2001

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Recommended Citation

Ryan, T. J., and C. T. Winne. 2001. Effects of hydroperiod on metamorphosis in *Rana sphenocephala*. *American Midland Naturalist* 145:46-53. Available from digitalcommons.butler.edu/facsch_papers/534/

Am. Midl. Nat. 145:46–53

Effects of Hydroperiod on Metamorphosis in Rana sphenocephala

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ABSTRACT.—Hydroperiod, the time a temporary pond holds water, is an important factor influencing recruitment in amphibian populations and structuring amphibian communities. We conducted an experiment to test the effect of hydroperiod on metamorphic traits of the southern leopard frog (*Rana sphenocephala*), a common amphibian in the southeastern United States. We reared larval *R. sphenocephala* in artificial ponds at a density of 32 larvae per tank (initial volume = approximately 650 liter). We dried the tanks according to natural patterns, using three different hydroperiods (60, 75 and 90 d). Experimental hydroperiods had a significant effect on the number of metamorphs and the length of the larval period, but not on overall survival (larvae + metamorphs) nor size at metamorphosis. Our findings confirm a pattern observed in field studies and are similar to results of experimental investigations of closely related ranid frogs. Our results demonstrate that relatively small differences in hydroperiod length (*i.e.*, as little as 15 d) may have large effects on juvenile recruitment in *R. sphenocephala*.

INTRODUCTION

Biotic factors such as interspecific competition and community composition (Morin, 1983), intraspecific competition (Scott, 1990) and predation (Wilbur, 1987; Fauth and Resetarits, 1991) influence the survival to, and timing of, metamorphosis in amphibians. Abiotic factors can be just as important. Paramount among these is hydroperiod (Semlitsch *et al.*, 1996), the time between the filling and drying of temporary ponds and other seasonal wetlands. Temporary aquatic habitats are frequently used by many amphibians as nuptial-natal sites because they feature high primary productivity (Odum, 1969; Wassersug, 1975) and fewer potential predators because fish and other species requiring relatively permanent aquatic habitats are excluded (Heyer *et al.*, 1975; Woodward, 1983).

The importance of hydroperiod on metamorphosis in various amphibians has been determined by field observations (Tevis, 1966; Calef, 1973) and experimental manipulations (Semlitsch, 1987; Semlitsch and Wilbur, 1988; Parris, 2000). Frequently the timing of metamorphosis is regarded as a key determinant of adult fitness, as the age and size at metamorphosis affect patterns of maturation and survival (Smith, 1987; Semlitsch *et al.*, 1988). Observational (*e.g.*, Semlitsch *et al.*, 1996) and experimental (*e.g.*, Wilbur, 1987) studies suggest that hydroperiod may have an effect beyond that of the individual by determining community composition through mediating biotic factors (*e.g.*, inter- and intraspecific competition and predation).

The southern leopard frog *Rana sphenocephala* (=*R. utricularia*) is ubiquitous throughout the southeastern United States, breeding in a diversity of aquatic habitats (Conant and Collins, 1998). Throughout much of its range, breeding is biannual, occurring in the fall and again in the spring when temporary ponds fill with rain (Caldwell, 1986). In general,

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tadpoles require about 3 mo to complete larval development (Martof *et al.*, 1980; Ashton and Ashton, 1988), but longer larval periods resulting from overwintering are not uncommon (Collins and Lewis, 1979). For example, on the Savannah River Site (SRS; Aiken and Barnwell counties, SC), tadpoles resulting from fall breeding overwinter in the ponds and face little risk of pond drying before metamorphosis (Gibbons and Semlitsch, 1991). Tadpoles of spring breeders, however, frequently must complete metamorphosis before the pond dries in the spring/summer. During a 16-y study of an amphibian community on the SRS, Semlitsch *et al.* (1996) found that longer hydroperiods produced significantly more metamorphic *R. sphenocephala* juveniles than did ponds with shorter hydroperiods. This result, however, is confounded because of a complex relationship between hydroperiod, juvenile recruitment and other variables, such as the initial larval density and the density of predatory salamanders. Because of the correlated factors, it is not clear how hydroperiod *per se* influences metamorphosis in this species. In this study we used a mesocosm approach to eliminate potentially confounding variables such as intra- and interspecific competition and predation and focussed on hydroperiod and its effect on metamorphic success of *R. sphenocephala.*

MATERIALS AND METHODS

We used 12 1000-liter polyethylene cattle tanks as artificial ponds. Each tank was equipped with a moveable internal standpipe that allowed us to manipulate water levels. On 2 April 1999 we filled each tank to a depth of 40 cm with untreated well water (approximately 800 liter). After filling, each tank was covered with a mesh fiberglass screen that remained in place throughout the experiment to prevent colonization by predatory insects (*e.g.*, odonates) and other anurans. We added approximately 180 liter aged rainwater with natural algal colonies to each tank on 5 April. Each tank received an equal-sized aliquot of concentrated zooplankton mixture collected from several local Carolina bays on 3–5, 7, 9, 10 and 13 April. We added 1.5 kg (dry) leaf litter to each tank on 5 April and an additional 1.0 kg on 7 April; we soaked each aliquot in water for at least 48 h prior to addition to encourage rapid settling. Finally, we lowered the water level in each tank to a uniform depth of 35 cm (approximately 650 liter) on 13 April.

We collected a freshly oviposited (<12 h) clutch of *Rana sphenocephala* eggs at Carolyn's Bay on 1 April and again on 6 April 1999. Carolyn's Bay, located on the SRS in Aiken Co., SC, is a 0.49 ha Carolina bay with a herbaceous substrate dominated by cut grass (*Leersia hexandra*), water millfoil (*Myriophyllum heterophyllum*), water shield (*Brasenia schreber*) and water lily (*Nymphaea odorata*). The pond fills with rain in the fall or winter and dries in the spring or summer, and the hydroperiod varies considerably among years (R. D. Semlitsch, pers. comm.). In exceptional cases the pond retains water throughout an entire year (*e.g.,* the bay held water continuously from winter of 1995 until September 1996; also *see* Winne and Ryan, 2001) or extremely short hydroperiods (*e.g.,* <2 mo in fall 1996). During this study we also observed *Rana capito, Bufo americanus, Ambystoma talpoideum* and *Notophthalmus viridescens* using this bay as a nuptial-natal site. Egg masses were maintained in the laboratory in polyethylene containers filled with water from Carolyn's Bay until hatching commenced. Newly hatched tadpoles were haphazardly assigned and introduced to tanks on 7 April (n = 10 per tank) and 12 April (n = 22 per tank); the staggered introduction of tadpoles reflects asynchronous breeding activity of *R. sphenocephala* at Carolyn's Bay.

We used three hydroperiods (60 d, 75 d and 90 d), each replicated four times, for a total of 12 tanks. The hydroperiods were within the normal range observed at *Rana sphenocephala* breeding sites in this region (Semlitsch *et al.*, 1996). They also posed a "challenge" to larval *R. sphenocephala,* as the shortest hydroperiod was about 1 mo short of the typical age at metamorphosis during spring breeding in this region (D. E. Scott, pers. comm.). The drying rate (approximately 1 cm/d, lowered every 5 d) was based on observed changes in maximum depth at Carolyn's Bay over the summer of 1997. We achieved the desired tank depth by bailing water by hand, then adjusting the standpipe to maintain a constant water depth.

After day 30, the tanks were visually surveyed every day for the presence of metamorphs [=individuals in the process of metamorphosis with all four limbs present; approximately Gosner stage 42 (Duellman and Trueb, 1986)]. We also placed four plastic minnow traps in each tank to passively collect metamorphs. Minnow traps were checked thrice daily (approximately 0700, 1300 and 1900 h). Metamorphs with tails equal to or longer than the length of the body were returned to the tank, while metamorphs with tails shorter than the length of the body were taken to the laboratory until tail resorption was completed. Following complete tail resorption, the length of the larval period (d), mass (to the nearest 0.01 g) and snout-vent length (to the nearest 0.1 mm) were recorded for each juvenile. On the final day of any given hydroperiod we drained each tank and thoroughly searched the leaf litter, collecting any remaining metamorphs and the surviving tadpoles. Metamorphs with tails *longer* than the length of the body were counted as tadpoles, as survival of these individuals is highly unlikely in terrestrial environments because the tail hinders terrestrial locomotion (*e.g.,* Wassersug and Sperry, 1977) and other characters (*e.g.,* mouth parts, respiratory organs) are incompletely developed (Emerson, 1986). Other metamorphs were treated as described above.

The mean responses of *Rana sphenocephala* in each tank were the independent units of analyses (Hurlbert, 1984). We used a single-factor analysis of variance (ANOVA) to test the effect of hydroperiod on overall survival (tadpoles and metamorphs at the end of the hydroperiod). The proportion of survivors was angularly-transformed (arcsine of the square root) to achieve normality. We used a multivariate analysis of variance (MANOVA) to test for the effect of hydroperiod on the three response variables: the proportion of individuals in each tank completing metamorphosis (angularly-transformed), the length of the larval period (inverse-transformed) and length of the larval period (log-transformed). All transformation rendered the data normally distributed and appropriate for parametric tests. Univariate ANOVAs were used to evaluate each response independently. We used analysis of covariance (ANCOVA) on log-transformed mass with log-transformed SVL as a covariate to account for the potentially confounding effect of body length (SVL) on mass. We report Type III Sums of Squares for the MANOVA and ANCOVAs. All analyses were conducted using SAS (version eight).

RESULTS

There were no significant differences in overall survival among the different hydroperiod treatments ($F_{2,9} = 0.1396$, $P = 0.87$), with per tank survival ranging from 43.75% to 84.38% [treatment mean % (1 SE): 60 d = 63.3 (0.026); 75 d = 64.1 (0.070); 90 d = 58.6 (0.089)]. The multivariate response of metamorphs to hydroperiod was significant (MANOVA: Wilks' $\lambda = 0.0509$; $F_{6,14} = 8.01$; $P = 0.0007$); univariate ANOVAs indicate that this result is due to the effect on age at metamorphosis (ANOVA: $F_{2,9} = 25.65$; $P = 0.0002$) and proportion metamorphosing (ANOVA: $F_{2,9} = 20.11$; $P = 0.0005$), but not mass at metamorphosis (ANOVA: $F_{2,9} = 0.52$; $P = 0.61$). About 90% of survivors completed metamorphosis in the 75 and 90 d tanks, as compared to less than 35% in the 60 d tanks (Fig. 1A). Metamorphs from the 60 d tanks were significantly younger than those in the 75 d and 90 d (Fig. 1B). There were no significant differences in SVL-corrected mass at metamorphosis [ANCOVA: $F_{2,6} = 3.50$, $P = 0.10$; uncorrected treatment means g (1 SE) 60 d = 2.3 (0.11); 75 d = 2.1

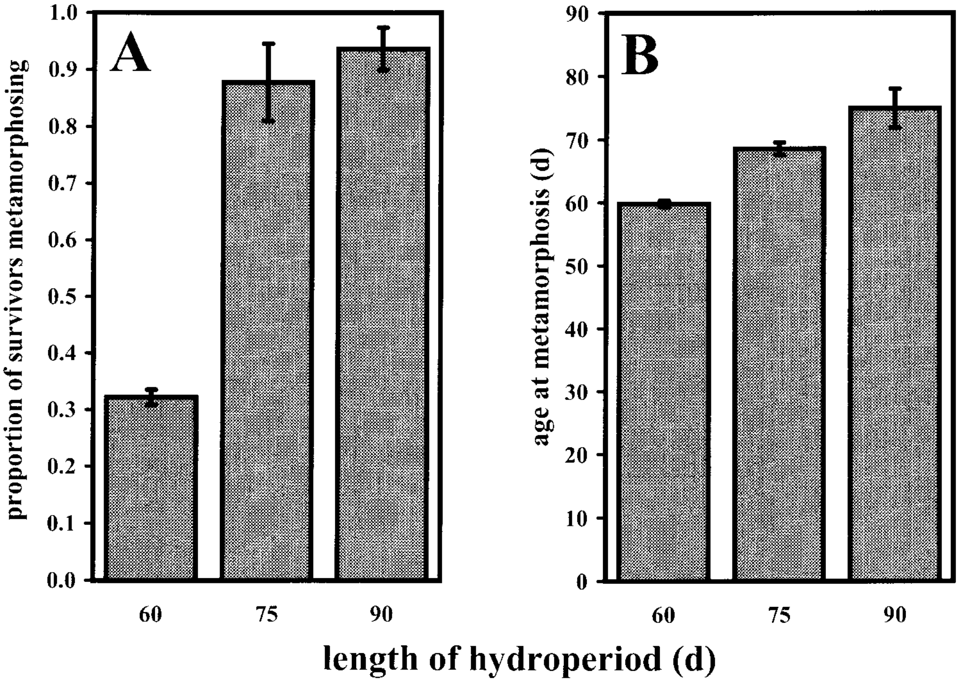

FIG. 1.—Effect of hydroperiod on: A) the mean proportion of survivors completing metamorphosis and B) their mean age at metamorphosis in *Rana sphenocephala*. Error bars equal 1 SE

(0.23); 90 d = 2.2 (0.08)]. The interaction between hydroperiod and SVL was likewise not significant (ANCOVA: $F_{2,6} = 3.49$, $P = 0.10$), indicating that metamorphs from each treatment had a similar mass-SVL relationship (Fig. 2).

DISCUSSION

The tadpoles used in this study came from two different clutches oviposited 5 d apart. Asynchronous reproductive activity is common in *Rana sphenocephala* (*e.g.*, Caldwell, 1986) and likely contributes to intrapopulation variation in metamorphosis and juvenile recruitment. Our experiment was not designed to test whether differences among sibships in the timing of oviposition, hatching or addition affected timing and size at metamorphosis. It is possible, for example, that the metamorphs in the 60 d tanks were all or mostly representatives of the first clutch oviposited. However, all experimental units (tanks) were treated identically; each tank in each treatment received the same number of hatchlings from either clutch on the same day.

Hydroperiod duration did not affect survival in the tanks. However, hydroperiod treatments influenced the production of juveniles, with significantly fewer individuals completing metamorphosis in 60 d than in 75 d and 90 d tanks. Age at metamorphosis had a similar pattern; juveniles from the 60 d hydroperiod had significantly shorter larval periods than those from the other treatments. If the population of *R. sphenocephala* breeding at Carolyn's Bay is representative of other populations in this region, hydroperiod length is probably the most critical determinant of juvenile recruitment (Pechmann *et al.*, 1989; Semlitsch *et*

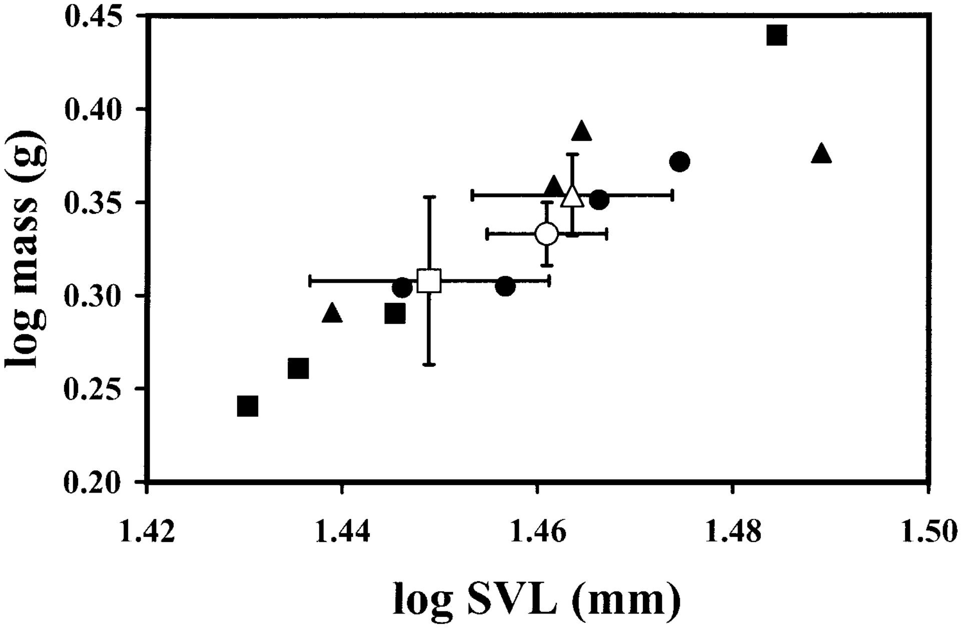

FIG. 2.—Effect of hydroperiod on the relationship between snout-vent length (mm; log-transformed) and mass (g; log-transformed) in metamorphosed *Rana sphenocephala*. Each filled point represents a tank mean, open symbols represent treatment means (error bars equal 1 SE): triangles = 60 d; squares = 75 d; circles = 90 d hydroperiods

al., 1996). The biannual breeding cycle of *R. sphenocephala* places different demands on tadpole growth and development depending on when breeding occurs. Tadpoles produced in fall are less likely to experience pond drying, as they hatch shortly after pond filling and during the period of greatest hydrological stability (*i.e.,* ponds are less likely to dry in the fall/winter than in to spring/summer). These tadpoles overwinter and initiate metamorphosis the following spring (Caldwell, 1986; Gibbons and Semlitsch, 1991), well after achieving a minimum age and/or size for metamorphosis. Tadpoles hatching in spring, however, face the temporal instability of the larval habitat (Semlitsch, 1987; Newman, 1989; Parris, 2000). A *relative* hydroperiod of at least 75 d (*i.e.,* measured from the time of hatching rather than pond filling) is required for the maximal production of *R. sphenocephala* juveniles during spring breeding. Whether tadpoles produced from fall and spring breeding react to pond drying in a similar manner is unknown, although there is no *a priori* reason to expect them to react dissimilarly.

The effects of environmental conditions on size at metamorphosis are mediated through correlated effects on the timing of metamorphosis. Theoretical treatments (such as Wilbur and Collins, 1973) predict a positive correlation between the length of the larval period (*i.e.*, age at metamorphosis) and size at metamorphosis. This result has been demonstrated in several experiments (Semlitsch and Wilbur, 1988; Newman, 1989; Semlitsch and Reyer, 1992; Denver *et al.*, 1998). Despite the clear effect of hydroperiod on the number of juveniles produced and their age at metamorphosis in the present study, we found no effect of hydroperiod on the condition (SVL-corrected mass) of the metamorphosing juveniles. This is consistent with other artificial pond experiments that investigated the importance of hydroperiod in other species of the *Rana pipiens* complex. Parris (2000) found that, while hydroperiod length influenced survival to metamorphosis in Missouri populations of *R. blairi, R. sphenocephala* and their hybrids, no effect was detected on size at metamorphosis. In leopard frogs, body size at metamorphosis is apparently most heavily impacted by intraspecific and interspecific competition (Parris and Semlitsch, 1998; Parris *et al.*, 1999). In the absence of density effects, mass at metamorphosis does not appear to be affected by hydroperiod, regardless of age at metamorphosis (Parris, 2000; this study), although Parris found correlated effects of size and age at metamorphosis in a laboratory experiment using ranid tadpoles. We detected significant developmental plasticity in the length of the larval period, but not a correlated change in body size.

Our results demonstrate that *Rana sphenocephala* tadpoles accelerate development in response to pond drying; the result is early metamorphosis, which shifts the timing of metamorphosis but *does not* reduce overall growth. Changes in some critical life history characters (such as size at metamorphosis and maturation) within populations may occur in nature, but often depend on the effect(s) of other factors, such as competition and predation (Wilbur, 1987; Scott, 1990; Fauth and Resetarits, 1991; Semlitsch *et al.*, 1996). The longer larval periods experienced by overwintering *R. sphenocephala* tadpoles may result in appreciable differences in body size at metamorphosis, and differences in age and size at metamorphosis within an annual cohort are likely accounted for by the biannual breeding cycle.

Our study demonstrates adaptive plasticity in the timing of metamorphosis and confirms that hydroperiod may affect the production of juvenile *Rana sphenocephala* in the Upper Coastal Plain of South Carolina. Moreover, these results have clear implications for management for *R. sphenocephala* in particular, and other amphibians in a more general sense, by emphasizing the role of hydroperiod in determining the success of metamorphosis in larval amphibian populations/communities. The size of metamorphosing juveniles appears to be unaffected by variation in hydroperiods. We suggest that in years marked by early pond drying, the most prominent effect on *R. sphenocephala* populations is a reduction of juvenile recruitment into the adult population rather than diminished size at metamorphosis. Although long-term field studies (*e.g.,* Semlitsch *et al.*, 1996) strongly suggest that both biotic and abiotic factors are important in determining the timing of metamorphosis, exactly how pond drying influences other factors remains unclear. Additional experiments will be required to determine the extent that biotic interactions (inter- and intraspecific competition and predation) are impacted by pond drying in shaping patterns of metamorphosis in *R. sphenocephala* (*see* Parris, 2000).

Acknowledgments.—We thank M. J. Parris for counsel on hydroperiod treatments, *Rana* husbandry in cattle tanks and for an astute review of the manuscript. K. A. Buhlmann, J. W. Gibbons and an anonymous reviewer also made valuable comments on the manuscript. The authors thank the Savannah River Ecology Laboratory Education Program for support in the form of Long-Term and Short-Term Graduate Research Fellowships (for the first and second authors, respectively). This research was supported by Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation.

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SUBMITTED 3 JANUARY 2000 ACCEPTED 19 JULY 2000