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Journal of the Arkansas Academy of Science, Vol. 43 [1989], Art. 31 FEMALE REPRODUCTIVE TRAITS OF THE SOUTHERN LEOPARD FROG, RANA SPHENOCEPHALA (ANURA: RANIDAE), FROM ARKANSAS

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ABSTRACT

Reproductive characteristics of female southern leopard frogs (*Rana sphenocephala*) were studied from specimens collected in northeastern Arkansas. Egg masses collected from breeding habitats were also examined. A communal ovipositional site was discovered in March 1988 and possessed over 75 egg masses within a 3 m square area. Clutch size from necropsied females averaged 2958.7 eggs, whereas number of eggs per egg mass averaged 2106.5 eggs. Egg diameter averaged 1.76 mm and was similar to populations reported previously in Texas. Positive correlations existed between mean ovum size and female body size and between clutch size and female body size. Negative correlations were found between mean egg diameter and mass per egg as each varied with number of eggs per egg mass. The reproductive traits of *R. sphenocephala* are basically similar to other ranid species.

INTRODUCTION

In many anuran species with complex life cycles, patterns of reproduction are explainable by a clarification of quantitative relationships that exist between female reproductive parameters (Salthe and Duellman, 1973; Salthe and Mecham, 1974). When viewed together along with reproductive mode and other life history traits, these patterns reveal predictable reproductive strategies (Duellman and Trueb, 1986). Salthe and Duellman (1973) showed that even among taxonomically-diverse anuran species, positive correlations are found between ovum size and female body size or snout-vent length (SVL), clutch size and female SVL, and ovum size and size of hatchlings. A negative correlation between clutch size and ovum size is generally the rule (for specific exceptions, see Collins, 1975). These correlations may also characterize intraspecific size-fecundity relationships (Duellman and Trueb, 1986; Berven, 1988).

The southern leopard frog (Rana sphenocephala), a member of the R. pipiens complex of the family Ranidae, is distributed throughout the southeastern and southcentral United States. This common species generally breeds in the late winter or early spring, but the species may breed at all times of the year in the southern portions of its range (Conant, 1975; Mount, 1975). Two distinct breeding periods (early fall and late winter) were observed by Caldwell (1986) in South Carolina. McAlister (1962) reported on variation in ovum size in Texas populations, while Caldwell (1986) summarized information on selection of egg deposition sites and on communal oviposition in South Carolina. General accounts on eggs are mainly limited to egg mass size (Smith, 1961; Rugh, 1964; Mount, 1975; Martof et al., 1980; Johnson, 1987). In the present study, I investigated the reproductive biology of R. sphenocephala specifically to address relationships among several female reproductive parameters.

MATERIALS AND METHODS

Nearly all of the female R. sphenocephala examined in this study (n = 36) were collected from late January to mid-March, 1987 in northeastern Arkansas. Several additional specimens were taken during the same period in 1985 and 1986. Specimens were killed in a dilute chloretone solution, fixed in 10% formalin, and stored in 70% ethanol. Snout-vent lengths (SVL) of females were recorded from preserved animals using vernier calipers. On 12 March 1988, egg masses (n =49) were collected from three locations in Craighead County, Arkansas. These sites were separated by no more than 30 km, and each was situated adjacent to (and just outside) the eastern levee of the St. Francis River (see Breeding Habitat in RESULTS).

Clutch size of each gravid female as well as egg mass size were calculated by estimation using gravimetric techniques. Ovaries were removed from the abdominal cavity and placed into vials of 70% ethanol. At the start of mensuration, each ovary was blotted and allowed to briefly air dry. The entire ovary was then weighed on an analytical balance; this was followed by the weighing of a sub-sample of ova (approximately 150) from the same ovary. Clutch size was estimated by dividing the ovarian mass (to the nearest 0.01 g) by the sample mass and multiplying this quotient by the number of ova in the sub-sample. The value obtained in this manner overestimates the number of ova per ovary because of the additional mass within the ovary (trapped interstitial water and ovarian stroma). To determine a percent error, all ova of a single ovary from six specimens were counted. (Ovaries were air dried and gently pulverized to release ova.) The average percent error was 21.9 ± 3.6 (range, 16.9 to 29.7). This percentage was deducted from the original overestimated number of ova and yielded a final clutch size for each female. The estimation of the number of eggs per egg mass was conducted in a similar fashion. Egg masses were removed from 10% formalin, blotted dry, and allowed to briefly air dry. Each egg mass was weighed (to the nearest 0.01 g); then, as above, a sub-sample of eggs was weighed, and the number counted. The total number of eggs was derived by dividing the mass of each egg mass by the mass of the sub-sample and multiplying by the sub-sample egg count. Unlike the estimation of clutch size, no percent error was determined mainly because evaporation of water was negligible during the rapid preparation and weighing of each sub-sample.

Egg diameters were determined by measuring 10 eggs for each of the above egg mass sub-samples using an ocular micrometer (see Berven, 1988). Following preservation, yolked ovarian follicles were highly irregular in shape and were, therefore, not measured in the present study. Specimens and egg masses are deposited in the Arkansas State University Museum of Zoology. Statistical data (means) are accompanied by \pm two standard errors.

RESULTS

Breeding Habitat

The breeding habitats of R. sphenocephala observed in this study were located at the margins of cultivated fields. These tracts of land sloped toward the levee of the St. Francis River and created lentic pools of temporary water. Globular egg masses of approximately 10 cm in width and 5 cm in depth were found laid either attached to submergent and emergent vegetation or, at some sites where no vegetation existed, were laid unattached. Water depth generally varied from 20 cm to 50 cm. All egg masses collected for study were taken from small, isolated egg clusters of from two to five egg masses. Eggs of these masses had begun embryonic development, but they had not progressed beyond gastrulation. On 12 March 1988, a communal egg deposition site was discovered (Fig. 1). This site was found within several km of other collection sites visited on the same day. The breeding habitat differed from other areas mainly by the abundance of vegetation (predominantly broom sedge, *Andropogon* sp.). Over 75 egg masses were counted in an open area (Fig. 1B) of approximately three square m. The eggs were hatching at this site and were left undisturbed; however, by early June, the site was completely dry as a result of severe drought conditions, and the population was decimated.



Figure 1. Breeding habitat of *Rana sphenocephala* in northeastern Arkansas. A. Communal breeding site, 3.5 km W Childres, Craighead County. Levee of St. Francis River in background. B. Close up of foreground in A showing a large aggregate of egg masses in approximately 25 cm of water.

Reproductive Parameters

The average clutch size of 39 female *R. sphenocephala* ranging from 51 to 89 mm SVL was 2958.7 \pm 328.7 (range, 1700-5537), whereas clutch mass averaged 6.68 \pm 1.02 g (2.37-15.37). The number of eggs per egg mass averaged 2106.5 \pm 171.7 (1289-3366). The mass per egg (22.82 \pm 3.61 mg; 15.73-52.02) was over 20 times as great as the average ovum mass (2.00 \pm 0.28 mg; 1.40-3.66). Egg diameter averaged 1.76 \pm 0.04 mm (1.40-2.52) and was similar to the values reported by McAlister (1962) in Texas. Average mass of 49 egg masses was 65.31 \pm 4.54 g (20.29-105.32).

Quantitative Relationships

The relationships between average ovum mass and female SVL and

clutch size and female SVL are shown in Fig. 2; each reveals a significant positive correlation. The coefficient of determination in Fig. 2A $(r^2 = 0.55)$ predicts that most of the variability in ovum mass is accounted for by female SVL; however, in Fig. 2B $(r^2 = 0.50)$, only 50% of the variation in clutch size is a result of female SVL.



Figure 2. Relationships between average ovum mass and snout-vent length (A) and clutch size and snout-vent length (B) in *Rana* sphenocephala from northeastern Arkansas. r = correlation coefficient.

In Fig. 3, average egg diameter (A) and mass per egg (B) are shown as a function of the number of eggs per egg mass; these relationships indicate inverse (negative) correlations with only the latter being significant. The coefficient of determination in each case (0.04 and 0.22, respectively) indicates that the variability in these *y*-axis parameters (i.e., egg diameter and egg mass) is not explained by their linear relationship to the number of eggs in each egg mass.

In Fig. 4, the relationship between the number of eggs per egg mass is plotted as a function of mass of each egg mass. Although the correlation is positive and significant, the coefficient of determination (0.44) predicts that only 44% of the variability in egg mass number is accounted for by increases in the mass of egg masses.

The clutch size-clutch mass relationship (not shown) reveals a significant positive correlation (r = 0.79; n = 36; P < 0.01) and can be inferred from the relationships in Fig. 2. The coefficient of determination (0.62) predicts that 62% of the proportion of variability in clutch size is attributed to its relationship to clutch mass.



Figure 3. Relationships between average egg diameter and number of eggs per egg mass (A) and mass per egg and number of eggs per egg mass (B) in *Rana sphenocephala* from northeastern Arkansas. r = correlation coefficient.



Figure 4. Relationship between the number of eggs per egg mass and the mass of each egg mass in *Rana sphenocephala* from northeastern Arkansas. r = correlation coefficient.

DISCUSSION

Caldwell (1986) hypothesized that R. sphenocephala resorted to communal egg mass deposition to gain a thermal advantage for the development of eggs and embryos (a behavioral adaptation common in several northern latitude ranid species; see Seale, 1982; Waldman and Ryan, 1983) during cold weather breeding. She also stated that during warm weather, isolated egg masses are laid. The presence of appropriate materials to which R. sphenocephala can attach egg masses will also influence the choice of egg mass deposition site. In my study, the location of the communal ovipositional site appeared to be related to the vegetational characteristics of the breeding habitat; i.e., the communal site was heavily vegetated, whereas the collection sites for isolated egg masses were sparsely vegetated. Male choice of calling sites within the actual breeding grounds could determine where females will eventually lay eggs (Wells, 1977). Although a male lek mating system has been proposed for R. catesbeiana (Emlen, 1977), no studies have shown that this is the case in R. sphenocephala.

The reproductive traits of female R. sphenocephala in northeastern Arkansas indicate that body size varied positively with ovum mass (and inferred ovum size) and clutch size (and inferred clutch mass). Collins (1975) found similar relationships in R. sylvatica, Hyla versicolor, and Bufo americanus in Michigan as did Berven (1988) for R. sylvatica in Maryland. My data support the general conclusions of Salthe and Duellman (1973); yet, contrary to Salthe and Duellman (1973), my data predict that clutch size in R. sphenocephala is positively correlated to ovum size (also found by Collins, 1975). Larger females produce larger clutches with larger ova than do smaller females. Recently, Berven (1988) indicated that an inverse relationship existed between mean clutch size and egg size in all size classes of R. sylvatica. In other words, he found that younger females produced larger clutches with smaller eggs (vice versa in older females). In R. sylvatica, this would suggest that younger females oviposit their entire complement of eggs into a single mass, whereas older females oviposit more than once in a breeding period (as stated by Davis and Folkerts, 1986). I did not age the R. sphenocephala in my study (although skeletochronological aging techniques are available for R. pipiens; see Leclair and Castanet, 1987) and, therefore, cannot make direct inferences about age classes as did Berven (1988). However, an examination of Fig. 3B shows an inverse relationship between mass per egg and number of eggs per egg mass which tends to support his findings. Presumably, as female R. sphenocephala age and grow larger, they begin to partition their clutches into several egg masses which would exhibit large egg size. Small-to-intermediate sized females would oviposit their entire clutches of small eggs. If these assumptions are valid, one would expect to find egg masses exhibiting a minimal number of eggs but would also exhibit a continuum of size, mass, or volume from small to large. This condition is demonstrated in Fig. 4 as there is a four-fold increase in mass per egg mass (approximately 20 g to 80 g) with no accompanying increase in minimal egg number.

CONCLUSIONS

Significant positive correlations exist between ovum size and female SVL and clutch size and female SVL in the southern leopard frog, *R. sphenocephala*, from northeastern Arkansas. Negative correlations occur between mean egg diameter and size of egg mass and mass per egg and size of egg mass (the latter being significant). Smaller females appear to lay egg masses of a larger size and have smaller eggs, whereas larger females partition their clutches into several masses (each with relatively large eggs). A communal oviposition site observed in this study differed from other egg-laying sites by a preponderance of submergent vegetation. Reproductive traits in *R. sphenocephala* are basically characteristic of other ranid species and most anurans.

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