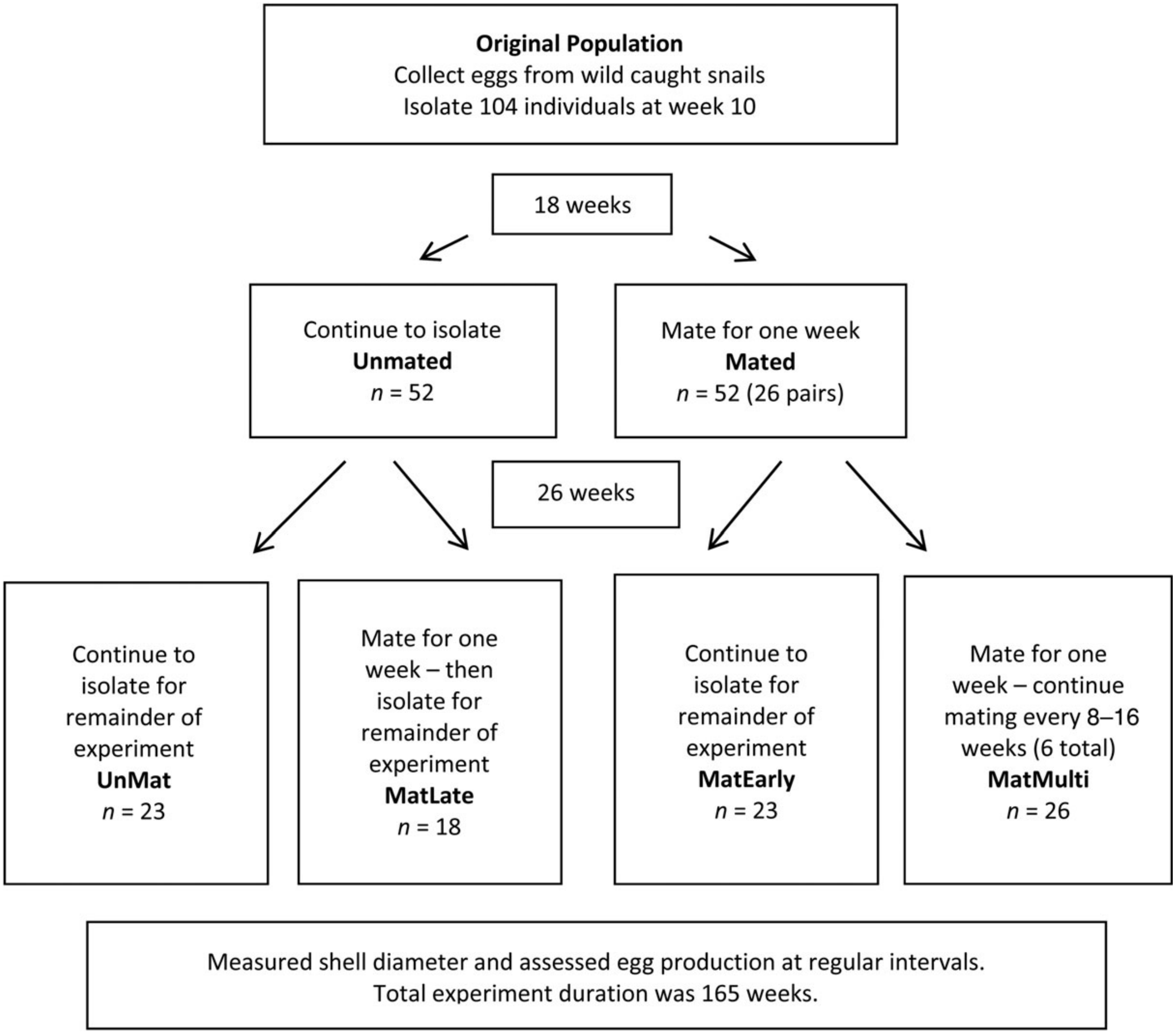

Figure 1. Overview of experimental design.

number of eggs in each mass every 3–4 d, transferring adult snails to new cups weekly. For each snail, we then calculated the number of egg masses laid, the average number of eggs per mass for the 3-week period and the total number of eggs laid. These measures over 3 weeks are good predictors of egg production for 7 weeks, the time between our measurements and the next mating cycle (unpublished data). Occasionally egg masses were not completely intact (most likely disturbed by the snail) and in these cases we counted the mass but omitted that mass from calculations of average eggs per mass. For each treatment group we calculated the mean (1) number of egg masses, (2) number of eggs per mass and (3) total number of eggs per snail laid over the 3-week period. When we calculated egg production, we included all egg masses, even those with only 0–2 eggs per mass (in some cases a mass was produced but included no eggs). For each 3-week session of egg counting, we also calculated the total number and percentage of individuals in each treatment group laying eggs. For these calculations, we counted as egg layers only individuals laying three or more eggs per mass in at least two egg masses.

Statistical analysis of egg production, body size and growth

All analyses of egg production, body size and growth were done in IBM SPSS v. 22. Several snails were omitted from all analyses: two snails in the original Mated group that never laid any eggs; seven snails in the MatLate group that never produced any eggs; four snails with either unusually-shaped shells (which made measurement inaccurate) or that had been dropped and died early in the experiment and one snail in whose cup we found parasites.

Baseline shell diameters were compared among snails in the four treatment groups before any manipulations (week 10) using a one-way ANOVA to confirm that groups were not biased in initial size. To compare growth among treatment groups we also used one-way ANOVAs of growth over four consecutive intervals. We compared growth from weeks 10–18 among the four treatment groups to confirm that there were no differences among groups prior to treatments. Between weeks 18 and 26 (Phase I), we compared growth between the original isolated (Unmated) and Mated (including both MateEarly and MatMulti snails) groups. During weeks 25–42 (Phase II), when MatLate and MatMulti snails were producing large numbers of eggs, but MatEarly snails were producing fewer eggs, and weeks 42–68 (Phase III), when MatMulti snails were still producing eggs but MatLate snail reproductive output had significantly slowed, we compared growth among all four treatment groups (Unmat, Matearly, MatLate and MatMulti). We ended the analysis after week 68, when 85% of the snails were still alive; death of snails after this point significantly reduced sample sizes. Six snails were omitted from the body-size analyses because they died early and would have limited the overall analysis of growth (one in the MatEarly group, two in the MatMulti group and three in the UnMat group).

Initial egg production (weeks 19–21) was analysed using a one-way ANOVA to rule out potential differences within subgroups of Mated snails. To determine whether a delay in reproduction would result in increased reproductive output, we compared long-term egg production in MatEarly and MatLate snails using a repeated-measures ANOVA. Although the first mating opportunity for the MatLate group was 8 weeks after that for the MatEarly group, we considered their egg production relative to time post-mating to determine whether the quantity or patterns of egg production differed. To assess the effect of increased body size on egg production due to growth during the 8-week delay, we compared the number of egg masses laid, the average number of eggs per mass and the total number of eggs laid in MatEarly and MatLate snails during the first 3-week period after mating using a one-way ANOVA with body size as a covariate. Finally, to assess the effect of single *vs* multiple mating opportunities on reproductive output, we compared the patterns of egg production in the snails mated only once (MatEarly and MatLate) with egg production in the snails mated to multiple partners (MatMulti) from first mating to 59 weeks (several weeks after snails in the first two treatments were no longer producing significant amounts of eggs) using a repeated-measures ANOVA. We omitted the three individuals from these analyses (one in the MatEarly group and two in the MatMulti group) that died before 59 weeks.

Sperm storage

To estimate the duration of sperm storage, we documented the time of mating and last instances of egg production in individual snails from the two groups mated only once during their lifetimes and subsequently isolated (MatEarly and MatLate). This was possible because *H. trivolvis* is an almost exclusively-outcrossing species (Escobar *et al.*, 2011), so the time to allosperm exhaustion could be estimated without being masked by a switch to selfing. We counted as last egg production the final week of the 3-week measurement period when a snail had laid at least three egg masses with at least two eggs per mass with no gaps of no egg deposition in a previous measurement period.

Longevity

Finally, to assess the relationship between reproductive output and longevity, we documented when each snail died and carried out a survival analysis (R statistical software, v. 3.2.1, analysis package: survival). We generated survival curves and compared survival among treatment groups using a chi-square analysis. There were no censored data, because all snails were followed from the beginning of the experiment until their death.

RESULTS

Growth patterns

Although there were no differences among groups in initial body size (one-way ANOVA: $F_{3,81} = 0.605$, $P = 0.61$; overall $\bar{x} = 4.93 \pm 0.11$ mm) or growth in the pretreatment phase (weeks 10–18) of the experiment ($F_{3,81} = 0.93$, $P = 0.43$; overall $\bar{x} = 6.3 \pm 0.10$ mm), once snails were paired and started to produce eggs, growth was typically slower for snails laying eggs (Fig. 2 and Table 1). During Phase I of the experiment, between 18 and 26 weeks, growth of Mated snails that were laying eggs was significantly less than that of the Unmated snails (Fig. 2A; ANOVA: $F_{1,83} = 8.12$, $P = 0.005$). When a subset of these unmated snails (MatLate) were mated 8 weeks later (week 26) and began to produce eggs, their growth slowed substantially (Fig. 2B). Furthermore, as the group of snails mated once at 18 weeks (MatEarly) decreased egg production, their growth increased. During this phase of the experiment (Phase II, weeks 26–42), there were significant treatment effects on growth (ANOVA: $F_{3,81} = 15.54$, $P < 0.001$); specifically, growth of MatMulti and MatLate snails was significantly less than that of the MatEarly and UnMat snails (post hoc Tukey tests). Once the MatLate snails stopped laying eggs (Phase III, weeks 42–68), their growth was significantly greater than UnMat snails, but no different from that of snails in the other treatment groups (Fig. 2B; ANOVA: $F_{3,81} = 3.52$, $P < 0.02$). After 68 weeks, sample sizes decreased as snails began to die and no significant differences in growth were evident among groups (Fig. 2C).

Egg production

Unmated snails: Of the 23 snails isolated before sexual maturity and never mated (Unmat), 39% (9 individuals) never laid any egg masses. The remaining 61% (14 snails) laid a total of 39 egg masses ($\bar{x} = 2.8 \pm 0.6$) containing 61 eggs ($\bar{x} = 1.6 \pm 0.3$ eggs per mass) during the almost 2-year period when we counted eggs. Although we did not formally assess embryonic survival, we never observed any hatched snails.

Mated snails: The 49 snails mated at 18 weeks (Mated, later divided into subgroups MatEarly and MatMulti) laid an average of 20.8 ± 0.8 egg masses in the first 3-week measurement period, approximately one egg mass per day (Fig. 3A). Egg masses contained an average 22.9 ± 0.6 eggs and ranged from 13.6 to 32.6 among individuals (Fig. 3B). There were no significant differences between the two subgroups in the number of egg masses laid (one-way ANOVA: $F_{1,47} = 0.75$, $P = 0.391$), the number of eggs per mass (one-way ANOVA: $F_{1,47} = 3.39$, $P = 0.07$) or the overall number of eggs (one-way ANOVA: $F_{1,47} = 0.10$, $P = 0.775$). These snails continued to lay eggs but, for those mated only once (MatEarly), egg production decreased over time (see below for details).

At 26 weeks, the MatLate snails were mated for the first time. When egg production was subsequently measured in all three groups during weeks 27–29, we noticed that snails in the MatLate group produced larger egg masses than we had observed in the snails mated earlier (MatEarly or MatMulti). In fact, the average number of egg masses laid (Fig. 3A; repeated-measures ANOVA: $F_{1,39} = 5.08$, $P = 0.03$) and the number of eggs per mass (Fig. 3B; repeated-measures ANOVA: $F_{1,39} = 9.76$, $P = 0.003$), were significantly larger in the MatLate snails than in the MatEarly snails throughout their egg-laying period, resulting in a significant difference in total number of eggs produced (Fig. 3C; repeated-measures ANOVA: $F_{1,39} = 8.39$, $P = 0.006$). These differences are explained by the differences in body size of the snails in this treatment, which had grown significantly more than the snails mated 8 weeks previously. When body size is used as a covariate, there were no significant differences between the MatEarly and MatLate snails in the mean number of egg masses laid (one-way ANOVA: $F_{1,37} = 2.25$, $P = 0.14$), number of eggs per mass (one-way ANOVA:

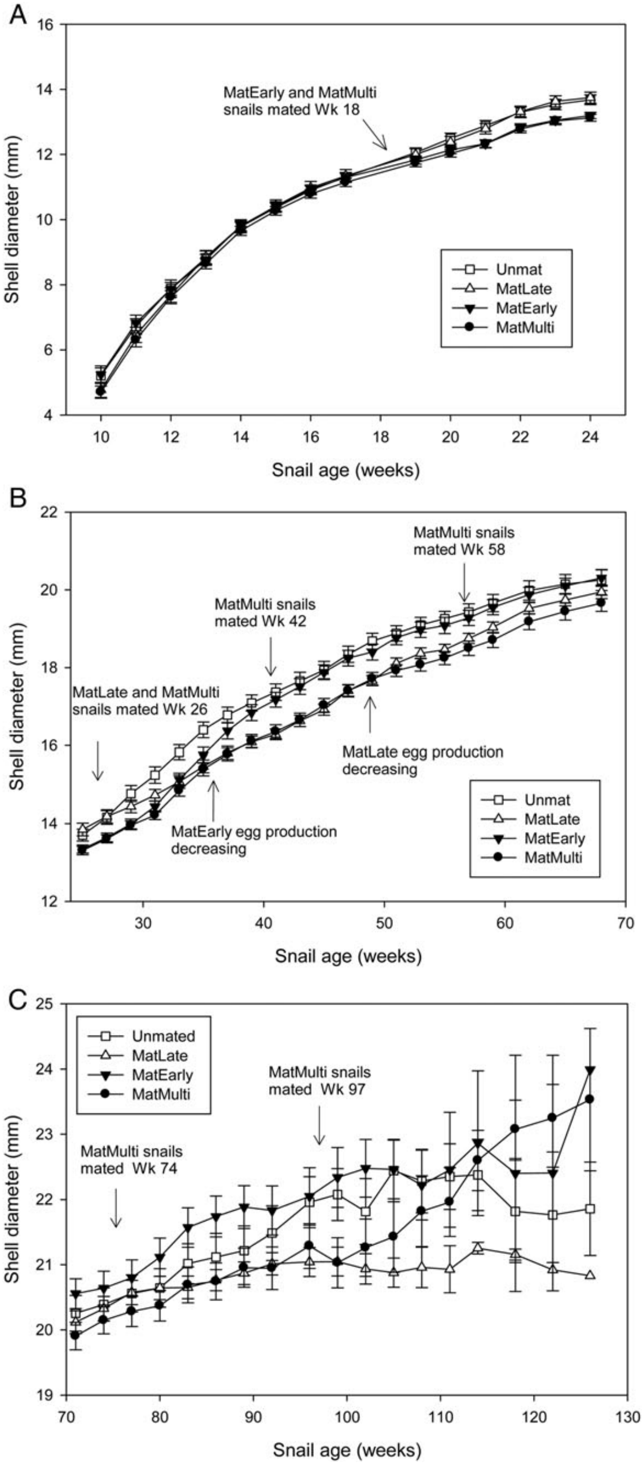

Figure 2. Body size of *Helisoma trivolvis* snails with different reproductive opportunities. The average shell diameter was calculated for snails in four treatment groups—Unmat snails were isolated for their entire lives, MatEarly snails were mated once at 18 weeks, MatLate snails were mated once at 26 weeks and MatMulti snails were mated at 18, 26, 42, 58, 74 and 97 weeks. Data are shown on three panels to visualize differences. **A.** 10–24 weeks **B.** 25–68 weeks **C.** 69–126 weeks. Error bars represent ± 1 standard error.

$F_{1,37} = 0.25$, $P = 0.62$) or overall number of eggs (one-way ANOVA: $F_{1,37} = 0.65$, $P = 0.43$) during the 3-week period after their first mating. Like the MatEarly snails, the egg production of MatLate snails also decreased over time.

In contrast, snails exposed to new mating partners every 8 (and later 16) weeks (MatMulti) laid about 1 egg mass per day with 19–25 eggs per mass for an average duration of 44.5 ± 3.5 weeks. By 67 weeks (48+ weeks later than for snails mated only once) 50% of the snails in this treatment group were still laying eggs. By 75 weeks (56 weeks after their first mating), 40% of the snails were still producing eggs, now at a lower rate of 17 masses per 3 weeks with an average 9 eggs per mass. At 77 weeks postmating (week 96), only one snail was producing egg masses, about one every 3 d, and the average number of eggs per mass was 6. No snails produced eggs after this observation period. Repeated-measures analysis indicates that the pattern of total egg production over time (19–67 weeks) differs significantly among treatments (repeated-measures ANOVA: $F_{2,61} = 18.29$, $P < 0.001$); specifically, the patterns of egg production are similar for snails in the MatEarly and MatLate treatments, but significantly different from that of the MatMulti snails, which continued to lay large numbers of eggs until week 67.

Sperm storage

The pattern of egg production for snails afforded a 1-week mating opportunity and then isolated allowed estimation of sperm storage. For those snails mated once at 18 weeks (MatEarly), there was a dramatic drop in the number of eggs per mass (from 22.9 to 6.5; Fig. 3B) 16 weeks after mating (week 34), but little change in the number egg masses laid (from 20.8 to 18; Fig. 3A); by 24 weeks postmating (week 43) the average number of egg masses laid had dropped to 2.75 with less than 2 eggs per mass (Fig. 3). Thirty-two weeks after mating, egg production was negligible. This pattern was remarkably similar in the MatLate snails, also mated only once but 8 weeks later. By 16 weeks postmating (week 42) the average number of eggs per mass had declined to 7.5, but snails were still producing an average of 21 egg masses in the 3-week period. By 24 weeks after mating (week 50), the average number of egg masses had dropped to 5 with 1.5 eggs in each mass. For these snails with 1-week-long mating opportunity (MatEarly and MatLate), the average duration of egg production was 16.8 ± 0.8 weeks.

Longevity

There were no significant differences in longevity among snails that were isolated and never mated, those that mated once (either early or later in life), and those that had multiple mating opportunities and continued to lay eggs throughout their lifetimes (Fig. 4; $\chi^2 = 0.5$, df = 3, $P = 0.923$). Nor was there any significant relationship between the total number of eggs an individual laid and their longevity ($R^2 = 0.03$; ANOVA: $F_{1,89} = 2.79$, $P = 0.099$). Snails lived an average of 95.3 weeks (1.8 years) with lifespans ranging from 24 to 165 weeks.

DISCUSSION

These data demonstrate a significant short-term trade-off between female reproductive output and somatic growth in a *Helisoma trivolvis* population derived from wild-caught snails, but no long-term cost of reproduction on longevity. The results confirm our earlier observations on a growth and reproduction trade-off in a long-time laboratory population (Norton & Bronson, 2006), while also documenting the duration and amount of egg production in snails subject to different mating regimes throughout their lifetimes. We confirm earlier studies on the low level of self-fertilization in this freshwater hermaphrodite and have used this negligible incidence of selfing to estimate the duration of sperm storage without the use of genetic markers.

Table 1. Initial snail size and growth during the experiment.

Treatment	n	Initial size (10 weeks)	Pretreatment growth (10–18 weeks)	Phase I growth (18–26 weeks)	Phase II growth (26–42 weeks)	Phase III growth (42–68 weeks)
Unmat	21	5.05 ± 0.25	6.20 ± 0.19	2.44 ± 0.10^{a}	3.74 ± 0.17^{b}	2.81 ± 0.22^{a}
MatLate	18	4.78 ± 0.23	6.57 ± 0.21	2.51 ± 0.17^{a}	2.43 ± 0.13^{a}	3.66 ± 0.16^{b}
MatEarly	22	5.11 ± 0.24	6.13 ± 0.22	2.09 ± 0.10^{b}	3.84 ± 0.17^{b}	3.13 ± 0.15^{a,b}
MatMulti	24	4.78 ± 0.18	6.40 ± 0.17	2.17 ± 0.11^{b}	3.02 ± 0.16^{a}	3.29 ± 0.18^{a,b}

Growth was calculated for each individual as the shell diameter on the last week of the interval minus shell diameter at the beginning of the interval. Unmat snails were isolated for their entire lives, MatEarly snails were mated once at 18 weeks, MatLate snails were mated once at 26 weeks and MatMulti snails were mated at 18, 26, 42, 58, 74 and 97 weeks. Values are mean (mm) ± 1 standard error. Lowercase letters indicate significant differences between means.

Short term trade-offs between growth and reproduction

The overall patterns of growth and reproduction we observed support the hypothesis that a significant cost of reproduction for *H. trivolvis* is reduction in somatic growth. Although all of the snails we observed grew during the experiment, when snails were actively laying eggs their growth slowed and their body sizes were generally smaller than those of contemporaries that were not reproducing.

Although there were no differences in growth among the four groups during weeks 10–18 (before treatments), Mated (MatEarly and MatMulti) snails grew less between weeks 18 and 26 (Phase I) than Unmated (Unmat and MatLate) snails (Fig. 2 and Table 1), as predicted. During weeks 25–42 (Phase II), when MatLate and MatMulti snails were producing large numbers of eggs, but MatEarly snails were producing fewer eggs, the pattern shifted: MatLate and MatMulti snails grew less than those in the Unmat and MatEarly treatment groups. From weeks 42 to 68 (Phase III), when MatMulti snails were still producing eggs, but reproductive output of the MatLate snails had significantly slowed, MatLate snails grew significantly more that the Unmat snails.

This pattern is similar to that observed in other pulmonates. Koene & Ter Maat (2004) compared sizes of isolated and grouped *Lymnaea stagnalis* and found that snails that did not lay eggs were larger and heavier than those producing egg masses (see also Koene, Loose & Wolters, 2008). In a field study of three pulmonate species, Russell Hunter (1961) observed that during times when individuals reached sexual maturity and began egg production, growth slowed. In our laboratory, when snails were provided with unlimited resources, they could easily shift their allocation of energy between growth and reproductive output, adjusting to current conditions. Those snails with no partner could allocate energy towards maintenance and growth, whereas those that were reproductively active supported egg (and initially sperm) production. When sperm stores were depleted in those snails that were mated just once, they could begin to compensate for lost growth as they decreased egg production.

This plasticity is expected in species, such as *H. trivolvis*, that are subject to varying environmental conditions, as shallow ponds may dry up and availability of food and mates is uncertain (Russell Hunter, 1961, 1978). Two freshwater snails (*Stagnicola elodes* and *Physella gyrina*) both showed changes in allocation to growth and reproduction in response to dietary restriction (Rollo & Hawryluk, 1988). *Stagnicola elodes* decreased reproductive output while maintaining growth, while *P. gyrina* maintained reproductive rate, but grew less. Although their strategies differed, the trade-offs were clear. The influence of environmental factors on growth and reproduction has also been well documented in *L. stagnalis*. Snails subject to shorter day lengths laid more eggs, grew more slowly and reached smaller ultimate lengths than those exposed to more hours of light (Zonnefeld & Kooijman, 1989). When starved, snails exposed to longer day lengths stopped reproducing, whereas snails at shorter day lengths continued to reproduce and they thus show differences in loss of dry weight.

It has been suggested that phenotypic plasticity in growth and reproduction may have been selected for in freshwater species, as the responses demonstrated here and described above appear to be common in the field (Russell Hunter, 1961). Selection may thus have resulted in genotypes that allow for these responses, particularly in organisms with indeterminant growth (Roff, 1992). Although the present study did not address resource limitation, our findings are consistent with these considerations. If snails can alternately channel resources to somatic or reproductive function, they may be able to recover from resource limitation or scarcity of mates, and thus suffer little long-term consequence.

Although we have demonstrated short-term trade-offs between growth and reproduction in snails subject to controlled mating regimes, it is not clear how these treatments may have influenced energy allocation directly. Measurements of food intake, as well as egg size and quality (viability, for example) would greatly enhance our understanding of this process. Furthermore, assessing the costs of female *vs* male reproductive effort (see De Visser *et al*., 1994) would be of particular interest in these hermaphrodites.

Consequences of delayed reproduction

A key prediction of life-history theory is that delayed reproduction should result in greater numbers of offspring, since individuals that mate later in life would have accumulated more resources and grown to a larger size (Ghiselin, 1969). This prediction appears to be borne out in our experiment. Over the course of their *c*. 16 weeks of reproductive output from a single mating, the total number of egg masses, eggs per mass and total number of eggs were consistently higher in snails that had been forced to delay reproduction. This increased productivity after delayed reproduction can be accounted for by their increased size relative to the early-mating snails; when body size is added as a covariate, these differences are no longer significant. This is consistent with previous work demonstrating that body size accounts for 19% of the variation in overall egg production and 24% of the variation in the number of eggs per mass in this species (Norton & Bronson, 2006). While this outcome highlights one immediate advantage of delaying reproduction, there may be a physiological disadvantage to delaying reproduction, since 7 of the 26 snails in the late mating group laid no eggs at all after being placed with another individual for a week (they were thus omitted from further analysis). In contrast, all but 2 of the 52 snails mated at 18 weeks produced offspring, a result significantly different from that expected by chance (Fisher's Exact test $P < 0.004$). In the field, we might expect that any delay in reproduction would have fitness consequences, since

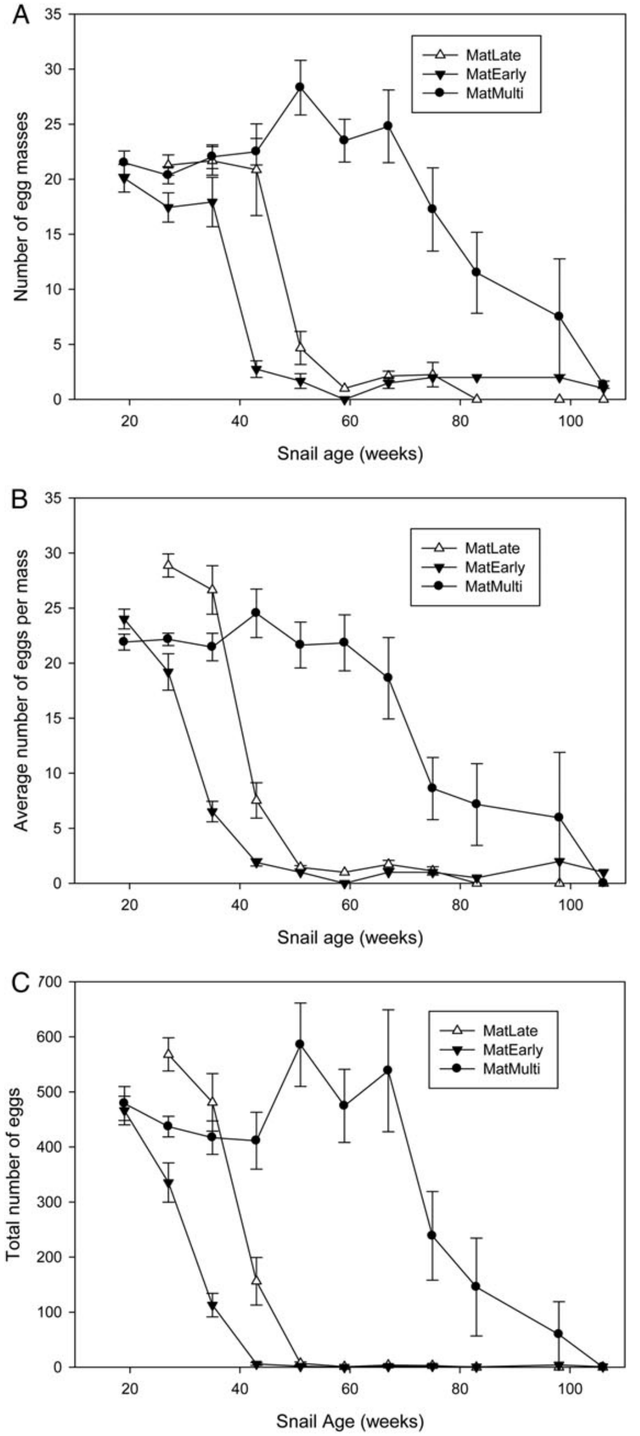

Figure 3. Egg production of snails with different reproductive opportunities. Three-week egg production of snails in the reproductively-active treatment groups was measured every 8 weeks, beginning at 19 weeks. MatEarly snails were mated once at 18 weeks, MatLate snails were mated once at 26 weeks and MatMulti snails were mated at 18, 26, 42, 58, 74 and 97 weeks. **A.** Average number of egg masses deposited per snail. **B.** Average number of eggs per egg mass. **C.** Average overall number of eggs per snail. Error bars represent ± 1 standard error. UnMat snails laid very few eggs and were omitted from these figures.

food can be scarce, predators are common and ponds can be transient (Dillon, 2000), so any time delay would increase the risk of death before reproducing.

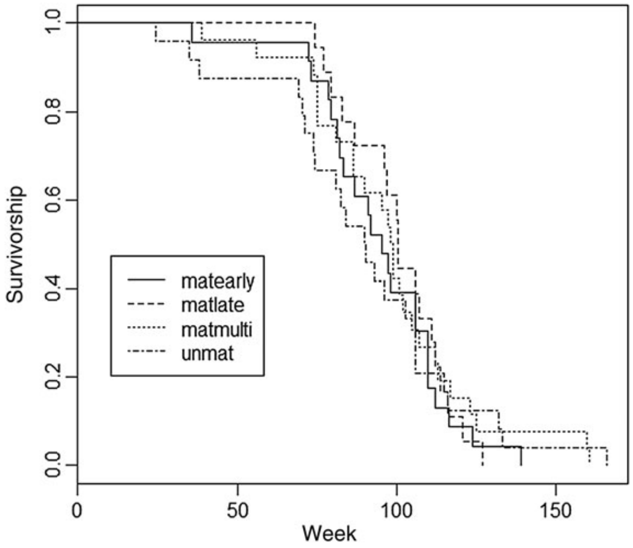

Figure 4. Survival curves for snails with different reproductive opportunities. Survival (proportion of individuals remaining) was calculated for each of the four groups of snails until all snails had died. Unmat snails were isolated for their entire lives, MatEarly snails were mated once at 18 weeks, MatLate snails were mated once at 26 weeks and MatMulti snails were mated at 18, 26, 42, 58, 74 and 97 weeks.

Egg production

The rate of egg-mass production in our population (based on the initial 3 weeks of egg production in the MatEarly and MatMulti snails) is similar to that documented in a laboratory population in 2005 (1.2 egg masses per day; Norton & Bronson, 2006). The number of eggs per mass (20.8 ± 0.8), however, is much greater than found in the laboratory population (12.7 ± 0.9). The differences in eggs per mass may be due to differences in origin of the populations—the current sample represents snails whose parents were wild collected, while the 2005 population had been maintained in the laboratory for many years. It is also possible that sperm depletion (see below) may have resulted in the smaller size of egg masses in the previous experiment, since snails in that study were sampled from a large aquarium population, with the time of mating unknown.

Self-fertilization in H. trivolvis

Although self-fertilization is fairly common in pulmonate snails, previous studies have documented low levels of self-fertilization in *Helisoma* species (Paraense & Correa, 1988; Jarne, Vianey-Liaud & Delay, 1993; Escobar *et al.*, 2011). Our study confirmed a low incidence of egg production by isolated *H. trivolvis* monitored for their entire lives. Because the Unmat snails laid so few (probably nonviable) eggs, we conclude that self-fertilization is rare enough to be considered almost nonexistent in this population.

Sperm storage

Sperm storage has been documented in a wide variety of animals (Neubaum & Wolfner, 1999; Birkhead, Hosken & Pitnik, 2011), ranging from less than 48 h in mammals, weeks to months in most insects, and between 4 and 7 years in some reptiles. Sperm storage has often been quantified by measuring the length of time an individual continues to produce viable offspring after a single mating opportunity. In pulmonates, this measure is complicated by the fact that many are hermaphrodites with the capacity for self-fertilization. In these cases, the paternity of offspring has been determined by using genetic markers such as pigment differences (e.g. Cain, 1956), electrophoretic variants (e.g. Wethington & Dillon, 1991) or more recently microsatellite markers (Nakadera, Blom & Koene, 2014). Because of the low selfing rate in *H. trivolvis*, we were able to estimate sperm storage times from snails isolated after a 1-week mating opportunity.

Durations of sperm storage in pulmonate snails are usually on the order of 5–60 d (7–8 weeks), with some individuals producing outcrossed eggs more than 100 d (14 weeks) after their sole copulation (Wethington & Dillon, 1991; Nakadera *et al*., 2014). Our estimates of sperm storage in *H. trivolvis* are much greater, of the order of at least 16 weeks in the MatEarly and MatLate snails. These longer durations may have been selected for as a result of the general self-sterility in *Helisoma* species (Paraense & Correa, 1988; Escobar *et al*., 2011; see above) and may indicate a larger capacity in the reproductive structures for allosperm storage. Because sperm storage allows maintenance of continued fecundity without mating, it should be particularly advantageous in species incapable of self-fertilization, which may experience isolation resulting from fluctuating environmental conditions (Dillon, 2000). These differences may also be due to the number of inseminations, which could be tested by manipulating the number of matings allowed.

The physiological nature of sperm storage in pulmonate snails is not well understood (Koene *et al*., 2009). When snails stop laying eggs, it is not clear whether the available pool of sperm has been used up (by fertilizing eggs) or if the maximal survival time has been reached (Cain, 1956). *Helisoma* seem programmed to lay egg masses on a regular basis, about one per day, as long as they have sufficient sperm. The first sign that sperm are being depleted is a decrease in the number of eggs deposited in each egg mass and then, several weeks later, a decrease in rate of egg-mass production.

No long term cost of reproduction on longevity

Contrary to our initial expectations, we saw no effect of the four mating treatments on longevity. Life-history theory maintains that a significant cost of reproduction is continued survival (Stearns, 1976) and predicts a negative relationship between reproductive output and longevity. Calow (1979) suggests several physiological mechanisms to explain this negative association. In general, reproductive effort is presumed to be costly, from sperm and seminal fluid and/or egg production and delivery to energy expended during courtship and copulation, which may weaken organisms and make them more susceptible to environmental hardship or other stresses. The earliest experimental studies showed increased longevity in *Drosophila* with reduced or absent ovaries due to either heat treatments or mutations (Maynard Smith, 1958) and subsequent work has documented numerous cases of reduced lifespans in reproductive individuals relative to those that are either nonreproductive or have reduced fecundity (reviewed in Bell & Koufopanou, 1986; Roff, 1992). A notable example is work on *L. stagnalis* where snails that were isolated or mated only once had significantly higher survivorship than snails that were mated repeatedly or had continuous access to partners (Hoffer *et al*., 2012). This pattern was also seen in starved snails under short-day conditions, which ceased reproduction and had longer survival times (Zonnefeld & Kooijman, 1989). In our case, isolated snails laid very few eggs and were expected to outlive their counterparts that mated at least once (typically as both male and female) and produced large numbers of eggs. The snails afforded multiple mating opportunities (6 weeks, each with a different partner) during their lifetime, most of which continually produced eggs (and presumably sperm along with seminal fluid) for almost a year, were expected to have even more reduced lifespans. But there were no differences in survival among any of the treatments.

Although classic life-history theory predicts trade-offs between reproductive output and lifespan due to competing energy demands, recent work on the genetic and physiological mechanisms controlling reproduction and longevity has challenged this notion. Edward & Chapman (2011) have reviewed the work on physiological mediators of trade-offs and presented evidence that classic 'Y models' of resource allocation (that energy must be shunted in either one direction, growth/maintenance—or another, reproduction) are not sufficient to explain observed relationships among life-history traits. Eliminating reproduction altogether (often by mutations influencing germ-line development) does not always increase longevity and not all mutations that increase lifespan reduce fecundity. It is possible that physiological mechanisms that control the short term trade-off between growth and reproduction in this species do not influence overall survival, or that snails may have traded reproduction or growth for longevity so that costs are hidden when comparing treatments.

Another likely explanation for the absence of a measurable effect of reproduction on longevity is that the laboratory environment, with plentiful food and lack of predators, may have removed most stressors. In fact, Bell & Koufopanou (1986) noted that most of the demonstrations of cost that they reviewed occurred under conditions of insufficient resources. Our experimental design limited us to studying the intrinsic costs of reproduction and not the ecological costs that may be accrued because organisms are at risk from external factors (Edward & Chapman, 2011). In the field, there may in fact be measurable long-term consequences of increased reproductive effort and we encourage studies that shed light on these relationships.

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