Reproductive Biology of the Mud Salamander, *Pseudotriton montanus*, in Western South Carolina

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The reproductive biology of *Pseudotriton montanus* was studied for two populations in the Piedmont of western South Carolina. Males reached sexual maturity during the first year following metamorphosis, and attained breeding condition in their second, third or fourth summer of life, depending on the duration of the larval phase and on the season of metamorphosis. Males produced sperm annually, with evacuation of the testes beginning in late July and continuing through the autumn. All mature males taken from August through November had vasa deferentia packed with sperm. Females were found to oviposit initially in the autumn or winter at four or five years of age, and thereafter on an irregular schedule. Clutch size increased as a function of body size, and ranged from 77 to 192 eggs/female. In comparison with *Pseudotriton ruber*, *P. montanus* shows more rapid growth, earlier maturity and higher fecundity. Such a life-history strategy may be a consequence of *r*-selection in response to intrinsically hazardous and uncertain environments. It is proposed that the irregular female reproductive cycle of *P. montanus* is a facultative adaptation which prolongs life and provides for iteroparity under conditions of variable juvenile and constant adult mortality, yet allows for high fecundity, especially in the initial reproductive year, under overall conditions of *r*-selection.

*Pseudotriton montanus* is one of many plethodontid salamanders for which essential life-history information has been lacking. The mud salamander occurs primarily in lowland environments in the southeastern United States, where it inhabits muddy springs and streams and swampy pools and ponds (Dunn, 1926; Conant, 1957, 1958). Brimley (1925, 1944) indicated that the species has an autumn and winter egg-laying period in North Carolina, and provided rather sketchy data on its egg-laying habits. Netting and Goin (1942), in their description of *P. m. floridanus*, gave brief notes on the natural history of this form; later, Goin (1947) described a clutch of eggs found in January in a Florida population, and noted the presence of at least two size groups of larvae in this population in the winter months. Fowler (1946) had previously located eggs of *P. montanus* in December in Maryland. Robinson and Reichard (1965) reported an apparent mating for the species in September in Kentucky. More recently, I compared fecundity with that of closely-related sympatric species, and showed that females of *P. montanus* produce extremely large egg clutches for a plethodontid (Bruce, 1969). A later study indicated that the larval period of *P. montanus* is shorter than that of *P. ruber*, but that, because of more rapid growth in the former, the two species metamorphose at nearly the same size (Bruce, 1974).

The present study was undertaken to determine the reproductive parameters for populations of *P. montanus* in western South Carolina, and to use the data obtained in evaluating the evolutionary significance of life-history variation among primitive plethodontid salamanders.

**MATERIALS AND METHODS**

This report is based on 231 metamorphosed specimens of *P. montanus* collected from two populations in the Piedmont of western South Carolina, mainly in the warmer months, between 1962 and 1972. The salamanders were collected by hand, in daytime, from bottomland habitats adjacent to muddy springs, streams and swampy areas. Specimens were preserved within several days after collection; they were killed by immersion in a Chloroform solution, and were then hardened in 8% formalin. Measurements of snout-vent lengths (SVL), to the nearest millimeter, were recorded for formalin-preserved specimens, and were taken from the tip of the snout to the anterior edge of the cloacal opening.
The reproductive organs of all individuals were examined by dissection. For females, measurements of diameters of ovarian oocytes and oviducts were made to the nearest 0.1 mm with an ocular micrometer in a dissecting microscope. The size of the largest oocyte was noted for each female, as were the diameters of the oviducts midway along the length of the ovaries. Counts of yolked oocytes were made for females designated gravid, as defined below. For males, the course of the spermatogenic cycle was studied by squash preparations of testis stained with iron acetoacarnine. In addition, longitudinal sections of testis were prepared histologically for ten males taken between April and September. The presence or absence of sperm in the vasa deferentia was determined by microscopic examination of squash preparations or, more easily, by observing exuding seminal fluid from torn pieces of vasa deferentia. These methods allowed recognition of the following reproductive categories:

**Females.**—Females designated immature had small, compact ovaries in which the oocytes were non-yolked and small (≈ 0.7 mm); their oviducts were straight and thin (0.2–0.3 mm). In mature females the ovaries either contained large, yolked oocytes or, if the oocytes were small (0.6–1.0 mm), had a more stretched, less compact appearance than in immature individuals. Moreover, the oviducts of mature females showed at least a hint of convolution, and were thicker (≈ 0.4 mm) than those of immature females. Within the mature category, those females with oocytes ≥ 2.0 mm were designated gravid; for these all of the large, yolked oocytes in both ovaries were counted, and the count was taken as an estimate of the clutch size in the forthcoming egg-laying season. The reliability of clutch size estimates was insured by restricting oocyte counts to gravid females, since in non-gravid, mature individuals it was difficult to distinguish oocytes of the next clutch from those destined for later clutches.

**Males.**—Males which had thin, cord-like testes and thin, straight vasa deferentia were considered immature; both structures were unpigmented in such individuals. In mature males the testes were larger, their shape varying with the phases of the spermatogenic cycle, with only the evacuated regions showing concentration of dark pigment. The vasa deferentia of mature males were convoluted and lightly pigmented. Mature males were separated into non-breeding and breeding categories by the absence or presence respectively of sperm in the vasa deferentia. Cloacal papillae were seen in all males judged mature according to the condition of the testes and vasa, but were not evident in immature males.

The age estimates of metamorphosed *P. montanus* given in this report were based on the literature concerning the season of oviposition and the larval period. Ages were determined from the time of oviposition, estimated as November (Brimley, 1925; Fowler, 1946; Goin, 1947). The larval period is ordinarily 14–20 months but may be prolonged for 26–32 months, in either case extending from a winter hatching date to a summer metamorphic period (Bruce, 1974). Thus by inspecting the body-size distributions of metamorphosed individuals from the spring and summer months, it was possible to estimate ages at which sexual maturity was reached in both males and females.

All of the specimens have been placed in the vertebrate collection of the Department of Zoology, Duke University.

**STUDY AREAS AND HABITATS**

The two study areas were located 6 km apart in the upper Piedmont of South Carolina. One area, known as Newry, was 1 km northwest of the town of that name, near the confluence of Cane Creek and Little River, in Oconee County. This site has been destroyed by the impoundment of the Little River, and is now covered by the waters of Lake Keowee. The second area, Clemson Forest, was within a research forest owned by Clemson University, about 7 km north of Clemson, in Pickens County. The elevation at both areas was about 200 m.

The habitats of *P. montanus* at Newry (Stream A) and at Clemson Forest (Sixmile Creek) have been described in a previous report (Bruce, 1974); however, it is noteworthy that at both study areas the species was found in damp, muddy habitats adjacent to sluggish springs and streams and swampy sloughs and ponds within bottomland deciduous forests. Most metamorphosed individuals were taken from burrows in the soft, damp mud along the banks of these watercourses. The burrows were usually nearly vertical structures, opening to the surface at least several centimeters above water level, with the opening often concealed by surface litter. The burrow diameter was
Fig. 1. Snout-vent lengths of metamorphosed males of *Pseudotriton montanus* according to reproductive condition and collection date. Pooled data, 1962-72, Newry and Clemson Forest. Crosses = immature; light circles = mature, non-breeding; dark circles = mature, breeding. Each small symbol represents one individual and each large symbol represents two individuals.

typically just slightly greater than the salamander in occupancy. Although many burrows were located within a meter of the edge of a stream or swamp, other mud salamanders were found in burrows in the adjacent bottomland, as far as 15-20 m from the nearest surface water. The burrows led downward into complex series of water-filled channels which provided access to the adjacent watercourses. Individuals of *P. montanus* were positioned with their heads at or just below the surface opening of the burrow; upon exposure they tended to withdraw downward, but could nearly always be captured by hand digging a side channel into the lower part of the burrow and forcing the animal upward through the surface opening. As captive mud salamanders kept in the mud of their native habitats readily built complex series of burrows and subsurface channels, it appeared that these salamanders ordinarily construct their own burrows rather than use those abandoned by other animals. Occasionally, metamorphosed individuals of *P. montanus* were taken under logs and leaf piles in the damp bottomland or, more rarely, were found submerged in the bottom debris of the streams and swamps. However, small, recently-transformed individuals seemed to have less proclivity for burrowing, and were more often found concealed under leaves and debris at the margins of the watercourses.

The collecting period at Newry extended
from 1962 through 1968, ending just prior to the final destruction of the habitat and flooding of the drainage basin. Throughout this period water levels in the stream and swamp in which *P. montanus* occurred appeared stable. However, at Clemson Forest, where collections were made from 1965 through 1972, most of the habitat was dry in late August and early September in both 1970 and 1972. At these times numerous adults of *P. montanus* were taken from burrows in the bed of the slough and swamp which had been filled with water at other seasons. Only limited field work was done from October through March, and few metamorphosed individuals were taken in the cooler months.

**Reproductive Biology of Males**

Based on the criteria given earlier, males were designated immature or mature, and those in the latter category were recognized as either non-breeding or breeding. Except for a 56 mm mature individual collected on 19 November 1966, the 108 metamorphosed males in the overall sample were taken from 11 April through 13 September over the 10-year study period. As the body-size distributions of males in each reproductive class according to collection date were similar at Newry and Clemson Forest, the data of the two localities were pooled (Fig. 1).

Most of the small males (< 50 mm SVL) taken from 10 July through 13 September had undoubtedly metamorphosed in the current summer, inasmuch as they were similar in size to large larvae, and as males of this size were not, with one exception, collected before July in any given year. A few small males (45–47 mm SVL) were mature and in breeding condition; it is uncertain whether they had metamorphosed early in the current summer or represented slower-growing individuals which had transformed in the previous year. However, in the small male (45 mm SVL) collected on 27 May 1972, which had probably just transformed in a year when larval growth had been rapid (Bruce, 1974), the testes were expanded posteriorly and packed with secondary spermatogonia. As this was similar to the condition of the testes in some larger males collected in May of this and other years, the recently-transformed individual would probably have matured sperm later in the summer of 1972. Perhaps males which metamorphose early in the year undergo initial spermatogenesis in the same year, whereas those which metamorphose late delay spermatogenesis until the next year. In any case, the size distributions suggested that males invariably mature by the year following metamorphosis. Thus if most larvae metamorphose in their second summer, the age of first reproduction in males is either 20 or 32 months, determined from oviposition in November to attainment of breeding condition in July of the second or third year. For those laggard males which delay metamorphosis until their third summer, first reproduction would occur at either 32 or 44 months.

The spermatogenic cycle, as studied by squashes and longitudinal sections of the testes, showed little annual variation. Most males collected in April and May had the testes enlarged posteriorly, filled mainly with secondary spermatogonia, but with primary spermatocytes often present in the posterior ampullae. Some males taken in late May showed meiotic activity in the posterior-most ampullae of the testes. By mid-June the testis was usually of nearly uniform diameter over its entire length, spermatids or sperm were present in the most posterior ampullae, and the meiotic wave had progressed anteriorly about one-third the length of the testis. Evacuation of the testis, beginning at the caudal end, was first noted in late July; by this time the meiotic wave had passed two-thirds the length of the testis in most males examined. In August and September the testis was typically club-shaped; meiosis was completed in most individuals; and the expanded anterior section was generally filled with sperm, except for a short zone of spermatids in the anterior-most ampullae. The caudal section of the testis in these males had been reduced to a narrow cord of evacuated ampullae in which secondary spermatogonia were beginning to proliferate posteriorly.

As noted above, evacuation of the testes began in late July. From mid-August through mid-September all mature males had vasa deferentia packed with sperm, as did the single male taken in November. The vasa deferentia were empty in males collected in other months. Thus the potential breeding season is at least of four months duration, from midsummer to late autumn. No courtship activity was observed, although on several occasions in late summer a male and female were taken from the same burrow. The observation by Robinson and Reichard (1965) of an apparent September mating of *P. montanus* in Kentucky is further
evidence of a late summer and autumn mating period in this species.

**Reproductive Biology of Females**

The total sample of *P. montanus* from both localities included 125 metamorphosed females, all but one of which were collected between 11 April and 13 September over the 10-year study period. As noted earlier, females were separated into immature and mature categories, and those in the latter class were designated non-gravid or gravid according to the vitellogenic phase. The body-size distributions of females in each reproductive category in respect to date of collection were similar for the two localities, and the data were therefore pooled (Fig. 2).

Immature females represented at least two age classes. The smaller individuals (35–48 mm SVL) taken from July into September had probably metamorphosed in the current summer, inasmuch as they were near or within the size range of large larvae, and as metamorphosed individuals of similar size were absent in the samples taken before July. Larger immature females (> 50 mm SVL) were taken throughout the sampling interval of each year, including the spring months preceding the summer metamorphic period. Such females must have transformed in the previous year or earlier. The wide gap in snout-vent length between recently-transformed and mature females suggested that females do not ordinarily attain maturity in the summer following metamorphosis. Thus, as most larvae metamorphose in their second summer, it follows that females do not become mature until at least their fourth year, such that the minimum age of first ovi-
position is four years. Accordingly, females which delay metamorphosis to the third summer would not be expected to reproduce initially until five years of age.

No gravid females were taken from April through June. These were first encountered in early July, and increased in frequency through the remainder of the summer. For all mature females the sizes of the largest oocytes were plotted against collection dates (Fig. 5). Most gravid females taken in late summer contained oocytes in the range of 2.0–2.8 mm in diameter, with only two individuals having eggs exceeding 3.0 mm. If the observed maximum of 3.5 mm was close to the egg size at oviposition, then the course of vitellogenesis portrayed in Fig. 5 showed that most gravid females would probably not have been ready to lay until November. This estimate of the oviposition season is in agreement with that arrived at from data on hatchling larvae (Bruce, 1974).

From spring through summer there was a progressive separation of females into the two distinct categories observed in late summer, i.e., non-gravid females with tiny non-yolked oocytes and gravid females with large yolked oocytes. By late summer, mid-August through mid-September, all of the females scheduled to reproduce in autumn of the current year had achieved a gravid state. The non-gravid females of late summer tended to lie toward the middle of the size range of mature females, and, from the condition of their reproductive organs, it seemed unlikely that immature individuals were mistakenly included in this category. Thus, some mature females taken in the summer were not scheduled to oviposit in the forthcoming autumn, indicating that females do not necessarily reproduce annually. Although females may possibly follow a biennial schedule of oviposition, the proportion (25%) of non-gravid individuals in the late summer sample is more suggestive of an irregular cycle, with females occasionally foregoing reproduction in a given year, perhaps as a facultative response to slow recovery from reproduction in the preceding year.

Inasmuch as deposited egg clutches of *P. montanus* could not be found, estimates of clutch size were obtained from counts of yolked oocytes in the ovaries of gravid females. Such counts were obtained for 30 of the 32 gravid females in the total sample; in 2 individuals only a few oocytes were enlarged to 2.0 mm,
and a precise count of those oocytes destined for the next clutch could not be made. An initial plot of egg number against snout-vent length showed that variance tended to increase with increasing snout-vent length; therefore, the relationship was treated according to a power function as a regression of log_e egg number on log snout-vent length (Fig. 4). The logarithmic transformation reduced the heterogeneity of variance; moreover, since it is more likely that clutch size varies rectilinearly with body volume rather than with body length, the logarithmic transformation is applicable when body length is used as the index of body size. Although there was a wide scatter of points around the regression line ($r^2 = 0.0429$), the regression coefficient, $b = 1.932$, was very significant ($t = 4.126, P_{28} < 0.001$), and it can be concluded that clutch size increases as a function of body volume in these populations of *P. montanus*.

The regression data do not readily convey the fact that females of this species produce exceptionally large clutches. The actual oocyte counts ranged from 77 to 192 ($N = 126$, $n = 30$). This is considerably greater than the clutch sizes of other plethodonts of similar body size and comparable mode of life; e.g., *Pseudotriton ruber* (Bruce, 1969), *Gyrinophilus porphyriticus* (Bruce, 1969, 1972a) and various species of *Desmognathus* (Tilley, 1968).

**DISCUSSION**

The information on the life cycle of *P. montanus* presented herein is of interest in comparison with that for related species, especially *P. ruber*, the only other member of the genus. Although the two species are sympatric over a large area and may share habitats, *P. ruber* is a more upland form and shows less departure from the ancestral springbrook environment in Appalachia than the lowland *P. montanus*. Whereas the life cycles of the two species have many similarities, *P. montanus* shows earlier maturity (Bruce, 1968), greater fecundity (Bruce, 1969) and more rapid larval growth (Bruce, 1972b, 1974). In contrast to the irregular female reproductive cycle of *P. montanus*, females of *P. ruber* apparently follow a strict annual schedule; thus, all of 12 mature females of the latter species taken at Newry and Clemson Forest in the summer months were gravid, and similar findings were obtained from larger Blue Ridge samples of this species (Bruce, 1968, 1969).

These life-cycle differences may conveniently be examined within the concepts of *r* and *K*-selection introduced by MacArthur and Wilson (1967), and subsequently elaborated upon by others (e.g., Gadgil and Bossert, 1970; Hairston, Tinkle and Wilbur, 1970; Roughgarden, 1971). Briefly, *r*-selection is selection for high productivity, arising under conditions where population density is often severely reduced by drastic increases in the death rate and/or decreases in the birth rate, such that the population is ordinarily in an expanding phase, well below carrying capacity, and with plentiful resources. These conditions are typically associated with unstable habitats and harsh, seasonal climates. The life cycles of *r*-selected populations will emphasize rapid growth, early maturity, high fecundity, and short life. In contrast, *K*-selection is selection for insensitivity of productivity to density, as expected in crowded populations, perpetually at or near carrying capacity, and thus expe-
riencing scarcity of resources. This mode of selection is characteristic of populations in stable habitats under mild climates, wherein catastrophic reductions in density are rare; the life-history correlates are slow growth, late maturity, low fecundity, and long life.

Because of the relative aspects of r- and K-selected life cycles, the most fruitful comparisons of life-history strategies in reference to the mode of selection will be among populations of the same species. For plethodontid salamanders such studies have been made for *Gyrinophilus porphyriticus* (Bruce, 1972a) and *Desmognathus ochrophaeus* (Tilley, 1973). The extension of these comparisons to closely-related species requires more care, but may yield insights into phylogenetic trends. Thus the differences between *P. ruber* and *P. montanus* enumerated above suggest that the latter has experienced greater r-selection in adaptation to more hazardous environments of the southeastern lowlands. Such hazards may be represented by greater predatory threats in lowland versus upland environments, and by less stable habitats in the former. For example, the late summer and autumn drying of lowland watercourses occupied by larval *P. montanus* at Clemson Forest has not been observed for the more stable larval habitats of *P. ruber* at this locality or elsewhere (Bruce, 1972b, 1974). Such drying, if a more general feature of the larval habitats of *P. montanus* than of *P. ruber*, could represent a source of catastrophic, density-independent mortality which has acted to generate r-selection in the former species.

It is likely that the more terrestrial postmetamorphic phase of *P. montanus* is less affected by seasonal drying than the aquatic larva. Moreover, it is probable that the smaller larvae, in open-water habitats, experience higher mortality through predation than the larger adults, occupying narrow mud burrows. Thus, if morphological, physiological, and ecological limitations prescribe a more uncertain mode of life for the pre- than for the post-metamorphic phase, then the latter component of the population may often exist below carrying capacity in environments having abundant resources, within which selection would favor an r strategy achieved through early reproduction and high fecundity (Gadgil and Bossert, 1970), as observed in this study. At the same time, if recruitment via metamorphosis often fails to saturate the adult environment, selection would act to reduce the duration of the larval period (Istock, 1967); thus, the higher larval growth rate and shorter larval phase of *P. montanus* versus *P. ruber* may represent the effect of such selection upon the former (Bruce, 1974).

Although females of *P. montanus* exhibit high fecundity, they do not reproduce on a precise annual schedule. The cycle is probably facultatively irregular, with individual females skipping reproduction in years when recovery from reproduction in the preceding year has been slow. If a high reproductive effort early in life is advantageous for this species, then failure to reproduce in a given year seems anomalous. However, the production of large egg clutches may make such a demand on the somatic resources of the female, that the cost of delaying the next reproduction is sometimes more than balanced by the profit in increased survivorship and growth from delay, especially since fecundity increases with age through the relationship between body size and clutch size. Such a strategy would be favored if survivorship increases with age (through increased body size), and is intrinsically higher in mature individuals than in prereproductive.

It is also possible that facultatively irregular reproduction has been selected for in females through a relationship between the duration of the larval phase and differences in uncertainty in survival of pre- and post-metamorphic individuals. Since the larval phase of *P. montanus* is greater than one year (Bruce, 1974), a single catastrophic mortality could eliminate the progeny of a single female produced in two successive years. Facultative reproduction, by increasing female survivorship, would thereby prolong adult life and help insure the production of offspring over a lengthy span. Thus the female reproductive cycle may be keyed to the time spent in the larval phase, in which mortality is likely to be higher and more density-independent than in the postmetamorphic phase.

It is noteworthy that males of *P. montanus* reproduce annually and mature at an earlier age than females. The obvious explanation of the differences between the sexes is that reproductive costs are lower in males, and that annual reproduction beginning at an early age has only a negligible effect on male survivorship and growth.

The argument advanced here for *P. montanus* is similar to that of Murphy (1968), who demonstrated that under conditions of unstable juvenile mortality and stable adult mortality
there can be selection for long life and multiple reproduction. Hairston, Tinkle and Wilbur (1970) criticized Murphy's computer simulations, and argued that iteroparity is ordinal. The consequences of a lengthy period of either density-dependent regulation or population decline. However, these authors cited the need for empirical studies, beyond the meager data provided by Murphy, of the relationship between iteroparity and variation in prereproductive survivorship. In the particular case of *Pseudotriton*, iteroparity is apparently characteristic of both species, but may be sustained under different circumstances. Thus *P. ruber* is envisioned as a K-strategist, occupying stable habitats under density-dependent regulation in all phases of the life cycle. In contrast, *P. montanus* is seen as a r-strategist, living in less stable habitats, but with iteroparity retained in females via facultative reproduction as a consequence of differential larval and adult mortality within a life cycle in which the larval phase is more than one year. Although I provided rough estimates of survivorship for larval *P. ruber* (Bruce, 1972b), such data are otherwise lacking for *Pseudotriton*, and may be difficult to obtain for these elusive salamanders.

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