

Maternal Body Size and Condition Predict Measures of Reproductive Success and Future Reproductive Allocation in Territorial Eastern Red-Backed Salamanders

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Female fitness is often influenced by trade-offs in energy allocated to reproduction or self-maintenance that is dependent upon the life history of the organism. Maternal body size and condition are factors that are often positively correlated with clutch size and offspring size. We investigated whether maternal body size (snout-vent length, SVL) and condition (residual of mass on SVL) could predict measures of reproductive success including clutch size, offspring body size (SVL), and offspring survival in the territorial Eastern Red-backed Salamander (*Plethodon cinereus*). Brooding females and their eggs were collected and then housed in a controlled laboratory setting. We recorded the number of eggs found with each female and separated offspring from females once hatched. At 40 d after hatching, we measured the SVL and mass of females and juveniles. Maternal SVL, but not condition, was positively related to clutch size and offspring SVL; however, there was no significant relationship between clutch size and offspring SVL, indicating no trade-off between number and body size of offspring. In the laboratory, survival of the offspring to 185 d post-hatching was positively related to maternal SVL and negatively related to maternal condition. Also, the mean SVL, measured on day 40, of the offspring that survived to 185 d was significantly greater than the SVL of the offspring that did not survive, indicating a benefit of larger offspring body size. Finally, subsequent egg production (number of new oocytes produced by females following the current reproductive bout) was positively related to maternal SVL and condition (measured 185 d after hatching their previous clutches). These results suggest that larger maternal body size and condition of Eastern Red-backed Salamanders provide increased reproductive success through the number and body size of offspring produced in current or subsequent reproductive bouts and in offspring survival. Large body size in territorial animals like *P. cinereus* provides benefits for holding high quality territories as well as for increased reproductive success.

DIFFERENTIAL acquisition of limited resources for reproduction will occur when some individuals in a population have a competitive advantage over others and are able to sequester a larger proportion of those resources (e.g., in territorial species). Brockelman (1975) modeled the expected fitness of parents relative to territorial quality, predicting that in territorial species, selection for greater parental investment per offspring should occur. Brockelman's model assumes size-related advantages to the offspring (such as increased survivorship) that translate into increased fitness of the offspring and, ultimately, of the parents (e.g., Ferguson and Fox, 1984; Sinervo, 1990; Krist, 2011). In many territorial species, larger individuals often have an advantage in contests resulting in access to higher quality territories (e.g., Riechert, 1978; Mathis, 1990; Candolin and Voigt, 2001; Kohlsdorf et al., 2006). Thus, larger individuals should have access to better territories and be capable of investing more resources into reproduction resulting in larger clutch sizes and/or larger offspring.

In many species, parental investment in reproduction often increases with maternal body size (Lim et al., 2014; Rollinson and Rowe, 2016), with larger females producing more numerous and/or larger offspring than smaller females (e.g., Wilbur, 1977; Kaplan and Salthe, 1979; Stewart, 1979; Ford and Seigel, 1989); other models, such as condition-dependent provisioning and parental size constraints, may also be important in explaining this relationship (Congdon and Gibbons, 1987; Ljungström et al., 2016; Rollinson and Rowe, 2016). Additionally, in iteroparous species, older females may invest more into reproduction than younger

individuals (Pianka, 1976), and when growth is indeterminate, body size and age should be directly related such that the oldest individuals are usually the largest. Thus, larger, older individuals may exhibit increased investment in reproduction compared to smaller, younger individuals (Clutton-Brock, 1991; Kindsvater et al., 2012).

We tested the hypothesis that maternal body size and condition affect measures of reproductive success (defined as a female's production of offspring in a single reproductive bout) including clutch size, offspring body size, and offspring survival using a well-studied population of the territorial Eastern Red-backed Salamander, *Plethodon cinereus*. This species is completely terrestrial, inhabiting temperate forests of northeastern North America. Adult males and females aggressively defend territories containing cover objects (rocks and logs) that provide access to moisture and prey during dry periods between rainfalls (reviewed by Jaeger et al., 2016). Adults of larger body size are generally more successful than smaller adults in gaining higher quality territories (Mathis, 1990) containing the greatest numbers of high quality prey (Gabor, 1995) making body size a good predictor of territorial quality. For adult females of *P. cinereus*, the two most important factors regulating access to resources are (1) their success in gaining and holding territories (reviewed by Jaeger et al., 2016) and (2) the abundance of high quality prey therein (Gabor and Jaeger, 1995); thus, body size should impact the resources available for reproduction.

At our research site at Mountain Lake Biological Station (MLBS), Virginia, mating occurs from September–May, except during winter (our observations). Individuals mature in 2–4

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y, with females reproducing biennially starting at 3.5 y (Petranka, 2010; Jaeger et al., 2016). Females usually lay a clutch of eggs (range 3–14 eggs) every second year under rocks and logs (Sayler, 1966; Ng and Wilbur, 1995) and brood their clutches for at least six weeks (May–September) during which they rarely leave their nest sites to forage (Ng and Wilbur, 1995; Peterson, 2000). Following hatching, the neonates remain near their mothers for up to three months (Gillette, 2003). This brooding period can be costly because brooding females do not forage for prey and lose a significant amount of mass that results in insufficient reserves to produce a clutch in the next year (Ng and Wilbur, 1995; Yurewicz and Wilbur, 2004), and females in better condition (e.g., more mass for a given body length or having intact tails) also produce more and larger maturing oocytes than females in worse condition (Fraser, 1980; Yurewicz and Wilbur, 2004; Evans et al., 2020). However, brooding provides protection that is beneficial to the eggs including increased survival and larger hatchling size (Heatwole, 1961; Highton and Savage, 1961; Crespi and Lessig, 2004; Tornick, 2010). Post-oviposition maternal care, such as brooding behavior, may be related to body size, age, or condition of the female. In a cross-fostering experiment, Crespi and Lessig (2004) found that brooding foster-females of *P. cinereus* with larger body sizes produced larger hatchlings than did smaller foster-females, regardless of the body size of the genetic mother from which the eggs originated.

Because resources available for reproduction are often limiting for this species (Jaeger, 1972, 1980), trade-offs among fitness variables are likely to occur. Theoretically, differences in acquisition of resources by females should lead to differences in the amount of energy allocated to self-maintenance (for survival and future reproduction) vs. energy allocated to a current clutch of eggs, including the number and size of the offspring (Pianka, 1976; Stearns, 1992). The body size of offspring can, in turn, impact their probability of survival (e.g., Krist, 2011). Our experiment focused primarily on the impact of maternal body size and condition on measures of current reproductive success, including clutch size, offspring body size, and differential offspring survival among females, as well as the impact of maternal body size and post-brooding condition on future reproductive potential. We hypothesized that those females that are larger in body size (SVL) and in better condition (residual of mass regressed on SVL) are able to allocate more resources into reproduction than are smaller females such that maternal SVL and condition will both be positively related to clutch size, offspring SVL, and offspring condition. Additionally, maternal SVL is positively related to future allocation to reproduction (measured as the number of new developing oocytes produced following a brooding event), but maternal condition after brooding is negatively related to future allocation to reproduction. Finally, we compared the size of offspring within a clutch that survived or did not survive to test the hypothesis that offspring that are larger in size (SVL) are more likely to survive than smaller offspring.

MATERIALS AND METHODS

Collection, maintenance, and measurement of salamanders.—We collected 54 brooding females and their eggs from under cover objects (rocks and logs) at MLBS from 13 June–7 July 1992. All females collected were considered territorial,

because they were found under cover objects, although territorial quality should be diverse (e.g., different sized cover objects; Mathis, 1990, 1991). Females were housed on a natural photoperiod at 16–19°C in individual Petri dishes (14.5 × 1.5 cm) that were lined with filter paper and wetted with filtered spring water. The clutches were kept with the females with which they were found until hatching to increase egg survival (Highton and Savage, 1961; Crespi and Lessig, 2004). Almost daily visual inspections revealed that most females were brooding their eggs (i.e., they were in contact with the eggs and usually curled around them) except that two of the females ate their eggs. We did not feed the females while they were brooding, because females rarely leave their eggs to forage and only feed opportunistically (Highton and Savage, 1961; Bachmann, 1984; Ng and Wilbur, 1995). Females and eggs were transported to the University of Louisiana, Lafayette on 14–15 July 1992, where they were kept under a natural photoperiod at 18–19°C for the duration of the study. Positions of females with clutches were randomized on shelves in the laboratory to prevent position bias.

Hatching occurred from 5 August–2 September 1992. Usually all the eggs in a clutch would hatch within 1–2 d after the emergence of the first young, but hatching of all eggs in some clutches took as long as 4 d. Once eggs hatched within a clutch, we moved neonates into separate Petri dishes (9 × 1 cm) lined with filter paper dampened with aged tap water. We randomized positions of neonates on shelves in the laboratory to reduce position bias. Neonates were not fed for 40 d after hatching because (1) they had a large supply of yolk visible through the body wall that was visible until 30–35 d post-hatching; (2) in at least some species of plethodontid salamanders with aquatic larvae, individuals do not begin to feed for several weeks to several months after hatching (reviewed in Nussbaum, 1985); and (3) we did not want to risk harming neonates by handling them at an earlier age. Forty days after hatching (14 September–12 October 1992), we measured each neonate's snout–vent length (SVL: from the tip of the snout to the posterior angle of the vent), caudal length (CL: from the posterior angle of the vent to the tip of the tail), and mass (g). We measured length as described in Wise and Buchanan (1992). Mass was determined by first blotting each neonate on absorbent tissue for 5 s before measuring them (all individuals showed signs of emptying their bladders) and then obtaining the individuals' empty-bladder masses.

For the duration of the study, neonates were fed and able to consume *Drosophila (Sophophora) melanogaster* and *Tubifex*. Females were also fed a larger species of fruit fly (*D. virilis*). Individuals were fed *ad libitum* and the filter paper was changed weekly.

Predictive measures of current reproductive success.—We determined the relationship between maternal variables (SVL and condition) and clutch size using multiple regression ($n = 52$). Maternal condition was measured as the residual of maternal empty-stomach mass regressed on SVL in mm (Schulte-Hostedde et al., 2005) as in other studies of female condition in salamanders (e.g., Harris, 2008; Riedel et al., 2012). Those females with larger positive residuals were in better condition (were more massive for a given body length) than those with larger negative residuals. We used the standard multiple regression procedure (SPSS) to evaluate the effect of each

independent variable after the effects of all others had been removed (Tabachnick and Fidell, 2001). The effects of maternal SVL and condition on hatch date were determined in a similar manner.

We examined the ability of maternal SVL, maternal condition, and clutch size to predict offspring size (mean SVL within a clutch) and offspring condition at 40 d post-hatching using multiple regression. Specifically, we regressed (1) mean offspring SVL (in mm) within a clutch and (2) mean condition of offspring within a clutch on maternal SVL (in mm), maternal condition, and clutch size. In these analyses, we used only clutches in which neonate survival was 100% at 40 d after hatching ($n = 43$) to eliminate the effect of differential survival based on offspring SVL. We did not include hatch date as a variable in this analysis, even though hatch date may impact resident condition (we measured condition in females that were already brooding eggs for different periods of time leading to different hatching dates, and females do not actively forage while breeding, which may impact condition) because hatch date was not a significant predictor of maternal condition ($R^2 < 0.001$; $B = 0.025$; $t = -1.002$; $P = 0.322$).

Differential survival of offspring may occur as a result of investment by females in each offspring (*sensu* Clutton-Brock, 1991; Crespi and Lessig, 2004). We determined the effect of maternal SVL, maternal condition, and clutch size on the number of neonates per clutch that survived to 185 d using multiple regression ($n = 52$).

Body size of the juveniles may be an important factor for survival, so we compared the SVL of siblings that did and did not survive to 185 d post-hatching using a paired t -test. We compared the mean offspring SVL (in mm) at 40 d of those that survived to 185 d and those that did not survive to 185 d within a clutch as the repeated measure (pair) to control for maternal effects. We did not include clutches in which all neonates survived or all died (because we compared within clutches), resulting in $n = 34$ clutches.

Predictors of future reproduction.—The future production of eggs (a measure of future reproduction) may be affected by current reproductive effort, so at 185 d (7 February–12 March 1993) we re-measured maternal SVL and condition and counted the number of new, maturing oocytes of females after brooding. Developing oocytes were visible by pressing each female between two panes of plastic and shining a light from a fiber-optic source through the body cavity (as in Fraser, 1980). In *P. cinereus*, oocytes maturing in a given reproductive bout most likely develop simultaneously (Fraser, 1980), so the maximum number of eggs that will be produced in the next reproductive bout can be determined by counting developing oocytes, although it is possible that some of the developing oocytes in females we examined had not yet reached a large enough size to be visible (Fraser, 1980). We regressed the number of visible oocytes on maternal SVL, original maternal condition (from the first brooding period), maternal condition after brooding, and clutch size of the original brood. Maternal condition after brooding was used as a measure of the potential investment females could make to reproduction for the next reproductive bout. Several females were excluded from the analysis because the number of new oocytes was ambiguous (difficult to see all the eggs) and one female died ($n = 48$ females used in the analysis).

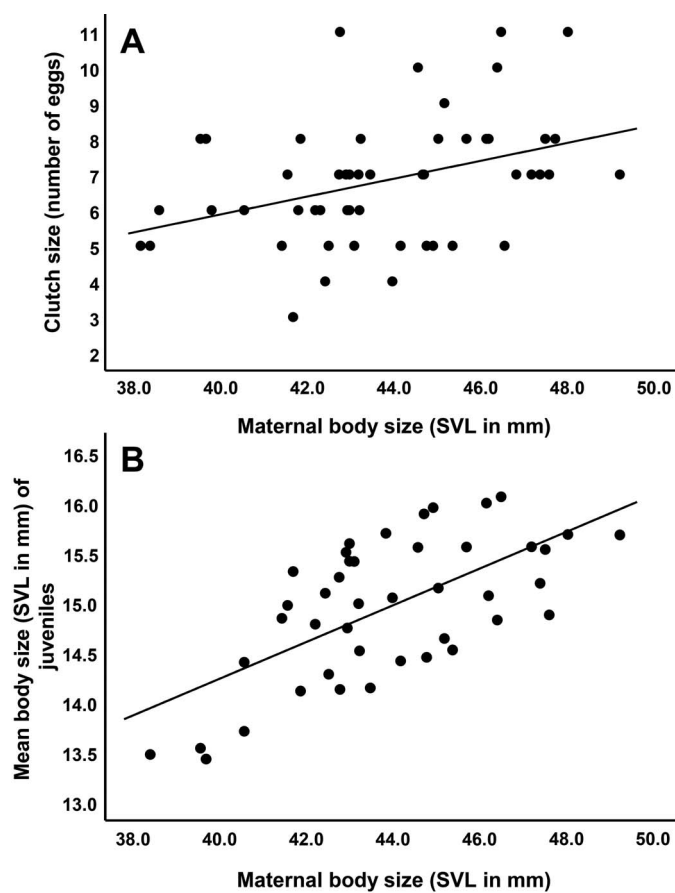


Fig. 1. The regression of (A) clutch size (number of eggs) and of (B) mean juvenile size (measured as mean snout–vent length, SVL, in mm per clutch) on maternal body size (SVL in mm).

RESULTS

Measures of current reproductive success.—The mean SVL of brooding females was 43.69 ± 2.69 mm (± 1 SD) and ranged from 38.15–49.21 mm. Clutch sizes ranged from 3–11 eggs with a mean of 6.8 ± 1.8 eggs (± 1 SD). Clutches hatched during 5 August–2 September 1992.

The overall model for the multiple regression of clutch size on maternal SVL and condition was significant ($R^2_{\text{adj}} = 0.115$; $F_{2,49} = 4.318$; $P = 0.019$). While maternal SVL contributed significantly to the model ($B = 0.251$, $t = 2.870$; $P = 0.006$), maternal condition did not ($B = -2.856$; $t = -0.633$; $P = 0.530$). As predicted, clutch size was positively correlated with maternal SVL (Fig. 1A). In a separate multiple regression analysis, we found that maternal body size and condition did not predict hatch date, defined as the date juveniles began to emerge from the eggs ($R^2_{\text{adj}} = 0.004$; $F_{2,49} = 1.102$; $P = 0.340$).

When regressing the mean offspring SVL on maternal SVL, maternal condition, and clutch size, the overall model was significant ($R^2_{\text{adj}} = 0.388$; $F_{3,39} = 9.884$; $P < 0.001$). However, only maternal SVL was a significant predictor of mean offspring SVL, showing a positive relationship ($B = 0.194$; $t = 5.259$; $P < 0.001$; Fig. 1B). Condition of the female ($B = -0.755$; $t = -0.740$; $P = 0.464$) and clutch size ($B = -0.035$; $t = -0.733$; $P = 0.468$) were not significant predictors of mean offspring SVL. A multiple regression also indicated that the model for the regression of the mean (per clutch) condition of juveniles on maternal SVL, female condition, and clutch

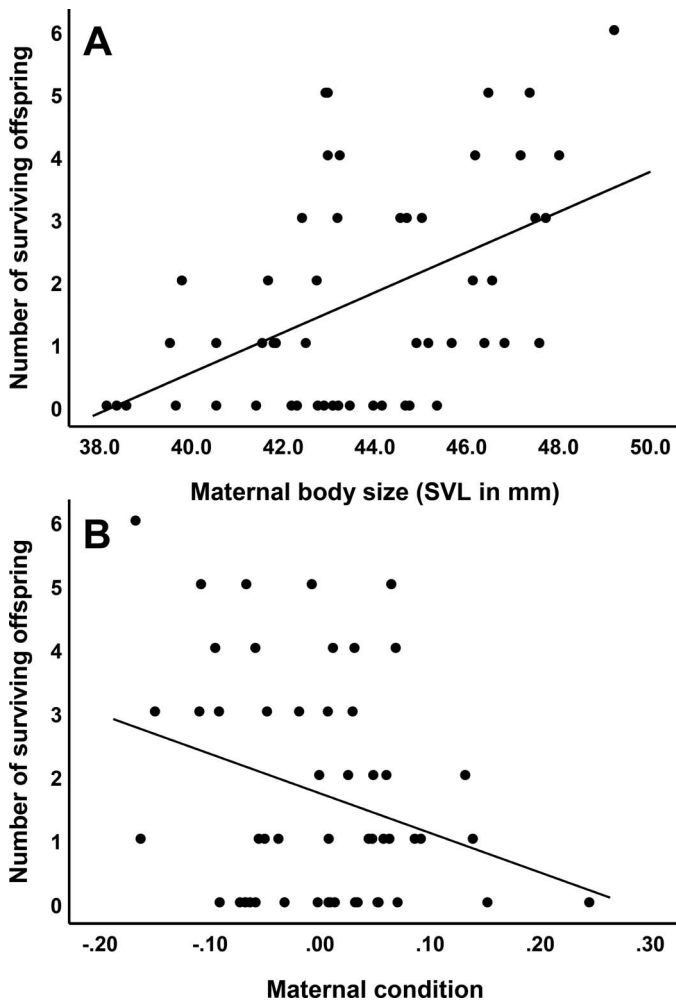


Fig. 2. The regression of number of surviving offspring (at 185 d post-hatching) on (A) maternal body size (SVL in mm) and (B) maternal condition (residuals of the regression of maternal mass, mm, on maternal body size, SVL in mm).

size was not significant ($R^2_{\text{adj}} = -0.009$; $F_{3,39} = 0.869$; $P = 0.465$).

Over the duration of the study, the percent survival of juveniles based on original clutch sizes was 24.8%. Numbers of neonates surviving per clutch ranged from 0–6 juveniles (0–86%), with a mean survivorship of 1.7 ± 1.8 juveniles (± 1 SD). The multiple regression model for number of juveniles surviving from each clutch on maternal SVL, maternal condition, and clutch size was significant ($R^2_{\text{adj}} = 0.297$; $F_{3,48} = 8.171$; $P < 0.001$). SVL of females was positively related to offspring survival, measured as number of offspring surviving per clutch ($B = 0.289$; $t = 3.476$; $P = 0.001$; Fig. 2A), while condition of females was negatively related to the number of offspring surviving ($B = -5.990$; $t = -2.368$; $P = 0.022$; Fig. 2B). Clutch size ($B = 0.127$; $t = 1.011$; $P = 0.317$) was not a significant predictor of the number of juveniles that survived. SVL of juveniles also affected their survival. In paired analyses of the mean size of siblings that survived versus those that did not survive to 185 d after hatching, surviving siblings were significantly larger in SVL at 40 d after hatching than were siblings that did not survive ($n = 34$; $\bar{X}_{\text{difference}} = 0.269$, $\text{SD}_{\text{difference}} = 0.316$, $t = 4.96$, $P < 0.001$; Fig. 3).

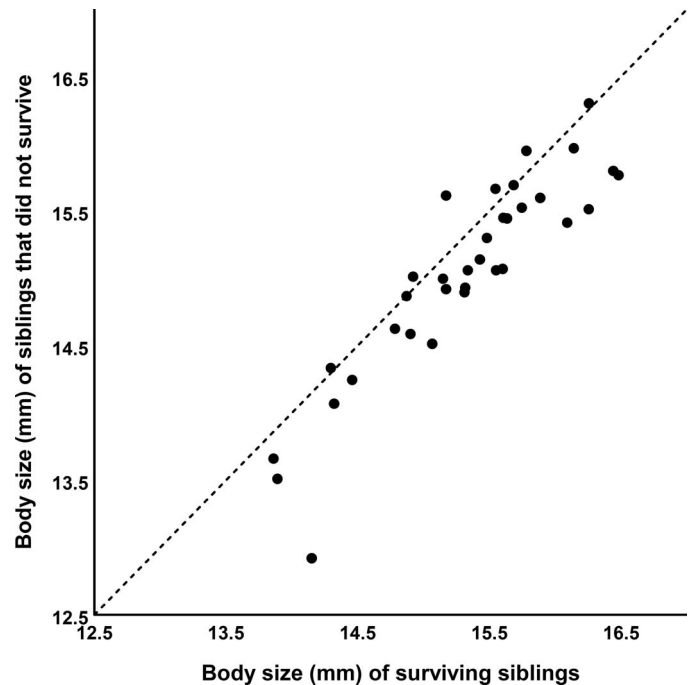


Fig. 3. The body size of siblings that did and did not survive to 185 d after hatching. Each point represents the mean SVL of siblings from a single female that did or did not survive. The diagonal line represents the hypothetical situation in which the body size of siblings that did and did not survive are equal.

Allocation to future reproduction.—The overall model using multiple regression of the number of new oocytes at 185 d regressed on maternal SVL, initial maternal condition, maternal condition after brooding, and most recent clutch size was significant ($R^2_{\text{adj}} = 0.328$; $F_{4,43} = 6.737$; $P < 0.001$). There was a positive relationship between the number of new oocytes and maternal SVL ($B = 0.406$; $t = 2.930$; $P = 0.005$; Fig. 4A) and maternal condition of the female 185 d following brooding of her original clutch ($B = 5.622$; $t = 3.732$; $P = 0.001$; Fig. 4B). Maternal condition while brooding the previous clutch ($B = 0.711$; $t = 0.153$; $P = 0.879$) and previous clutch size ($B = -0.072$; $t = -0.408$; $P = 0.685$) did not significantly predict the number of new oocytes produced by females.

DISCUSSION

Territoriality can evolve when its fitness benefits outweigh the costs of territorial defense (Kauffman, 1983). For species like *P. cinereus* that defend feeding territories (Jaeger et al., 1982; Mathis, 1991), females may be increasingly reproductively successful when occupying more profitable (e.g., energy-rich) territories and should compete intrasexually, and in some cases intersexually, for these territories. Females of *P. cinereus* are territorial and defend territories that provide access to prey and shelter (Horne, 1988; Mathis, 1991). Larger females (measured using SVL) are often more successful in gaining and holding higher quality territories (Mathis, 1990) and thus should be more reproductively successful than smaller females. We tested the prediction that larger territorial females of *P. cinereus* should have greater reproductive success (i.e., produce more offspring with greater survival) than smaller territorial females. Additionally, with

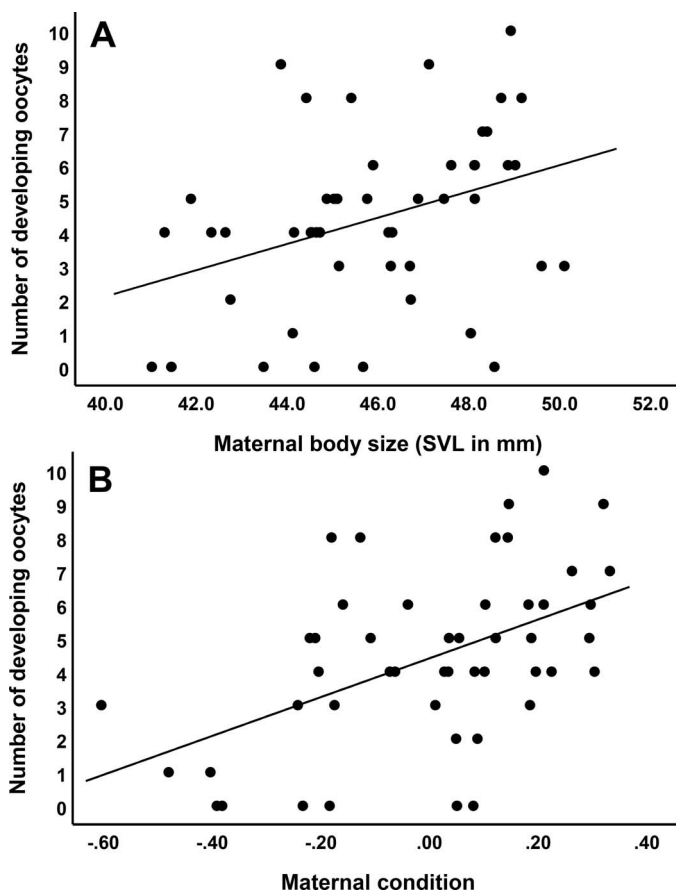


Fig. 4. The regression of number of developing oocytes (visible through the body wall) produced by females on (A) maternal body size (SVL in mm) and (B) maternal condition (residuals of the regression of maternal mass, mm, on maternal body size, SVL in mm).

limited resources available for reproduction, there are trade-offs between current reproductive output and future reproductive potential (Ng and Wilbur, 1995; Yurewicz and Wilbur, 2004). Thus, we examined the relationship between maternal SVL and condition and current and future reproduction; females with larger body sizes, and those in better condition (i.e., heavier for a given body length), may be able to invest more in both current and future reproduction than smaller females.

In our study, females of *P. cinereus* showed increasing investment in reproduction with increasing body size; clutch sizes and mean offspring SVL were both positively related to maternal SVL. Field studies suggest that larger salamanders have higher quality territories (larger cover objects and cover objects with more numerous high quality prey) and are in better condition than smaller salamanders (Mathis, 1990; Gabor, 1995). Thus, larger females should be able to invest more in reproduction than smaller females.

Maternal SVL was a good predictor of clutch size; larger females produced larger clutches. We measured clutch sizes as the number of eggs present at the time of collection, which may have underestimated the original clutch sizes. However, this underestimation may be minimal, given that the mean original clutch size was not smaller than the mean number of developing oocytes in females used in this study, and clutch sizes were similar to those found in other studies (e.g., Yurewicz and Wilbur, 2004; Evans et al., 2020). The observed

relationship between maternal SVL and clutch size may be due to (1) greater number of eggs produced by larger females (e.g., Nagel, 1977; Lotter, 1978), (2) better protection of eggs (from desiccation, fungal or bacterial infections, and predation) by larger females (Crespi and Lessig, 2004), and/or (3) some other attribute not measured in our study. We did find a positive relationship between maternal SVL and number of developing oocytes for females, suggesting the importance of maternal body size in influencing clutch size that is independent of post-oviposition egg loss. Regardless of the mechanism involved (i.e., greater production and/or protection), our study demonstrates that at MLBS in Virginia, larger territorial females have larger clutches. This relationship is frequently found across diverse taxa (Lim et al., 2014; but see Milanovich et al., 2006 for a discrepancy in *P. albagula* and Evans et al., 2020 for a discrepancy in *P. cinereus*).

In our study, larger females not only produced larger clutches but also produced larger offspring (measured as SVL at 40 d post-hatching) demonstrating that there is no trade-off between clutch size and offspring size in territory-holding females. Larger SVL of offspring enhanced their survival (to 185 d post-hatching). In a comparison of siblings (to control for maternal effects), offspring that survived to 185 d post-hatching were significantly larger in SVL (measured at 40 d post-hatching) than those that did not survive to 185 d. Juveniles in our study were not exposed to the risks of desiccation, predation, or starvation that are possible in the natural habitat, but survival rates were still low (24% at 185 d after hatching). Survival rates were not impacted by hatching mortality because eggs were kept with females and most hatched successfully. We pose several hypotheses to explain the low survivorship of juveniles. H1: Temperature in the laboratory (18–19°C) did not mimic the natural environment, especially during the winter, when temperatures fall to near freezing and salamanders overwinter underground (Vernberg, 1953; Taub, 1961). Although temperature in the laboratory was not excessively high (Taub [1961] found that *P. cinereus* in natural enclosures did not avoid temperatures in this range), Bobka et al. (1981) found that this range of temperatures reduced adult assimilation efficiency of prey (*Drosophila*) by 20%. Reduced assimilation efficiency may have impacted juvenile survival, although individuals do not seem to forage during winter months in nature and emerge emaciated in the spring (Fraser, 1976). Our juveniles did not appear to be emaciated as a result of their diet. H2: Juveniles in our study were not fed their natural prey (e.g., collembolans) but were fed *Drosophila* and *Tubifex*. Although Jaeger et al. (2016) reported that a sole diet of *Drosophila* resulted in very low mortality (<1%) among adults kept for over 18 months in the laboratory, juveniles may require different or a greater variety of nutrients to support growth. H3: Juveniles were exposed to novel pathogens existing in the laboratory (Buchanan and Jaeger, 1995). However, we did not see high female mortality in our study. H4: Survival in the laboratory was similar to, or higher than, that for juveniles in the forest at MLBS. No published estimates are available for the overwinter survival rates of neonates in the natural environment to which we can compare our result. However, mortality may be high over the winter season in nature for adults (Vernberg, 1953; Taub, 1961), and Gillette (2003) found that no uniquely marked juveniles at MLBS were found again after their first winter, suggesting low survivorship. H5: Differential fitness among females impacts juvenile survivorship. For

Table 1. The predicted lifetime number of juveniles produced by females that live in low, average, or high quality food territories. The last row in the table provides a scenario for a female that is small in SVL for her first clutch, medium in size for the second, and large for her third clutch.

Territorial quality	Maternal SVL	Original brooding condition	Condition when developing oocytes	Original clutch size	Number (percent) of juveniles surviving	New oocyte number	Lifetime number of eggs	Lifetime number of juveniles
Low	Small	Poor	Poor	6.0	1.3 (22%)	1.8	13.8	2.5
		Median	Poor		0.6 (10%)	1.8	13.8	1.2
		Good	Median		0.3 (5%)	3.5	15.5	0.7
Average	Medium	Poor	Poor	6.7	2.1 (31%)	3.0	16.4	4.6
		Median	Median		1.5 (22%)	4.7	18.1	3.8
		Good	Good		1.1 (16%)	6.1	19.5	3.2
High	Large	Poor	Median	7.8	3.5 (45%)	6.0	21.6	9.3
		Median	Good		2.9 (37%)	7.4	23.0	8.5
		Good	Good		2.5 (32%)	7.4	23.0	7.3
Low to high	Small to large	Median	Median	6.9	1.7 (25%)	4.7	18.6	4.3

example, Figure 2A indicates that while 29 females produced 0–1 surviving juveniles, four females produced 5–6 survivors.

The number of offspring that survived per clutch in the laboratory was positively related to maternal SVL. Condition of the female was also a good predictor of offspring survivorship; however, maternal condition was inversely related to offspring survivorship, such that a greater number of offspring survived when females were in poorer condition during brooding. This suggests a greater reproductive investment by females that are large or in good condition either while developing eggs or while brooding them. Females of *P. cinereus* brood eggs for long periods of time (approximately 2 months; Highton and Savage, 1961; Crespi and Lessig, 2004) with little food intake, and brooding can negatively affect maternal growth (Ng and Wilbur, 1995). Thus, a large investment into a single reproductive bout increases survival of offspring but can negatively affect the future reproductive investment of females. In our study, the number of new oocytes that were visible through the body wall of females after 185 d since hatching of their last clutch was positively related to maternal condition. Evans et al. (2020) also found a positive relationship between tail length (their measure of condition) and number of developing oocytes and clutch size. We conducted a *post hoc* analysis using tail length as a main factor, and found no significant relationship with tail length and either developing oocyte number or clutch size. Fraser (1980) predicted that the number of eggs that will be laid by females of *P. cinereus* during the next reproductive season is determined during the first year following the current reproductive season, and is dependent upon female condition (early in the second year of ova maturation). Our results suggest that the reproductive investment of females during one season can adversely affect the number of offspring that she is able to reproduce in the next season, although females with larger SVLs had an advantage over smaller females in clutch size and oocyte production, a result comparable with Fraser (1980). Increases in body size (SVL) and condition contributed to increased number of oocytes indicating that body size (perhaps due to physical constraints, such as length of body for eggs, or some other advantage to larger size; Congdon and Gibbons, 1987) as well as condition (more resources that can be invested in oocyte production) contribute to egg production. Overall, it

is beneficial for females of *P. cinereus* to be larger in SVL and have a higher quality feeding territory.

A heuristic model to predict the impact of maternal body size (SVL) and condition (relative mass) on lifetime reproductive output can provide insight into the impact of differential reproductive success and generate predictions that may be tested in the field (Table 1). In our model, we categorized females as small, medium, or large (Table 1) based on the median SVL of females in the following size ranges: (1) from the smallest to the first quartile (small females); (2) between the first and third quartiles (medium-sized females); and (3) from the third quartile to the largest (large females). For condition estimates we used a similar process to categorize females as in (1) poor condition, (2) median condition, or (3) good condition (Table 1). Based on past studies, we assume that larger females occupy larger territories and have access to more food resources (Mathis, 1990; Gabor, 1995). Thus, residents of large territories should be large in size and be in better condition than smaller females when developing eggs for the next breeding period. Larger females with high quality territories should be able to return to relatively good condition more quickly when producing new oocytes and have more developing oocytes, whereas smaller females with lower quality territories will produce fewer eggs and potentially be in worse condition following a brooding event and when producing new oocytes and have fewer developing oocytes. To determine original clutch sizes for the model (Table 1), we used original maternal SVL, the only significant predictor of clutch size in our data, in a regression of original clutch size on original maternal SVL ($n = 52$; $R^2_{adj} = 0.120$; $F_{1,51} = 7.975$; $P = 0.007$; using the equation of the regression line for clutch size = $0.251(\text{maternal SVL}) - 4.139$). For our model (Table 1) we used small (40.54 mm), medium (43.22 mm), and large (47.65 mm) maternal sizes based on quartiles (described above). For future clutch size, we used the number of developing oocytes in our females (new oocyte number, Table 1). We used both maternal SVL and condition at 185 d as predictors of new oocyte number because these were the significant predictors in our original analysis. The regression of new oocyte number on maternal SVL and condition at 185 d ($n = 48$; $R^2_{adj} = 0.355$; $F_{2,45} = 13.933$; $P < 0.001$) produced a regression line of number new oocytes = $-13.528 + 0.391$

(maternal SVL) + 5.793 (condition 185 d after brooding). For our predictions in Table 1, we used small (42.70 mm), medium (45.83 mm), or large (49.13 mm) maternal SVL and poor (−0.237), median (0.050), or good (0.302) condition (residuals) based on quartiles (described above). The original maternal SVL and condition of the females significantly predicted the survival of juveniles to 185 d in our original analysis, so we used the regression line from the multiple regression of number of juveniles surviving to 185 d regressed on original maternal SVL and condition to predict number (and percent) of surviving offspring ($n = 52$; $R^2_{\text{adj}} = 0.296$; $F_{2,49} = 11.741$; $P < 0.001$), with a regression line of number juvenile surviving = $-12.323 + 0.321$ (maternal SVL) − 6.225 (condition after brooding first clutch). For our predictions in Table 1, we used small (40.54 mm), medium (43.22 mm), and large (47.65 mm) maternal SVL and poor (−0.092), median (0.008), and good (0.070) condition (residuals) based on quartiles (described above). In our model, we also assumed that females in high quality territories would be in either median or good condition when developing oocytes, whereas females in average territories would be in either good or median condition, and those in low quality territories would be in either median or poor condition when developing oocytes. Females at MLBS probably do not produce offspring until they are 3 yr of age (Petranka, 2010; Jaeger et al., 2016) and lay eggs every other year (Sayler, 1966; Ng and Wilbur, 1995). To determine lifetime reproduction, we conservatively assumed only three clutches (ages 3, 5, and 7 yr) per lifetime of a female based on a maximum age estimate of 8 yr using skeletochronology by Leclair et al. (2006) for a Canadian population of *P. cinereus*. However, this estimation for longevity may be very conservative; Staub (2016) cautioned that skeletochronology may underestimate maximum age because growth rates slow considerably in older animals making discrimination of ages of older animals more difficult. Leclair et al. (2006) also stated that the age estimates were difficult in salamanders exceeding 6 yr, and they discarded 3.5% of their salamanders (12 of 342 individuals) because they could not reliably determine age. Staub (2016) suggested that these may represent older individuals in the population because at older ages, growth is slower and rings are not distinct. Although studies of longevity in *P. cinereus* using other techniques such as mark-recapture (capture-recapture) or observation in captivity have not been published, similar western species of *Plethodon* observed in captivity lived for 11–13 yr (*P. vehiculum* and *P. elongatus*) and an eastern species, *P. hubrichti*, lived for 36 yr in captivity (Staub, 2016). Thus, we assumed that females of *P. cinereus* are able to produce offspring through 8 yr of age, a seemingly conservative estimate, and used the average of the original clutch and the number of new oocytes as the clutch size for a single breeding period and multiplied this number by 3 to get lifetime production of offspring. In our estimate for lifetime number of juveniles, we used the survivorship percent from our laboratory data based on lifetime production of eggs. Based on our model, larger females that inhabit the best territories should produce 7.3–9.3 offspring over their lifetime depending on condition, whereas females inhabiting average territories should produce only 3.2–4.6 offspring depending on condition (Table 1). Those females in poor quality territories do far worse, producing only 0.7–2.5 offspring during a lifetime; thus, females that inhabit smaller, poorer quality territories may

not produce a sufficient number of offspring for replacement (Table 1) unless their initial investment in reproduction is substantial, resulting in poor condition following brooding. Other consequences of poor condition after brooding, however, are not considered in our model, such as survival over winter, where having fat stores is important (e.g., Fitzpatrick, 1976), making future long-term field studies important in determining the impact of brooding intensity on female fitness. Finally in our model, it is likely that over a female's lifetime, as she ages and grows, she is able to move from a small, to medium, to large territory. Under this scenario (Table 1) by averaging the values, a female obtaining successively better territories would produce around 4.3 offspring during a lifespan of 8 years. For our model estimates, we assumed that we sampled females from low, medium, and high quality territories by turning a variety of cover objects. However, it is possible that females in the highest quality territories (e.g., large logs that could not be moved) or those in the lowest quality territories or without territories (sub-optimal cover objects or underground) may not have been sampled. Also, our lifespan estimates may have been underestimated. Thus, the disparity in reproductive success over the lifespan of a female may be more severe than our model predicts, such that lifetime production of juveniles may be closer to 0 for females in the lowest quality territories and well over 9 for those in highest quality territories.

In summary, females of *P. cinereus* with larger SVLs produce more and larger offspring, showing no evidence of a trade-off between clutch size and offspring SVL. These larger offspring have a higher probability of survival at least under laboratory conditions, indicating that females producing larger offspring will have relatively higher fitness gains than females producing smaller offspring. Thus (1) territorial females with larger SVLs produce more and larger offspring that survive longer than smaller territorial females and (2) larger territorial females, and those in better condition following a reproductive bout, produce more oocytes in preparation for the next reproductive episode. However, even for smaller offspring, higher investment in a current reproductive bout may increase lifetime reproductive success in production of total number of juveniles. In territorial animals like *P. cinereus*, large body size affects the ability to acquire and hold a territory and translates directly into increased reproductive success that reinforces the further evolution of territorial behavior.

DATA ACCESSIBILITY

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