

Are there interactive effects of mate availability and predation risk on life history and defence in a simultaneous hermaphrodite?

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Abstract

Encountering mates and avoiding predators are ubiquitous challenges faced by many organisms and they can affect the expression of many traits including growth, timing of maturity and resource allocation to reproduction. However, these two factors are commonly considered in isolation rather than simultaneously. We examined whether predation risk and mate availability interact to affect morphology and life-history traits (including lifetime fecundity) of a hermaphroditic snail (*Physa acuta*). We found that mate availability reduced juvenile growth rate and final size. Predator cues from crayfish induced delayed reproduction, but there were no reduced fecundity costs associated with predator induction. Although there were interactive effects on longevity, lifetime fecundity was determined by the number of reproductive days. Therefore, our results indicate a resource-allocation trade-off among growth, longevity and reproduction. Future consideration of this interaction will be important for understanding how resource-allocation plasticity affects the integration of defensive, life-history and mating-system traits.

The life history that an organism employs can be viewed as a strategy for partitioning resources among fitness functions of growth and reproduction. Given some allocation of resources to reproduction, the mating system (i.e. the degree of inbreeding from self-fertilization to outcrossing) can have important fitness consequences by directly affecting the transmission of genetic variation. Therefore, an organism's life history and mating system are intimately connected and both play an important role in determining reproductive success. In addition, both the life history and mating system of an organism may be affected by intra- and interspecific ecological interactions that alter the allocation of resources to growth and reproduction. The avoidance of predators and the search for mates are two such interactions that most organisms face in natural communities. Although variation in predation risk and mate availability is ubiquitous and despite many examples of inducible defences and mating-system plasticity, we are only beginning to consider the ways that these factors may interact.

Inducible defences have been demonstrated in plants, animals and protozoans and have served as a fruitful

model system for exploring the ecology and evolution of adaptive phenotypic plasticity (Tollrian & Harvell, 1999; Relyea, 2005). Commonly, predators induce defences that have fitness costs in prey such as reduced growth rate or fecundity thereby favouring inducible rather than constitutive expression of defensive traits (Tollrian & Harvell, 1999). It is important to consider such effects because the induction of a defence can alter allocation of resources between growth and reproduction and costs may not be incurred until late in ontogeny. For example, theory predicts that prey should respond to small-size-selective predators by delaying reproduction in favour of growth to a size refuge (Stearns & Koella, 1986). This prediction has been tested and supported by empirical studies (e.g. Crowl & Covich, 1990; Hoverman *et al.*, 2005). Although many studies of inducible defences have examined the fitness consequences of expressing a defence, no animal studies to our knowledge have examined the effects of expressing a defence on longevity and lifetime fecundity.

In addition to defence, the mating system employed by an organism can have direct fitness consequences. The benefits of inbreeding include the maintenance of favourable gene complexes (in the context of local adaptation) and the transmission advantage of selfing (Fisher, 1941; Jarne & Charlesworth, 1993). However, inbreeding increases homozygosity, which can result in

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inbreeding depression if partially recessive, deleterious alleles are segregating in the population (Jarne & Charlesworth, 1993). Conversely, outcrossing can reduce inbreeding depression, but at the cost of decreased gene transfer (Goodwillie *et al.*, 2005). Thus, there is a fitness trade-off between selfing and outcrossing that can favour plasticity in the mating system. Indeed, it has been suggested that in the event of low mate availability, a self-fertile hermaphrodite from a population harbouring inbreeding depression should delay selfing for a period of time after achieving reproductive maturity to find a mate and avoid the costs of selfing (Lloyd, 1992; Goodwillie *et al.*, 2005). However, at the end of this 'waiting time', the organism proceeds with self-fertilization if no mates are present. A recent model predicts that the waiting time should be longer with strong inbreeding depression and efficient resource reallocation to future reproduction (Tsitrone *et al.*, 2003a). This model has been tested and supported in hermaphroditic animals (Tsitrone *et al.*, 2003b; Schjørring, 2004; Escobar *et al.*, 2007; but see Schärer & Wedekind, 1999) suggesting that mate availability can influence resource allocation between growth and reproduction in an adaptive fashion.

Clearly, mate availability and predation risk affect many of the same traits. Both mate availability and predation risk can influence individual reproduction (e.g. timing of reproduction) and may potentially affect the mating system. Interestingly, the manner in which organisms respond to predation risk and mate availability may interact in potentially important ways. For example, if simultaneously hermaphroditic organisms respond to the presence of a small-size-selective predator by delaying reproduction, this delay may affect how long individuals will delay selfing. In other words, the predator-induced delay in reproduction may affect the length of the waiting time in the absence of mates. If the waiting time is altered by predation risk, the mating system may be altered as well. Additionally, when individuals have limited mate availability, resources may be allocated differentially to growth instead of reproduction (Tsitrone *et al.*, 2003a, b). If this differential allocation results in increased growth, a larger and therefore more defended phenotype can be achieved, thereby providing a benefit in the event of predator colonization. Alternatively, the manner in which organisms respond to variation in predation risk and mate availability may be additive, not interactive, but we currently lack data to evaluate these alternatives. Here we investigate the potential interaction between predation risk and mate availability for morphology and life-history traits including total lifetime reproduction. Based on previous work, we predict that the availability of mates will lead to early reproduction whereas the presence of predator cues will induce a delay in reproduction. In addition, we predict a trade-off between growth and reproduction.

Methods

Study system, animal collection and rearing

We examined the effects of predation risk and mate availability on morphology and life history in the freshwater snail *Physa acuta* (Pulmonata, Basommatophora). This snail is a simultaneous hermaphrodite that has been widely used for studying predator-induced plasticity in morphology, behaviour and life history (Crowl & Covich, 1990; DeWitt *et al.*, 1999, 2000; Turner *et al.*, 1999; Tsitrone *et al.*, 2003b). Specifically, snails display fast growth and narrow shell apertures that appear to increase survival in the presence of small-size-selective, shell-entry predators such as crayfish. *Physa* detects predators via water-borne chemicals (Crowl & Covich, 1990; DeWitt *et al.*, 1999), which allows investigators to examine the inductive effects of predators without changes in prey density. *Physa* has also been widely used in studies of mating interactions, mating system expression and the effects of inbreeding depression (e.g. Jarne *et al.*, 2000; Facon *et al.*, 2006). Because *P. acuta* is easy to culture and has a short generation time (i.e. < 3 months), it is an ideal species to use for studies of longevity and lifetime fitness.

Adult *P. acuta* snails were collected at Geneva pond #3 in north-west Pennsylvania, USA (41°35'N; 80°14'W) on 23 January 2006. Snails were transported to the University of Pittsburgh (Pittsburgh, PA) within 2 h and isolated in 1-L plastic containers for oviposition. The experimental room was held at 22 °C with constant 12-h light/dark cycles during hatching and the subsequent experiment. Containers were checked daily for eggs and 65 snails were chosen that laid eggs on 29 January (hereafter considered day 0 for determining snail age). *Physa acuta* is a preferential outcrosser (Jarne *et al.*, 2000) and can store sperm for long periods of time (e.g. up to 3 months; Dillon *et al.*, 2005) so we assume that all the progeny of these wild-caught snails were outcrossed. Adults were removed from containers and dissected to assure that the specimens were *P. acuta* [*P. acuta* is superficially similar to other co-occurring *Physa* species (e.g. *Physa gyrina*) and positive identification needs to be made based on male genital morphology; Wethington, 2004]. Hatching began on 10 February (age = 12 days) and all snails were fed ground *Spirulina* (O.S.I. Marine Lab, Inc., Burlingame, CA, USA) *ad libitum*. From the 65 ovipositing snails, 10 families were randomly selected for use in the experiment. All water used was carbon filtered and UV irradiated.

Experimental design

Individual snails were reared in 1-L plastic containers (filled with 1 L of water) under a completely randomized design, employing a factorial combination of two predator treatments (predator cues present or absent) and two mate-availability treatments (mate available or not,

i.e. isolation). Each treatment was replicated 10 times, yielding 40 experimental units. To equalize genetic differences and potential maternal effects among the treatments, one individual from each of 10 families was used in each treatment (i.e. $n = 10$). Individual snails were added to the containers on 3 March 2006 (age = 33 days; initial mass < 1 mg), the predator-cue treatment was initiated on 6 March and the mate-availability treatment began on 10 March (i.e. treatments were applied for approximately two-thirds of the snail's juvenile period). Throughout the experiment, snails were fed three times per week and water was changed weekly. The experiment was conducted for the entire life of the snails (age at death ranged 72–212 days) to determine longevity and lifetime fecundity.

The predator treatment was implemented by adding water that had been conditioned by a pond-dwelling crayfish (*Procambarus acutus*) that is native to the region and co-occurs with *P. acuta*. Crayfish ($n = 15$) were held individually in 10-L plastic tubs containing 3 L of water. Three times per week, we collected 1 L of crayfish-conditioned water from each tub, discarded the remaining 2 L, re-filled the tubs with 3 L of fresh water and fed the crayfish 150 mg of laboratory-reared *P. acuta* and rabbit chow *ad libitum* (crayfish are omnivores). After pooling the 15 L of predator-cue water, we removed 400 mL of water from each experimental unit assigned the predator treatment and replaced it with 400 mL of predator-cue water. Therefore, the predator-cue concentration in each experimental unit was 20 mg of consumed *Physa*/L. Snails in the no-predator treatment had 400 mL of water removed three times per week and replaced with 400 mL of fresh water. Predator cues break down, so this static-renewal treatment was implemented to maintain constancy in perceived predation risk.

Mate availability was manipulated without rearing individuals under different densities. Snails in the no-mate-available treatment remained in isolation throughout their entire life whereas snails in the mate-available treatment had a marked, sexually mature *P. acuta* added to their container three times per week for 3 h at a time (Tsitrone *et al.*, 2003b). Mates were selected from laboratory cultures that were all founded from the same population and represented > 30 families (i.e. isofemale lines). These lines were consistently mixed throughout the experiment and mates were cultured together. Therefore, each time a mate was added, experimental snails potentially had access to a different mate. Mates were marked with fast-drying red nail polish, which is an effective and harmless marking technique (Henry & Jarne, 2007). As the majority of oviposition occurs at night (Duncan, 1975), it is unlikely that the mates oviposited during these conjugal visits. This duration of mate availability was sufficient to allow copulation of snails and reciprocation of gender roles (Facon *et al.*, 2006; J.R. Auld, personal observation).

Morphological measurements and analysis

To assess plasticity in shell morphology at the same point in ontogeny, we weighed each snail and took a digital picture using a Canon PowerShot A300 camera on 11 April (age = 72 days). Images were viewed using Optimas (Bothell, WA, USA) and four shell measurements were recorded: shell length, shell width, aperture length and aperture width (measured at the maximum for each snail). Shell thickness was also measured to the nearest 0.01 mm with digital calipers at the leading edge of the shell. To standardize morphological measurements for differences in overall size, we conducted a MANCOVA with ln-transformed mass as a covariate and shell dimensions as response variables (shell thickness was not correlated with mass ($r = 0.074$, $P = 0.653$), so it was not corrected for size). The MANCOVA included predator and mate treatments as fixed effects and the assumptions of the MANCOVA model were verified, including the absence of treatment-by-response variable interactions (i.e. all treatment slopes were parallel). We saved the residuals from the MANCOVA and subsequently used the sum of each individual snail's residual plus the estimated marginal mean (i.e. the mean estimated from the model, including the effects of mass as a covariate) for each treatment as our response variables. This procedure produces estimates of shape variables that are adjusted to remove the effects of overall size and has been successfully used in previous studies of morphological plasticity (e.g. Hoverman *et al.*, 2005). All statistical analyses were performed using SPSS (v. 11 for Mac; SPSS Inc., Chicago, IL, USA).

To provide a comparison with previous studies (e.g. DeWitt *et al.*, 1999, 2000), we analysed the aspect ratio of shell and aperture traits (i.e. length divided by width) in addition to analysing the shape variables independently. We calculated aspect ratios based on size-independent and un-adjusted measures of shell and aperture dimensions and found these two methodologies to be qualitatively identical. We report test statistics based on the analysis of aspect ratios calculated with un-adjusted shell dimensions.

Life-history/reproductive response variables and analyses

The experiment lasted the entire life of the snails to measure a complete set of life-history traits including age/size at first reproduction, growth rate, longevity and lifetime fecundity. Experimental units were checked daily for egg masses and the number of oviposited eggs was counted weekly. During each weekly egg counting, the number of eggs that failed to hatch was also counted to determine egg-hatching success. Individuals were placed in new containers weekly so that we could easily count eggs and evaluate egg hatching. Snails were blotted dry and weighed weekly (to the nearest mg), when they produced their first egg mass (i.e. size at first

reproduction) and at death (i.e. size at death). We assessed the effects of our treatments on the allocation of resources between growth and reproduction by comparing growth rate prior to reproduction (i.e. juvenile growth rate) with growth rate during reproduction (i.e. adult growth rate). Juvenile growth rate represents the size at first reproduction divided by the age at first reproduction. Adult growth rate was calculated as the difference between size at death and size at first reproduction divided by the difference between age at death and age at first reproduction. As most snails reproduce up until the day they die, these measures provide a linear estimate of how resource allocation to growth changes when snails initiate reproduction. We also quantified the fraction of total growth that occurs prior to initiating reproduction (i.e. size at first reproduction divided by size at death; SFR/SD) as an additional means of determining how resource allocation between growth and reproduction differs among our treatments.

We used a MANOVA to examine treatment effects on 17 traits: size-independent morphology (i.e. shell length, shell width, aperture length and aperture width), shell aspect ratio, aperture aspect ratio, shell thickness, age at first reproduction, size (mass) at first reproduction, age at death (i.e. longevity), size at death, the number of reproductive days (age at last reproduction – age at first reproduction), the total number of eggs laid, the proportion of total eggs that hatched, juvenile growth rate, adult growth rate and the proportion of final size attained prior to reproduction. All life-history/reproduction variables except the three growth variables were ln-transformed prior to analysis (except the egg-hatching

proportion which was arcsine-square root transformed). When multivariate effects of our treatments were significant, we examined univariate effects of the treatments on each variable independently. In an effort to control for multiple testing while balancing the risk of type I and type II errors, we used the methods suggested by Verhoeven *et al.* (2005) to estimate the false discovery rate. This methodology was initially suggested by Benjamini & Hochberg (1995) as a more powerful means of controlling for multiple testing than the traditional Bonferroni/sequential – Bonferroni tests (Verhoeven *et al.*, 2005). When univariate tests were significant, we conducted mean comparisons using *t*-tests to examine specific comparisons between a pair of treatments (e.g. between mate and no-mate treatments within the no-predator treatment). Two snails were excluded from the final analysis; one of which proved to be a statistical outlier in terms of growth and reproduction whereas the other never reproduced. Inclusion of the available data from either of these two snails did not qualitatively affect the outcome of the analyses.

Results

The MANOVA included 17 response variables and revealed significant multivariate effects of predator ($F_{17,18} = 3.023$, $P < 0.05$) and mate ($F_{17,18} = 2.255$, $P < 0.05$) treatments. The predator-by-mate interaction was nonsignificant ($F_{17,18} = 0.720$, $P > 0.05$). However, univariate tests revealed a significant univariate predator-by-mate interaction for shell thickness, the age at death and the number of reproductive days (Table 1).

Trait	Predator		Mate		Predator × mate	
	$F_{1,34}$	<i>P</i>	$F_{1,34}$	<i>P</i>	$F_{1,34}$	<i>P</i>
Shell length	3.724	0.062	1.668	0.205	0.788	0.381
Shell width	0.034	0.855	0.005	0.945	2.066	0.160
Shell aspect ratio	0.769	0.387	0.652	0.425	0.223	0.640
Aperture length	0.001	0.971	3.893	0.057	0.012	0.912
Aperture width	3.787	0.060	0.001	0.975	0.275	0.604
Aperture aspect ratio	3.896	0.057	1.612	0.213	0.503	0.483
Shell thickness	6.421	0.016	0.006	0.938	4.968	0.033
Age at first reproduction	34.836	< 0.001	3.164	0.084	0.063	0.803
Size at first reproduction	17.038	< 0.001	7.948	0.008	1.383	0.248
Age at death	3.011	0.092	1.645	0.208	7.862	0.008
Size at death	0.341	0.563	9.014	0.005	0.198	0.659
Reproductive days	2.344	0.135	0.282	0.599	5.741	0.022
SFR/SD	20.28	< 0.001	0.011	0.917	0.419	0.522
Total eggs laid	3.495	0.070	0.147	0.703	2.833	0.102
Egg-hatching proportion	0.585	0.450	1.975	0.169	0.294	0.591
Juvenile growth rate	2.668	0.096	5.684	0.023	0.411	0.526
Adult growth rate	5.968	0.020	0.683	0.414	1.867	0.181

Table 1 Results of 17 univariate tests showing predator, mate and interactive effects on each of the variables included in the MANOVA.

Boldface values denote significant tests after controlling for the false discovery rate (see text for details). SFR/SD is size at first reproduction divided by size at death (i.e. the proportion of total mass attained prior to reproduction).

Morphology

In our examination of morphology, predator cues did not affect shell width and aperture length, although there was a tendency for snails reared with predator cues to have longer shells and narrower apertures than snails reared without predator cues (Table 1). The presence of mates did not affect morphology although there was a tendency for snails with mates to have reduced aperture length than snails reared without mates. Previous studies on predator-induced morphology in freshwater snails have used the ratio of length to width (i.e. aspect ratio; DeWitt *et al.*, 1999, 2000) to describe shell shape. We calculated this statistic for both shell and aperture traits and found no treatment or interaction effects on shell aspect ratio. There were no mate or interaction effects on aperture aspect ratio, but consistent with the tendency for predators to induce relatively narrow apertures, predators tended to increase the aperture aspect ratio.

Predator cues caused an average 13% increase in shell thickness. However, we detected a univariate interaction for shell thickness. This resulted because snails with mates showed a 25% increase in shell thickness with predator cues (mean \pm SE: 0.28 ± 0.01 and 0.35 ± 0.02 mm, no-predator and predator induced, respectively; $t_{17} = 3.305$, $P = 0.004$) whereas snails without mates showed no change in shell thickness with predator cues (0.31 ± 0.01 and 0.32 ± 0.02 mm, no-predator and predator induced, respectively; $t_{17} = 0.220$, $P = 0.828$).

Life history and reproduction

Predation risk and mate availability affected the allocation of resources to life-history traits including growth and the timing of maturity. Predator cues induced larger age and size at first reproduction (Fig. 1a,b; Table 1). In addition, mate availability reduced size at first reproduction and size at death (Fig. 1b). There were no significant predator or mate effects on total lifetime fecundity or the egg-hatching proportion.

We observed evidence for a predator-by-mate interaction for age at death and the number of reproductive days. These interactions resulted because in the no-predator treatment, snails without mates lived 35% longer than snails with mates ($t_{17} = 2.479$, $P = 0.024$), whereas in the predator treatment there was no mate effect ($t_{17} = 1.344$, $P = 0.197$; Fig. 1a). In the mate treatment, snails lived longer when exposed to predator cues than when not exposed to predator cues ($t_{17} = 3.819$, $P = 0.001$), but snails reared without mates were not affected by predator cues ($t_{17} = 0.664$, $P = 0.515$). A somewhat similar pattern emerges for the predator and mate effects on the number of reproductive days, which is presumably correlated to longevity (Fig. 1c). These patterns of differential longevity and reproductive lifetime produced the pattern of fecundity observed in our treatments.

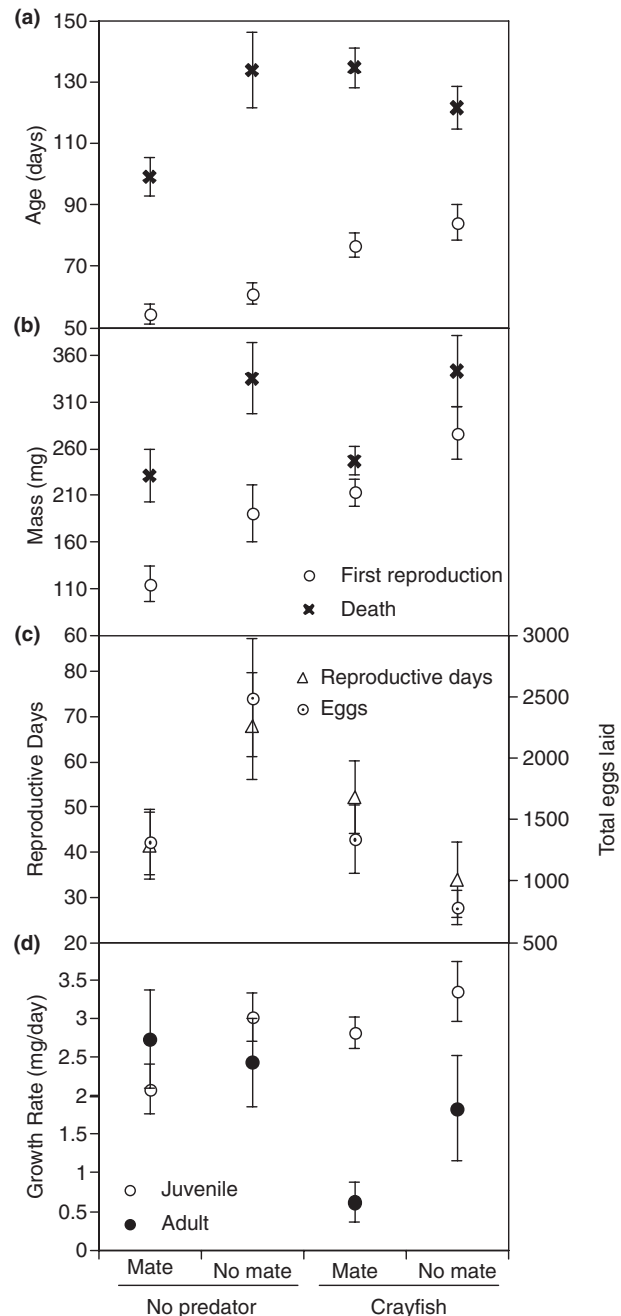


Fig. 1 The effects of predation risk and mate availability on several life history traits in *Physa acuta*. (a) Age and (b) mass at first reproduction and death [symbols are the same in panels (a) and (b)]. (c) The number of reproductive days (age at last reproduction – age at first reproduction) and total lifetime fecundity (total number of eggs laid). (d) Juvenile and adult growth rates (see text for details). Data were transformed prior to analysis and symbols represent mean ± 1 SE.

We explored how predator cues and mate availability altered resource allocation to growth and reproduction by comparing juvenile and adult growth rates. Although

predator cues did not affect juvenile growth rate, predator-induced snails did experience reduced adult growth rate compared with snails without predator cues (Fig. 1d). Alternately, snails with mates experienced reduced juvenile growth rate compared with snails without mates, but there was no mate effect on adult growth rate. There was no predator-by-mate interaction for these measures of juvenile and adult growth rate. Additionally, by dividing size at first reproduction by size at death, we found that snails reared without predator cues initiated reproduction when they were approximately 55% of their final mass whereas snails exposed to predator cues obtained approximately 85% of final mass before reproducing. There was no mate effect or interaction for this measure of growth prior to reproduction.

Discussion

Although previous studies have reported plasticity in response to predation risk and mate availability, this is apparently the first time they have been considered together. By doing so, we can evaluate an interaction that may occur under natural conditions where both predation risk and mate availability are variable. It is imperative to consider both factors over ontogeny because predation risk and mate availability affect resource allocation between growth and reproduction and the ultimate consequences on lifetime fitness should be evaluated.

Predator cues did not affect overall shell shape, but did affect shell thickness. DeWitt *et al.* (2000) reported that snails respond to crayfish cues by producing an elongate shell (i.e. increased ratio of length to width). Consistent with these findings, we found a marginally nonsignificant increase in the aperture aspect ratio with predator cues. Although previous studies have not examined changes in shell thickness, increased shell thickness is likely an important defence; indeed, in additional research with *Physa* from the same population, we have found that crayfish can more easily crush and kill noninduced snails than crayfish-induced snails (J.R. Auld & R.A. Relyea, unpublished data). Therefore, the predator-induced increase in shell thickness may be an adaptive anti-predator response.

Predator cues caused snails to delay reproduction, which is consistent with theoretical predictions (Stearns & Koella, 1986) and previous empirical observations (e.g. Crowl & Covich, 1990; Hoverman *et al.*, 2005) that size-selective predation can affect resource allocation between growth and reproduction. Snails reared without predator cues initiated reproduction when they were 55% of their final mass whereas snails reared with predator cues obtained 85% of final mass before reproducing. As predator cues did not affect size at death, these predator-induced snails had lower growth rates during reproduction (i.e. lower adult growth rates) than snails reared without predator cues. Taken together, predator

cues altered the timing of reproduction in ways that cascade to alter the patterns of growth.

Although reduced growth and/or fecundity are potential (and commonly mentioned) costs of expressing an inducible defence (Tollrian & Harvell, 1999), we find no evidence for such costs in our experiment. However, delayed reproduction can be viewed as a potential cost of expressing a predator-induced phenotype since delayed reproduction leads to a longer generation time. Although several studies have examined the effects of predators on reproduction (namely in *Daphnia*; e.g. Black & Dodson, 1990; Tollrian, 1995; Scheiner & Berrigan, 1998), these previous studies have yielded mixed results concerning a fecundity cost; predators often induce delayed reproduction, but fecundity either increases or decreases. In a previous study with a different species of freshwater snail (*Helisoma trivolvis*; Hoverman *et al.*, 2005), crayfish predators induced delayed reproduction and decreased fecundity of snails (all snails were reared with available mates), but this experiment did not last the entire life of the snails. Note that if we had terminated our experiment before the snails died, we would have arrived at similar results. Our study appears to be the first animal study to examine the consequences of an inducible defence over the entire lifetime. However, it is difficult to assess how these results can be extrapolated to field conditions where individuals may not live as long. In general, our approach provides a relatively complete understanding of the potential effects of an inducible defence expressed over the entire lifespan and more studies of this type will greatly contribute to our understanding of the costs and benefits of plastic defences.

Mate availability had strong effects on the total amount of growth. Although isolated and mated snails started reproduction at approximately the same age, isolated snails had larger mass at first reproduction than mated snails. Therefore, isolated snails grew at a faster rate prior to reproduction (i.e. faster juvenile growth rate). One potential explanation for this difference in allocation to growth is that mated snails may have invested more resources in male function than isolated snails. Indeed, theoretical models predict that male allocation should increase with mate availability and that completely selfing individuals should only produce enough sperm to fertilize their own ovules (Charlesworth & Charlesworth, 1981; Charnov, 1982). Many hermaphroditic organisms increase male allocation with mate availability (e.g. Raimondi & Martin, 1991; de Visser *et al.*, 1994; Koene *et al.*, 2006). Although this hypothesis is consistent with established theory, it remains speculative and will require further investigation.

Previous studies on simultaneously hermaphroditic animals (including *Physa*) have used reproductive effort and success of isolated and mated individuals to study aspects of the mating system. Past studies, all without predator cues, have found that preferentially outcrossing individuals experience reduced fecundity and progeny

survival when isolated (Jarne *et al.*, 1991, 2000; Doums *et al.*, 1996), whereas preferentially selfing individuals experience increased fecundity when isolated (Wedekind *et al.*, 1998; Gutiérrez *et al.*, 2001a,b). Interestingly, studies that have observed reduced fecundity by isolated snails also reported a long waiting time prior to self-fertilization (Jarne *et al.*, 1991, 2000) whereas studies documenting high fecundity of isolated snails report little or no waiting time (Gutiérrez *et al.*, 2001a,b). In contrast to previous research on different populations of *P. acuta* (Wethington & Dillon, 1997; Tsitrone *et al.*, 2003b; Escobar *et al.*, 2007), we did not observe a significant effect of mates on the age at first reproduction, however isolated snails tended to reproduce after mated snails. Importantly, additional research on *P. acuta* has demonstrated substantial among-population variation in waiting time (J. Escobar, B. Facon, P. Jarne, J. Goudet, & P. David, unpublished data). Therefore, our data are not inconsistent with the model of Tsitrone *et al.* (2003a) predicting a waiting time in outcrossing species.

Research on a diverse array of taxa has demonstrated a general trend that reproductive value gradually declines following the initiation of reproduction (i.e. senescence; Rose, 1991). Evolutionary theory of senescence predicts that longevity should be negatively related to growth rate (Metcalf & Monaghan, 2003). In our study, mate availability reduced juvenile growth rate and final size, but the consequences for longevity depended on predation risk. We also found that predation risk induced delayed reproduction, and subsequently, mated snails lived longer under predation risk than mated snails under no predation risk. Without predator cues, where snails initiated reproduction at relatively small size, longevity was reduced due to mating. Comparatively, with predator cues, where snails initiated reproduction at relatively large size, longevity was not affected negatively by mating. This suggests that mating and initiating reproduction at a relatively small size can have detrimental effects on longevity. These findings are in agreement with studies on the effects of mating on longevity in insects (Mishra & Mishra, 2005; Maklakov *et al.*, 2007).

Conclusions

Our results demonstrate that predation risk and mate availability can affect morphology and life history in *P. acuta* and although some traits exhibit additive effects of these treatments, we have some evidence for an interaction between predation risk and mate availability. In this study, we quantified total fecundity, which represents complete male and female fitness for isolated snails, but only female fitness for mated snails. Individuals reared in isolation should maintain a sperm supply large enough to fertilize their own eggs and engage in mating if a mate shows up, but this sperm storage is most likely never depleted as in the case where individuals encounter mates. Therefore, male allocation

is likely to be higher in an individual reared with available mates and increased allocation to male function may result in decreased growth ability. Despite rearing individuals under *ad libitum* food conditions, our results are indicative of a trade-off among growth, longevity, male reproduction and female reproduction. Therefore, these trade-offs are likely to be stronger under more realistic, food-limited conditions. We have shown that mate availability and predation risk act together to influence resource allocation and senescence and future studies should be designed to evaluate these trade-offs over the entire lifespan. Additionally, a number of our non-significant results may be indicative of a lack of power. Future experiments with increased sample size will reveal whether the patterns described here are robust. In summary, the mating system (i.e. outcrossing when mated and selfing when isolated) had dramatic consequences for the expression of several life-history traits; most notable were the effects on growth and longevity. Reciprocally, the expression of life-history traits may influence mating-system expression if a trade-off among growth, reproduction and sex allocation occurs under natural circumstances.

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