Population Structure and Life History Attributes of Syntopic Populations of the Salamanders *Desmognathus aeneus* and *Desmognathus wrighti* (Amphibia: Plethodontidae)

**KEVIN J. HINING**1,2 AND RICHARD C. BRUCE1,3,*

**Abstract** - We investigated population structure and life history traits in syntopic populations of *Desmognathus aeneus* and *Desmognathus wrighti* in the southern Nantahala Mountains, NC. The two species occupy similar microhabitats in the spring months, occurring in clumps of moss and damp leaf litter and under stones and logs in deciduous forests bordering streams and seepages. We examined the reproductive organs in living individuals taken in large samples in early spring. The goal was to determine sex and reproductive status. We confirmed these assessments by dissections of small numbers of preserved individuals collected in the same months of later years. The sample of *D. wrighti* included first-year juveniles (8 mo), second-year juveniles and adult males (20 mo), and third-year and older adults (≥ 32 mo). Similarly, the sample of *D. aeneus* contained first-year (11 mo), second-year (23 mo), and third-year and older (≥ 35 mo) individuals, in the same reproductive categories as identified in *D. wrighti*. The age differences reflect different oviposition times, estimated as 1 May in *D. aeneus* and 1 August in *D. wrighti*. In both species, we estimated that males attain sexual maturity in their second or third year, and that females usually oviposit initially at age 3 years. There is considerable overlap in body size between species. Within species, adult females average larger than males, in part a consequence of earlier maturation of males, but maximum sizes of males and females are similar.

**Introduction**

*Desmognathus aeneus* Brown and Bishop (Seepage Salamander) and *Desmognathus wrighti* King (Pygmy Salamander) are miniaturized salamanders, the smallest of the nineteen species of *Desmognathus*, and the only members of the genus that lack a free-living larval stage and undergo direct development. Although largely allopatric, their ranges overlap in southwestern North Carolina (Harrison 1992, 2000). *Desmognathus wrighti* is restricted to intermediate and high elevations of the southern Blue Ridge; *D. aeneus* to intermediate and lower elevations, from the southwestern corner of the southern Blue Ridge, southwestward into the Piedmont, with disjunct populations in the Fall Line Hills of Alabama.

The life history of *D. aeneus* has been studied by Harrison (1967), Jones (1981), and Valentine (1963). Although hatchlings may emerge from the egg capsules with external gills, they apparently do not feed in the gilled stage,
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and resorb the gills within a few days. Inasmuch as hatchlings are metamorphosed in regard to other traits, the life history mode is considered to be direct development (Marks 2000, Marks and Collazo 1998). Organ (1961a,b) reported that in *D. wrighti* the embryos resorb the gills prior to hatching, and emerge from the egg capsules as fully metamorphosed juveniles. Although Marks and Collazo (1998) noted that hatchlings of *D. wrighti* may sometimes retain gills, they are otherwise developmentally advanced (Collazo and Marks 1994). Thus, like *D. aeneus*, the life history mode of *D. wrighti* is categorized as direct development.

The present study stemmed from our discovery that both species were abundant in a small watershed in the southern Nantahala Mountains, with no apparent separation by habitat. This provided an opportunity to investigate and compare habitat use, population structure, and some associated life-history traits in syntopic populations of the two species. Our principal goals were (1) to determine ages and body sizes at first reproduction in males and females relative to reproductive phenology, (2) to evaluate the pattern of sexual size dimorphism in these species, and (3) to compare our findings with those of other studies dealing with allopatric populations of the two species.

Methods

Study area

Our study area was located at the lower end of Park Creek, near its confluence with the Nantahala River, in the southern Nantahala Mountains, Macon County, NC. We sampled along a 1-km stretch of Park Creek, over an elevational range of 950–1000 m. Park Creek is a medium-size, fast-flowing stream bordered by steep slopes supporting hemlock-cove hardwood forest. There is a dense understory of rhododendron along the margins of Park Creek. Numerous seepages and first-order streams drain the adjacent slopes. An abandoned logging road parallels Park Creek throughout the study area.

Field and laboratory procedures

We collected the principal samples on 11 days between 20 March and 29 April 1998. Sampling on successive days was conducted progressively in the upstream direction to minimize the chance of recapturing salamanders following release of earlier captures. A total of 101 *D. aeneus* and 124 *D. wrighti* were taken by hand in daytime by turning surface litter (rocks, logs, leaves) and lifting clumps of moss on stream banks, along seepage margins, and in the surrounding forest. Most sampling was conducted within 10 m of streams, with occasional searches to 20 m. We recorded capture locations, weather conditions, and observations of other species of salamanders.

Salamanders were returned to the laboratory, where they were kept in a refrigerator at 10 °C. For measurement and evaluation of reproductive status, individuals were placed in a clear plastic bag, restrained by fingers, and first measured with vernier calipers for snout-vent length (SVL) from the tip of the snout to the posterior margin of the cloacal aperture. Each individual was measured 2–3 times and an average taken. Some individuals were measured
by both investigators to check for accuracy. Following measurement, the salamander was placed on the stage of a dissecting microscope, venter up, and a beam from a fiber-optics illuminator was directed through the body cavity, following the procedure of Bruce (1988). The internal organs were visible, and sex and reproductive status could be determined. The salamanders were subsequently returned to the study area and released within four days of capture. Mortality from capture and handling was < 1%.

In order to verify our assessment of reproductive status in the 1998 sample, we collected, preserved, and dissected small numbers of *D. aeneus* (n = 19) and *D. wrighti* (n = 19) in late March and April of 2000 and 2001. The specimens were killed by immersion in a 5% solution of MS-222, measured for SVL, fixed in 10% formalin, and later transferred to 70% ethyl alcohol. We examined the testes and vasa deferentia in males, and the ovaries and oviducts in females. In addition, we made squash preparations of the vasa deferentia and testes of males, which we examined microscopically for the presence of sperm. Although we counted testis lobes in the dissected specimens, we were unable to obtain accurate counts in the living specimens of the 1998 sample.

In all statistical tests, significance was evaluated at $\alpha = 0.05$.

**Results**

**Habitat use**

Of the 101 *D. aeneus* and 124 *D. wrighti* captured on 11 days during the 1998 sampling period, 40% and 32%, respectively, were captured on the last two sample days of 24 and 29 April. This may have been a result of greater above-ground activity as temperatures increased through the spring. Most individuals of both *D. aeneus* and *D. wrighti* were found within a 10-m swath on both sides of Park Creek and its tributaries. Only two individuals, both *D. wrighti*, were found between 10 and 20 m from a stream. *Desmognathus aeneus* and *D. wrighti* shared habitats with *D. ocoee* Nicholls (Ocoee Salamander), *Plethodon shermani* Stejneger (Red-legged Salamander), and *Eurycea wilderae* Dunn (Blue Ridge Two-lined Salamander). In wet areas close to stream and seepage margins, adult *D. aeneus* and *D. wrighti* were often found next to juvenile *D. ocoee*. In drier areas, further from stream and seepage margins, both adult and juvenile *D. aeneus* and *D. wrighti* were observed under the same cover objects within a few centimeters of *P. shermani* and *E. wilderae*. Other plethodontid salamanders observed at Park Creek were *Desmognathus marmoratus* (Moore) (Shovel-nosed Salamander), *D. quadramaculatus* (Holbrook) (Black-bellied Salamander), *D. monticola* Dunn (Seal Salamander), *Gyrinophilus porphyriticus* (Green) (Spring Salamander), and *Pseudotriton ruber* (Latreille) (Red Salamander).

Although we did not attempt to quantify habitat use, we observed no obvious difference in habitat utilization between the two species. Both species were found in damp leaf litter, in moss, and in moist depressions under logs and rocks along the margins of seepages and streams outward into the forest. For both species, the most frequented habitat appeared to be small depressions of the soil surface beneath leaf litter. Individuals of each species
were often found together, e.g., six *D. wrighti* taken within a 1-m² area on 13 April. In each species, juveniles were often found in groups of 3–4 individuals, while adults appeared to be more solitary and spatially scattered. Furthermore, on three of the 11 sample dates, individuals of both species were found within 10 cm of each other under the same cover object—three adult *D. aeneus* and 1 juvenile *D. wrighti* on 13 April, one adult of each species on 15 April, and two adults of each species on 24 April.

**Reproductive categories**

We attempted to differentiate sexually immature and mature individuals of both sexes. The immature category includes individuals that had not reproduced previously and were apparently not scheduled to reproduce in the current year, whereas the mature category refers to individuals that had reproduced previously and/or were scheduled to reproduce in the current year. In plethodontid salamanders, where reproduction involves sperm transfer from male to female via a spermatophore, followed by sperm storage in cloacal spermathecae of the female for weeks or months prior to oviposition, reproduction in a given year has different meanings for the two sexes; i.e., spermatogenesis and mating in males, and fertilization and oviposition in females, which are not necessarily contemporaneous.

Females of both species were scored as immature if the ovaries contained small, pale, translucent follicles, and if the oviducts were narrow and straight. In mature females, the follicles were larger, vitellogenesis was in progress, and the oviducts were wider and usually convoluted. In the 1998 sample, some small individuals were difficult to sex; we assumed that most of these were immature females, in which the ovaries were translucent because the follicles lacked yolk. This was verified by our later dissections of females in the 2000–2001 samples.

In males of both species, individuals considered immature had small, lightly-pigmented (gray) testes, and the vasa deferentia were narrow, straight, and whitish. In contrast, males scored as mature had darker gray or black testes, and the vasa deferentia were usually wide, coiled, and dark gray or black. In the dissected specimens, none of the males scored as immature, but all of those judged mature on gross examination had sperm in the vasa deferentia; these findings supported our assessment of living males in the 1998 sample.

**Age indexing**

We located one female of *D. aeneus* brooding a clutch of 13 eggs on 23 April 1998. Average diameter of the eggs was 2.3 mm. Otherwise we did not locate clutches of either species. The larger females of *D. aeneus* were gravid, with follicles enlarged to 1.8–2.2 mm in diameter; we assumed these females would oviposit in the next month, i.e., later in April or May. Inasmuch as the species is known to have a spring oviposition season (late April–early May) in the southern Blue Ridge (Harrison 1967, Jones 1981, Marks and Collazo 1998), we estimated 1 May as the modal date for oviposition at Park Creek and assigned ages from this estimated date of entry of the individual into the population as an egg.
In southern Virginia, Organ (1961a,b) found eggs of *D. wrighti* with late embryos in mid-October, with hatching following on 19–25 October. He estimated that oviposition had occurred in August. Although egg clutches of *D. wrighti* have not been reported in southwestern North Carolina, in the mature females sampled in April at Park Creek, maximum follicle diameters were only 1.4 mm and the eggs were probably not fully yolked. This and other evidence (see below) suggests later oviposition-hatching dates for this species than for *D. aeneus*; we have estimated 1 August as the modal date of oviposition and have assigned ages accordingly.

**Size distributions**

Our spring sample of *D. wrighti* sorted into three relatively well-defined size classes (Fig. 1A), which we have interpreted as age classes, i.e., first year (8 mo), second year (20 mo), and older (≥ 32 mo). A small number of tiny (6–8 mm) juveniles formed a discrete class of first-year individuals, undoubtedly hatchlings of the previous year. Although the middle size class showed a wider range of SVL (11 to ≈ 18 mm), its apparent unimodality (mode = 14 mm) reflected the presence of mainly 20-mo individuals. It included immatures of both sexes, plus a few mature males at the upper end of the distribution. Given that our dissections of the 2000–2001 samples showed that spermatogenesis and spermiation may be attained in males of *D. wrighti* as small as 16 mm, we conclude that some males may attain sexual maturity during their second year, and virtually all by the third year, as shown by their presence in the class of larger individuals. Nearly all the members of the latter class (≥ 19 mm SVL) were sexually mature.

In females of *D. wrighti*, follicle size in individuals scored as mature varied from 0.7 to 1.4 mm. Because our samples were taken several months prior to the oviposition season, it is unclear whether all the mature females would have been ready to oviposit in the current year. However, one female having follicles of 0.9 mm had a spermatophore in her vent. Given that all females in the largest size class were mature, it seems likely that females of *D. wrighti* ordinarily initiate vitellogenesis by their third year and oviposit at age 3 years.

The size distribution in the 1998 sample of *D. aeneus* was more obscure than that of *D. wrighti*, but the general pattern was similar (Fig. 1B). We interpreted the distribution as containing three age classes that overlapped in SVL: first-year juveniles (11 mo, modal SVL = 12 mm), second-year juveniles and adult males (23 mo, modal SVL = 19 mm), and third-year and older adults (≥ 35 mo, modal SVL = 24–25 mm).

In *D. aeneus*, the size class representing second-year individuals included immatures of both sexes, as well as mature males, but no mature females. The smallest mature male in the sample was 15.9 mm. The later sample of individuals we dissected included two small males: a 15.4-mm immature, and a 15.5-mm individual with sperm in the testes and adjacent sections of the vasa deferentia. Thus, we feel confident that our assessments of smaller males in the 1998 sample were correct. The data suggest that males mature and reproduce in either their second or third year at Park Creek.

Among females of *D. aeneus*, maturation probably occurs in most individuals in the third year, with first reproduction at an age of three years.
Three females (all 21 mm SVL) had slightly yolked follicles (0.5–0.9 mm). They fell toward the upper end of the intermediate size class, and were considered 23-mo individuals that were scheduled to oviposit the next year at age 3 years. Thus, they were scored as immature. All of the larger females (≥ 23 mm) had follicles enlarged to 1.5–2.1 mm. They were considered to be ≥ 35 mo, and were probably scheduled to oviposit in the next month of the current year. This interpretation assumes that females oviposit annually, as reported by Harrison (1967).

The larger sizes of first-year and second-year individuals of *D. aeneus* than of *D. wrighti*, the greater overlap in size between these age classes in *D. aeneus*

![Figure 1](image_url)
than in *D. wrighti*, and the higher proportion of second-year mature males of *D. aeneus* than of *D. wrighti*, are probably consequences of the earlier oviposition-hatching schedules of the former species. The greater similarity in body sizes between the species in the mixed age class of older individuals probably reflects progressive slowing and eventual cessation of growth following maturation, as in other species of *Desmognathus* (Bruce et al. 2002).

Our methodology precluded precise counting of yolked follicles. We were able to obtain accurate measurements of maximum follicle diameters in most mature females. In *D. aeneus*, variation in follicle diameter was strongly correlated with capture date \( (r = 0.87, \text{df} = 17, p < 0.001) \), but not with SVL \( (r = 0.34, \text{df} = 17, p = 0.16) \). In other words, females captured in late April tended to have larger follicles than those of late March–early April. In contrast, in mature females of *D. wrighti*, the correlations between follicle diameter and capture date \( (r = 0.26, \text{df} = 18, p = 0.27) \) and follicle diameter and SVL \( (r = 0.22, \text{df} = 18, p = 0.26) \) were both non-significant. The difference between species may reflect the later oviposition season of *D. wrighti*.

The body size characteristics of adult *D. aeneus* and *D. wrighti* are summarized in Table 1. Within each species, the variance in SVL was significantly greater in males than in females (*D. aeneus*: \( F = 4.80, \text{df} = 23, 23, p < 0.001; D. wrighti*: \( F = 4.03, \text{df} = 30, 21, p < 0.002 \)). For this reason, we compared mean SVLs with Welch’s approximate t-test (Sokal and Rohlf, 1995). In both species, the mean SVL of females was significantly greater than that of males (*D. aeneus*: \( t_s = 7.36 \text{ vs. } t_{0.05} = 2.07, \text{df} \approx 23; D. wrighti*: \( t_s = 4.68 \text{ vs. } t_{0.05} = 2.05, \text{df} \approx 27 \)). Between species, within sexes, the variances did not differ in either comparison. Although adult males of the two species did not differ significantly in SVL \( (t = 1.16, \text{df} = 53, p = 0.252) \), there was a significant difference in mean SVL between adult females \( (t = 4.41, \text{df} = 44, p < 0.001) \).

**Discussion**

We find the coexistence of *D. aeneus* and *D. wrighti* at Park Creek remarkable. Both are abundant within the study area and broadly overlap in habitat. The species are similar in size and share life-history traits. Their courtship behaviors are essentially identical and differ from those of other *Desmognathus* (Verrell 1999). Differences include the phenology of oviposition and nesting (spring–early summer in *D. aeneus*, late summer–autumn in *D. wrighti*). The species may also differ in nesting sites. Females of *D. aeneus* usually oviposit in clumps of moss, less frequently under logs, in the

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<td>males</td>
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vicinity of seepages (Harrison 1967, Jones 1981, Valentine 1963). In contrast, insofar as is known, *D. wrighti* females oviposit in beds of saturated gravel in the banks of headwater streams (Organ 1961a). The species also differ in behavior; our observations agree with those of others that *D. aeneus* is a relatively sluggish, slow-moving salamander, whereas *D. wrighti* is more active and agile, and is more prone to climbing into the vegetation above the forest floor (Hairston 1949, 1987; Harrison 1967; Organ 1961b).

Although *D. aeneus* and *D. wrighti* may be in competition at Park Creek and elsewhere in the southern Nantahalas, field experiments, like those of Hairston (1986, 1987) would be required to test this hypothesis. It is possible that the populations are not in equilibrium and that one is undergoing replacement by the other. *Desmognathus wrighti* was first recorded in the Nantahala Mountains in 1969 (Tilley and Harrison 1969), but now seems to be widespread at the southern end of this mountain range, including the Coweeta Creek watershed (Bruce 1996 and unpublished data, Bruce et al. 2002), where Hairston (1986) failed to find it in the early 1980s. Thus, *D. wrighti* may be undergoing expansion at the southern end of its range.

In reference to life history, Harrison (1967) estimated, based on size-frequency data, that sexual maturity is attained in *D. aeneus* at two years in both sexes, at SVLs of 18–19 mm, but that females do not oviposit until three years of age. His data showed that females follow an annual reproductive cycle. These findings are in agreement with ours, although it seems likely that at Park Creek some males delay maturation until the third year. It is difficult to estimate the age when males reproduce (i.e., transfer spermato- phores) initially, given that we do not know the extent of the courtship-mating season. Nevertheless, it is presumably less than a whole number of years inasmuch as there is usually a lag between mating and oviposition in plethodontid salamanders. In all nine mature males that we dissected in the late March–April samples of 2000 and 2001, the testes were empty but the vasa deferentia were packed with sperm, indicating that males were in breeding condition in early spring and perhaps for some months before.

In southwestern Virginia, Organ (1961b) estimated that males of *D. wrighti* mature at approximately 3.5 years and that females oviposit initially at five years. However, he estimated that all five species of *Desmognathus* in his study area matured at these same ages, whereas later skeletochronological studies have shown that larger species in the genus tend to mature later than smaller species (Bruce et al. 2002, Castanet et al. 1996). At Park Creek, we estimate that in *D. wrighti*, like *D. aeneus*, males attain reproductive maturity in the second or third year, and females usually oviposit initially at three years. Our first-year individuals of *D. wrighti* in the April sample were slightly smaller (mean SVL = 7.4 mm) than first-year individuals (mean SVL = 8.4 mm) collected by Bernardo (2000) in June in the Great Smoky Mountains. He considered them to be hatchlings of the previous autumn.

*Desmognathus aeneus* and *D. wrighti* are among the smallest salamanders. The adult sizes reported herein lie within the range of adult body sizes in the neotropical genus *Thorius* Cope (Bruce 2000), which are considered the world’s smallest urodèles. In other, larger species of *Desmognathus*, males mature at younger ages and smaller sizes than females, but outgrow and/or
outsurvive females, and thus reach larger maximum sizes than females (Bruce 1993, 2000). Larger male size may be an outcome of sexual selection involving male-male aggression in contests for females (Bruce 2000), as documented in *D. ocoee* (Houck 1988, Houck and Francillon-Vieillot 1988). However, the situation may be different in *D. aeneus* and *D. wrighti*. In *D. aeneus*, Harrison (1967) reported that males and females were nearly identical in size; in *D. wrighti*, Organ (1961b) noted that males were larger, but Crespi (1996) found that females were larger in a majority of 14 populations. Our study at Park Creek showed that adult females average larger than males (in part a consequence of earlier maturation in males), but that maximum sizes of males and females are essentially the same in both species.

Obviously, elucidation of many aspects of ecology and life-history in syntopic populations of *D. aeneus* and *D. wrighti* require more detailed, longer-term studies than ours. For example, given the similarities in body size and courtship behavior (Promislow 1987, Verrell 1999) of the two species, it would be of interest to determine the extent and overlap of their courtship and mating seasons in allopatric and sympatric populations. In addition, mating experiments, like those on *D. ocoee* by Houck (1988) and Houck and Francillon-Vieillot (1988), designed to test for sexual selection by female choice and/or male-male competition, could provide insight regarding the apparent lack of sexual size dimorphism in *D. aeneus* and *D. wrighti* relative to other species of *Desmognathus*. Finally, the nature and degree of competitive interactions between the species will require long-term experimental manipulation of populations, similar to those conducted by Hairston (1986) in other *Desmognathus* assemblages.

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**Literature Cited**


