Age Distribution and Longevity in the Ramsey Canyon Leopard Frog, *Rana subaquavocalis*

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ABSTRACT.—Phalangeal elements from 80 individuals from two populations of the Ramsey Canyon leopard frog, *Rana subaquavocalis*, were sectioned, stained and then examined to estimate individual ages and to determine population age structure and growth curves. Large adults from the Ramsey Canyon population exceeded 100 mm in body length and had as many as 10 resting lines indicating that they were 11 years postmetamorphosis. In contrast the majority of the 38 specimens from the Barchas Ranch population were small, under 70 mm in body length, and were five or less years old. Growth curves for both populations were constructed based on body size and the number of resting lines in bone sections. Growth rates for males in the Ramsey Canyon population are lower than females. The population age structure revealed in this study has implications to conservation efforts. Our findings suggest that the Ramsey Canyon population was composed largely of reproductive and very old reproductive individuals whereas at the time of analysis the Barchas Ranch population was larger and contained mostly prereproductive individuals.

During the summer of 1988 large ranid frogs were discovered in Ramsey Canyon in the Huachuca Mountains, Cochise Co., southeastern Arizona. They were subsequently recognized as a new species, *Rana subaquavocalis*, by Platz (1993). Currently they are known to occur only from this small mountain range, making it the geographically most restricted species among the seven described leopard frogs in the United States. Until recently they existed in natural plunge pools created by water flow along Ramsey Creek at an elevation of approximately 1600 m. However, most of the Ramsey Canyon population now inhabit a 15 x 15 m man-made concrete tank adjacent to the creek (Fig. 1, Site 1). Subsequent survey work uncovered a second breeding population on the Barchas Ranch (Fig. 1, Site 3), less than 6 km away. At the time of this study, a total of four localities (Fig. 1) were known to have adults, but only the two indicated above have a sustained history of successful breeding.

Adults at the Ramsey Canyon study pond (Site 1) are large compared to most species of leopard frogs. Some females reach 120 mm snout-urostyle length (SUL) and males, although smaller, often reach 90 mm and sometimes 100 mm SUL. Amphibians appear generally to have indeterminate growth (Duellman and Trueb, 1986) although the rate decreases greatly after sexual maturity. The Barchas Ranch population (Site 3) appeared to consist of much smaller individuals, and observations in June–July of 1992 indicated that tadpoles all metamorphosed at this time, in contrast to the repeated observation of tadpoles overwintering at the Ramsey Canyon site (Platz, unpubl. data).

The large body size of adults at Ramsey Canyon suggested that they might be quite old because of indeterminate growth at a low rate. This question, the smaller body sizes at the Barchas Ranch, along with the limited distribution and very small population size (an estimated number of fewer than 100 adult individuals) prompted the present study to determine the age structure of these two populations. Small population size and limited sites and additional evidence of recent declines in other leopard frog species in Arizona (Clarkson and Rorabaugh, 1989; Sredl and Howland, 1994) make obtaining population age structure and longevity data critical issues.

METHODS AND MATERIALS

Ramsey Canyon frogs (N = 42) were hand captured on 27 April and 25 May 1990 and 8 July 1991 to obtain toe samples. Toes were obtained from the Barchas Ranch population (N = 38) on 8, 16, and 21 June 1995. Individuals were sexed on the basis of the presence of well-developed external vocal sacs (only in the case of animals larger than 80 mm SUL) and measured for body length (SUL) from the tip of the snout to the base of the urostyle and recorded to the nearest mm. The distal two phalangeal elements of the fourth toe from the right hind foot were
surgically removed, placed in 10% formalin, and stored until sectioned. Skeletochronological studies followed the protocol of Hemelaar and Van Gelder (1980). Toes were decalcified in 5% nitric acid for 5 to 6 and left in water overnight. The mid-diaphyseal region of the distal phalangeal element of each toe was then sectioned at 20 μm using a Spencer model 880 freeze microtome. The resulting sections were mounted on slides in an albumen-glycerin preparation and stained in Cabisco's Delafield Hematoxylin for 10 min. Stained slides were examined under a compound microscope to determine the number of resting lines (RLs). Age estimates were then determined by counting RLs which appear as darker stained rings separating lighter bands of bone material. The resulting age estimates were used in conjunction with body size to examine variation in size in relation to age and to compare population age structure between the Ramsey Canyon and Barchas Ranch sites. To compare populations, growth parameters (von Bertalanffy, 1938) were fitted to growth curves using the nonlinear procedure utilizing the Powell variant of the Levenberg-Marquardt methodology (Micromath Scientist, 1995).

The equation form used was: \[ S_t = S_{max} - (S_{max} - S_0)e^{-k(t-t_0)} \], where: \( t \) = age estimate in years, \( t_0 \) = age at metamorphosis, set at 0.5 yr, \( S_0 \) = size at age \( t \) (in mm), \( S_{max} \) = asymptotic maximum size, \( S_n \) = size at age \( t_n \), \( k \) = growth coefficient. Population census data from 1990 through 1995 were examined to determine trends at the Ramsey Canyon site.

**RESULTS**

Age estimates based on the number of RLs were obtained from preserved toes of 22 adult males and 20 adult females from Site 1. The results from Site 1 (Fig. 2 A–B) indicate that adults may reach 10 yr of age postmetamorphosis. Males (Fig. 3) ranged from 2 to 10 yr postmetamorphosis. Eleven of 22 (50%) were five or more years postmetamorphosis. Female adults (Fig. 3) ranged in age from 3 to 10 yr postmetamorphosis and a larger proportion of the female adults, 15 of 20, (75%) reached or exceeded five years postmetamorphosis. Both males and females vary substantially in body size within most age groups. Age estimates (Fig. 4) based on phalangeal sections from 38 Barchas Ranch specimens (Fig. 1, Site 3) revealed a population composed mostly of small individuals. Body length measurements ranged from 40 to 110 mm SUL. As was the case for Site 1, the age classes at Site 3 showed considerable range in body size and overlap among adjacent age groups precluding the use of SUL values as a good indicator of age.

Age classes were plotted for both sexes from Site 1 along with all individuals from Site 3 (Fig. 5). Seventy-eight percent of those from Site 1 were age four or more years postmetamorphosis. Males were best represented among the 3 to 5 yr age classes while females were better represented among the 6, 7, and 8 year classes. In contrast, at Site 3, 87% were age 1 to 4 yr postmetamorphosis. The two common age classes were almost evenly split between 2 and 4 yr postmetamorphic individuals. Mean age class sizes were used to determine growth curves (Fig. 6) for male and female individuals from Site 1 and for all individuals from Site 3. Growth rates appear to be higher at Site 1 compared to Site 3. At Site 1 the growth trajectory differed between the sexes with females growing on the average to larger sizes at a given age. This trend is apparent as early as age 2. Although we were unable to sex individuals from Site 3, the composite of all individuals produced a curve which fell below that of both males and females from Site 1.

**DISCUSSION**

Among the 42 adults from Ramsey Canyon, 47% were age 6 yr or older including one male and one female with 10 RLs, confirming that large adults in this population are quite old for leopard frogs. This is in sharp contrast to LeClair and Castanet (1987) for *R. pippens*. The majority of their specimens (77%) were in the 1 or 2 yr age categories (\( k = 1.4 \) yr). Only one among the 52 individuals in their study lived to be 4 yr and another to be 5 yr old. Adult *R. subaquavocalis* from the Ramsey Canyon site have a
Fig. 2. Cross section of phalangeal elements of a R. subaquavocalis. Fig. 2A represents individual 4/27/90-22, 90 mm SUL, male from Site 1; 2B represents AMNH 136099, 116 mm SUL, female from Site 1. Fig. 2C represents individual 6-16-95-10, 59 mm SUL, from the Barchas Ranch (Site 3); 2D represents individual 6/16/95-9, 60 mm SUL, from Site 3. Numbered arrows identify resting lines (RLs) indicating the number of years postmetamorphosis.

Mean postmetamorphic age of 4.95 yr and thus an average longevity 3.5 times that of R. pipiens from LeClair and Castanet's study. Even if young of the year (RLs = 0) are removed from their data (N = 10), the mean longevity is still approximately 1.5 yr.

Our findings are pertinent to the life history of R. subaquavocalis. Numerous egg masses were laid at the Ramsey Canyon site during the spring and summer of 1995 (Platz, unpubl data). By 23 June the total was 19 clutches. All of the 16 adults occupying this site exceeded 85 mm SUL. Repeated visits to the Barchas Ranch site failed to record any evidence of breeding activity through 23 June of the 1995 breeding season. All but four individuals at this site were under 70 mm SUL. It therefore seems likely that animals do not generally reach sexual maturity before 6 yr postmetamorphosis. LeClair and Castanet (1987) reported that R. pipiens mature at 2 yr of age, which is consistent with Merrell's findings (1977) for fairly dense populations of this species in Minnesota. Both of these studies found that individuals with body sizes as small as 46 mm to 50 mm SUL were sexually mature. Thus sexual maturity is reached much later in life for R. subaquavocalis when compared to R. pipiens. Growth rates are much lower in R. subaquavocalis compared with LeClair and Castanet's population of R. pipiens. Of those at Barchas Ranch with 5 RLs (N = 4) the mean was 66.5 mm SUL which is essentially the same body length for their 2 yr old R. pipiens (LeClair and Castanet, 1987). It seems clear therefore that R. subaquavocalis not only mature at a much later age than R. pipiens but also grow more slowly. Our findings for R. subaquavocalis are also quite different from another North American rand of somewhat larger body size, R. catesbeiana. Turner (1960) reported that individuals from New
York populations may reach 100 mm at the end of their second postmetamorphic season, and Howard (1978) has reported that females are sexually mature at 3 yr postmetamorphosis.

Studies which compare body size with age estimates using skeletochronology often show a positive correlation. However some do not and positive correlation may be true for one sex and not the other. Hemelaar (1983) reported positive correlations for Bufo bufo for both sexes as did Ryser (1988) for R. temporaria. Höglund and Säterberg (1989) found positive correlation between body size and age for female B. bufo but not for males. Studies utilizing mostly or only males have also shown positive correlations including those of Leclair and Castanet (1987) for R. pipiens, and Lykens and Forester for Pseudacris crucifer (1987). Platz and Lathrop (1992) found no correlation in their study of P. maculata and P. triseriata. Although the majority of studies do show positive correlation, some are weak and typically size overlap among age classes is too extensive to permit the use of body length as an indicator of age. However, Daugherty and Sheldon (1982) reported reasonably distinct size classes among younger cohorts of Ascalaphus truei.

The growth form is similar for males and females (Fig. 6) at the Ramsey Canyon site until about age 1 to 2 yr but beyond this for a given age females are larger on the average than males. Our findings are quite similar in this respect to those of Ryser (1988) for R. temporaria. Data reported by LeClair and Castanet (1987)
for *R. pipiens* are suggestive of this same trend although their study contained only a small sample of females.

Although the sample size is small, there is a marked range of body sizes of frogs from Barchas Ranch having 1 resting line. This may reflect either of two conditions: (1) nutritional and/or genetic differences which are manifested during the larval or premetamorphic period of life (Halliday and Verrell, 1988), or (2) bone remodeling (LeClair and Castanet, 1987). Resorption of endosteal bone eradicates inner layers, reduces the number of apparent RLs and results in an under reporting of age estimates. The uneveness of the medullary cavity representing a 2 yr old (Fig. 2C) is indicative of this condition. Endosteal bone resorption could, therefore, in the present study, increase the range of body sizes attributed to 1 yr old individuals. This phenomenon has been documented in a number of amphibians. What portion of a population undergoes enough resorption to eliminate one or more RLs varies considerably among species. LeClair and Castanet (1987) review bone resorption and report the frequency in *R. pipiens* at 17% for their study, although it was reported by Regnier (1983), in LeClair and Castanet (1987), to be as high as 72% in 3 yr old *R. lessonae*.

Evidence of recruitment into the Ramsey Canyon population has been minimal for the last six years (Platz, unpubl. data). No juveniles were seen during intensive field work at this site in the spring and summer of 1995. Among specimens toe clipped in 1990 and 1991 only four individuals were encountered (not included in graphs) which, on the basis of body length (52–57 mm SUL), would be considered to be juveniles. In contrast Site 3 was composed of mostly young animals of smaller body size suggesting that recruitment had been substantial over the past four years.

Our findings have implications to current and future conservation efforts. Over the past six years the population numbers at the Ramsey Canyon location have dropped each year (Fig. 7) from an initial census of more than 90 individuals to a total of 18 adults and one subadult (Platz, unpubl. data). Nightly surveys during the spring and summer of 1995 indicated 16 adults resident in the Ramsey Canyon pond; an additional two adults and one subadult were known to reside in the lower reach of Ramsey Creek. Reports by Hoven Riley (pers. comm.) from the Ramsey Canyon Preserve headquarters confirm that all 19 were in the pond at Site 1 by September 1995.

Based on our age profiles, most (if not all) of the frogs in this population are now of reproductive age and many are in their last years with no evidence of natural recruitment from overwintering tadpoles for the last two years. From a genetic standpoint this population has to be considered small with all of the concomitant implications resulting from erosion of genetic variability through genetic drift and the risk of local extinction as adults die.

The Barchas Ranch population was the larger of the two sites by late summer of 1995, (an estimated 50 individuals; 38 were toe clipped). However, genetically it should also be considered small. As a largely prereproductive population, if survivorship among Barchas Ranch individuals were to remain high, then within two years there should be a considerable number of tadpoles and new metamorphs. If survivorship remains low, then this population would be in more jeopardy in terms of numbers of sexually mature adults in the next two years than the Ramsey Canyon site. Neither of these scenarios address two critical issues which remain unknown: (1) intraspecific competition for food resources which are finite but have not been studied; (2) the risk of loss of genetic variability over time through genetic drift and inbreeding making them more vulnerable to disease and other harsh changes in the environment. After completion of this manuscript Eric Wallace (pers. comm.) indicated to us that the duck pond (Site 3 in this report) on the Barchas Ranch which contained the young frogs went dry in early summer of 1996. Even though the pond was replenished with water later in the summer, repeated visits have so far failed to locate any of the young individuals marked during our work.

In summary sexual maturity in *R. subaratovalis* is delayed considerably compared to other ranid examples and growth rates are low. Based

![Figure 7](image-url)  
**FIG. 7.** Census data from the Ramsey Canyon pond population from 1990 to 1995. Numbers in parentheses represent spring and summer residents (N = 16) and 3 additional individuals which spent the summer in Ramsey Creek, and returned to the pond in early fall.
on the total number of egg masses (N = 28) seen at Site 1 over the entire breeding season of 1995 and the body sizes of residents, the Ramsey Canyon population was by that time mostly if not totally composed of sexually mature individuals. On the other hand three observations suggest that the Barchas Ranch population was primarily composed of prereproductive individuals: (1) small body size; (2) the absence of secondary sexual characters (swollen thumb pads; evidence of vocal sacs) among small individuals but normally seen in large, mature males; (3) the fact that there was no evidence through late June 1995 of any breeding activity that season.

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