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Reproductive Allometry in Three Species of Dusky Salamanders

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***Desmognathus* comprises 21 currently recognized species of salamanders in eastern North America. Assemblages of 3–6 species occur in the Appalachian Mountains, wherein the larger species are more aquatic and the smaller more terrestrial. Adaptive divergence along the habitat gradient from stream to forest involves variation in such life-history traits as age and size at metamorphosis and maturation, survival, propagule size, and fecundity. In this study I examined the tradeoff between egg size and clutch size in North Carolina populations of *D. santeetlah*, *D. ocoee*, and *D. aeneus*, with special emphasis on the latter two species. Traits evaluated included standard length, body mass, trunk volume, egg size, clutch size, and clutch volume. For *D. aeneus* and *D. ocoee,* regressions of log-transformed values of body mass and trunk volume on standard length, and trunk volume on body mass, indicated strong similarity between the species in those body proportions that ostensibly constrain female reproductive effort. In all three species bivariate linear regressions of log-transformed values of clutch dimensions on body size suggested little correlation between egg size and body size within species, although larger species had larger eggs, larger clutches, and greater clutch volumes. An apparent interspecific tradeoff between egg size and clutch size in *D. aeneus* and *D. ocoee* suggested a common pattern in the relationship between body size and reproductive effort in these species. The results provided further evidence of a high level of life-history invariance in the genus *Desmognathus*, embodied in tradeoffs, which may stem from morphological conservatism related to specializations of the musculo-skeletal system.**

REPRODUCTIVE allometry has been defined as ‘‘the relationship between a mother's morphometrics and the morphometrics of her respective reproductive output including nest, clutch and/or hatchlings'' (Murray et al., 2013). Landmark studies of reproductive allometry in salamanders were conducted by Salthe (1969), Kuramoto (1978), and Kaplan and Salthe (1979). All three detected apparent tradeoffs between clutch size and propagule size in a wide range of species. Salthe (1969) singled out *Desmognathus* for special consideration, given that the data showed that the clutch volume to body volume ratio remained constant over the range of body sizes, in contrast to salamanders generally, where it tended to decrease with increasing body size. A re-evaluation of the Salthe (1969) and Kaplan and Salthe (1979) analyses, carried out by Wells (2007: chapter 10), generally supported the earlier authors' findings.

The relationship among body size, clutch size, and propagule size has often been treated under optimality models, such as that formulated by Smith and Fretwell (1974). Those authors argued that offspring fitness is a function of propagule size, whereas maternal fitness is a joint function of propagule size and number. Van Noordwijk and de Jong (1986) have shown graphically that within species positive or negative correlations, or lack thereof, between such life-history traits as number and size of propagules may depend on the magnitude of variation in both resource acquisition and allocation. Later, they incorporated a genetic analysis into their acquisition–allocation model (de Jong and van Noordwijk, 1992). Bernardo (1996) invoked the role of maternal effects to question the assumptions of optimality models of propagule size. Subsequent studies have examined the complexities of the tradeoff between clutch size and offspring size in numerous taxa (e.g., Christians, 2000; Hendry et al., 2001; Warne and Charnov, 2008; Brown and Shine, 2009). Nussbaum (2003) summarized a wealth of data on egg size in salamanders; he briefly commented on the tradeoff between clutch size and propagule size but provided no analysis. Davenport and Summers (2010) compared the relationship between egg size and clutch size in pond- and stream-breeding salamanders; their results suggested but did not confirm a tradeoff.

If both egg number and egg size are functions of body size, the relationship between fecundity and standard length (SL) can be expressed as $\ln \mathit{egg\ number} = \ln a + b \cdot \ln SL$, and that of egg size and standard length is $\ln \mathit{egg\ volume} = \ln c + d \cdot \ln SL$. Solving each equation for standard length, and equating them yields the relationship $\ln \mathit{egg\ number} = (\ln a - (b \cdot \ln c/d)) + (b/d) \ln \mathit{egg\ volume}$, wherein $b \neq 0$ and $d \neq 0$ (Roff, 2002). This equation embodies any tradeoff between egg number and egg size. Assuming a geometric constraint on fecundity, if body proportions increase isometrically, and in the absence of a tradeoff with egg size, the expected value of b is 3.0, given that egg number reflects a volume. However, if egg size also increases with SL ($d > 0$), a value of b less than 3.0 would represent a tradeoff between these traits.

The present study is designed to address the tradeoff between clutch size and propagule size among several species of *Desmognathus*. For the intraspecific evaluation, I examined data on body size, egg size, and clutch size in *D. aeneus*, *D. ocoee*, and *D. santeetlah* in populations from several adjacent mountain ranges in southwestern North Carolina. The main objective was to examine the relationship between female body size and egg size within species. To this end, I evaluated two unpublished data sets generated in studies by Beachy (1988, 1993) on *D. aeneus*, *D. ocoee*, and *D. santeetlah,* and Bruce (1990) on *D. ocoee*.

At the interspecific level, I conducted a morphometric comparison of *D. aeneus* and *D. ocoee* in populations from the southern Nantahala Mountains of North Carolina, in the context of differences between these species in body size and reproductive parameters. Although *D. ocoee* and *D. aeneus* are not considered close relatives within *Desmognathus* (Beamer and Lamb, 2008), they overlap broadly in microhabitat use in the southern Nantahalas (pers. obs.), and ostensibly have similar feeding habits (Donavan and Folkerts, 1972; Huheey and Brandon, 1973; Jones, 1981). *Desmognathus aeneus* reproduces earlier in the year than *D. ocoee* (Bruce, 2009); however, I have observed late clutches

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of the former and nearby early clutches of the latter on the same dates in late July. Although females of both species often nest in clumps of moss, the nests of *D. ocoee* are typically located adjacent to a small stream, whereas those of *D. aeneus* may be further from water (Harrison, 1967; Jones, 1981; Bruce, 1990). In the southernmost ranges of the southern Blue Ridge, including the Nantahalas, both species appear to have an annual reproductive cycle, such that most females usually deposit a single clutch of eggs in successive years (Harrison, 1967; Tilley, 1980; Bruce, 1996, 2009, 2013; Hining and Bruce, 2005).

In *D. aeneus* and *D. ocoee* the number of eggs in field clutches tend to be slightly less than counts of yolked follicles in the ovaries of gravid females (e.g., Harrison, 1967:table 2; Bruce, 1996:table 1). This difference may in part be a consequence of follicular atresia (Aranzábal, 2003), but especially of egg mortality in the nest, including oophagy of infected eggs by the attending female parent (Forester, 1979). Tilley's (1972) account of egg mortality in *D. carolinensis* and *D. ocoee* is instructive in this regard, as is Hom's (1987) study of *D. fuscus,* which has similar nesting habits. Thus, counts of yolked ovarian follicles may be a more reliable indicator of female reproductive investment or ''clutch size'' than number of eggs in field clutches, especially late clutches.

In the present study I have examined size-mediated differences between *D. aeneus* and *D. ocoee* in clutch size, egg size, and clutch volume, as well as morphometric variation in characters most directly related to these traits. Although female reproductive effort is often evaluated by the gonadosomatic index, defined as clutch weight relative to body weight (Roff, 2002), in the present study, in the absence of reliable data on clutch weight, I define gonadosomatic index as clutch volume relative to trunk volume. Given that the trunk encloses the body cavity, which contains the gonads, a geometric constraint on clutch volume may be directly imposed by the volume of the trunk. Thus, a principal objective herein is to evaluate the contribution of tradeoffs among body size, clutch size, egg size, and clutch volume to differences in annual reproductive effort between *D. aeneus* and *D. ocoee*, as measured by the gonadosomatic index. These results, together with the earlier findings on female size and egg size in *D. aeneus*, *D. ocoee*, and *D. santeetlah*, are examined in the broader context of symmetry in life-history variation in the genus *Desmognathus*.

MATERIALS AND METHODS

To evaluate evidence for intraspecific relationships between female body size and propagule size, I drew on unpublished data from a study by Beachy (1988, 1993) on variance in egg size in several species of salamanders, including three desmognathines, *D. aeneus*, *D. ocoee*, and *D. santeetlah.* In addition, I have further evaluated data on *D. ocoee* from an earlier study of mine (Bruce, 1990). Beachy's (1993) samples of *D. ocoee* and *D. santeetlah* were taken in the Balsam and Great Smoky Mountains of Swain, Haywood, and Jackson counties, North Carolina, whereas that of *D. aeneus* was collected in the Nantahala Mountains, Macon County, North Carolina. My sample of *D. ocoee* was taken in the Wolf Creek watershed of the Cowee Mountains, Jackson County, North Carolina. All three mountain ranges lie within the southwestern section of the southern Blue Ridge Province. I attempted no interspecific analysis of these data because of the disparate sources of the samples and the small sample sizes.

In each study clutches of eggs and attending females were collected in the field, as described by Beachy (1993) and Bruce (1990), and removed to the laboratory. Standard lengths (SL) of living females were measured from the tip of the snout to the caudal end of the cloacal slit to the nearest 0.1 mm. The diameters of living eggs were also measured to the nearest 0.1 mm. ''Egg'' is defined as an embryo of Harrison developmental stage 30 or earlier. Beachy (1988, 1993) reported that in these species there is no perceptible increase in size over that of the uncleaved egg until after stage 30. Egg clutches having embryos of later stages were not included in the analyses. For consistency, values of all variables were log-transformed. For all four of these datasets I regressed (1) ln clutch size on ln SL and (2) ln mean egg diameter on ln SL, and calculated (3) Pearson correlation coefficients of ln mean egg diameter versus ln clutch size. The females and their clutches were subsequently released at the sampling locales.

For the evaluation of differences between *D. aeneus* and *D. ocoee*, I sampled populations of these species in the southern Nantahala Mountains in Macon County, North Carolina, in the adjacent watersheds of the upper Nantahala River and Coweeta Creek. These species are members of larger assemblages of *Desmognathus*, comprising six species in the former and five species in the latter watershed (Bruce, 2011, 2013). Unfortunately, the other species could not be included in the current study because of insufficient data on the parameters of interest. The samples of both species were taken on various dates in all seasons of the year between 2006 and 2013. The samples from which trunk volumes were estimated were taken in 2011–2013, and egg measurements were made on samples of clutches from 2013 only. For each species I pooled the samples of the Coweeta Creek and Nantahala River watersheds, inasmuch as I could not discern differences in either species in any of the parameters in question between samples from the two watersheds.

I conducted a morphometric analysis of three variables that presumably correlate with reproductive effort, namely standard length (SL), body mass (BM), and trunk volume (TVOL). The first two variables were measured for 106 *D. ocoee* and 91 *D. aeneus*. Trunk volumes were estimated for a subset of the above samples, 59 *D. ocoee* and 57 *D. aeneus*. Standard lengths to the nearest 0.1 mm and masses to the nearest mg were recorded for living salamanders anesthetized in 1% MS-222. Individuals were lightly blotted with paper toweling before weighing. For trunk volume, I measured the length of the trunk (trl) from the middle of the gular fold to the posterior end of the cloacal slit, and trunk widths at the axillae, mid-trunk, and groin, all to the nearest 0.1 mm on living, anesthetized individuals. In assuming that the trunk is circular in cross section, with radius = width/2, I fitted a quadratic to these measurements, $y = ax^2 + bx + c$, where y = radius and x = length. I then estimated trunk volume, by the method of circular discs, $TVOL = \pi \int_0^{trl} (ax^2 + bx + c)^2 dx$. Given that the trunk tapers more strongly at the caudal than at the cranial end, the coordinates of the vertex of the parabola described by the quadratic lie cranial to the mid-trunk measurement. This introduced very slight errors only into the volume estimates. I did not attempt to estimate the volume of the body cavity itself. I log-transformed the values of SL, BM, and TVOL, and

Table 1. Variation in female body size, clutch size, and egg size in nests of three species of *Desmognathus*. Included are clutches in which embryos are at Harrison stage 30 or earlier in development.

	Attending females							
		SL (mm)		Clutch size		Egg diameter[1]		
Species/Population	*n*	mean	SD	mean	SD	mean	SD	Source[2]
D. aeneus								
Nantahala R.	10	23.8	1.41	11.6	2.50	2.59	0.14	A
D. ocoee								
Wolf Cr.	8	36.0	2.51	12.6	3.85	3.06	0.29	B
Wolf Cr.	9	37.3	2.06	14.4	1.59	–	–	C
Balsam Mts.	8	40.9	1.41	15.8	3.37	3.55	0.30	A
D. santeetlah								
Great Smoky Mts.	26	42.6	2.95	19.7	4.06	3.58	0.36	A

[1] The mean is that of the mean egg diameters of the *n* clutches.
[2] (A) Beachy, 1993 & unpublished data. (B) Bruce, 1990 & unpublished data. (C) Bruce, 1996 & unpublished data.

evaluated separately the between-species regressions of ln BM on ln SL, ln TVOL on ln SL, and ln TVOL on ln BM with ANCOVA.

Following measurement, many of the salamanders were rinsed in spring water, revived, and subsequently released at multiple sites within the study area. However, specimens from the earliest samples and most gravid females (16 *D. aeneus*, 58 *D. ocoee*) were euthanized in MS-222, measured, and then fixed in 10% formalin. They were later transferred to 70% ethyl alcohol, and the reproductive organs were examined by dissection. Counts of yolked ovarian follicles (FOL) were made in females having follicles $\geq$1.5 mm in diameter.

Egg clutches and attending females of *D. aeneus* and *D. ocoee* were collected in late May and late July, 2013, respectively, at several sites in the Nantahala River/Coweeta Creek study area. For each female, standard length, trunk dimensions, and mass were recorded, as outlined above, on living, anesthetized individuals. The egg clutch was weighed to the nearest milligram, and the diameter of each egg was measured to the nearest 0.1 mm. I assumed that the eggs were spherical and estimated volume as $(4/3)\pi r^3$. Although I adopted Beachy's (1988) criterion of Harrison stage 30 as the stage at which embryos begin to increase in size, all the clutches that I included in my evaluation of egg size had embryos at Harrison stage 19 or earlier (Harrison, 1969), equivalent to Marks and Collazo's (1998) stage 15 in *D. aeneus*. Following measurements, each female was reunited with her clutch, returned to the collection site, and placed under moss near a seepage (*D. aeneus)* or at the margin of a small stream (*D. ocoee)*.

For field clutches, clutch volume (CVOL) in mm^3 was calculated as the sum of the estimated egg volumes. For gravid females, clutch volumes were estimated as the product of follicle counts and the mean volume of deposited eggs/species.

Statistical and mathematical procedures were carried out with SYSTAT 10.2 and SYSTAT 12 (Systat Software, Inc.), and Mathematica7 (Wolfram Research). Statistical significance was evaluated at $\alpha = 0.05$.

Preserved specimens were deposited in the herpetological collection of the North Carolina Museum of Natural Sciences. For samples accessioned by lot, sample sizes are given in parentheses. *Desmognathus aeneus*: NCSM 78251 (2), 78257 (2), 80463–80470, 80474–80475, 80484 (10), 80492–80493, 81319 (13), 81320 (6). *Desmognathus ocoee*: NCSM 78248 (32), 78253 (69), 78256 (29), 78259 (27), 78260 (25), 80461–80462, 80471–80472, 80478 (13), 80479–80483, 80491(18), 80494–80500.

RESULTS

Data on attending females and eggs in field clutches of *D. aeneus*, *D. ocoee*, and *D. santeetlah* from the Beachy (1988, 1993) and Bruce (1990) datasets are provided in Table 1. In all samples the regressions/correlations of (1) ln egg number on ln SL, (2) ln mean egg diameter on ln SL, and (3) ln mean egg diameter versus ln egg number were nonsignificant, except for that of ln mean egg diameter on ln SL in Wolf Creek *D. ocoee* ($\ln \text{mean egg diameter} = -2.52 + 1.02 \cdot \ln SL$, $F_{1,6} = 10.13$, $P = 0.019$, $R^2 = 0.628$). A complete set of plot scatters is shown for the largest sample, that of *D. santeetlah* (Fig. 1); the plots of ln mean egg diameter on ln SL only are shown for the other samples, as this was the principal relationship of interest (Fig. 2).

For the 2006–2013 samples of Nantahala Mountain *D. aeneus* and *D. ocoee*, the regressions of both ln BM and ln TVOL on ln SL generated regression coefficients close to the predicted value of 3.0, and that of the regression of ln TVOL on ln BM was close to the predicted value of 1.0 (Table 2). In the ANCOVA of ln BM on ln SL, there was no significant interaction between species and ln SL ($F = 0.263$, df = 1, 193, $P = 0.609$), nor a significant difference between species in ln BM ($F = 0.296$, df = 1, 194, $P = 0.587$) when adjusted for the difference in the covariate, ln SL ($F = 9829$, df = 1, 194, $P < 0.0005$). Similarly, in the ANCOVA of ln TVOL on ln SL the interaction of species and ln SL was nonsignificant ($F = 0.170$, df = 1, 112, $P = 0.681$); the difference in ln TVOL between species was marginally nonsignificant ($F = 3.262$, df = 1, 113, $P = 0.074$) when adjusted for the difference in ln SL ($F = 3883$, df = 1, 113, $P < 0.0005$). Thus, common regression lines provided good fits to the pooled data of these two relationships (Fig. 3A, B). The ANCOVA of ln TVOL on ln BM yielded a nonsignificant interaction between species and ln BM ($F = 0.171$, df = 1, 112, $P = 0.680$), but a significant difference between species in ln TVOL ($F = 6.140$, df = 1, 113, $P = 0.015$) when adjusted for

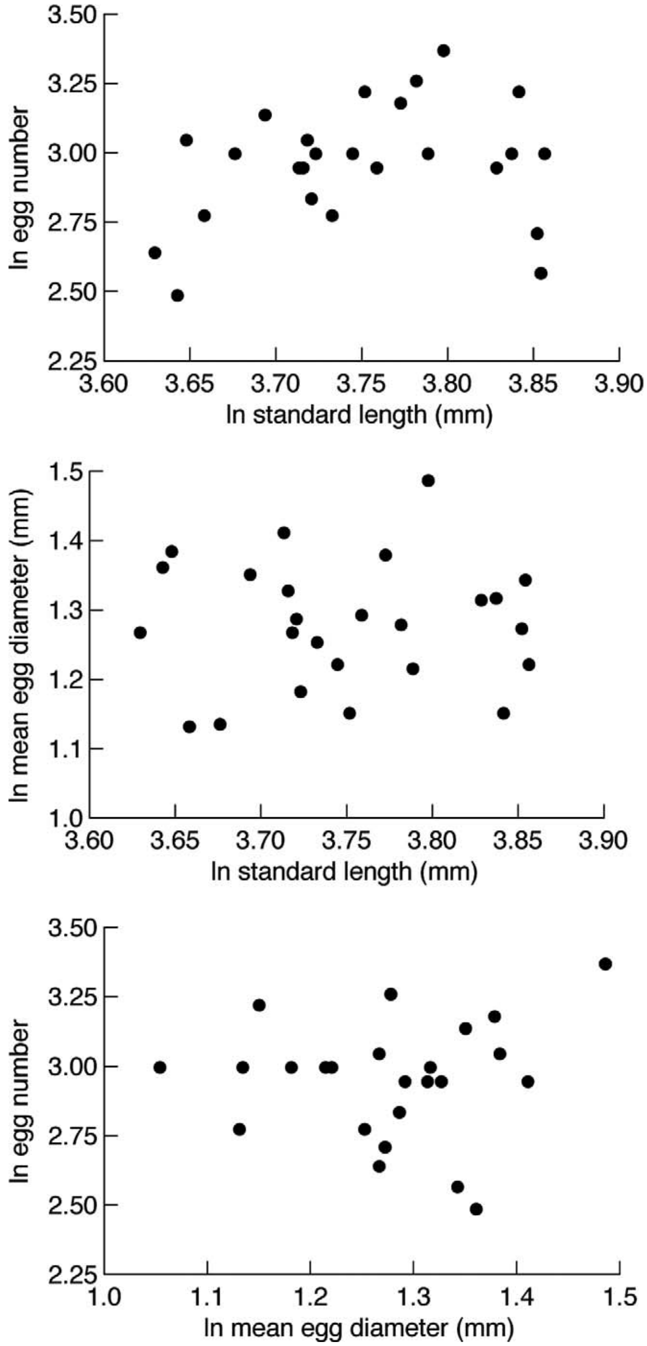

Fig. 1. Plots of egg number, egg size, and body size in 26 nests of *Desmognathus santeetlah.* There were no significant correlations between the variables in any of the plots.

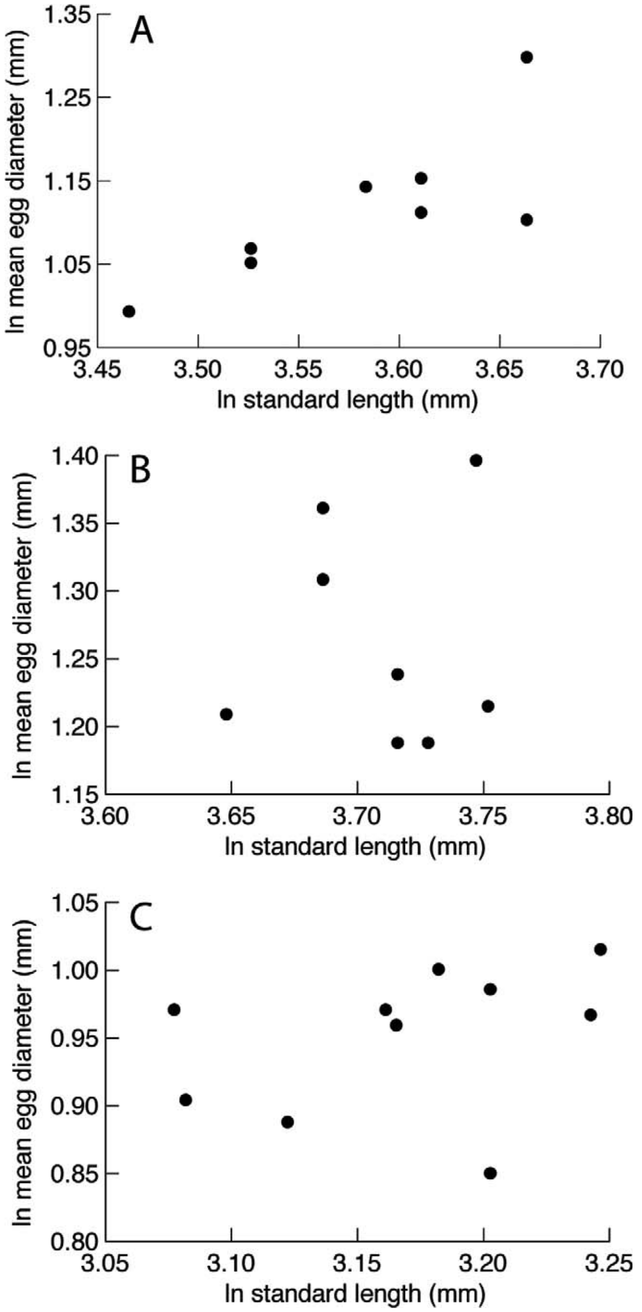

Fig. 2. Plots of egg size versus body size for (A) Wolf Creek *D. ocoee*, (B) Balsam *D. ocoee,* and (C) Nantahala *D. aeneus*. As noted in the text, only the slope of the Wolf Creek *D. ocoee* regression is significant.

the difference in ln BM ($F = 8322$, df = 1, 113, $P < 0.0005$). Nevertheless, given the similarity in the regression equations (Table 2), a common regression line provided a close fit to the pooled data of the two species (Fig. 3C). Thus, whereas large adults of *D. ocoee* may be more than five times more massive than those of *D. aeneus*, the two species have very similar body proportions in the characters that were evaluated.

For the samples of gravid female *D. aeneus* and *D. ocoee*, follicle number increased with standard length in both species, although the values of the regression coefficients were less than the expected value of 3.0 under a geometric argument (Table 3, Fig. 4A). As there was no overlap in body dimensions (SL, BM, TVOL) between gravid females of the two species, the statistical results for the covariates are omitted from the ANCOVAs. In evaluating the regressions of ln FOL on ln SL with ANCOVA, I found no significant interaction between species and ln SL ($F = 0.001$, df = 1, 70, $P = 0.981$), but the difference between species in ln FOL was significant ($F = 13.33$, df = 1, 71, $P < 0.0005$) when adjusted for the body size difference. In other words, for a given body size, females of *D. aeneus* are expected to have higher fecundity than those of *D. ocoee*.

Data on the 2013 field clutches of *D. aeneus* and *D. ocoee* are presented in Table 4. In the total sample of eggs from clutches having early embryos (≤Harrison stage 19), I calculated mean egg diameters: *D. aeneus*, $n = 70$, mean±SD = 2.66±0.176 mm; *D. ocoee,* $n = 79$, mean±SD =

Table 2. Regressions of the morphometric variables: SL (standard length in mm), BM (body mass in mg), TVOL (trunk volume in mm^3). R^2 = coefficient of determination.

Species	*n*	Regression equation	R^2
D. aeneus	91	ln BM = −3.830 + 2.930 ln SL	0.983
D. ocoee	106	ln BM = −3.742 + 2.899 ln SL	0.979
Pooled	197	ln BM = −3.746 + 2.901 ln SL	0.989
D. aeneus	57	ln TVOL = −4.554 + 3.005 ln SL	0.985
D. ocoee	59	ln TVOL = −4.356 + 2.965 ln SL	0.959
Pooled	116	ln TVOL = −4.652 + 3.044 ln SL	0.985
D. aeneus	57	ln TVOL = −0.714 + 1.041 ln BM	0.990
D. ocoee	59	ln TVOL = −0.706 + 1.050 ln BM	0.983
Pooled	116	ln TVOL = −0.816 + 1.064 ln BM	0.992

3.46±0.258 mm. For these samples, assuming spherical eggs, I estimated the volume of each egg as $(4/3)\pi r^3$, and calculated the means: *D. aeneus*, n = 70, mean±SD = 10.03±1.99 mm^3; *D. ocoee*, n = 79, mean±SD = 21.95±5.07 mm^3. Although the number of nests was small, neither species showed a trend for egg size to increase with size of the female parent (similar to the earlier findings given above). For each species, the mean number of eggs/clutch was less than the mean number of yolked follicles/gravid female (*D. aeneus*: 9.1 vs. 12.7; *D. ocoee*: 15.7 vs. 16.9, respectively).

Egg mortality may explain in part the observation that in these relatively small samples of egg clutches the ratios of clutch mass to female mass and clutch volume to trunk volume were highly variable and uncorrelated with female size (Table 4). Thus, I have used counts of mature ovarian follicles in estimating female reproductive investment. On the assumption that egg size is not strongly correlated with female body size within a population, I have multiplied average egg volume/species (*D. aeneus*: 10.03 mm^3, *D. ocoee*: 21.95 mm^3) by the number of yolked follicles in the ovaries of each gravid female, and used these derived values as indices of individual clutch volume. The effect on the regressions was to increase the elevations but leave the slopes unchanged vis-à-vis the regressions of ln FOL on ln SL. Thus, as in the latter regressions, the slopes of the plots of ln CVOL on ln SL were nearly identical (Table 3, Fig. 4B); an ANCOVA showed no significant interaction between species and ln SL ($F < 0.0005$, df = 1, 70, $P = 0.983$), but a significant difference between species in ln CVOL (F = 4.724, df = 1, 71, P = 0.033) when adjusted for the difference in ln SL. To evaluate the gonadosomatic index, and given that trunk dimensions were not measured for females in the earlier samples, I estimated trunk volume for each gravid female from the regressions of ln TVOL on ln SL (Table 2, Fig. 3B), and plotted ln CVOL against ln TVOL. An ANCOVA showed no significant interaction between species and ln TVOL (F = 0.003, df = 1, 70, P = 0.955), and no difference between species in ln CVOL (F = 3.453, df = 1, 71, P = 0.067) when adjusted for the ln TVOL difference. A common regression line fitted to the pooled sample data (Fig. 4C) provided a close fit to the data. Thus, the incorporation of the interspecific difference in egg size into the two evaluations of female reproductive investment nearly eliminated the difference shown by the ln FOL on ln SL regressions (Fig. 4A).

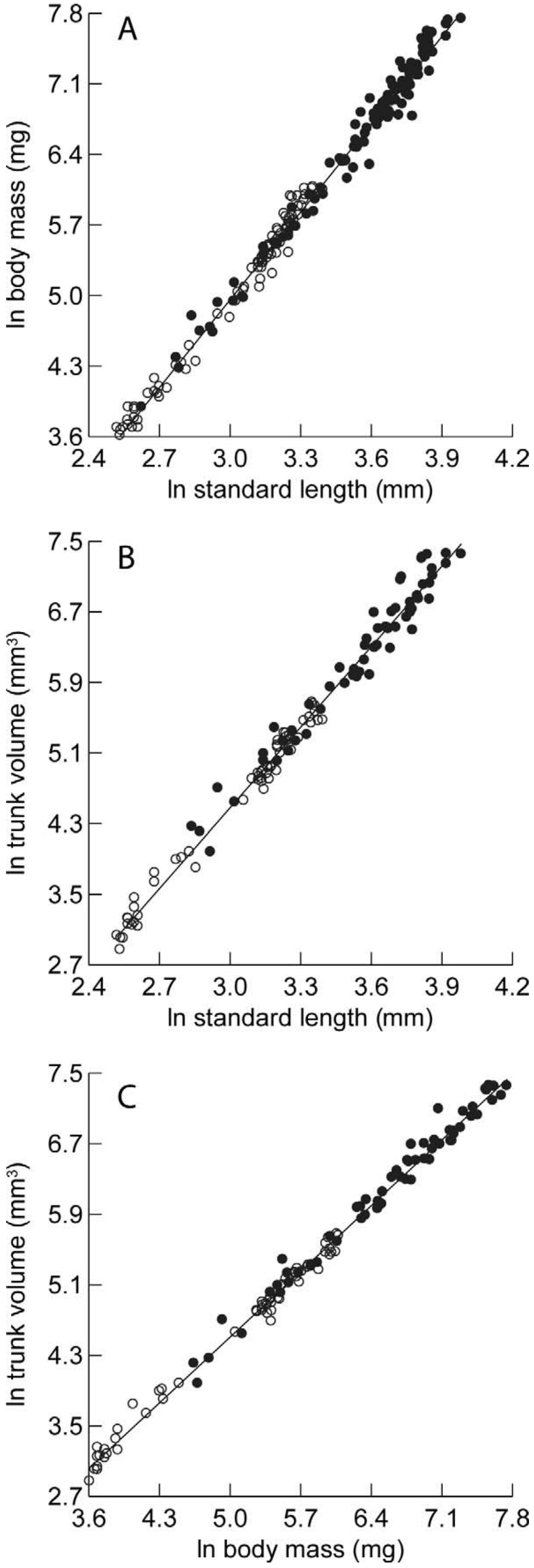

Fig. 3. Regressions of morphometric variables for pooled samples of *D. aeneus* (open circles) and *D. ocoee* (closed circles). A common regression equation is fitted to each dataset based on the results of ANCOVA, as reported in the text.

Table 3. Female reproductive investment in *Desmognathus aeneus* and *D. ocoee.*

Regression				
Sample	*n*	Regression equation	*P*	R^2
ln follicle number on ln standard length				
D. aeneus	16	$\ln \mathrm{FOL} = -3.205 + 1.754 \cdot \ln \mathrm{SL}$	0.012	0.375
D. ocoee	58	$\ln \mathrm{FOL} = -3.763 + 1.775 \cdot \ln \mathrm{SL}$	<0.001	0.336
ln clutch volume on ln standard length				
D. aeneus	16	$\ln \mathrm{CVOL} = -0.899 + 1.754 \cdot \ln \mathrm{SL}$	0.012	0.375
D. ocoee	58	$\ln \mathrm{CVOL} = -0.674 + 1.755 \cdot \ln \mathrm{SL}$	<0.001	0.336
ln clutch volume on ln trunk volume				
pooled	74	$\ln \mathrm{CVOL} = 0.843 + 0.762 \cdot \ln \mathrm{TVOL}$	<0.001	0.818

DISCUSSION

The intraspecific analyses of *D. aeneus*, *D. ocoee*, and *D. santeetlah* based on the Beachy (1988, 1993) and Bruce (1990) datasets suggest neither a tradeoff between egg size and egg number, nor a correlation between egg number and size of the female parent in clutches encountered in the field. The analyses of the relationship between egg diameter and female size were ambiguous, with no correlation in Nantahala *D. aeneus*, Balsam *D. ocoee*, and Great Smoky *D. santeetlah*, but with a significant correlation in the small sample of Cowee Mountain *D. ocoee*. However, females of the larger species (and the larger females of Balsam vs. Wolf Creek *D. ocoee)* tend to produce larger eggs. The comparative analyses of Nantahala *D. aeneus* and *D. ocoee* in the present study and in other studies of these and related species (Harrison, 1967; Tilley, 1972; Jones, 1986; Bruce, 1996, 2013) have all shown a positive correlation between number of yolked ovarian follicles and female body size.

The observation that counts of eggs in nests tend to be at least slightly lower than ovarian complements (Harrison, 1967; Tilley, 1972; Bruce, 1996) may be mainly a consequence of mortality in the nest and female oophagy (Tilley, 1972; Forester, 1979; Marks and Collazo, 1998). Thus, nest mortality may have contributed to the discrepancies in the current study between analyses of clutch size based on ovarian counts versus nest counts. A more refined analysis of larger samples is required to resolve this question.

The reliability of the product of follicle counts and egg size as an index of female reproductive investment requires further consideration. Atresia of follicles may occur at various stages of vitellogenesis, and the end products of atresia are apparently recycled by the female (Aranzábel, 2003). Moreover, following oviposition, oophagy of infected eggs by the female parent allows her to recoup part of her reproductive investment (Forester, 1979). If the effects of these factors on overall energy allocation are similar for *D. aeneus* and *D. ocoee* (and other desmognathines), follicle

Table 4. Body sizes of female parents and clutch dimensions in field clutches of *D. aeneus* and *D. ocoee* from 2013.

	Attending female[1]				Eggs[2]					
					Diameter (mm)				Clutch volume (mm^3)	Clutch mass (mg)
Species	No.	SL (mm)	Body mass (mg)	Trunk volume (mm^3)	*n*	Range	Mean	SD		
D. aeneus	1	29.3	408	236.0	12	2.5–3.0	2.76	0.098	132.6	224
	2	—	—	—	10	2.5–3.0	2.74	0.131	100.3	146
	3	24.6	288	178.7	3	2.8–2.8	2.80	—	—	—
	4	25.9	303	190.1	12	2.3–2.5	2.49	0.090	97.5	161
	5	25.6	290	196.7	8	2.3–3.0	2.70	0.204	83.9	102
	6	25.3	334	205.6	9	2.5–2.8	2.66	0.121	89.0	105
	7	24.7	282	188.1	9	2.3–2.8	2.50	0.138	74.6	81
	8	23.5	215	129.5	10	2.5–3.0	2.74	0.131	108.0	117
	9	23.2	224	154.2	9	2.7–3.2	2.76	0.157	108.2	110
D. ocoee	1	37.8	941	669.7	17	3.4–3.8	3.53	0.181	373.7	471
	2	43.3	1369	903.2	17	3.6–4.6	4.05	0.292	373.7	608
	3	—	—	—	14	3.6–4.3	3.93	0.202	307.8	443
	4	44.6	1463	971.5	16	3.6–4.1	3.81	0.173	351.8	450
	5	42.6	1106	761.6	18	3.1–3.4	3.28	0.116	395.7	326
	6	37.6	719	545.4	15	2.9–3.6	3.25	0.178	329.8	255
	7	39.4	1078	675.3	13	3.1–3.6	3.40	0.145	285.8	254

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[1] Attending females of *D. aeneus* no. 2 and *D. ocoee* no. 3 escaped.

[2] Clutch no. 1 of *D. aeneus* and clutch nos. 2 and 3 of *D. ocoee* had late embryos and were excluded from calculations of mean egg size and mean egg volume.

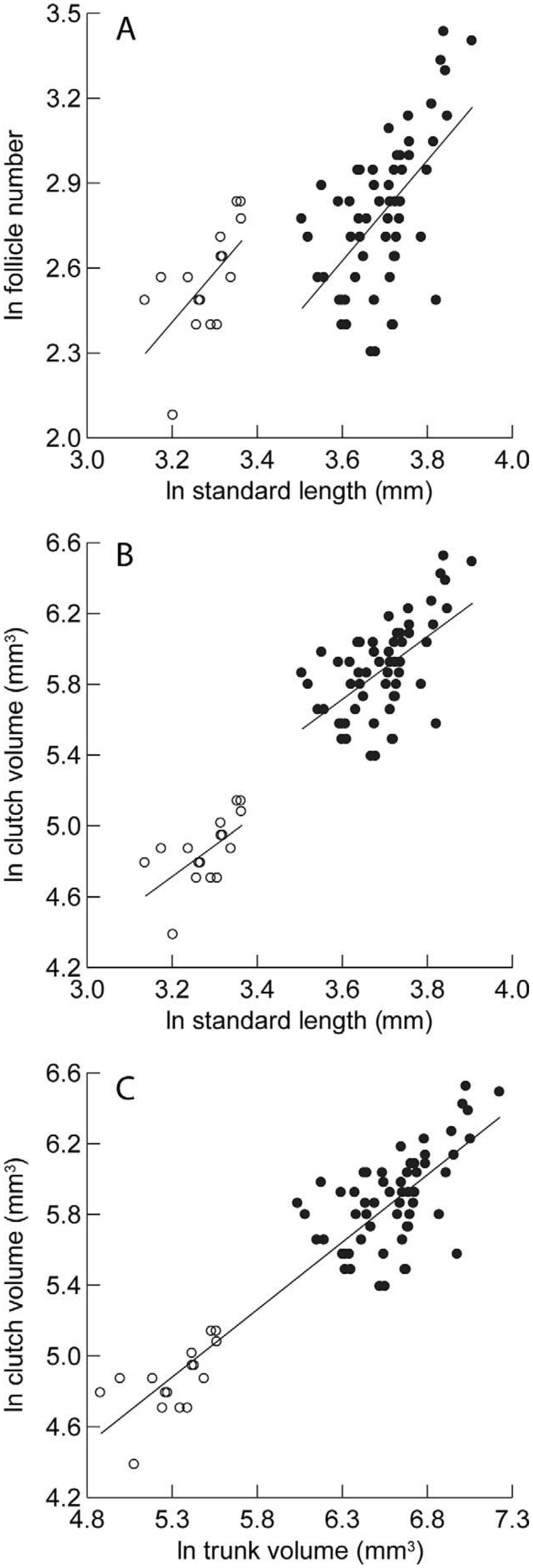

Fig. 4. Regressions of indices of female reproductive investment on body size in 16 *D. aeneus* (open circles) and 58 *D. ocoee* (closed circles).

counts may serve as an appropriate metric for a comparative assessment of female reproductive output in these species.

The finding that in both *D. aeneus* and *D. ocoee* the value of the regression coefficient of ln follicle number on ln standard length was less than 3.0 did not seem related to a tradeoff with egg size in either species. However, the samples of field clutches were small, and those data may have been inadequate to detect a tradeoff. A more extensive analysis would be required to test the tradeoff relationship under the Roff (2002) equation cited earlier, i.e., $\ln \text{egg number} = (\ln a - (b \cdot \ln c/d)) + (b/d)\ln \text{egg volume}$, which is applicable only if $d \neq 0$.

The relationship between female reproductive effort and body size is essentially identical in *D. aeneus* and *D. ocoee*, as expressed in the close fit of the common regression equation of ln CVOL on ln TVOL. Although a significant difference was found in the regression of ln CVOL on ln SL, the difference was small. That the slope of the regression of ln CVOL on ln TVOL ($b = 0.762$) was less than 1.0 indicates that the gonadosomatic index is greater in the smaller species, and declines with size, and ostensibly age, in both *D. aeneus* and *D. ocoee*, but as a function of body size rather than species, in accordance with a common relationship between reproductive output and body size in the two species. This may be characteristic of salamanders generally, as shown by Wells's (2007:fig. 10.22) reanalysis of Kaplan and Salthe's (1979) data, wherein the coefficient of the regression of ln clutch volume on ln body volume was 0.64. The high residual variance around the regression line of the *D. aeneus–D. ocoee* plot indicates that factors other than age and size strongly influence female reproductive effort in both species. In the context of the sampling protocols followed in this study, environmental factors might include climatological and resource variation associated with year of sampling, elevation, and microhabitat.

In Salthe's (1969) earlier comprehensive evaluation of reproductive allometry in salamanders, he reported that the clutch volume to body volume ratio in *Desmognathus* was essentially invariant among species over the body size range of the genus. It is difficult, however, to pinpoint his literature sources for the equation he derived for desmognathines (*clutch volume = 0.16 body volume*). Moreover, neither Kaplan and Salthe (1979) nor Wells (2007) commented on the special situation that Salthe (1969) discerned in *Desmognathus*.

The similarity in reproductive effort in *D. aeneus* and *D. ocoee*, which incorporates an egg size–egg number tradeoff, is part of a more comprehensive pattern of tradeoffs in such features as age and size at metamorphosis and maturation, growth rate, survival, and fecundity, which are correlated with evolutionary transformations in body size and other morphological traits in the genus *Desmognathus*, and are associated with adaptive divergence along the stream-to-forest habitat gradient (Bruce, 2011, 2013). In the latter publication, I suggested that life-history variation in *Desmognathus* conforms to Hubbell's (2001) model of fitness invariance, wherein related species of an assemblage show predictable covariance in life-history traits. Such a pattern may be an outcome of design constraints in morphology, as documented in a functional context in *Desmognathus* by Schwenk and Wake (1993). The body-size mediated tradeoff of egg size and clutch size between *D. aeneus* and *D. ocoee* conforms to such a model, and it is possible that this relationship applies generally within the genus, given the available data on variation in egg size and clutch size provided here and in Bruce (1990), Beachy (1993), Tilley and Bernardo (1993), Nussbaum (2003), Wells (2007), and references therein. It would be of interest to examine such tradeoffs, in the framework of the fitness invariance model, for the other desmognathine species that coexist with *D. aeneus* and *D. ocoee* in the southern Nantahala Mountains, as well as in multispecies communities of *Desmognathus* elsewhere.

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