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Comparative Reproduction of the Endemic New Mexico Plethodontid Salamanders, *Plethodon neomexicanus* and *Aneides hardii* (Amphibia, Urodela, Plethodontidae)

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ABSTRACT—*Plethodon neomexicanus* (Stebbins and Riemer, 1950) and *Aneides hardii* (Taylor, 1941) are members of the family Plethodontidae that are endemic to the Rocky Mountains of New Mexico. The active season for both species is mainly June, July, and August. *Aneides hardii* has a male reproductive cycle about 1 month ahead of *P. neomexicanus.* No male of *A. hardii* collected had his vasa deferentia packed with sperm, while the majority of males of *P. neomexicanus* had sperm-packed vasa deferentia. In *P. neomexicanus* oviposition occurs every other year, while in *A. hardii* oviposition may occur every 3 years. In *P. neomexicanus* mating occurs above ground in July and August and oviposition occurs between mid-August and the following spring. In *A. hardii* mating occurs below ground by the beginning of the active season, oviposition occurs in June and early July, and hatching occurs by August or early September. In *P. neomexicanus* one clutch of eggs contained seven eggs, with a range of 6.8 to 7.3 mm in diameter and in *A. hardii* six clutches averaged 4.7 eggs, with a range of 6.4 to 9.1 mm in diameter.

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INTRODUCTION

It is believed that the harsh climate associated with the evolution of the Rocky Mountains has left this mountain chain almost barren of salamanders. Three species of plethodontid salamanders have survived by adapting to long, harsh winters and short but wet summers. Two of the species, *Plethodon neomexicanus* (Stebbins and Reimer, 1950) and *Aneides hardii* (Taylor, 1941), are endemic to the southern range of the Rocky Mountains of New Mexico.

The two genera, *Plethodon* and *Aneides,* are so closely related that Wake (1966:67) postulated a direct origin of *Aneides* from *Plethodon. Aneides hardii,* considered the most primitive *Aneides,* is also the most *Plethodon*-like in appearance and habits.

Plethodon neomexicanus is known from 18 localities in Sandoval County and five locations in Los Alamos County at 7200 to 9000 ft. in the Jemez Mountains of New Mexico. Reagan (1972) provides a distribution map and a limited study of reproduction. Williams (1972) studied reproduction and ecology and Williams (1973) summarized all pertinent literature.

Precipitation averages for the area occupied by *P. neomexicanus* were supplied by the United States Forest Service. These monthly averages were taken at the Lee Ranch in the Jemez Mountains at an elevation of 8601 feet from 1924 to 1940. In the Jemez Mountains precipitation during July, August, and September comprises almost 49% of the average yearly total of 22 inches, with July (4.1 in.) and August (3.6 in.) alone comprising 35% of the total. Only 7% of the average yearly total occurs in June.

Aneides hardii is known from seven localities in three disjunct areas of the Sacramento

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Mountain range: three localities in the Capitan Mountains and two areas in the Sierra Blanca of Lincoln County and two localities in the Sacramento Mountains of Otero County. All collections were made between 8500 and 11,000 feet elevation. Eggs have been described by Johnston and Schad (1959), Lowe (1950), and Schwartz (1955). The account by Wake (1965) is still an almost complete summary of all pertinent literature.

Averages for precipitation for the general area occupied by *A. hardii* were taken from United States Weather Bureau records. Monthly averages were taken at Cloudcroft Lodge in the Sacramento Mountains at an elevation of 8827 feet from 1927 to 1971 for the 27 years for which data are available. Precipitation in the Sacramento Mountains during July and August comprises more than 44% of the average yearly total of 26 inches, with 5.8 inches in July and 5.6 inches in August. Precipitation during July, August, and September comprises almost 54% of the total, while only 7% of the average yearly total occurs in June.

MATERIALS AND METHODS

Collections of *Plethodon neomexicanus* were made during the summers of 1970, 1971, 1973, and 1974. The study area is located about 230 m south of N.M. Route 4 about 19 km west of Los Alamos in the Jemez Mountains, Sandoval County, New Mexico, at 8700 ft. elevation.

Collections of *Aneides hardii* were made during the summers of 1972, 1973, 1974, and 1976. The study area is located about 200 m north of the Sierra Blanca Ski Area just north of N.M. Route 532 in the Sierra Blanca, Lincoln County, New Mexico, at 10,000 ft. elevation.

All measurements of length in the text refer to snout-vent length and were taken from the tip of the snout to the anterior edge of the vent. Measurements were taken prior to fixation or shortly thereafter. Animals were killed in a chloretone solution, fixed in 10% formalin, and subsequently preserved in 55% isopropyl alcohol.

Testes, vasa deferentia, some ovaries, and spermathecas were prepared for histological examination. The tissue was fixed in 10% formalin, imbedded in paraffin, and sectioned at 10 microns. This method uses Harris' hematoxylin with cytoplasmic counterstains of Biebrich scarlet and orange II.

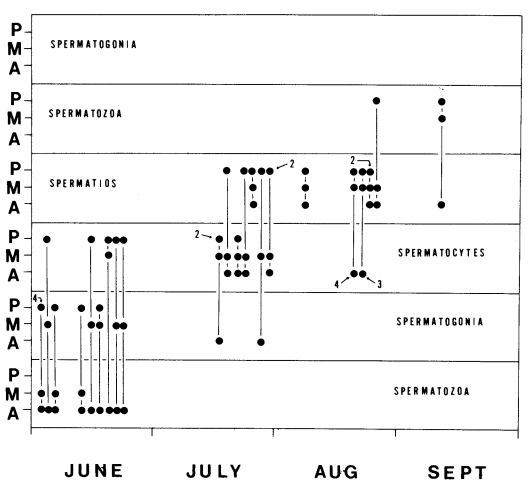

FIGURE 1. Reproductive cycle of male *P. neomexicanus* by region of testis. Each vertical line with three symbols represents one testis unless indicated otherwise. The regions of the testis are anterior (A), middle (M), and posterior (P).

Significance for the Student's *t*-test were based on 0.05 probability levels.

RESULTS

Male reproductive cycle in Plethodon neomexicanus.—The reproductive cycle is shown by region of testis for 33 males in Figure 1. Mature males have an average length of 55.2 mm, with a range of 47 to 63 mm.

In both *P. neomexicanus* and *A. hardii* spermatogenesis takes place in a caudocephalic wave beginning at the posterior end of the testis and passing slowly anteriorly. Secondary spermatogonia are probably formed in late fall and are present until late July. Primary spermatocytes are found from early June to late August, and spermatids are found from mid-July to mid-September. Secondary sper-

matocytes are rarely seen either in *P. neomexicanus* or in *A. hardii* and are considered transitory. Spermatozoa are first formed in late August and probably fill the entire testis by early fall. Sperm are transferred to the vas deferens in late fall or winter. *Plethodon neomexicanus* (23 of 27 males with data) has sperm in the vasa deferentia during its entire season from June through September.

Male reproductive cycle in Aneides hardii.—Figure 2 shows the reproductive cycle for 31 males. Mature males have an average length of 48.4 mm, with a range of 39 to 59 mm. Males of *P. neomexicanus* with an average length of 55.2 mm are significantly larger than males of *A. hardii*.

Secondary spermatogonia are first formed in late July and are present until the following spring. Primary spermatocytes are probably formed first in spring and are found until late July; spermatids are probably formed in late spring and are found until early September. Spermatozoa are first formed in mid-July and are probably transferred to the vas deferens in early fall. No male collected had abundant sperm in his vasa deferentia.

Female reproductive cycle in Plethodon neomexicanus.—Forty-one adult females averaged 56.2 mm in length, with a range of 49 to 67 mm.

Figure 3 indicates two classes of females separated by a theoretical line. Twenty-five females in the first class had a mean length of 56.0 mm (49-67 mm) and do not have sufficiently developed ova to lay eggs during the current active season. Females with maturing ova had ova that ranged in diameter from 1.1 mm in mid-June to 2.1 mm in mid-September. The number of eggs over 1.0 mm in diameter averaged 7.7, with about four in each ovary. None of the females in this group had spermathecas with an abundance of sperm, indicating none had mated during the current summer.

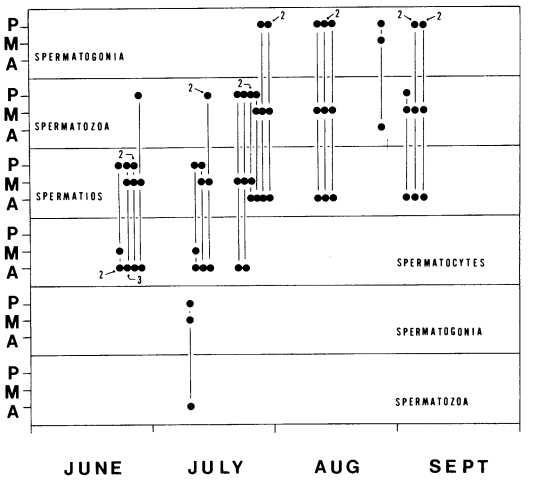

FIGURE 2. Reproductive cycle of male *A. hardii* by region of testis. Each vertical line with three symbols represents one testis unless otherwise indicated. The regions of the testis are anterior (A), middle (M), and posterior (P).

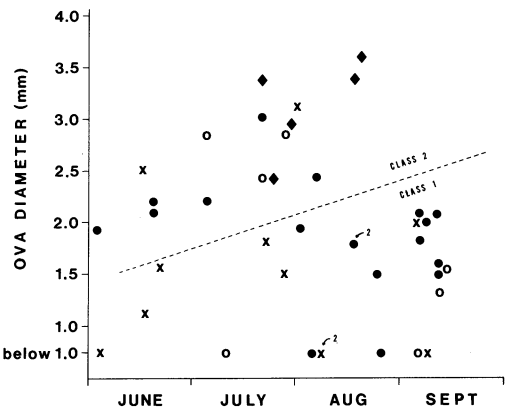

FIGURE 3. Two classes of females of *P. neomexicanus* separated according to average ova diameter. Each symbol represents one female unless otherwise indicated. Presence of sperm in the spermatheca is noted; open circle = no data, solid circle = no sperm, X = little sperm, and solid diamond = abundant sperm.

Sixteen females in the second class would mate and oviposit their eggs by the beginning of the next active season; these females are considered gravid. Females in this class had a mean length of 56.4 mm (52-63 mm) and were not significantly different in length from the first class of females. Ova diameters ranged from 1.9 mm in early June to 3.6 mm in late August. The number of maturing eggs remains about the same on the average (7.8 ova) with no significant difference from the average number of ova in the first class (7.7 ova). Only females with an average ovum diameter of at least 2.4 mm mated, with the earliest mating 17 July and the latest 20 August. From mid-July to mid-August five of eight gravid females with data had spermathecas packed with sperm. No gravid female was collected after 20 August.

No eggs have been found in the field. This indicates probably underground oviposition. One clutch laid in the laboratory in early June had seven eggs averaging 6.9 mm (6.8-7.3 mm) in diameter. The eggs were attached to the sides of pieces of wood by a stalk or peduncle.

Female reproductive cycle in Aneides hardii.—Seventy adult females averaged 44.5 mm in length, with a range of 36 to 53 mm. Females of *P. neomexicanus* with an average length of 56.2 mm are significantly larger than females of *A. hardii*.

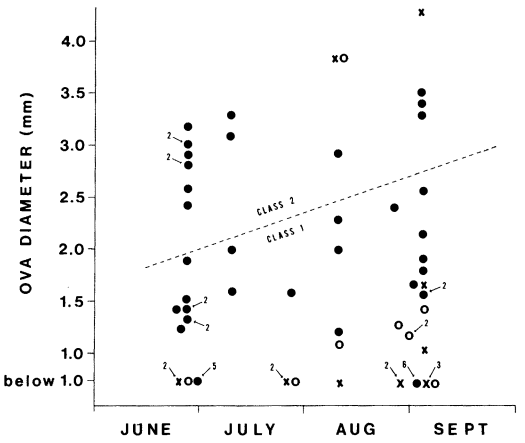

FIGURE 4. Two classes of females of *A. hardii* separated according to average ova diameter. Each symbol represents one female unless otherwise indicated. Presence of sperm in the spermatheca is noted; open circle = no data, solid circle = no sperm, and X = little sperm. No female had a spermatheca with abundant sperm.

Figure 4 indicates two classes of adult females separated by a theoretical line. Fifty-three females in the first class would not lay eggs during the current summer and had a mean length of 46.1 mm (36-53 mm). Ova diameters over 1.0 mm ranged from 1.2 mm in late June to 2.6 mm in early September. The number of maturing eggs averaged 10.1, with about five in each ovary. None of the females in this group had an abundance of sperm in their spermathecas. This class may consist of two types of females. Females of one type had oviposited, brooded, and returned to feeding. The second type possibly consists of females in the next active season after oviposition, together with younger females that are beginning to mature. Females of the second type are not considered gravid and would require yet another active season for maturation of eggs.

Seventeen females in the second class are considered gravid and would mate and oviposit their eggs by the beginning of the next active season. Females in this class had a mean length of 45.6 mm (40-59 mm) and were not significantly different from females of the first class. Ova diameter ranged from 2.4 mm in late June to 4.3 mm in early September. The female with the 4.3 ovum diameter and with only one maturing ovum is considered atypical and is excluded from results referring to the number of maturing ova. The average number of maturing eggs significantly decreased from 10.1 in the first class to an average of 7.7 (including the ovum of the atypical female, 7.3) in the second class, with about equal numbers in both ovaries. No female in this class had a spermatheca with abundant sperm.

Lowe (1950:94) found three eggs in a large, moist, decomposing Douglas-fir log on a steep north-facing slope in August. The eggs were stalked. Schwartz (1955:70) found 10 eggs and an accompanying female on 12 August. The eggs were suspended by a peduncle or stalk from the upper surface of a cavity in a Douglas-fir log. The eggs had an average diameter of 8.1 mm (7.4-9.1 mm). Johnston and Schad (1959:58) found four egg clusters, one on 14 July and three on 27 July. These clutches contained one, four, four, and six eggs with an average diameter of 6.4 mm. The eggs found in August were in advanced stages of development. From these three studies, eggs averaged 4.7 (disregarding the single-egg clutch, 5.4) per clutch and ranged from 6.4 to 9.1 mm in diameter. Eggs are peduncled and are brooded by females.

DISCUSSION

The time of optimum temperature and moisture for both species is July and August. During this time the habitat for *Aneides hardii* receives more rain (11.4 in.) than does the habitat of *Plethodon neomexicanus* (7.7 in.). For both species, June is a dry month, while

September is wetter for *P. neomexicanus* than for *A. hardii.* Reproductive cycles in both species are timed for maximum activity during July and August. *Aneides hardii* appears to be adapted to lower temperatures than is *P. neomexicanus.* The active season for *P. neomexicanus* is longer than for *A. hardii,* with a warmer June and a warmer and wetter September in the Jemez Mountains than in the Sacramento Mountains.

Male reproductive cycles.—The basic mechanics and sequence of testis maturation in *A. hardii* are the same as in *P. neomexicanus,* but the reproductive cycles are timed differently.

The first males of *A. hardii* collected in late June had a cycle much like that seen in a late July testis of *P. neomexicanus.* In *A. hardii* spermatocytes are last found in late July, 1 month ahead of the last occurrence in *P. neomexicanus.* Spermatozoa are first formed in the majority of males by late July in *A. hardii,* while first evidence of spermatozoa in *P. neomexicanus* occurs in late August. In both species, sperm are transferred to the vas deferens after the active season is over, in early fall in *A. hardii* and in late fall or winter in *P. neomexicanus.* No male of *A. hardii* collected had an abundance of sperm in the vasa deferentia, while the majority of males of *P. neomexicanus* had sperm-packed vasa deferentia. *Aneides hardii* would appear to mate underground before the next active season as suggested by Johnston and Schad (1959:582), while *P. neomexicanus* may mate any time during the active season and appears to do so in July and August.

Both species have an active season too short for completion of the spermatogenic wave and transfer of sperm to the vas deferens during the same active season. *Aneides hardii* used its sperm for mating before the beginning of the next active season, while during its entire active season *P. neomexicanus* keeps the vasa deferentia packed with sperm made during the previous active season. This pattern in *P. neomexicanus* is unlike that of most other species of *Aneides* or *Plethodon.*

Female reproductive cycle in Plethodon neomexicanus.—Plethodon neomexicanus with the longer active season places its reproductive effort into almost all of the eight eggs it is developing from the previous year. Reagan (1972:490) found an average of 7.7 ova in 45 adult females with no separation of classes; this closely agrees with my results. Based on only one clutch, the species may oviposit about the same number of eggs it develops. *Plethodon neomexicanus* produces few eggs for its length and has evolved one of the largest eggs (6.9 mm in diameter) in the genus *Plethodon.* In Reagan's study no females with enlarged follicles were found after the first week of August. In my study gravid females at the beginning of the active season had ovarian eggs that averaged 1.9 to 2.5 mm in diameter, and the diameter increased to 3.6 mm by late August. Mating occurs in late July and August and probably takes place above ground. No gravid female was found after 20 August.

Forty-one females in this study show that gravid females disappear from the surface by mid-August, and this is supported by the data from adult females in Reagan's study. Gravid females mate in July and August, and it appears that *P. neomexicanus* deposits its eggs underground between mid-August and the following spring. An August oviposition would place hatching in the fall after the active season. A spring oviposition may be more likely and this idea is supported by the fact that a female collected in August and maintained at 10 C in the laboratory deposited her clutch in early June.

Plethodon neomexicanus appears to oviposit every other year. If this is so, we should expect a little less than half the adult females collected to be gravid. A little more than half the females collected would include young females just maturing and older females that had oviposited the previous year. Of the adult females collected, 39% were considered gravid.

Female reproductive cycle in Aneides hardii.—Females not gravid have about 10 ovarian eggs over 1 mm in diameter but only about half are deposited. The shorter active season does not permit enough energy accumulation for maturation of all the eggs as does the active season of *P. neomexicanus.* At the beginning of the active season, gravid females have ovarian eggs that have a diameter from 2.6 to 3.2 mm and the eggs increase to an average of 3.5 to 4.3 mm in diameter by early September. Much of the energy is probably supplied by absorbing other eggs and concentrating reproductive effort into a few eggs. *Aneides hardii* produces one of the smallest clutch sizes and one of the largest eggs in any species of *Aneides.*

Mating probably occurs underground between active seasons. No male collected during the active season had his vasa deferentia packed with sperm, and no female collected during the active season had a sperm-filled spermatheca. Thus it is concluded that during the active season no mating occurred.

Aneides hardii takes at least two active seasons to accumulate sufficient yolk for oviposition. From my data it appears that three active seasons may required; females may lay eggs every 3 years. Gravid females do not mate during the active season and deposition of eggs occurs at the beginning of the next active season. Six clutches have been found in July and August, with the August clutches in advanced stages of development. Females brood their eggs and do not begin feeding until late July or August. In *P. cinereus* Sayler (1966:189) states that 1 year is required for eggs to reach a diameter of 1.3 mm, and the species appears to have a number of ova comparable to those of *A. hardii.* Spent females of *A. hardii* begin feeding toward the end of the active season and are not able to add much yolk to developing ova. Twelve of 30 females collected toward the end of the active season contained ova with diameters less than 1.0 mm. At the beginning of the next active season, the recently spent females still have ova diameters less than 1.5 mm and would appear to require this next active season to develop 10 ova to diameters of 2.5 mm. Still another active season would bring eight ova close to 4.0 mm in diameter. By similar reasoning as for *P. neomexicanus,* if females of *A. hardii* oviposit every 3 years, less than 33% of the adult females collected would be gravid. In this study 24% of the adult females collected were considered gravid.

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LITERATURE CITED

Johnston, R. F. and G. A. Schad. 1959. National history of the salamander, *Aneides hardii.* Univ. Kansas Pub. Mus. Natur. Hist. 10:573-585.

Lowe, C. H. 1950. The systematic status of the salamander *Plethodon hardii,* with a discussion of biogeographical problems in *Aneides.* Copeia 1950:92-99.

Reagan, D. P. 1972. Ecology and distribution of the Jemez Mountain salamander, *Plethodon neomexicanus.* Copeia 1972:486-492.

Sayler, A. 1966. The reproductive ecology of the red-backed salamander *Plethodon cinereus* in Maryland. Copeia 1966:183-193.

Schwartz, A. 1955. A clutch of eggs of *Aneides hardyi* (Taylor). Herpetologica 11:70.

Stebbins, R. C. and W. J. Riemer. 1950. A new species of plethodontid salamander from the Jemez Mountains of New Mexico. Copeia 1950:73-80.

Taylor, E. H. 1941. A new plethodont salamander from New Mexico. Proc. Biol. Soc. Washington 54:77-79.

Wake, D. B. 1965. *Aneides hardii,* p. 17.1-17.2. *In* W. J. Riemer (ed.), Catalogue of American Amphibians and Reptiles. Amer. Soc. Ichthol. Herpetol., Kensington, Maryland.

————. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. Southern California Acad. Sci. 4:1-111.

Williams, S. R. 1972. The Jemez Mountain salamander, *Plethodon neomexicanus,* p. 118-127. *In* Symposium on rare and endangered wildlife of the Southwestern United States. New Mexico Department of Game and Fish, Santa Fe, New Mexico.

————. 1973. *Plethodon neomexicanus,* p. 131.1-131.2. *In* W. J. Riemer (ed.), Catalogue of American Amphibians and Reptiles. Amer. Soc. Ichthol. Herpetol., Kensington, Maryland.

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