

DRAFT FINAL REPORT

Grant-in-Aid Project

Status Survey of the Ramsey Canyon Leopard Frog,

Rana subaquavocalis

by

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Project No. I94051

DISCLAIMER

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Site 1 is the type locality 7 km southwest of Sierra Vista (see Platz, 1993). Almost all of the frogs reside in a ground level concrete tank (15 x 15 m) and approximately 1.4 m deep. The tank is fed by an upstream pipe which brings water from Ramsey Creek down to the pond. Water from the 2-inch iron pipe can be supplemented under low flow conditions by output from a second pipe from an independent nearby spring. The pond outflow is directed back into Ramsey Creek over a small spillway located at the east end of the north wall of the tank. The pond experiences limited direct sunlight except for the summer months and cool water temperatures including temporary ice cover in some years in the coldest months of winter (mid-January to mid-February). Large sycamore trees (*Platanus wrightii*) shade the banks of the pond and provide a periodic source of fallen branches and leaf litter which accumulate on the bottom. The water is extremely clear except for seasonal (summer) filamentous algal blooms seen in some years and not others. No fish or other ranid frogs inhabit this site.

Site 2 is in the upper Brown Canyon to the northwest of Ramsey Canyon. The region known as "the Upper Box" is a plunge pool approximately 3 x 2.5 m and over 2 m deep. Although rather small in volume, it has had water in it each year that it has been examined since 1990. No fish or other ranid frogs inhabit this site.

Site 3 is a large earthen stock tank on the Barchas Ranch just below the entrance to Brown Canyon. It is approximately 30 m in diameter; shallow along the perimeter grading gently to a maximum of 1 to 1.5 m in depth near the center. It is fed by pipeline from a spring in the canyon and also by a well which is wind powered. The tank is rich with submerged aquatic plant life (*Chara* sp.) and during the warmer summer months accumulates some filamentous algal mat.

RAMSEY CANYON LEOPARD FROG -PART I

No fish or other ranid frogs inhabit this site, although *Hyla arenicolor* is known to inhabit rocky portions of the the upper canyon.

Site 4 is a small pool approximately 2 m in diameter, but quite deep (> 1 m) and is the result of earlier efforts to construct a mine shaft. It has fluctuated in water level to some extent (< 0.5 m) but has not gone dry since it was discovered in 1991 suggesting that it is near the water table level and is natural seepage. Because of its rocky nature, it is devoid vascular aquatic vegetation which often surrounds ponds and has not, during the survey time frame, had any filamentous algal mat. Despite these limitations it is frequented by numerous flying insects seeking water. It is devoid of fish and any evidence of other anuran species.

Site 5 represents Tinker Pond on the Fort Huachuca military base was confirmed as a locality in October 1994. Like the Barchas Ranch pond it is an earthen depression, in this case a water catchment basin, which traps and holds seasonal runoff. It has fluctuated in recent years in size greatly and can be at times the largest of the known sites both in terms of diameter (> 70 m) and depth (greater than 2 m). This tendency to fluctuate greatly in size is particularly evident this year which has been unusually dry. According to Sheridan Stone (pers. comm.) 150,000 gallons of water were trucked in during June 1996 to sustain it as aquatic habitat. On 7 May of 1996 it measured only 22.3 x 11.1 m (Eric Wallace, pers. comm.) At the present time, there is no significant shore vegetation and little is known about algal conditions. It was surveyed several times during 1994-96. Ramsey Canyon leopard frogs (160 most less than 50 mm SUL) were observed in July of 1995 by AZG&F staff. A bullfrog, but no fish were reported.

Anecdotal reports over the past five years (Tom Wood, pers. comm.) indicate that periodically an unidentified ranid frog, but probably not *R. catesbeiana*, has been seen in

Miller Canyon to the south of Ramsey Canyon. Most recently an unidentified species of leopard frog has been reported from a small yard pond (2 x 3 m) at a residence in south Sierra Vista 1.3 km east of the Barchas Ranch site (M. Sredl, pers. comm.). Neither of these two localities has been confirmed by the author. However a recent census by Eric Wallace (pers. comm.) indicated 8 individuals greater than approximately 85 mm snout urostyle length (SUL) were present at the Sierra Vista residential locality along with about 350 tadpoles of three different size ranges and two which were large enough to consider them to perhaps represent overwintered individuals. The general description of the frog and its close proximity to the Barchas Ranch make it very likely that they are *R. subaquavocalis*.

An additional population of leopard frogs of unknown identity exists in Leslie Creek, Cochise Co., east of the Huachucas. These were identified and verified to species during the course of this project and do not represent *R. subaquavocalis* and are discussed in detail in part IV of this report.

Site 1 has been monitored every year since 1990 and surveyed at least once a year and in the last three years at different times of the year. These efforts include counts of all postmetamorph sizes made at night using headlamps to obtain "eye shine" counts. Currently there are 19 postmetamorphs most of which are over five years old. Through the breeding season of 1994, 16 of these maintained residence in the concrete pond. Three others (two adults and one subadult) occupied a small plunge pool (2.0 x 0.8 m) just below the first foot bridge to the guest cabins at the Ramsey Canyon Nature Preserve. They remained there until September of that year and then joined the others at the concrete pond for the winter.

RAMSEY CANYON LEOPARD FROG -PART I

Site 2 has produced tadpoles at one time (summer 1991) but no juveniles were subsequently seen. Frogs in this pond are large, wary adults and submerge if approached. Early surveys indicated two or perhaps three individuals greater than 100 mm SUL. Eric Wallace (pers. comm.) reported sighting only one on 8 May 1996.

Site 3 was, as of the last survey in this study by the author, the largest population, at least in terms of total metamorphs. At last census in mid June of 1995, 38 individuals had been marked all but four of which are currently subadults and juveniles (under 75 mm SUL). Events of late spring and early summer of 1996 after the end of this study suggest that some or all of the members of this population may have died. Extremely dry weather and low water table caused the duck pond to dry out briefly. Eric Wallace (pers. comm.) noted only a muddy area near the center of the pond and no evidence of frogs on 13 June 1996. Even on his earlier survey on 7 May 1996 when some water still existed, there were no frog sightings. Examination of the south pond near the house did not reveal frogs either. It is still possible that some have survived and simply were not seen at either pond. Part II of this report contains details of the age structure and reproductive potential of this site.

During July of 1992, juveniles were observed in a temporary catchment basin on the Barchas Ranch to the north of the main dirt road near the east entrance. None were seen later in July nor at any time since then. Because water here is very shallow and of an extremely temporary nature, it is not listed as a permanent breeding site.

Site 4 has supported 3 to 5 large subadults from the time of discovery in 1991. In October of 1994 hatchling larvae from an egg mass were documented. None were seen as juveniles at later points in the spring and summer of 1995 and it has not been surveyed yet in 1996.

Site 5 supported reproduction in 1993 apparent from the October 1994 survey when a total of approximately 50 or so overwintered tadpoles were observed. One was subsequently reared through metamorphosis and verified to be a leopard frog. Census reports (Eric Wallace, pers. comm.) on 7 May 1996 and 11 June 1996 indicated 21 and then 34 large individuals respectively.

Populations at Sites 1 and 3 during the time frame of this investigation were the only well-established breeding populations. As of early July 1996 Site 5 may now be the largest one in terms of reproductive individuals followed by Site 1. The Barchas Ranch population (Site 3) will require continued close monitoring to determine whether or not it still represents an extant locality. Section II of this report provides age structure and growth form details for Sites 1 and 3.

Aquatic sites in Scotia Canyon were revisited three times during the course of this project (both at night and during the day), Bull frogs were observed on each occasion but no leopard frogs were seen during any of these visits. Sections of Bear Canyon south of the road leading to Nogales, Arizona were visited once during the course of this project. Examination of the plunge pools and stream did not reveal leopard frogs. Both of these localities represent "follow up" visits to earlier ones during the summers of 1990, 1991 and 1992, none of which turned up evidence of leopard frogs.

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- Platz, J. E. 1993. *Rana subaquavocalis*, a remarkable new species of leopard frog (*Rana pipiens* complex) from southeastern Arizona that calls under water. J. Herpetology 27(2):154-162.

II. Population Census and Age Structure

Introduction— 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 U. S. Until recently they existed in natural plunge pools created by water flow along Ramsey Creek at an elevation of approximately 1600 meters. Most of the Ramsey Canyon population now inhabit a man-made concrete tank 15 x 15 m adjacent to the creek. Subsequent survey work uncovered a second breeding population on the Barchas Ranch, less than 6 km away. At the time of this study, a total of five localities (See Part I, 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 site are large compared to most species of leopard frogs. Some females reach 120 mm snout-urostyle length (SUL) and males, although generally smaller, often reach 90 mm and some 100 mm SUL. Amphibians thus far studied appear to have indeterminate growth (Duellman and Trueb, 1986) although the rate decreases greatly after reaching sexual maturity. The Barchas Ranch population 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 well-documented phenomenon of tadpoles overwintering at the Ramsey Canyon site.

RAMSEY CANYON LEOPARD FROG - PART II

The large body size of adults at Ramsey Canyon suggested that they might be quite old because of indeterminate growth or alternatively were young and experienced rapid growth. This question, the smaller body sizes at the Barchas Ranch, along with the limited U. S. distribution and very small population size (an estimated number of less than 100 adult individuals) prompted the present study to determine the age structure of these two populations. Both individual longevity and population structure are particularly important given the evidence (Clarkson and Rorabaugh, 1989, Sredl and Howland, 1994) of leopard frog declines in Arizona.

Methods and Materials —

Individuals were hand captured, sexed (only in the case of animals larger than 80 mm SUL) and measured for body length (SUL) recorded to the nearest mm. The distal two phalangeal elements of the fourth toe from the right hind foot were surgically removed and 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 hours and left in water overnight. The mid-diaphyseal region of the distal

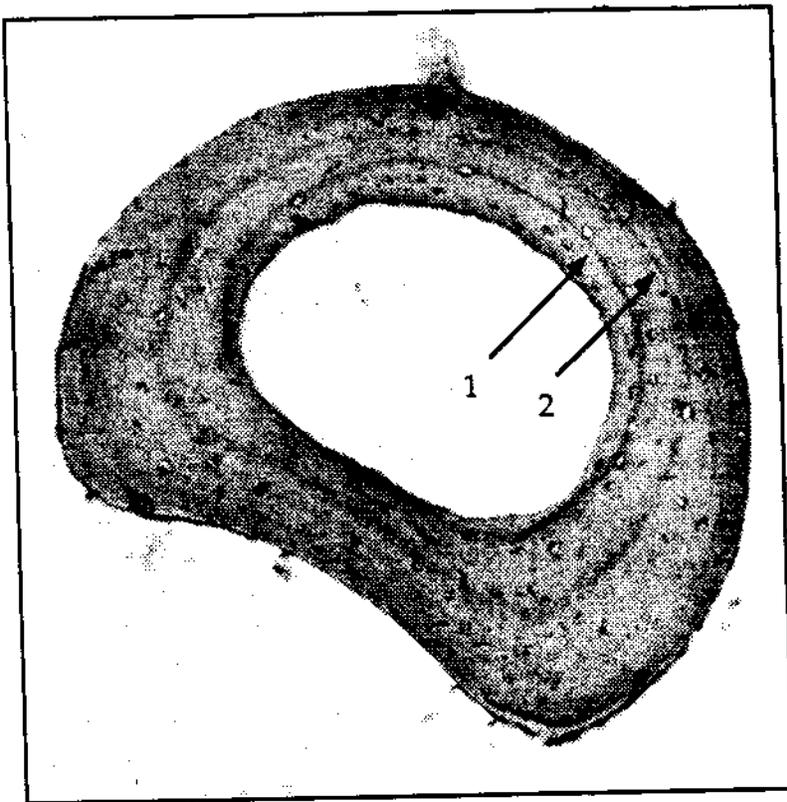


Fig. 1. Cross section of phalangeal element of a juvenile *R. subaquavocalis* from Barchas Ranch. Numbered arrows indicate RL 1 and 2 indicating that this individual is in its third postmetamorphic year.

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 minutes. Stained slides were examined under a compound microscope to determine the number of resting lines (RL). Age estimates were then determined by counting RL which appear as darker stained rings separating lighter bands of bone material (Fig. 1).

Results— Age estimates based on the number of RL were obtained from preserved toes representing 22 adult males and 20 adult females from Site 1. The results (Figs. 2 & 3) indicate adults may reach 11 years of age (10 RL + one year overwintering as tadpoles). Males (Fig. 2) ranged from two to ten years post metamorphosis. Eleven of 22 (50%) were five or more years postmetamorphosis. As Figure 2 indicates, there is a positive relationship between body size and number of RL ($r = 0.68$; $P = 0.002$).

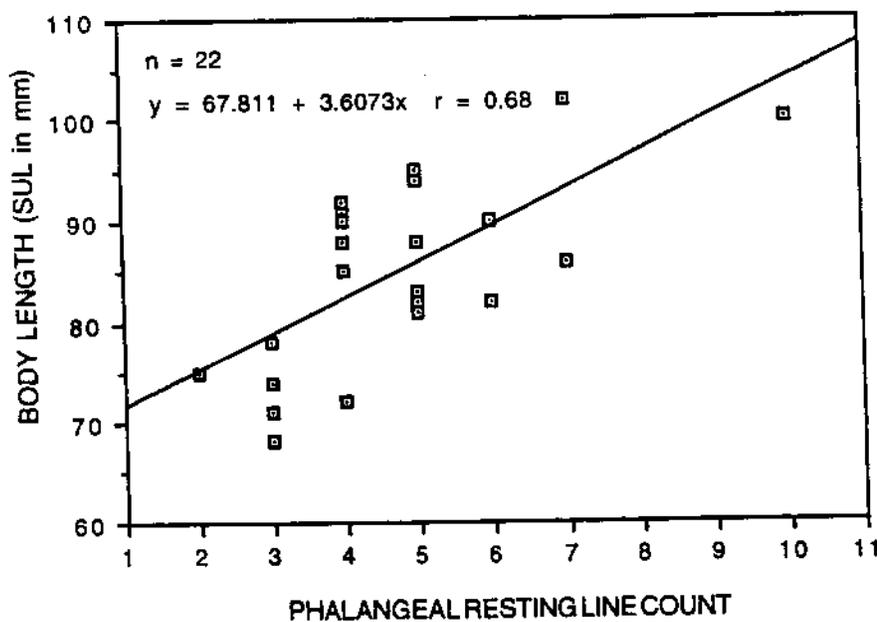


Fig. 2. Regression analysis of body length against number of phalangeal RL for 22 adult males from the Ramsey Canyon population from 1991-92 samples.

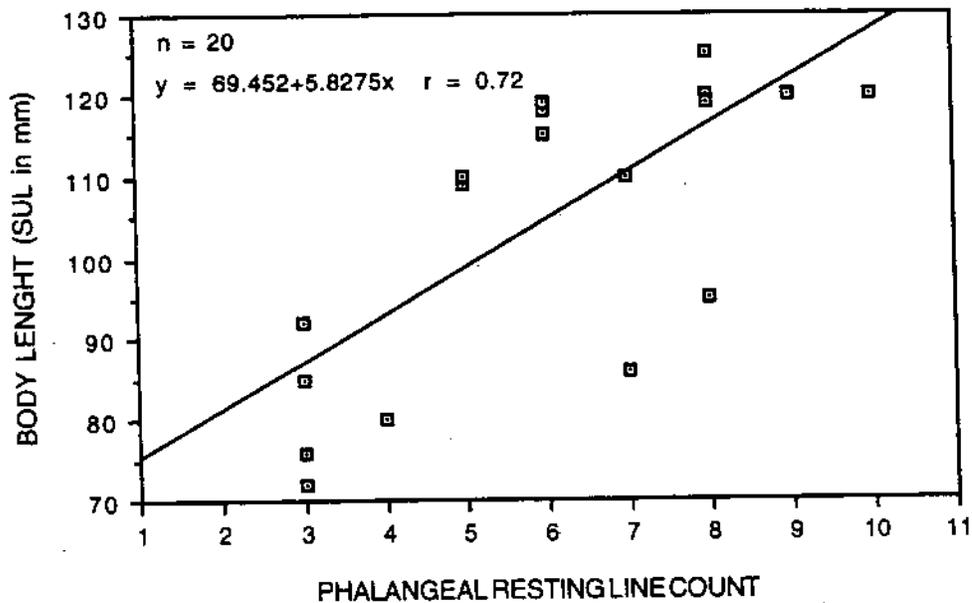


Fig. 3. Regression analysis of body length against number of phalangeal RL for 20 adult females from the Ramsey Canyon population from 1991-92 samples.

Female adults (Fig. 3) ranged in age from 3 to 10 years and a larger proportion of the female adults, 15 of 20, (75%) reached or exceeded 5 years postmetamorphosis. There is also a significant positive relationship between body size and age estimates for females ($r = 0.72$; $P = 0.003$) and as with males as well there was substantial variation in body size within any given age group precluding the use of SUL values as a good indicator of age. For example, among males a body size of approximately 85 mm SUL represents individuals as young as 4 and as old as 7 years postmetamorphosis.

To determine whether the growth form of males and females differed from each other data from the toes preserved in the summer 1990 ($n = 20$ males and 20 females) were compared. A t test of the slopes indicated that they were not significantly different ($df = 36$; $0.8 > P > 0.7$)

Age estimates (Fig. 4) based on phalangeal sections from 38 Barchas Ranch specimens (Site 3, see Part I, Fig. 1) revealed a population composed mostly of small individuals. Body length measurements ranged from 40 to 110 mm SUL with a mean of 60.6 mm. Again there was a strong positive relationship between SUL and the number of RL ($r = 0.791$; $P = 0.0001$). Variation in SUL for the age class with 1 RL was particularly marked.

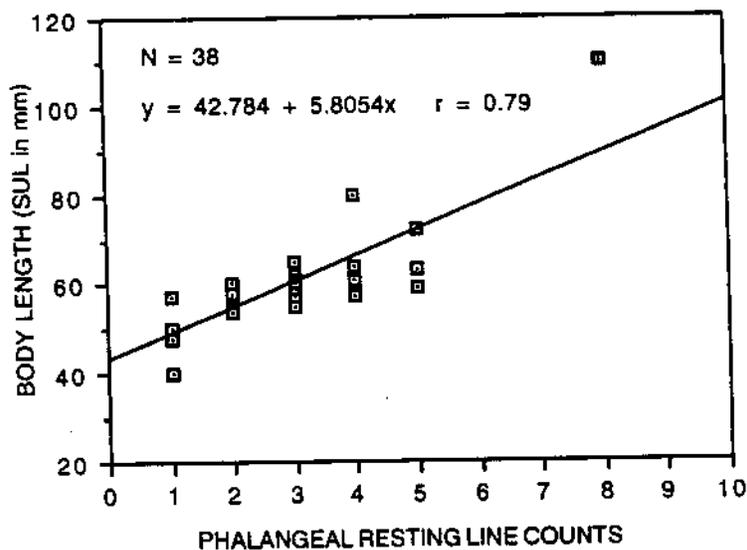


Fig. 4. Regression analysis of body length against number of phalangeal RL for N=38 individuals from the Barchas Ranch.

A t test of the slopes for the regression of body size against number of RL for the combined male and female adult data set for 1990 ($n=40$) from the Ramsey Canyon site and the Barchas Ranch data were compared and found not to be significantly different ($df = 72$; $0.5 > P > 0.3$). This suggests that despite the difference in age composition and possible ecological conditions, that the growth forms are similar for the two populations.

RAMSEY CANYON LEOPARD FROG - PART II

Discussion— Finding both male and female *R. subaquavocalis* specimens at the Ramsey Canyon site with 10 RL confirms that large adults in this population are quite old. Fully 62% of those at this site exceed 5 years postmetamorphosis. This is in sharp contrast to data published by LeClair and Castanet (1987) for *R. pipiens* in which age estimates placed the majority of specimens (77%) in the 1 or 2 year categories producing a population mean age of 1.4 years. In fact only 1 each among the 52 individuals in their study lived to be 4 and 5 years old. Adult *R. subaquavocalis* for the Ramsey Canyon site have a mean postmetamorphic age of 4.95 years and thus an average longevity 3.5 times that of *R. pipiens* from Leclair and Castanet's study. Even if young of the year (RL = 0) are removed from their data (N=10) the mean longevity is still only 1.5 years.

Additional observations 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. Every individual among the 16 adults occupying this site was in excess of 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. With the exception of 4 individuals at this site, all were under 70 mm SUL. It therefore seems likely that animals do not generally reach sexual maturity before 6 years postmetamorphosis. LeClair and Castanet (1987) reported *R. pipiens* becoming sexually mature at two years of age which is consistent with Merrell's findings (1977) for fairly dense populations of this same species in Minnesota. Therefore both of these studies indicate that individuals with body sizes as small as 46 mm to 50 mm SUL are sexually mature. The combined observations, similar growth rates for the Ramsey Canyon and Barchas Ranch sites and the fact that no breeding activities were noted at Barchas Ranch, indicate that 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*. The mean ($n=4$) of those at Barchas Ranch with 5 RL was 66.5 mm SUL which is essentially the same body length for their two year olds. It seems clear therefore that *R. subaquavocalis* not only matures at a much later age than *R. pipiens*, but also grows more slowly. These findings are also quite different from a larger North American ranid of similar 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 years postmetamorphosis.

Patterns relating body size to age estimates using skeletochronology often, but not always, show a positive correlation and it 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*, males. 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 *Ascaphus turei*.

The growth form (based on r values from the linear regression analyses) is similar for males and females at the Ramsey Canyon site but, for a given age, females are larger. This finding is similar to that of Hemelaar for *Bufo bufo* (1983). Data reported by LeClair and Castanet (1987) are suggestive of this same trend although their study contained only a small sample among females.

Although the sample size in the present study is small, there is a marked range of body sizes of those from Barchas Ranch bearing 1 RL. This may reflect one of two conditions: 1) nutritional and or genetic differences which are manifested during the larval or premetamorphic period of life; 2) the result of bone remodeling. The first of these two possibilities has been suggested previously by Halliday and Verrell (1988). If the second suggestion is true, resorption of endosteal bone would eradicate inner layers reducing the number of apparent RL resulting in an under reporting of age estimates which would in the present study increase the range of body sizes attributed to one year 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 RL 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 three year old *R. Lessonae*.

Evidence of recruitment into the Ramsey Canyon population has been minimal for the last 6 years. 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 4 individuals were encountered (not included in graphs) which on the basis of body length (52-57 mm SUL) would be considered to be juveniles. Two were 2 years and 2 were 3 years postmetamorphic, a further indication that variability in body size within age classes is substantial. This is apparent as early as age 3 at the Barchas Ranch site (Fig. 4) and also for age classes of adults seen at the Ramsey Canyon site (Figs. 2 and 3). In females, for example, individuals with 3 RL range from about 70 mm to greater than 90 mm SUL. A similar trend is apparent in older age groups and for males as well.

In *R. subaquavocalis* sexual maturity is delayed considerably compared to other ranid examples and growth rates are low. Based on the large number of egg masses seen at Site 1 during the spring and summer of 1995 and the body sizes of residents, the Ramsey Canyon population was by this time mostly if not totally composed of sexually mature individuals. On the other hand 3 observations suggest that the Barchas Ranch population was primarily composed of prereproductive individuals: 1) small body size; 2) the absence of secondary sexual characters among small individuals but normally seen in mature males; 3) the fact that there was no evidence through late June 1995 of any breeding activity that season.

Conservation Issues— Over the past 6 years the population numbers at the Ramsey Canyon location have dropped each year (Fig. 5) from an initial census of more than 90 individuals to a total of 18 adults and 1 subadult. Nightly surveys during the spring and summer of 1995 indicated 16 adults resident in the Ramsey Canyon pond;

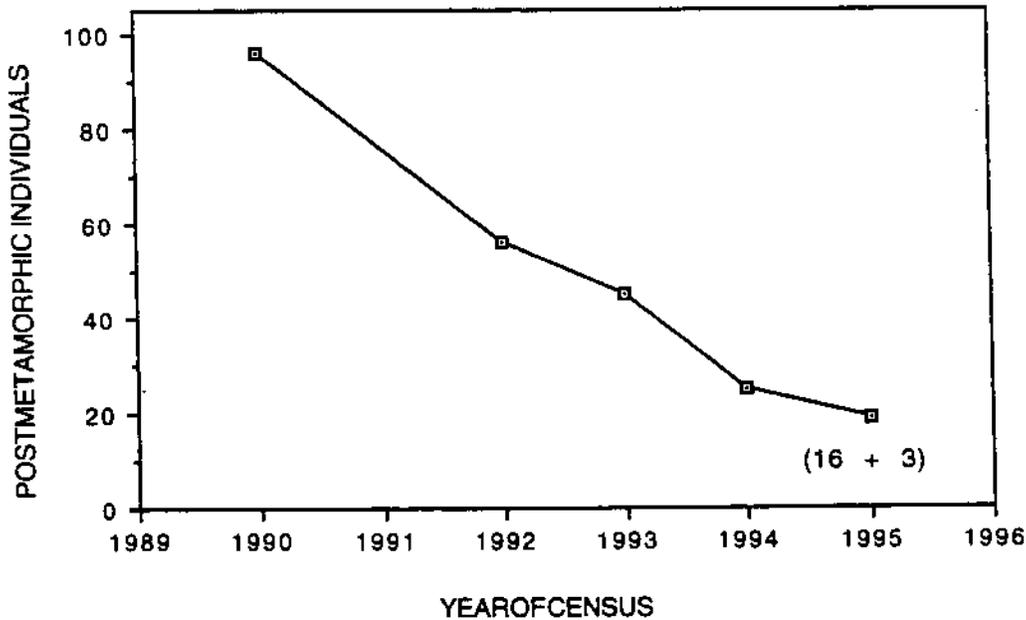


Fig. 5. Census data from the Ramsey Canyon pond from 1990 to 1995. The numbers in parentheses represent spring and summer residents (n=16) and 3 more individuals which spent the summer in Ramsey Creek, returning to the pond in early fall.

an additional 2 adults and 1 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.

The Barchas Ranch population was in late summer the largest of the 5 sites (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 remains high, then within two years there will be a considerable number of tadpoles and new metamorphs. If survivorship is low, then this population may be in more jeopardy in terms of numbers of sexually mature adults in two years time than is the Ramsey Canyon site. Neither of these scenarios address two critical issues: 1) competition for food resources which are finite but unknown; 2) the fact that over long periods of time small populations which lose genetic variability each generation through genetic drift are more vulnerable to disease and other harsh changes in the environment.

As a post log to this report Eric Wallace (pers. comm.) has indicated to me that the duck pond (Site 3 in this report) on the Barchas Ranch which contained the young frogs went dry in early summer. Even though the pond was replenished with water later in the summer repeated visits failed to locate any of the young individuals seen and marked during my work.

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III. Reproductive Biology

Introduction-- The Ramsey Canyon leopard Frog has a protracted season of reproductively related activities. Based on observations over the past 5 years at the Ramsey Canyon site males are known to begin to call as early as the end of March and continue to do so through late summer, in most seasons into August. The breeding season is essentially coincident with the calling season in that egg masses have been seen as early as the end of March in some years and continue to occur periodically into August.

Preliminary analyses of male vocalizations include four distinct call types and a rarer fifth type and are readily distinguished audibly and by oscillogram. One of these (a snore-like call) may be functionally diverse. All vocalizations are propagated while males rest on the substrate. At the Ramsey Canyon pond this is usually 1.0 to 1.4 m below the surface. Vocalizations at this depth are therefore inaudible in air. Under rare circumstances an occasional calling male is in extremely shallow water and an airborne signal is detectable but they were very weak at best. All underwater vocal signals with the exception of one type are however quite loud for leopard frogs. Acoustical measurements of calls propagated underwater were made with a mechanically coupled sound pressure level meter and easily reached 90 db at 1.0 m distance. Experimental underwater playback of recorded signals of calling males propagate to all parts of the concrete pond at the Ramsey Canyon site with little attenuation.

Methods and materials — All recordings of male vocalizations were obtained at the concrete lined pond on the Ramsey Canyon Preserve run by the Nature Conservancy. The pond is approximately 15 x 15 m with a depth of 1.0 to 1.4 m (Fig. 1). Significantly

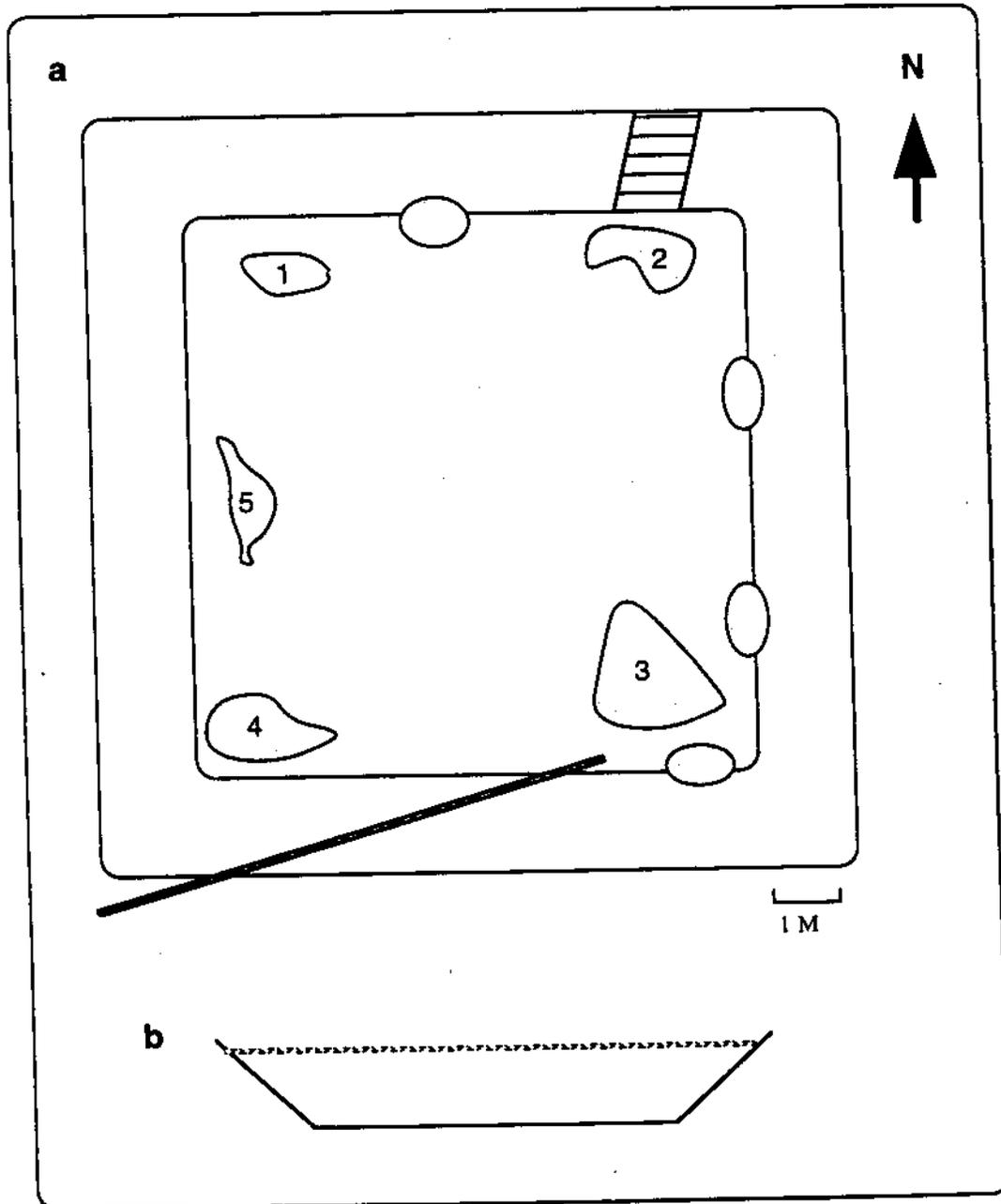


Fig. 1a. Represents an aerial view of the 15 x 15 m concrete tank in Ramsey Canyon. The numbered, shaded areas represent locations which were used for egg deposition during the 1995 breeding season. Fig. 1b. Represents a profile view of the pond and sloping walls.

the walls (Fig. 1b) slope in such a way that they tend to dampen echoes allowing exceptional high quality acoustical recordings. Male vocalizations were detected using a Sparton Electronics model PR 23 Navy hydrophone recorded on Marantz PMD 201 cassette recorders at standard tape speed (4.76 cm/s.). The hydrophone's extreme sensitivity and flat response curve through 10 kHz produced excellent recordings. All calls were subsequently digitized using a Farallon Corp. Macrecorder and either a Macintosh LC or Quadra 650 computer. The data capture rate from analog tapes was set at 22 kHz employing an antialiasing filter to prevent the misrepresentation of frequencies higher than 10.5 kHz as lower frequency components. Calls were analyzed with Sound Edit version 2.0.3 (Farallon Corp.) digital oscilloscope and sonograph software modules and Canary version 1.2 from Cornell University. All subsequent printed output of oscillograms, spectrograms and sonograms were obtained from the computer screen using Mainstay Software's Capture 4.02 software and then labeled prior to printing with Deskdraw version 3.03 from Zedcor Inc. Test comparisons made with a Kay Electrometrics model 6061 B Sonograph showed no differences in call parameters with the exception that time measurements are more easily and accurately read (digitally to 0.5 ms) from Sound Edit. In addition much weaker signals can be processed than those obtained by conventional sonograph.

Water and air temperatures were recorded to the nearest 0.1 C using a Bailey model BAT-12 telethermometer periodically during recording sessions and water temperature continuously at 4.0 hour intervals using a submersible data logger (Onset Computer Corp.). Males call very little during daytime so recording sessions began approximately at the point when full darkness had begun and routinely ran until 1 am after which calling activity decreased markedly on most, although not all, nights.

Results— Males have a vocal repertoire similar in complexity to other leopard frogs

RAMSEY CANYON LEOPARD FROG - PART III

recorded by Mecham (1971) and include snore-like calls (which may be either long or short) various abrupt sounds of short duration described by different authors as "rasps", "grinds" or "grunts". Choruses varied in size from two to five individuals. In earlier years when the population density was much higher many large males would sit silently on the bottom along the periphery and did not call.

A loud snore-like sound (Type I) is among the most prevalent offered (Fig. 2a). The call is long relative to the other types which have been recorded and quite variable

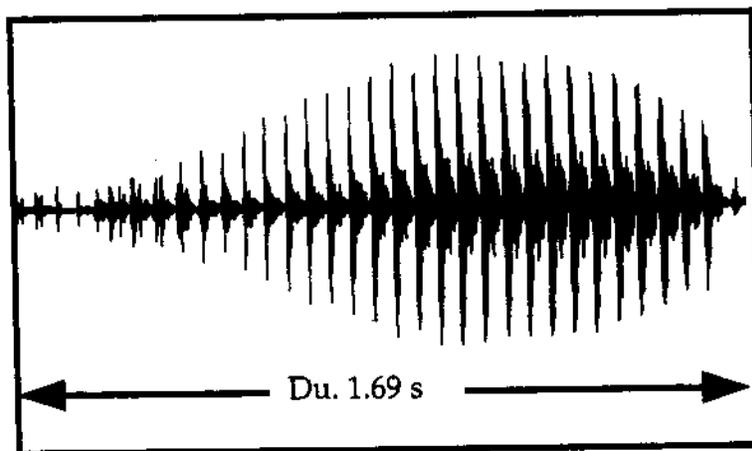


Fig. 2a. Represents an oscillogram of a typical Type I snore call recorded at the Ramsey Canyonpond at a water temperature of 15 C. Duration of the call (Du) is shown along the horizontal axis.

in length varying from 0.5 s to well over 3 s and on rare occasions approach 5 s.

Variation in duration seen in *R. subaquavocalis* has two sources.

Call duration is inversely related to temperature as it is generally in frogs (Duellman and Trueb, 1986). A second and larger source affecting call duration is present and represents intraindividual variation in call length discussed later. Type I calls are amplitude modulated and consist of a long series of pulses which rise in amplitude and then taper off toward the end of the call but more abruptly than during the rise as the call is initiated. Pulse rate varies directly with rising temperature as is the case for other

anurans thus far studied. At a temperature of 15 C the pulse rate is typically about 16 pulses/s.

Type I calls are harmonically rich (Fig. 2b) with a dominant frequency of about 1 kHz

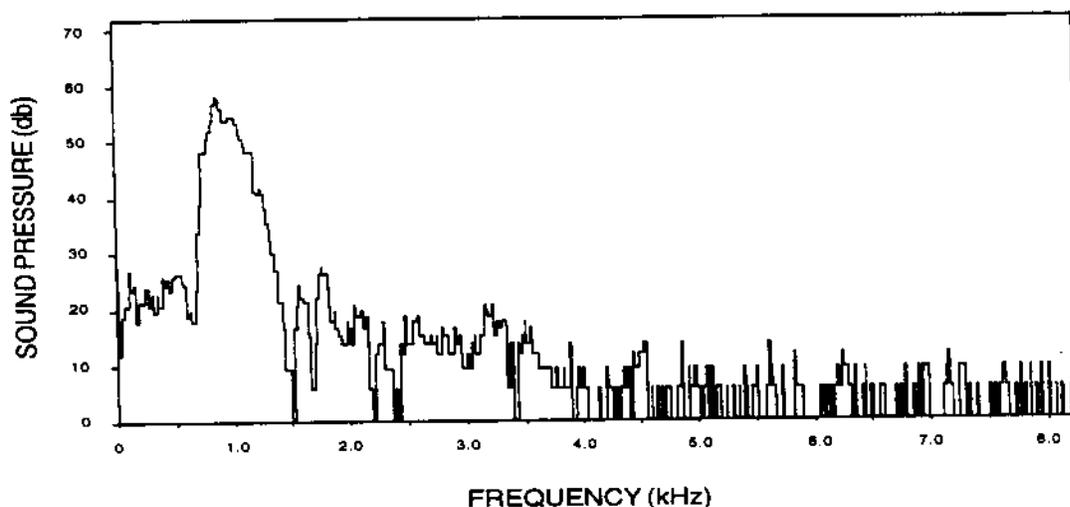


Fig. 2b. Represents a frequency spectrum of a single mid pulse from within a Type I call. Recorded at the Ramsey Canyon pond at 15 C.

but ranging downward to 0.7 kHz. Dominant frequency of calls in anurans is inversely related to body size suggesting that the lower value of 0.7 kHz represents a large male.

Although many species of anurans attempt to reduce call overlap with near neighbors (antiphonal calling), quite often the snore calls of the Ramsey Canyon leopard frog show call overlap between two males. Initial observations suggested that the vocal sacs did not inflate while males called, but observations during this study indicated that one or the other or both may inflate, although not as dramatically as in some species of ranids which routinely produce airborne calls.

Type I calls are offered at considerable amplitude. In addition to these, a less frequent but very soft version of snore has also been recorded. They are offered at a 3 to 10 fold decrease in intensity relative to Type I calls. Loud and soft snores are similar in pulse rate, but the latter are somewhat shorter in duration. The above observations, especially the greatly reduce sound intensity suggests that soft calls may be of

biological significance.

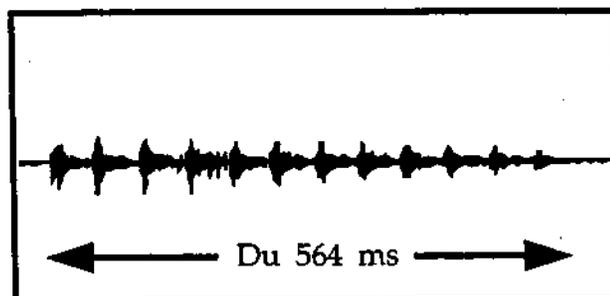


Fig. 3. Represents a Type II or soft snore call. Recorded at the Ramsey Canyon pond at a water temperature of 15 C.

Based on this possibility soft snores are here designated a Type II call (Fig. 3) and treated in more detail in the Discussion section.

Type III calls or rasps (Fig. 4) are quite common and may correspond to what McAlister (1962) called a "grind". Type III calls are consistently identifiable by oscillogram as short, abrupt sounds varying two-fold in length (0.14 to 0.31 s) at 15 C.

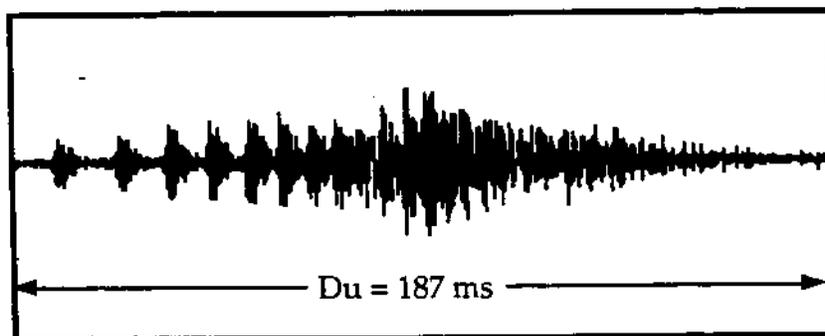


Fig. 4. Represents a Type III or rasp call. Recorded at the Ramsey Canyon pond at 15 C.

The term rasp is used to convey the sense of a higher pulse rate call than that of snores. In some (presumably smaller males and at higher temperatures), it is rapid enough to sound almost insect-like. In others with lower pitch it takes on a more duck-like "honk" and may even sound more like a short bark in some. Visual inspection of oscillograms of the leading half of the call (Fig. 4) revealed them to be composed of pulses

indistinguishable from those of the snore call and permitted counting of pulses, in some, if the call was expanded sufficiently in oscilloscope mode during analysis. In fact, a decent artificial rasp call was synthesized by repeatedly copying the same single pulse from a typical Type I snore call, adjusting it in relative amplitude, and placing each successive pulse closer to the next to match as they appear in Figure 4. Played back, the artificial and natural calls are nearly indistinguishable. This is not as apparent in lower amplitude calls which are also more insect-like, suggesting that it is not just that they were farther from the hydrophone, but may represent smaller males.

Chuckle calls, designated Type IV (Fig. 5), have been observed by others among leopard frogs in Texas (*R. blairi*) and in Arizona in *R. chiricahuensis* and *R. yavapaiensis*. Both visually in oscillograms and audibly this is a high amplitude, high energy, pulsed call. In *R. subaquavocalis*, it usually consists of a series of four pulses, but occasionally three, and on rare occasion only two are heard. Figure 5 is typical of most in that the

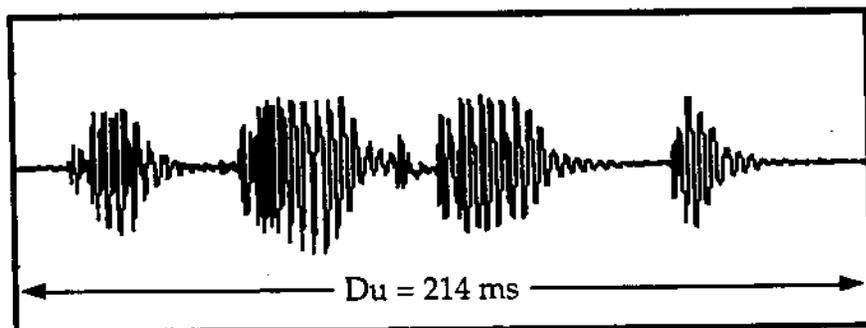


Fig. 5. Represents a typical Type IV or chuckle call. Recorded at the Ramsey Canyon pond at a water temperature of 15 C.

middle two pulses are of higher amplitude and greater duration, although both parameters vary considerably. Chuckle calls are also short, approximately 0.15 s in duration and the most highly conserved in length of the three common call types (Type I, III and IV) varying in duration by only 10 to 20 ms at 15 C. Like the rasp or Type III call, most of the high amplitude component lies near 1.0 kHz. Within the call sequences

analyzed, Type IV were the least common among Type I, III and IV.

A possible Type V call, designated as a click call (Fig. 6) is heard infrequently. It is a low amplitude call of very short duration (approximately 60 ms at 15 C). In wave

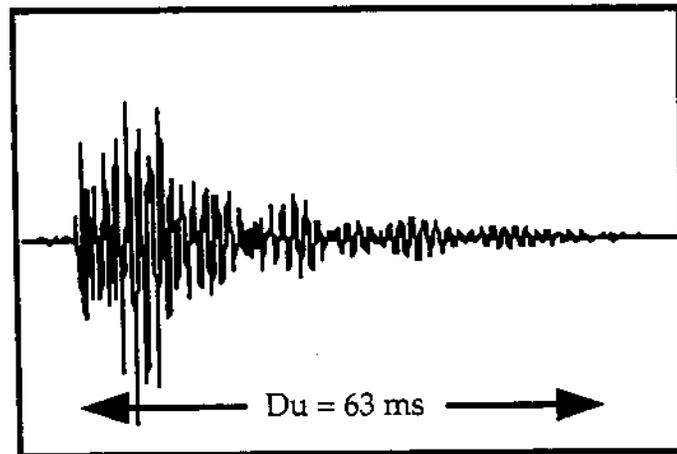


Fig. 6. Represents a Type V or click call. Recorded at Ramsey Canyon pond at a water temperature of 15 C.

form composition it is similar to one of the basic elements (a pulse) in a typical Type I or snore call, but is offered alone.

A common call type not heard so far in the Ramsey Canyon leopard frog is a release call, offered when one male clasps another. This would represent a sixth type of call. It can be artificially induced in the field by gently but firmly grasping an adult male with one's thumb and forefinger just posterior to the shoulders. The resulting call is structured like a snore call but different in sound and temporal properties. It has a much lower pulse rate and is shorter than Type I snores heard on a typical evening of recording at the Ramsey Canyon pond.

Discussion— To date, only limited attention has been given to the possible functions of the various call types produced by North American leopard frogs. Most workers have

presumed that the snore call, Type I, is used in mate attraction and that the various shorter call types are involved in territorial spacing or some form of male-male interaction. If the Ramsey Canyon leopard frog is similar to *R. temporaria* in Europe, then the snore call alone may be more diverse in function than commonly assumed. Walkowiak (1988) reported that males respond differently to long and short snore calls. He identified both a long and short snore call which were territorial in function along with an intermediate length snore used as an advertisement call.

The soft snores identified in this study as Type II calls were of low enough amplitude to question whether they were made by very small animals or represent large males, offering a soft version of the normal snore call. The latter is most likely given the following facts, observations and reports in the literature: 1) In most species of small leopard frog which call in the air, males can offer a substantially louder snore than the soft ones detected on my tapes. Spectrographic measurements of normal Type I snores range from 50 to 71 db on a relative scale, i.e. the entire set of calls was recorded and digitized at the same level, and normal calls fall within this range. The soft calls however were of a much lower amplitude, varying from 35 to 40 db or within 5 to 10 db of the background sound level in the pond of 30 db. Even Dytiscid beetle stridulations commonly accompanying night chorusing among the frogs were louder, ranging from 41 to 55 db relative to frog vocalizations. 2) The small pond size at Ramsey Canyon and the fact that calls do not attenuate as rapidly in water compared to air, suggests that the effect was not heavily influenced by distance to the hydrophone. (The maximum distance sound would have to travel is less than 20 meters even if reflected from a wall.) 3) Analysis of soft snores revealed dominant frequencies in the 0.9 to 1.0 kHz range, indicative of a large animal. McAlister (1962) reported a similar soft call in *R. berlandieri* in Texas populations. According to Wells (1988) soft calls have been reported for several dendrobatid frogs and within the family hylidae as well.

RAMSEY CANYON LEOPARD FROG - PART III

Current presumption suggests that low intensity calls would be advantageous for a male courting a nearby female because this strategy would be less likely to attract rival males than if a high intensity mating call was being offered. Although this strategy would work fairly well in airborne situations where males may be spread out, it is unlikely that it would go undetected by nearby males in the Ramsey Canyon pond. It might do so in an open aquatic system of great size, but only if males were spread out. To further support or eliminate this explanation would require simultaneous recordings and visual confirmation of the size and location of calling males.

Rasp and chuckle calls (Type III and IV) share two similar characteristics. They are both very short signals and contain a lot of energy. Based on this, it makes sense that they too may be involved in signaling nearby males. Davies and Halliday (1978) have shown that males in *Bufo bufo* make use of dominant frequency in assessing the potential size of males already in amplexus with females. The potential for males to exploit this information (call intensity and/or dominant frequency) to assess other nearby males seems likely, but little information exists for leopard frogs.

Male frogs among many species that have been studied also make a release call which is offered when a male clasps another male in amplexus. In at least one species of European ranid frog, *R. temporaria*, (Walkowiak, 1988) reported a release call produced by females and differing from that of males. These should be rare except on evenings of intense mating activity and have thus far not been documented for the Ramsey Canyon leopard frog.

Most other leopard frogs species, both male and female, are also known to make alarm cries. It is typically a cry of medium length (1 to 2 s or more) with an almost human scream-like quality. None were detected in this study, and this was not

surprising, given the reduced likelihood of predator detection and attack in an under water situation.

Methods and materials— The breeding season at the Ramsey Canyon site was closely monitored by the author through the spring and summer until 20 June 1995 and thereafter for egg mass deposition dates through August 1995 by personnel at the Nature Conservancy. This included water temperature records which were obtained from a submersible data logger (Onset Computer Corp.). The date of deposition of each egg mass was easily determined by daily inspection of the pond which was clear and free of emergent algal mats throughout the duration of this study. Surveys of the pond center (which lacked significant submerged branches) were made periodically by inflatable boat. The location of egg masses within the pond and depth in mm at the point of deposition were recorded for each clutch. After 48 hours selected egg masses were removed from the pond and measured volumetrically in ml. In two cases hand counts were made to obtain the total number of embryos for a given volume to allow estimates for the number of embryos in subsequent clutches. When deemed appropriate by Nature Conservancy staff, some clutches or portions of clutches were moved from the pond to a steel tank for rearing purposes. Mid-season clutches were allowed to develop unhampered in the pond to allow observations of predation. Late-season clutches were protected but remained within the pond during early development. "Wall counts" of overwintered tadpoles were taken after dark to estimate the number of second-year larvae after it was determined by inspection that almost all tadpoles remained near the perimeter of the pond after dark.

Results— Breeding activity began in early April and continued through 6 August 1995. Complete data on the first 19 egg masses laid in Areas 1-5 are depicted in Figure 1a. Each of these areas (1-5) contained dead tree branches submerged at various depths.

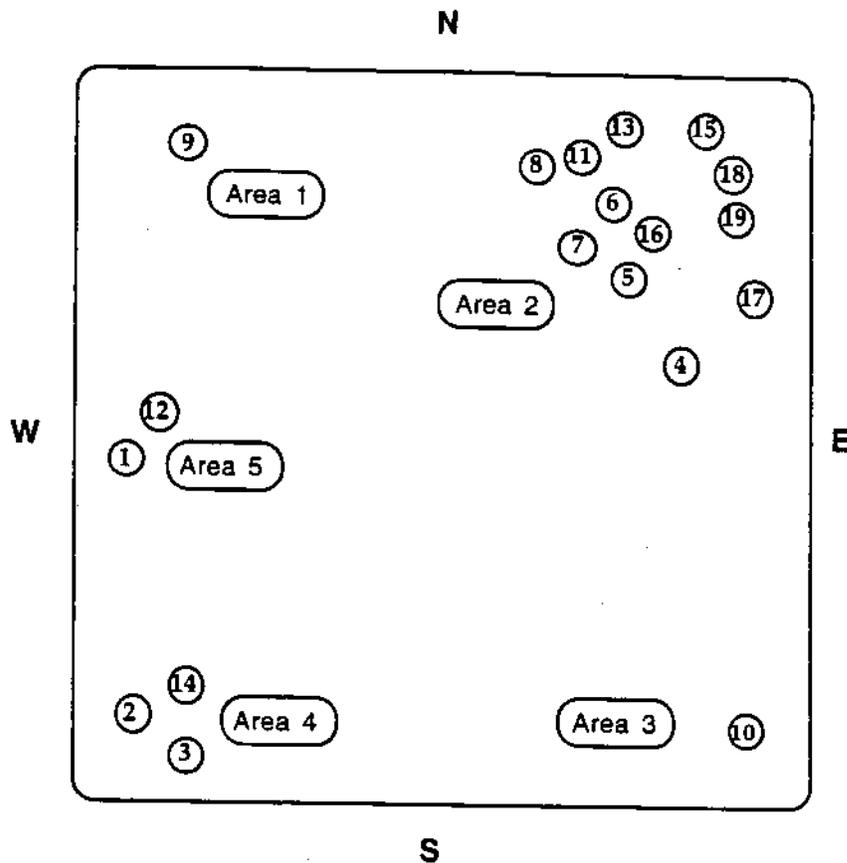


Fig. 7. Aerial view of the Ramsey Canyon pond indicating by area the location and identity of egg masses laid between 8 April and 16 June, 1995.

The location, by area, for each egg mass is shown in Figure 7. The first egg mass was laid on 8 April along the midwest wall (Fig. 7, Area 5) at 110 mm on a tree branch. The deposition of egg masses 2 and 3 followed (one each on 13 and 15 April) at similar depths. Both were laid in the southwest corner of the pond (Area 4, Fig. 7). No more egg laying activity occurred until 30 April when 5 masses (clutches 4-8) were visible in the northeast **corner** of the pond near the overflow. Again each was attached to submerged branches at depths ranging from 85 mm to 635 mm. A single egg mass (clutch 9) was laid in Area 1 during the next week. Then on 22 May three new clutches were observed. All were attached to branches at depths ranging from 140 to 320 mm. Of these clutch 10 represents the only one laid in the southeast corner despite the fact that submerged branches for attachment were abundant there. Three additional egg

masses were laid (one each) on 6, 11 and 13 June. Clutch 14 was the last to be deposited in the southwest corner of the pond attached to a submerged branch at a depth of 150 mm. The remaining two (clutches 13 and 15) were deposited in the northeast corner at a depth of 270 and 100 mm respectively. Clutches 16-18 were observed on 14 June and represent a third and last day of peak activity. All three were deposited on submerged branches somewhat deeper (400, 320, 270 mm) than those laid earlier that month. Egg mass 19 was deposited 2 days later in 120 mm of water in the same vicinity as clutches 13, 15-18.

Observations made during development indicate that mortality is high. Egg clutch 1 suffered heavily from predation by large conspecific tadpoles which had overwintered. Clutch 2 was measured by volume, separated and hand counted and subsequently moved to a steel tank as experimental material to obtain information about growth rates and to attempt rearing outside of a predatory environment. Clutches 3 through 12 were allowed to develop in place in the face of predation by conspecific overwintered tadpoles and insect larvae as well as the forces of high winds, which on occasion caused egg masses to break free from the branches they occupied. Intraspecific predation by second year larvae turned out to be extremely heavy; some clutches among those above were completely consumed. This generally took less than 48 hours; often only a single day. Egg masses 13-18 were removed and measured volumetrically. Embryos from egg mass 13 were counted by hand. These egg masses were protected at first by commercial plastic pint strawberry containers; two wired to each other to contain the eggs. These ultimately proved ineffective in deterring predation by large tadpoles which were able to gain access to the egg mass from the bottom side of the cages. Clutches 15-18 and eventually clutch 19 were, therefore, further protected by placing the strawberry cages within a netted enclosure made of sufficiently fine mesh to keep tadpoles out. This combination worked quite well and

hatching success was better than 95% in all cases. Clutches 20-27 were laid on the following dates: one on 25 June; three on 4 July; one on 14 July and two on 6 August. Because of the incomplete data for these last seven remaining egg masses, statistical

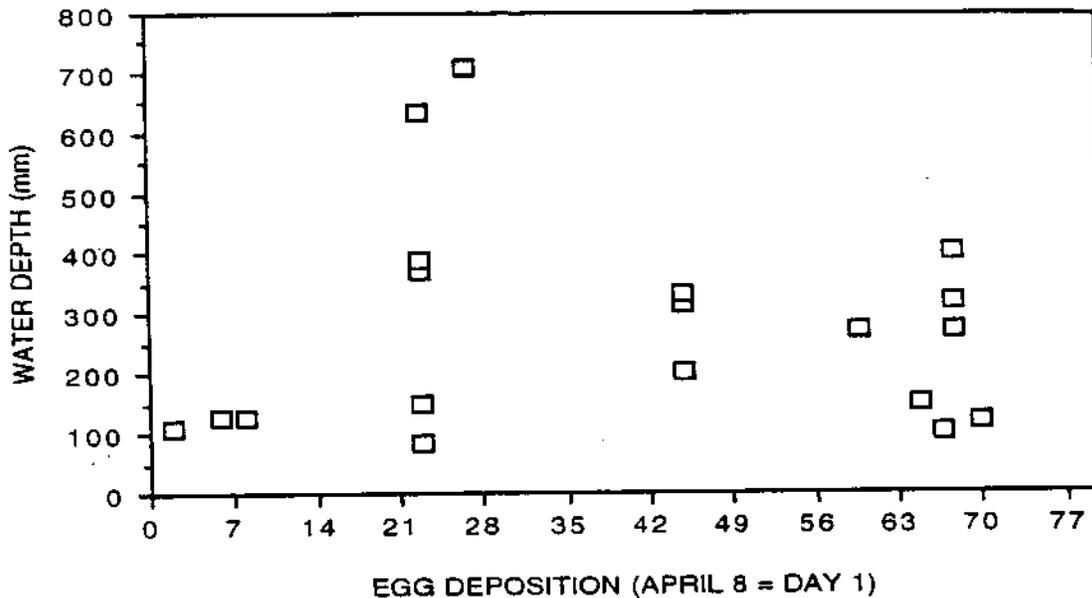


Fig. 8. Represents a scattergram of egg masses by depth below the water surface at the Ramsey Canyon pond. Each square represents a single egg mass.

analysis is restricted to clutches 1-19.

Discussion— Egg deposition in the Ramsey Canyon leopard frog involves attaching the egg mass to submerged objects. In every case at the Ramsey Canyon pond this turned out to be dead tree branches. The depth of deposition (Fig. 8) varied from a minimum of 110 mm to a maximum of 710 mm with a mean of 269 mm. All egg masses were laid **within** 1.5 meters of the pond perimeter. Figure 7 suggests that the distribution of egg masses among the five areas of submerged branches was not random. The northeast corner alone accounted for 12 of 19 (63%) of the total. Three more resided in the northern half, but to the west, for a total of 15 or 79% of all clutches. The latter total is significantly different from random deposition, with or without Yates correction for small sample size (with Yates, $df = 1$, $\chi^2 = 5.26$; $0.01 < P < 0.025$). The

most striking comparison is the low number of egg masses in the southeast corner (Area 3, Fig. 7) and Area 2 in the northeast corner. This disparity is also significantly different from random, with or without Yates correction for small sample size, (with Yates, $df = 1$, $\chi^2 = 7.69$; $0.001 < P < 0.01$) and it is this one which may help explain the paucity of egg masses in the southern half of the pond. Both Areas 2 and 3 have abundant submerged branches for deposition and similar maximum depth and yet only one egg mass among the total of 19 was laid in this corner. However Area 3 is the most shaded of the entire pond while Area 2 is sunlit for more time than the rest. Therefore, it seems most likely that some factor or factors connected to sunlight availability probably account for the pattern, although it is not clear how amplexic pairs would determine this. Egg deposition site preferences may include benefits to embryos while they are in the egg mass or later as small tadpoles. Preliminary experiments with egg masses at the Ramsey Canyon pond consistently show that core temperatures in full sunlight exceed the surrounding water temperature by over 2 C, which is enough to drive a convection current within the egg mass to bring through freshly oxygenated water according to Seymour and Bradford, (1995). Merrill (1977) made similar observations for egg masses of *R. pipiens* in Minnesota. In his case core egg mass temperatures were as much as 3 C higher than the surrounding water. He and others pointed out that elevated temperatures foster more rapid development. While this is true, Seymour and Bradford (1995) make a convincing argument that in large spherical egg masses it is the combination of large swollen jelly coats, which reduce embryo density, combined with melanistic torsos, which absorb heat, as the critical factors. Elevating clutch temperature draws water up through channels in the egg mass bringing in oxygenated water to embryos in the interior. It may also provide additional value once the embryos hatch in that algal concentrations may be more robust in better sunlight conditions. One could also propose some connection with the fact that the highest concentration of egg masses is in the same vicinity of the pond

outflow. However this seems a less likely explanation because often during the breeding season little or no water flowed out of the pond over the small spillway. This was true when flow from upstream was insufficient to keep up with evaporative losses and greater losses from a large crack in the bottom of the concrete tank which carried significant amounts of water out of the pond.

Clutch size varied from 1,200 to 2,040 embryos with a mean of 1,518 among the seven which were available to assess. Furthermore there was a statistically significant

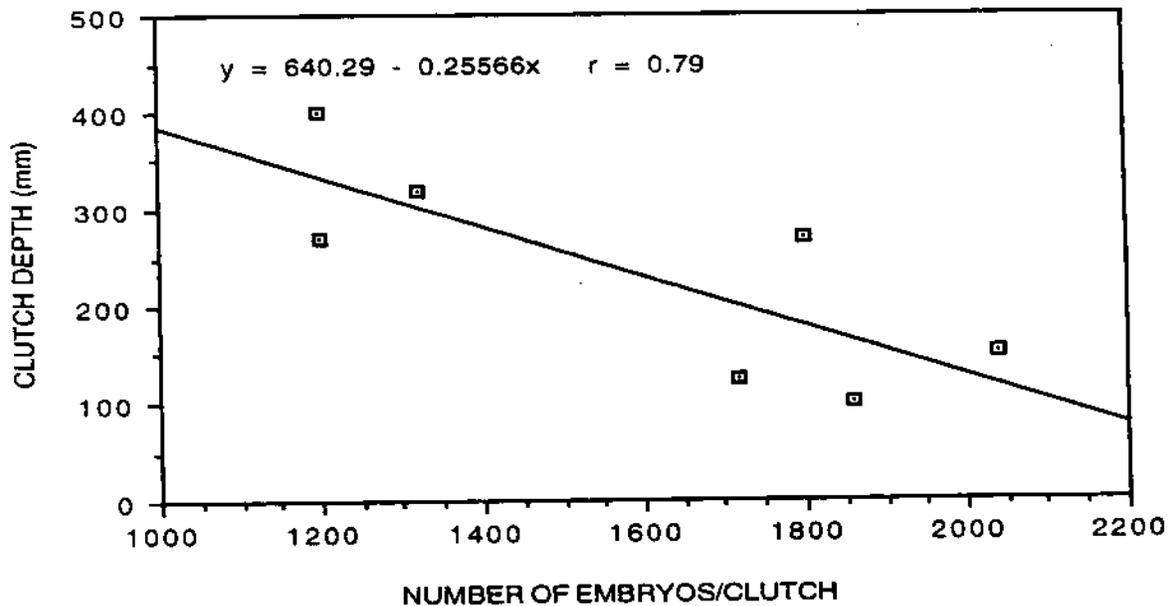


Fig. 9. Linear regression of egg mass depth against clutch size at the Ramsey Canyon pond during the spring and summer of 1995.

negative relationship ($r = 0.79$; $P = 0.05$) between the number of eggs in a clutch and the depth at which they were laid (Fig. 9). In general larger females lay larger clutches, which suggests that larger females were utilizing shallower water for oviposition sites. The average clutch size for the larger masses was 1,854 as opposed to 1,240. Clutches closer to the surface would be exposed to greater levels of incident light and for longer periods of the day than those at greater depths. Although left undisturbed and not counted, the two smallest egg masses seen were clutches 7 and 9 which were

respectively 635 and 710 mm below the surface. It might also be argued that depth of egg deposition was somehow correlated to water temperature which has two possible components of variation: 1) on a temporal basis as egg deposition episodes occur in an environment of increasing seasonal temperature from about 10 C in April at the time of the first clutch to 18 C at the end of the season. 2) Water temperature can vary with depth at any given time. To test the first hypothesis, depth of deposition of these same seven clutches was regressed against the water temperature on the day of each clutch was deposited (Fig. 10). There is no significant relationship between these two factors

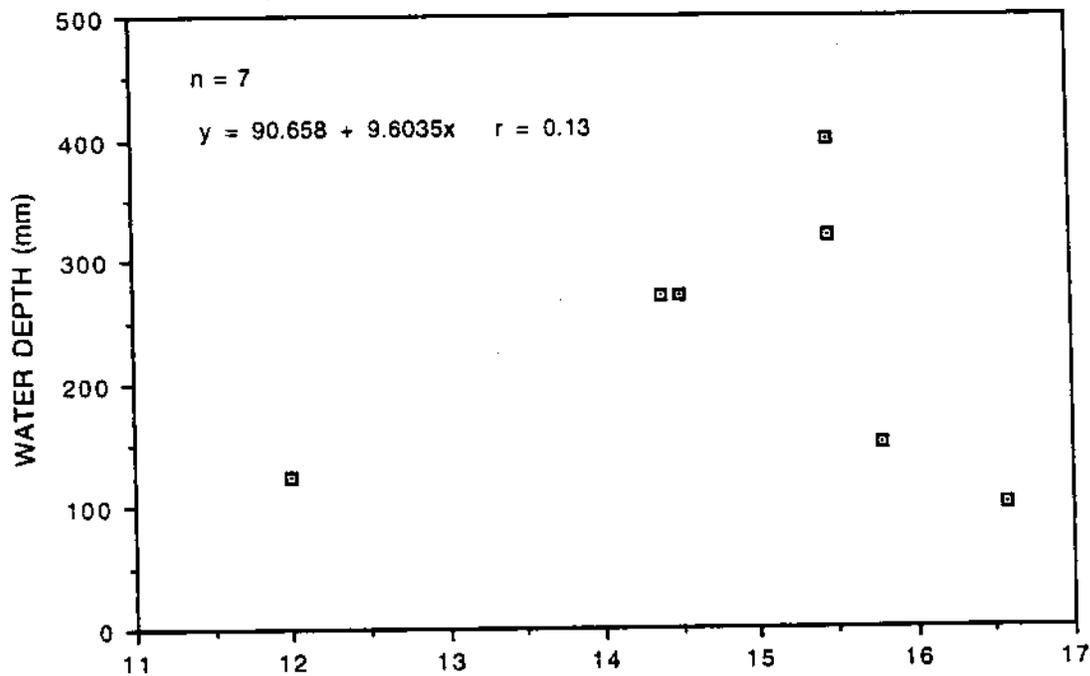


Fig. 10. Linear regression of water depth against water temperature on the night each of the egg masses in Figure 9 was laid.

($r = 0.13$; $P = 0.79$).

A similar test of hypothesis 1 utilizing egg masses 1-19 against the temperature of the water at the time of deposition (Fig. 11) supports the same conclusion ($r = 0.09$; $P = 0.70$).

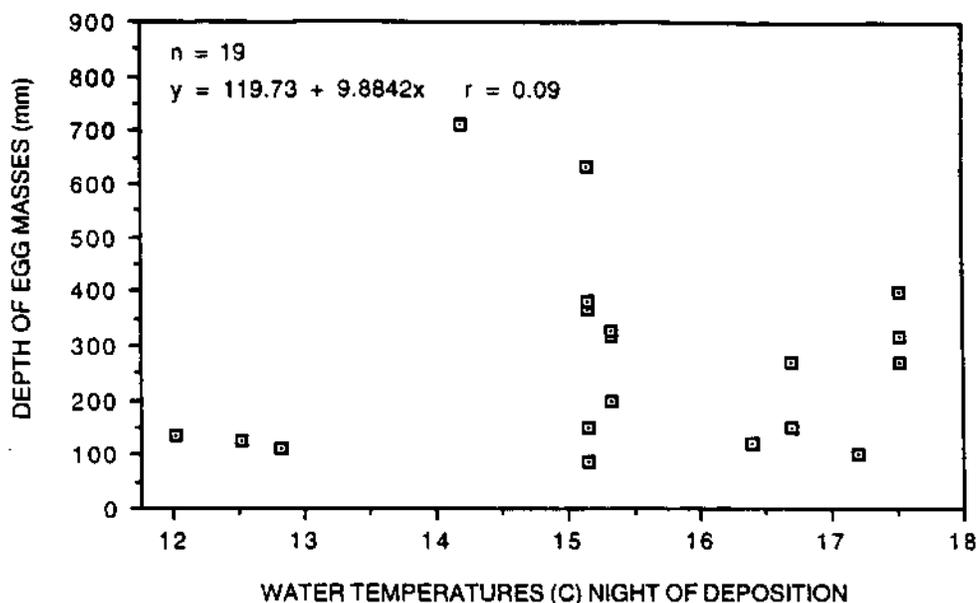


Fig. 11. Linear regression of egg mass depth against water temperature for all 19 egg masses at the Ramsey Canyon pond during the spring and summer of 1995.

The second hypothesis suggesting that amplexic pairs might make site choices on the basis of differences in vertical variation in water temperature the night of deposition is unlikely. Water temperatures taken by probe with a telethermometer (readable to 0.1 C) varied less than 1 C at various locations in the pond and by less than 0.5 C from shallow to deep positions. The high uniformity in temperature is the product of the shallow nature of the pond and the angle of incoming water from upstream along with denser air masses at night that bring cool breezes from higher in the canyon. All of these factors help keep the water in slow circulation and, therefore, well mixed.

The temporal pattern of egg deposition is one of clustered activity (Fig. 12). Three early egg masses laid on separate nights over the first week of the breeding season were followed by a 2-week break and then the first of 3 peaks in egg mass deposition on 30 April involving five clutches. After another single egg mass on 5 May a second peak in activity occurred on 22 May when 3 egg masses were deposited 23

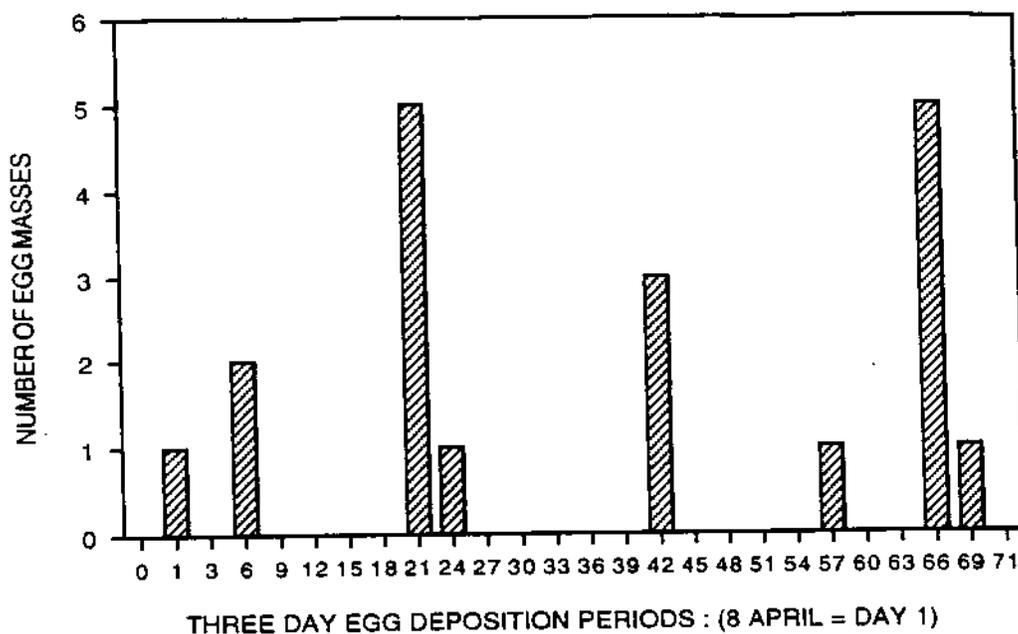


Fig. 12. Temporal pattern of egg mass deposition at the Ramsey Canyon pond during the spring and summer of 1995. Data were plotted for 3 day intervals.

days after the first peak. One egg mass was deposited in the week prior to the last peak in activity which involved 5 egg masses. Three of these were laid on the same night of a three day period whose midpoint was 14 June. The mid peak of this episode was 24 days after the second peak. The last egg mass followed quickly on 16 June. It therefore seems clear that egg laying which began on 8 April when the water temperature had reached 12 C occurred in regular peaks approximately 24 days apart with occasional nights when a single egg mass was laid. Fully 74% (14 of 19) of the egg masses were deposited on 6 nights contributing to the 3 peaks in activity.

Contrasting opinions by various workers suggest that breeding activity either is or is not related to rain. Breeding activity is not correlated with rainfall for the Ramsey Canyon leopard frog, but there is a pattern in relation to water temperature (Fig. 13). Each night ovipositing took place was at or shortly after a temperature maximum which was in each case preceded by a sharp drop in pond temperature. The solid triangles in Figure 13 mark the bottom of each downward temperature swing before the

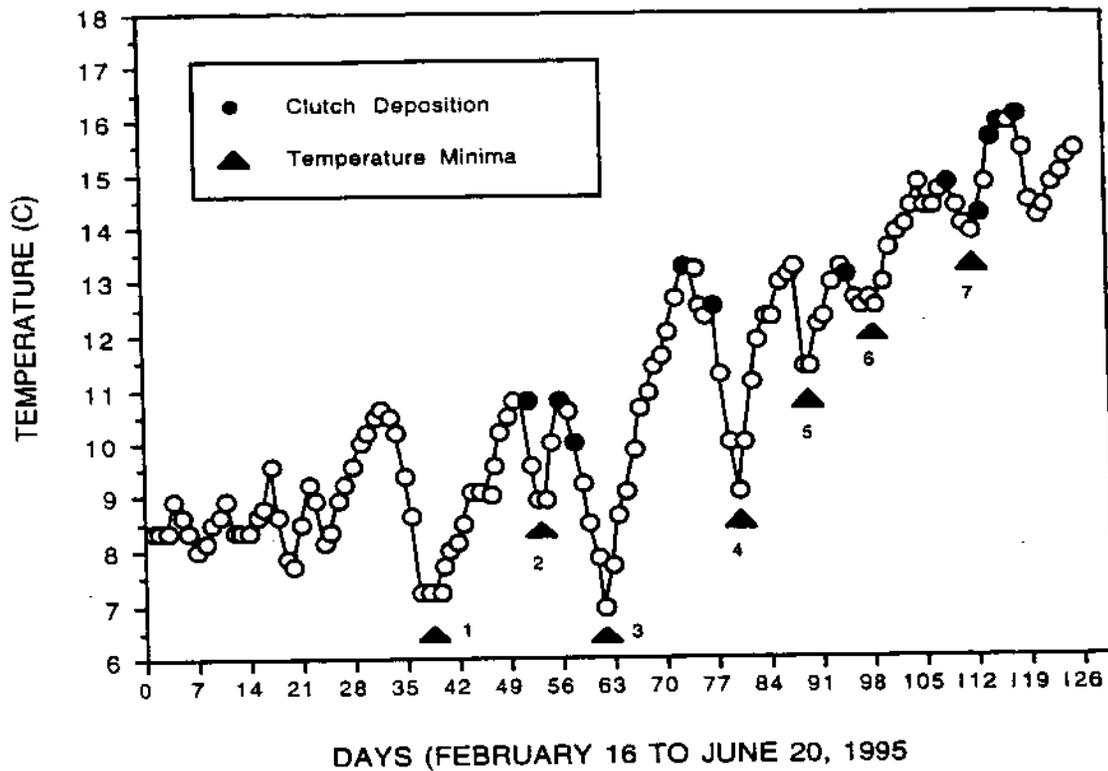


Fig. 13. Nightly low water temperature profile for the spring of 1995 at Site 1, Ramsey Canyon. Each open circle in the connected series represents the nightly low temperature (C). The solid circles (N = 11) represent nightly lows on evenings when oviposition took place. The solid triangles signify water temperature minima.

start of a rise. The only one among the 11 egg laying nights which does not appear to conform to this pattern is event 8 on 16 June (after low 7) which occurred just after a small drop (< 0.5 C) in water temperature. Even it may be a reaction to the larger upward trend, after the previous minimum (low 6) in water temperature begun several days earlier on 28 May, five days after egg deposition event 7. If so, it would appear that *R. subaquavocalis* may be stimulated by decreasing pond temperature requiring several days to actually ovulate and oviposit thereafter at temperatures above about 10 C. The mean number of days before egg deposition for the first 3 of these major low temperature episodes is 15 days and varies little (range 14.5 to 15.5). These involved a downward temperature greater than 3 C each reaching a minimum on days 39, 62 and 80, (triangles numbered 1, 3 and 4, Figure 13). The number of post low days prior to egg deposition is more difficult to define in relation to declines in water

temperature for the remaining five egg laying days. The drops in water temperature were less marked beyond day 91. However if the stimulant prior to egg deposition is assumed to be the sixth dip in water temperature (day 99) then the remaining egg mass deposition events range from 10 to 19 days after the last day of low 6 or an average of 15.2 days. Interpreting the last five egg deposition events as likely to be the result of low 6 instead of low 7 is based on the lag time which is so clear cut for lows 1 and 3; especially low 3. In each of these cases no egg deposition took place until 11 days had elapsed. It is also interesting that with one exception (the first egg mass after low 7) all other egg deposition events were at or near (<0.5 C) days of peak temperatures. If egg deposition event 8 (clutch 13 on 16 June) is actually a response to the previous water temperature cycle, then the mean time period in days between a low and egg deposition is 15.7 days based on five clusters of egg laying events.

Several factors prevented the direct determination of which females laid which egg mass. Foremost among these was the inability to follow amplexic pairs throughout the night. One episode of egg laying was captured on video during the day attesting to the challenge of obtaining these data. While amplexus had been initiated the evening before, time measurements taken from video tape late the next afternoon indicated that it took less than three minutes to deposit and fertilize the eggs. Therefore even though amplexus may be of long duration (8 to 20 hours) egg laying is a brief process. Nor was it easy to identify with certainty those which were seen in amplexus. This second factor was because the dorsum of frogs was too dark during a substantial portion of the breeding season to use individual spotting patterns to identify a particular individual. The position of the male during amplexus also obscured most of the dorsum of the female. However it was known that there were 11 adult females in the Ramsey Canyon pond through late June during which time 19 egg masses were deposited. These observations support the contention that many of the females laid a

minimum of two clutches. It would be intriguing to know whether those which double clutched did so on a regular (24 or 48 day) cycle.

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Each of the above taxa are distinct from one another morphologically (Platz, 1993) and diagnosable on the basis of a suite of characters which involve the following traits: Presence or absence of a light colored, complete supralabial stripe; continuous or discontinuous dorsolateral skin folds; coloration of the skin in the groin area; mottled or immaculate (cream colored) venter; presence or absence of vestigial oviducts in males; presence or absence of lighter halos around dark spots on the dorsum; and the presence or absence of numerous small light pigmented papilla on the rump region either side of the cloaca. Non-morphological characteristics of additional diagnostic value include differences in male vocalizations. Beyond the fact that male calls can be assessed for parameters which indicate that there are differences among species, it is also notable that male *R. subaquavocalis* offer their repertoire of calls from the bottom of the pond which makes them inaudible in air. All other species of U. S. leopard frogs are known to have airborne calls. Therefore the fact that male *R. subaquavocalis* call under water and females find and mate with them under these conditions implies the evolution of a distinctive mate recognition system.

At the time that the present work was undertaken the taxonomic affinities of *R. subaquavocalis* to other U. S. ranid frogs were unknown. On the basis of discontinuous morphological characters, *R. subaquavocalis* is most similar to *R. chiricahuensis* from the type locality (Platz, 1993), but differs in overall body proportions, and the calls of *R. chiricahuensis* are airborne signals. If electrophoretic comparisons between these two taxa support the morphological assessment, then *R. subaquavocalis* should be considered a member of the *R. montezumae* group (within the *R. pipiens* complex) as defined by Hillis and Davis (1986) (Fig. 2). This group currently includes *R. montezumae*, *R. megapoda*, *R. dunnii* and *R. chiricahuensis*. All four have airborne calls (Mecham, 1971; Platz and Mecham, 1979).

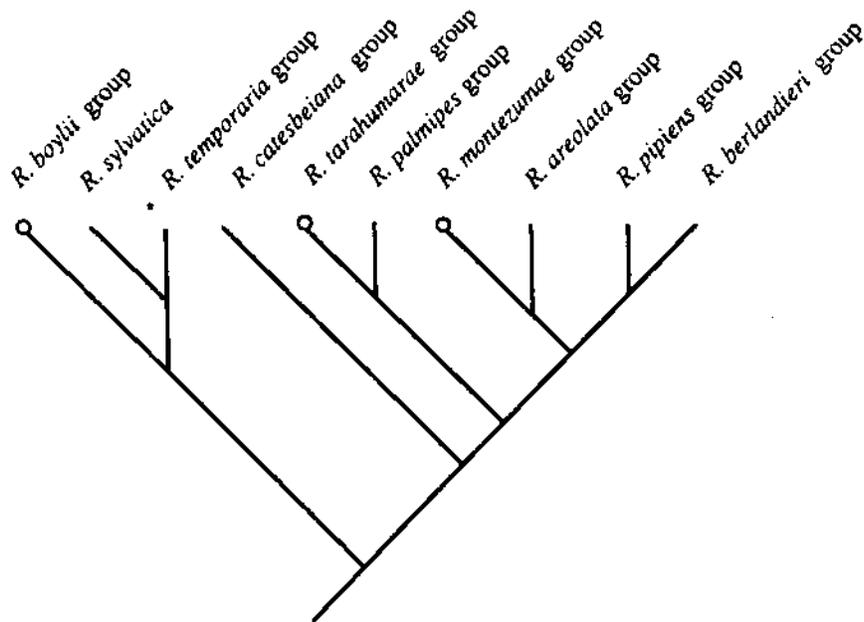


Fig. 2. Cladogram of major groups of ranid frogs from the U. S. and Mexico. * *Rana temporaria* represents the outgroup. After Hillis and Davis, (1988). Open circles represent groups in which underwater calls have been reported.

Most members of the *Rana boylii* group (*R. boylii*, *R. aurora*, *R. cascadae*, *R. muscosa*, and *R. pretiosa*) are less like *R. subaquavocalis* in general morphology and size. The exception is *R. aurora draytoni*, which extends into southern California and Baja California del Norte. *Rana subaquavocalis* and *R. a. draytoni* are both large frogs with well-developed vocal sacs and prominent dorsolateral folds but differ in coloration of the proximal, ventral surface of the hind limb and groin region. In *R. a. draytoni* the color is red compared to pigmentation in *R. subaquavocalis* which is yellow. They also differ in that *R. a. draytoni* males lack vestigial oviducts whereas *R. subaquavocalis* males possess them. More importantly three of the five species in the *R. boylii* group are now known to call under water: *R. aurora aurora* (Licht, 1969); *R. boylii* (Mactague and Northen, 1993); *R. muscosa*, (Northen, pers. comm.). Stebbins (1985) lists *R. cascadae* and *R. pretiosa* as calling in the air and under water. *Rana boylii* produces five under water vocalizations (Northen, pers. comm.) and is thus similar in call complexity to *R. subaquavocalis*. Less is known of the call repertoire of other members of this group.

Given these last observations, two explanations seemed plausible: 1) *R. subaquavocalis* closest affinities were to the *R. montezumae* group (based on external morphology and closer geographical proximity); 2) Although less likely, *R. subaquavocalis* might be more closely related to *R. a. draytoni* as a member of the *R. boylii* group. This hypothesis is based on published data by Licht (1969) indicating that *R. a. aurora* produce mating calls under water, morphological similarities discussed above and the proximity of *R. subaquavocalis* to southern California locales for *R. a. draytoni*. It is of further interest that herpetologists are divided as to whether *R. a. aurora* and *R. a. draytoni* represent two species. Electrophoretic, morphological and behavioral data (Hayes and Miyamoto, 1984) and electrophoretic data from Green, (1984) suggest that populations in the southern part of the range (*R. a. draytoni*) are distinct from the northern form (*R. a. aurora*). In addition, *R. a. draytoni* is quite variable in appearance (Mark Jennings, pers. comm.).

A second issue of affinities involves populations of ranid frogs within the state of Arizona found in two different regions. Those frogs along the Mogollon Rim of central Arizona were originally assigned to *R. chiricahuensis* based on call similarities with those recorded at the type locality at Herb Martyr Dam in the Chiricahua Mountains (Platz and Mecham, 1979). A second location, the headwaters of Leslie Creek, Cochise Co., AZ, also contains large, rugose specimens for which there were no electrophoretic, morphological or mating call data. The differences between Mogollon Rim specimens and *R. chiricahuensis* from the type locality (the former also lack vestigial oviducts in males) and the high degree of similarity between *R. chiricahuensis* and *R. subaquavocalis* therefore represent additional relationships which needed clarification.

The major thrust of this paper was to resolve the various relationships between *R. subaquavocalis* and various other Arizona and Pacific Northwest ranids. It is the

conviction of the author that ultimately an understanding of the phylogenetics is a prerequisite to making the most use of information comparing life history characteristics regardless of which of the two groups *R. subaquavocalis* is more closely allied to. This is in turn important because members of both groups in the West are known to be in decline.

Methods and materials— Specimens (tadpoles) of *R. subaquavocalis* and the undiagnosed leopard frog from Leslie Creek, Cochise Co., Arizona and metamorphs representing the Mogollon Rim species, *R. yavapaiensis*, and *R. chiricahuensis* used in electrophoresis were collected by hand capture or net. All were flash frozen in liquid nitrogen and then shipped to the lab on dry ice and stored at -70 C until the time of electrophoresis. Specimens (metamorphs) of *R. sylvatica*, *R. catesbeiana*, *R. clamitans* and *R. pipiens* were collected by T. Grudzien and frozen at -70 C in the lab. Tissues from the *R. boylei* group (*R. a. draytoni*, *R. boylei*, *R. muscosa*) were provided by B. Shaffer. Individuals of *R. temporaria* from Europe were purchased from Charles D. Sullivan Co., Nashville, TN and used as an outgroup for cladistic analysis.

For electrophoresis, skeletal muscle, liver, and heart tissues were dissected from juveniles and homogenized using the grinding buffer of Turner (1983). Gut contents were removed from tadpoles and the entire individual homogenized in grinding buffer. Tissue homogenates were stored at -70 C, thawed, and centrifuged at 13,000 rpm for 10 minutes prior to electrophoresis in 12% starch gels (60% Starch-Art starch, 40% U.S. Biochemicals starch). Allozyme electrophoretic methods and terminology follow those presented by Hillis et al. (1983) and Hillis and Moritz (1990). The products of 50 presumptive protein-coding loci were resolved as follows: ACOH (aconitase hydratase, EC 4.2.1.3), ACP (acid phosphatase, EC 3.1.3.2), ADA (adenosine deaminase, EC 3.5.4.4), G3PDH (glycerol-3-phosphate dehydrogenase, EC 1.1.1.8), ALD-1, ALD-2

(FBA) (fructose-biphosphate aldolase, EC 4.1.2.13), AK-1, AK-2 (adenylate kinase, EC 2.7.4.3), CAT (catalase, EC 1.11.1.6), CPK-1, CPK-2 (creatine kinase, EC 2.7.3.2), DIP (dipeptidase, 3.4.11), DLR (dihydrolipoamide dehydrogenase, EC 1.8.1.4), ENO-1, ENO-2 (enolase, EC 4.2.1.11), EST-1, EST-2 (esterase), FUMH (fumarase, EC 4.2.1.2), FBP (fructose biphosphate, EC 3.1.3.11), GDA (guanine deaminase, EC 3.5.4.3), AAT-1, AAT-2 (aspartate aminotransferase, EC 2.6.1.1), ALAT (alanine aminotransferase, EC 2.6.1.2), GAPDH (glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.12), G6PDH (glucose-6-phosphate dehydrogenase, EC 1.1.1.49), β GLUR (beta glucuronidase, EC 3.2.1.31), HEX-1, HEX-2 (HK) (hexokinase, EC 2.7.1.1), IDH-1, IDH-2 (isocitrate dehydrogenase, EC 1.1.1.42), LDH-1, LDH-2 (l-lactate dehydrogenase, EC 1.1.1.27), α MAN (alpha mannosidase, EC 3.2.1.24), ME-1, ME-2 (MDHP) (malate dehydrogenase, EC 1.1.1.40), MDH-1, MDH-2 (malate dehydrogenase, EC 1.1.1.37), MPI (mannose-6-phosphate isomerase, EC 5.3.1.8), PNP purine-nucleoside phosphorylase, EC 2.4.2.1), GPI-1, GPI-2 (glucose-6-phosphate isomerase, EC 5.3.1.9), PGM-1, PGM-2 (phosphogulcomutase, EC 5.4.2.2), PK (pyruvate kinase, EC 2.7.1.40), PRO (proline dipeptidase, EX 3.4.13.9), PROT (muscle protein), SOD (superoxide dismutase, EC 1.15.1.1), TPI (triose-phosphate isomerase, EC 5.3.1.1), XDH (xanthine dehydrogenase, EC 1.1.1.204), 6PGD (PGDH) (phosphogluconate dehydrogenase, EC 1.1.1.44). The results from the scored gels were analyzed using the BIOSYS-1 program (Swofford, 1981), MacClade ver 3.03 (Maddison and Maddison, 1992) and PAUP ver 3.1.1 (Swofford and Page, 1993).

Morphometric comparisons among Arizona species were made utilizing a data set of seven body measurements taken from preserved adult specimens representing *R. chiricahuensis*, from the type locality, populations from the Mogollon Rim and Leslie Creek, *R. pipiens*, *R. yavapaiensis*, *R. berlandieri* and *R. subaquavocalis*. These measurements included: snout-urostyle length (SUL), tibia-fibula length (TFL), head width (HW), head length (HL), height from naris to margin of upper lip (NLH),

internarial width (IW), and tympanum diameter (TD). All measurements were made with needle point dial calipers (Helios) and with the exception of SUL read to 0.05 mm. Data were subjected to stepwise multivariate discriminant analysis (SPSS Ver. 4.0, 1990).

Biochemical Results—The electrophoretic results obtained from the 50 presumptive loci were used to construct a data matrix using each of the loci as a systematic character and the alleles (relative mobility) as character states. A metric genetic distance matrix (Rogers Modified Distance, Wright, 1978) was calculated and used to construct a Distance Wagner tree (Farris, 1972) that estimated the phylogenetic relationships of the 12 ranid taxa (Fig. 3).

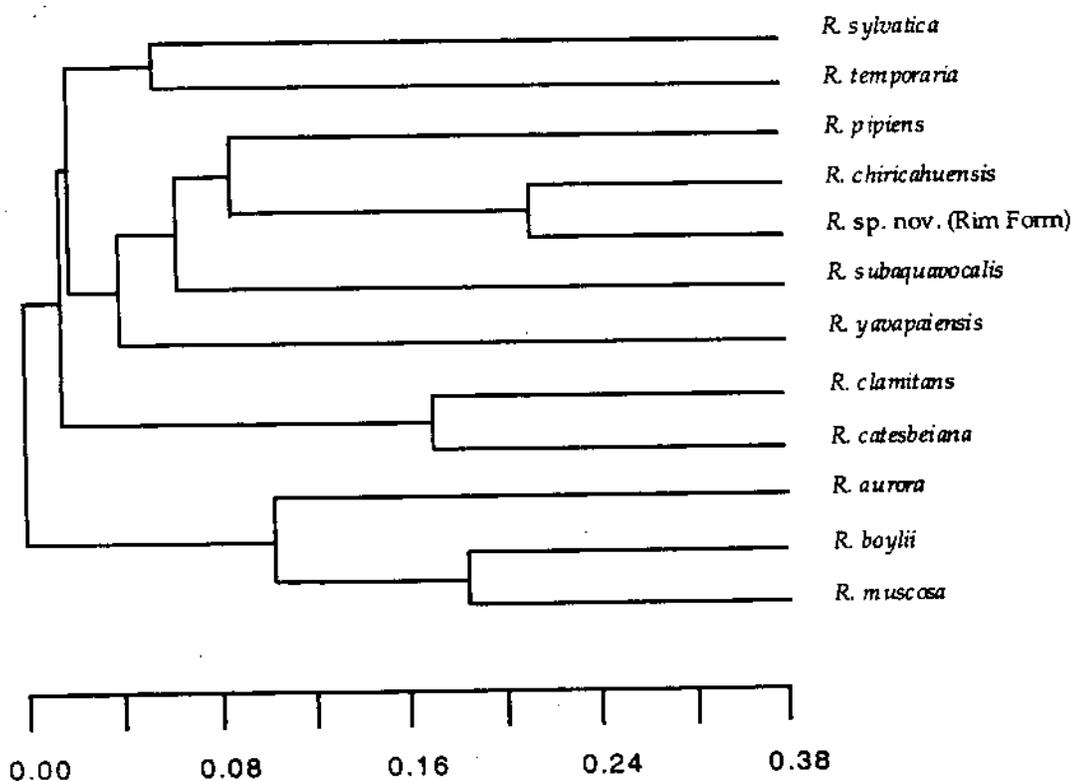


Fig. 3. Distance Wagner Tree representing relationships among the 12 taxa examined for 50 presumptive loci utilizing starch gel electrophoresis. Tissues representing *R. aurora* are from southern California, within the range of *R. a. draytoni*.

Cladistic analysis (PAUP ver. 3.1.1) of the 12 taxa assessed electrophoretically employed the branch and bound search method to obtain the most parsimonious trees. The shortest tree length involved 221 steps and resulted in 10 such trees. From these 10 trees a majority rule consensus tree was constructed (Fig. 4).

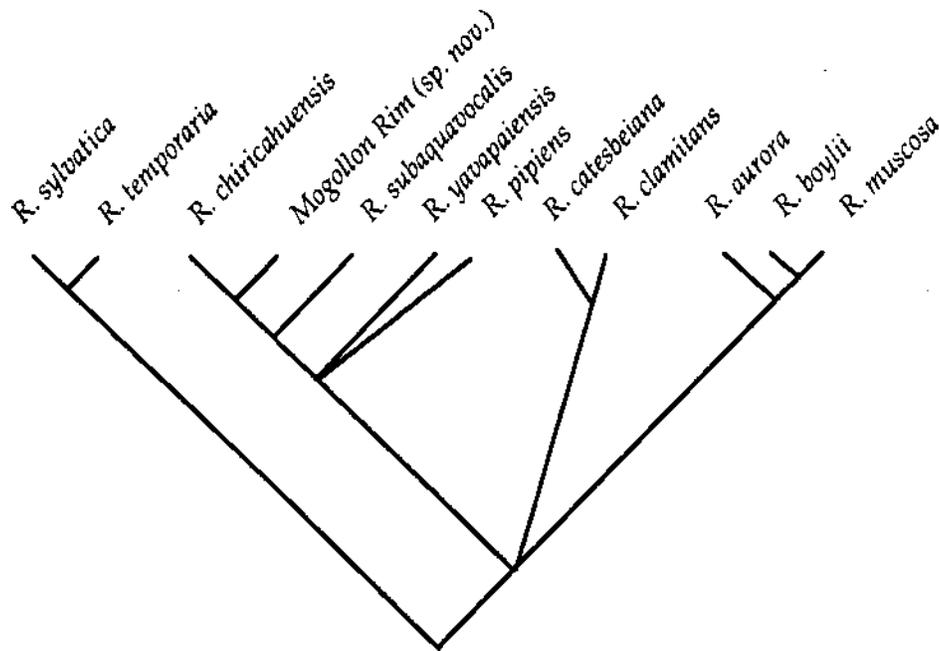


Fig. 4. Majority Rule Consensus tree constructed with PAUP analysis. The cladogram is based on electrophoretic results from the 50 presumptive loci used in this study to elucidate phylogenetic relationships among major groups of U. S. species of ranid frogs. *Rana temporaria* represents an outgroup species from Europe. The consensus tree is based on 10 trees each with a length of 221 steps.

Morphometric Results— Morphometric data for the seven body measurements were obtained from 161 adult individuals representing 10 populations including the undiagnosed population at Leslie creek (pop. 1) and three localities on the Mogollon Rim (pops. 3, 4, 5). Canonical discriminant function coefficients (Table I) indicate that the separation scheme was most influenced along the K_1 axis by variables 5 (NLH) and 6 (IW) and to a lesser extent by variables 1 (SUL) and 7 (TD). Variable 7 (TD), followed by 6 (IW) had the greatest influence on dispersion of means along the K_2 axis. The resulting two axis plot (Fig. 5) explains 80% of the variation in the data set (K_1 axis = 59%; K_2 axis = 21%) providing a robust separation of species by population means.

TABLE I. Grand Means and Eigenvectors from Multivariate Stepwise Discriminant Analysis.

	Trait	Grand mean	K ₁	K ₂
X ₁	SUL	68.30	0.8160790	-0.1193827
X ₂	TFL	37.61	0.4179172	0.7066834
X ₃	HW	25.42	-0.5808165	0.1446371
X ₄	HL	25.87	-0.5247260	0.6015087
X ₅	NLH	6.28	-1.813507	-0.6389471
X ₆	IW	4.67	1.766823	-1.496784
X ₇	TD	6.09	0.6324381	1.908798
Constant			-0.8504782	-1.116221

Discussion— The analysis of the electrophoretic data indicates that *R. subaquavocalis* clusters with two described species of leopard frogs native to Arizona (*R. pipiens* and *R. chiricahuensis* and a third form (sp. nov.) which occupies the Mogollon Rim country of central Arizona. These results are consistent with the supposition that *R. subaquavocalis* is part of a set of speciation events among Arizona leopard frogs rather than an eastern peripheral of *R. a. draytoni* whose nearest populations are in southern California. The typology from our tree is similar to that of Hillis and Davis (1986) based on an assessment of 18S ribosomal DNA sequences providing an additional set of traits congruent with this study.

The majority rule consensus tree obtained from our 10 shortest trees (Fig. 4) resolved all taxa with the exception of the branch order for *R. yavapaiensis* and *R. pipiens* and the placement of *R. catesbeiana* and *R. clamitans*. As was expected *R. a. draytoni*, *R. boylei* and *R. muscosa* grouped together. Our results indicate that *R. boylei* and *R. muscosa* are sister taxa which is in agreement with the electrophoretic assessment of Green (1984) based on 31 loci. The next closest relative to these two is *R. a. draytoni*. *Rana subaquavocalis* appears to be the sister taxon to the Mogollon Rim form (sp. nov.) and *R. chiricahuensis* from the type locality. Placement of *R. subaquavocalis* within this group of species, all native to Arizona, is consistent with geographical considerations as well and therefore should be considered to be a member of the alpha division of the *R. pipiens* complex of Hillis et al. (1983).

The wood frog, *R. sylvatica*, was genetically most similar to *R. temporaria* from Europe which is consistent with Green (1984). These two taxa are also similar morphologically and yet quite different from other North American ranids. Both of these two observations are consistent with Hillis and Davis (1986) and both their approach (using ribosomal DNA) and the electrophoretic data from this study suggest that the evolution of North American ranids may have involved two different invasions from old world stock.

Morphometrically *R. subaquavocalis* is the most distinctive element in Figure 5. On biochemical grounds its sister taxon is the undescribed species (Mogollon Rim form). Morphometrically these two are the least similar members of the alpha division of the *R. pipiens* complex as represented in this study. Population means among the Mogollon Rim localities along the K_1 axis are separated by a minimum of 6 standard deviations from *R. subaquavocalis* at the type locality. They also differ by over 4 standard deviations along the K_2 axis. The Mogollon Rim species is distinct from its

sister taxon *R. chiricahuensis* at five loci and also differs morphometrically (Fig. 5) although the differences along the K_1 and K_2 axes are less marked than those between the Mogollon Rim form and *R. subaquavocalis*. They are however on the same order of differentiation as that between *R. berlandieri* (pop. 10) and the two localities representing *R. pipiens* (pops. 8 and 9). *Rana chiricahuensis* populations (1 and 6) are also distinct morphologically from the Mogollon Rim form in that the latter lack vestigial oviducts in males. Two populations of *R. pipiens* from geographically distant sources were included in the morphometric assessment to provide information regarding intraspecific variation as an aid in interpreting the biological meaning of distances on

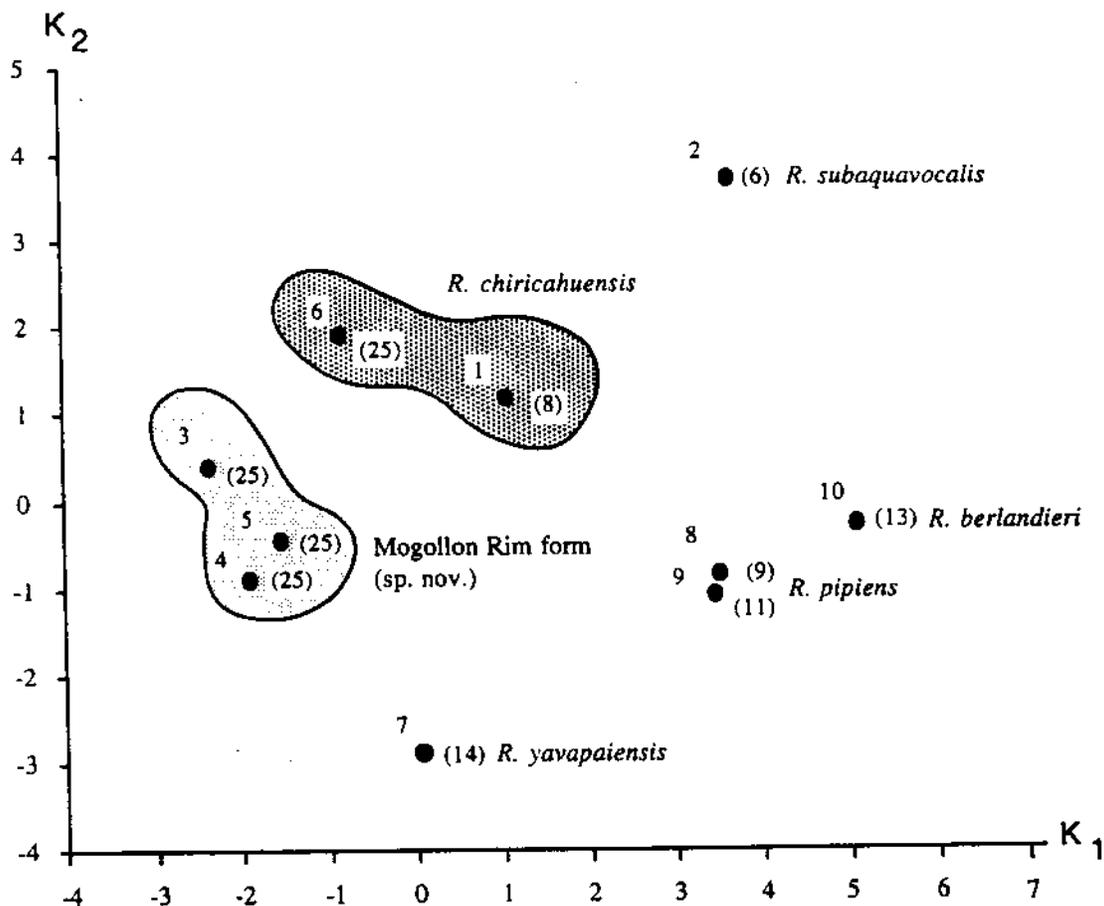


Fig. 5. Stepwise multivariate analysis of six species of leopard frogs occurring in Arizona. The K_1 and K_2 axes are marked in standard deviation units. Each population mean is represented by a solid circle. Numbers to the left and above each mean identify populations. Numbers in parentheses indicate sample size. The shaded areas represent populations which were both considered *R. chiricahuensis* at the time of original description (Platz and Mecham, 1979).

the K_1 and K_2 axes among species. This is important because multivariate stepwise discriminant analysis programs are by their inherent nature designed to produce algorithms which maximize between population differences. It is then up to the investigator to determine the biological meaning. The two centroids for *Rana pipiens* cluster very tightly despite the fact that one population is from Salt Lake City, Utah and the other from central Arizona over 600 km further south suggesting that the overall separation among species seen in Figure 5 functions as a useful additional indication of divergence among congeners.

Published information now exists indicating that the capacity to produce mating calls while submerged occurs in at least one species among several clades of ranid frogs in North America (Fig. 6) and some in Europe as well. However many such reports are anecdotal. Noting that males can vocalize while submerged as presently construed may encompass a number of categories. Some may have only limited or no real biological significance.

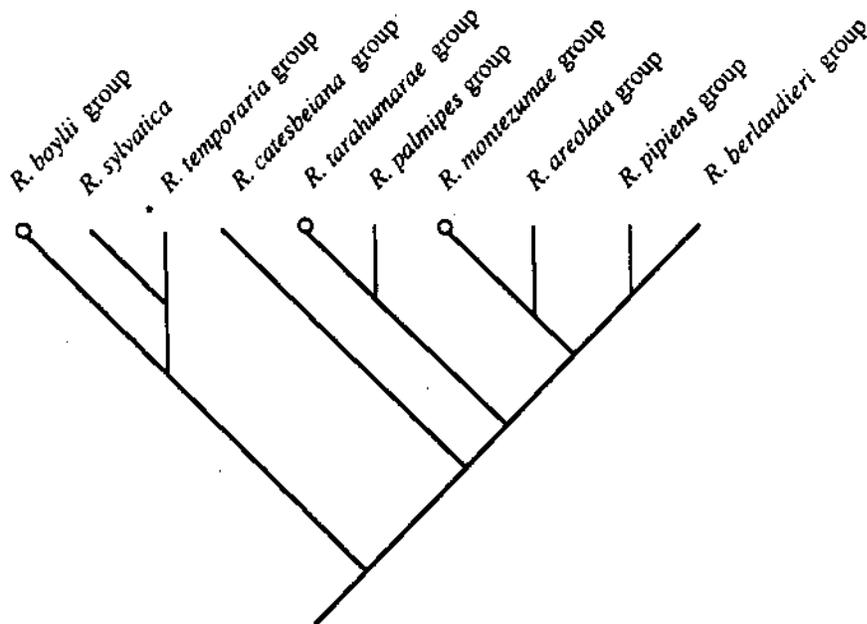


Fig. 6. Cladogram of major groups of ranid frogs from the U. S. and Mexico. * *Rana temporaria* represents an outgroup species from Europe. After Hillis and Davis (1986). Open circles represent groups within which under water calling behavior has been reported.

Category 1 includes species which have well-documented airborne vocal signals and yet males on occasion have been seen to call while submerged. Most involve a male in very shallow water and could be heard offering a muffled airborne signal. These may represent males at the peak of hormonal state during the breeding season attempting vocalization while still submerged. In category 1 most males typically call in the air. Males which call while submerged in these instances are therefore in competition with other males producing airborne signals to attract females seeking surface mates. Under these circumstances submerged males would be at a marked disadvantage because of extreme attenuation of signal incurred during the transition from one medium (water) to air, loss of signal directionality and reduced availability to approaching females. These factors strongly suggest that this male behavior (calling while submerged or partially submerged in shallow water) is not the result of an evolved component of a mate recognition system but is an epiphenomenon of limited biological significance.

Based on 4 summers of observations at the Ramsey Canyon pond *R. subaquavocalis* is representative of category 2 in which males call while submerged. In this species males call from the substrate, typically a meter or more below the surface of the water. Under these conditions several facts are striking: 1) All males are consistent in this behavior; 2) The water depth and reflective properties of the water surface prevent the propagation of the signal into the air; 3) Females prior to amplexus also spend much time submerged on the pond bottom; 4) amplexus is initiated from the pond substrate; 5) Neither males or females have ever been observed to spend time floating at the surface away from the pond perimeter or to swim across the pond at or near the surface at night during breeding activities. All of these observations are consistent with the evolution of an under water mate recognition system.

Category 3 would include species in which a large portion (or all) the males in a population produce calls either in the air or under water. Category 3 may prove to be significant biologically if most or all of the males call (under some set of circumstances) while submerged deeply enough to prevent airborne propagation of signals and females actively seek, locate and amplex with such males, and under different conditions (for example: water temperature, season, wind conditions) call from the surface and successfully engage in amplexus. Category 3 would suggest phenotypic plasticity in call propagation and mating behavior in both males and females. In order to document this mode of calling behavior and to understand its evolutionary merit one would need detailed studies which at the present time have not been undertaken.

To date under water calling has only been investigated in three species of North American ranids and only in one (*R. subaquavocalis*) has the depth of examination reached the level needed to determine whether it conforms to category 1, 2 or 3 throughout its range. *Rana a. aurora* may also be a category 2 species (L. Licht, pers. comm.)

Under water call propagation was examined from a cladistic perspective in order to determine whether it is more likely to be a phenomenon which evolved once or has a history as multiple evolutionary events. As Figure 6 indicates some form of under water calling is present in the *R. boylei* species group which is basal to the leopard frog complex and is seen as well within the *R. tarahumarae* and in the *R. montezumae* species groups. Three species within the *R. boylei* group from the Pacific Northwest are known to propagate calls under water as category 2 calls. These include *R. boylei*, *R. muscosa*, and *R. a. aurora*. Based on their phylogenetic relationships to each other as assessed in this study and others cited in this report it seems most likely that under water calling evolved at least once here and two more times independently among other North

American ranids: Once in the *R. tarahumarae* group and then again within the alpha division (*R. montezumae* group) of Hillis et al., (1983). It therefore seems more parsimonious to conclude for the present that under water call propagation has evolved independently a minimum of three times among North American ranids. The circumstances which favored the evolution of this behavior and the call mode category (1, 2 or 3) into which some species should be placed remain unknown. The latter of these two circumstances is readily amenable to elucidation with field work while the former is more difficult to test but represents a rich area for future work.

In summary morphometric assessment utilizing multivariate stepwise discriminant analysis has produced a discriminant function (Table I) which clearly separates Arizona populations of leopard frogs (Fig. 5) on the basis of species and is congruent with the consensus rule cladogram derived from the 50 biochemical markers. The major finding of fixed differences at five loci between the Mogollon Rim form and other described species of leopard frogs from Arizona, the morphometric differences and the lack of oviducts in males from the Mogollon Rim populations in contrast to their presence in *R. chiricahuensis* from the type locality all support recognition of the Mogollon Rim populations included in this study as a distinct new species. As Figure 4 indicates, the new species is the sister taxon to *R. chiricahuensis* (sensu stricto) and should, therefore, be regarded as a new member of the alpha division of the *R. pipiens* complex of Hillis et al. (1983).

The Leslie Creek population shares the same alleles that tissue samples from specimens from the Chiricahua Mountains possess, confirming that they represent the same species, *R. chirichauensis* (sensu stricto). As redefined, *R. chiricahuensis* would include those populations from Arizona and New Mexico conforming to the general characteristics in the original description (Platz and Mecham, 1979) which also possess

vestigial oviducts in males. Requiring males to possess vestigial oviducts removes those populations from the Mogollon Rim which lack this feature permitting reassignment to the new species. Recognizing the Mogollon Rim form as a new species brings the total number of leopard frog species in U. S. to eight, seven of which are now found within the borders of the state of Arizona. Three of these (*R. chiricahuensis*, *R. subaquavocalis*, and the Mogollon Rim form) are representatives of the alpha division and four are members of the beta division (*R. pipiens*, *R. blairi*, *R. yavapaiensis*, and *R. berlandieri*.) of the *R. pipiens* complex as described by Hillis et al. (1983). Of these, six have well-documented (published) airborne male vocalizations and one, *R. subaquavocalis*, is best regarded as a category 2 call mode species in which acoustical signals are propagated under water.

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APPENDIX I.

Specimens Utilized in Multivariate Stepwise
Discriminant Analysis in Figure 5

Specimens are on deposit at the following institutions: American Museum of Natural History (AMNH), Arizona State University Vertebrate Collection (ASU), University of Michigan Museum of Zoology (UMMZ), University of Utah Teaching Collection (UUTC). Sample sizes identify adult specimens and are represented by n. Numbers in parentheses following specific catalog numbers identify the series from which adults were taken to form part or all of a locality sample. Specimens held by the author are identified by initials, JEP. Localities (from Fig. 5) are identified in brackets, and specimen catalogue numbers for each species are as follows.

Rana chiricahuensis.—[1] Leslie Creek, 0.8 km above point where Hwy 181 crosses Crk., Cochise Co., Arizona. (n=9) ASU 32148-156. [6] Herb Martyr Dam, Cochise Co., Arizona.—(n=25) AMNH 100372-82; ASU 14866, 14869, 14877, 14889; UMMZ 150049-54; (n=4 of the 25 adults for locality 6 are from within the series) ASU (14853-89).

Rana subaquavocalis.— [2] Ramsey Canyon, Cochise Co., Arizona.—(n = 6) AMNH 136096-101.

Mogollon Rim form (sp. nov.) — [3] New Tank, Coconino Co., Arizona.—(n=25) ASU 14966-68; 14974-97; [4] Jones Crossing, Coconino Co., Arizona.—(n=25 from within the series) ASU (14751-824); [5] Luna Lk., Apache Co., Arizona.—(n=25 from within the series) ASU (14608-47).

Rana yavapaiensis.—[7] Tule Creek, Yavapai Co., Arizona.—(n=25) AMNH 117632-39; UMMZ 174839-44; (n=14 of the 25 from locality 7 are from within the series) ASU (15123-42).

Rana pipiens.—[8] Salt Lake City, Davis Co., Utah.—(n=9) UUTC; [9] Daves Tank, Coconino Co., Arizona.—(n=11 from within the series) ASU (14652-79)

Rana berlandieri.—[10] San Angelo, Tom Green Co., Texas.—(n=13) JEP.