Pakistan Journal of Biological Sciences 3 (10): 1784-1789, 2000
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Variation in Developmental Duration and Metamorphosis of the Green Toad, *Bufo viridis* in Temporary Ponds as an Adaptation to Desert Environment

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Abstract: The green toads, *Bufo viridis* breed in temporary desert ponds of variable duration and exhibit extreme plasticity in the timing of metamorphosis. Pond duration depended on initial depth and frequency of rainfall. Tadpoles in shorter duration ponds metamorphosed earlier than tadpoles in longer duration ponds. The larvae complete their development rapidly before the pond dried, but resulted in small size at metamorphosis. The effects of changing resource availability on the timing of metamorphosis were examined by raising the larvae in a factorial field experiment manipulating food level, larval density and pond duration. At constant pond duration, tadpoles at constant high food level metamorphosed earlier at largest sizes. At low food level, tadpoles metamorphosed at a uniformly small size, but varied between treatments in the time required to reach that size. Tadpoles from both decreasing density and low food treatments metamorphosed earlier than tadpoles from increasing density and low food treatments. These results support the idea that tadpoles can respond adaptively to resource variation.

Key words: Developmental duration, metamorphosis, *Bufo viridis*, desert environment

Introduction

Environmental variability affects processes at all levels and scales of ecological organization. Local variation, for example, probably accounts for much within population variation in birth and death rates. Incorporating environmental variation into theories of life histories, population dynamics and community organization requires a detailed understanding of how environmental factors influence physiology, development, behaviour and the resulting life histories of individuals (Dunham et al., 1989).

The study of larval amphibian development has provided considerable insight into the ecology and evolution of organisms in heterogeneous environments (Wilbur, 1980, Travis, 1983, Semlitsch and Gibbons, 1985; Werner, 1986; Newman, 1992; Shi and Ishizuya-Oka, 1996). The aquatic larval stage of the amphibian life cycle is considered an adaptation to transient productive habitats (Wassersug, 1975; Wilbur, 1980; Semlitsch et al., 1988). Many amphibian species breed in temporary ponds that are sporadically filled by rain and then dry at different rates. The rate of pond drying depends both on the geological characteristics of the pond and climatic factors (Bragg, 1965). In the desert habitats desiccation is arguably the single most important environmental factor affecting larval survivorship, and species that breed in such ponds have evolved several traits that allow successful development. For instance, these species tend to have short periods of development compared with species that breed in more permanent environments (Low, 1976; Denver, 1997). In addition, larvae of several species have been shown to accelerate metamorphosis in response to habitat desiccation. The plasticity in larval amphibian development may be adaptive in such ephemeral environments (Steeves, 1989; Newman, 1992).

The effects of resource level have been a major focus in all studies of amphibian ecology, particularly for the larval stage (Alford and Harris, 1988; Ludwig and Rowe, 1990; Pfennig et al., 1991). Resource availability may be especially critical in ephemeral desert ponds, where larvae must acquire sufficient energy to grow and develop before the pond dries. In these ponds, low food level or high density in combination with short pond duration may be a major cause of mortality, because tadpoles may be unable to metamorphose before a pond dries (Wilbur, 1987; Pfennig, 1990). Variation among ponds in density, food level, and duration may generate considerable variation in larval survival and size at metamorphosis (Collins, 1979; Smith, 1983; Pfennig et al., 1991; Hussein, 1995). Size at metamorphosis may affect juvenile physiology of performance (Taigen and Pough, 1985; John-Adler and Morin, 1990), survivorship (Pfennig et al., 1991) and size at maturity (Jarr, 1990). Variation in the larval environment, therefore, may be a major contributor to variation in individual fitness and population dynamics (Newman, 1988; Berven, 1990). Because the green toads, *Bufo viridis* breed in ephemeral desert ponds of variable duration, exhibit a rapid development time and have strong responses to environmental variation, tadpole’s of this species provide an excellent model system to study the ecological and evolutionary consequences of environmental variability.

The present study was conducted to manipulate single aspects of the environment that accompany habitat desiccation in order to determine the degree of plasticity in developmental response. On the other hand, little attention has been paid to identification of the proximal environmental cues that signal a drying pond so, the effects of pond drying and growth history were determined. The larvae were also raised in a factorial experiment manipulating food level, larval density and pond duration under field conditions to test several potential environmental cues that tadpoles respond to deleterious changes in their larval habitat.

Materials and Methods

Study site and species: The study site is Burg El-Arab region, 60 km west of Alexandria on the northwestern coastal region of Egypt (Fig. 1). The climate, soils and water resources of this region can be qualified as semiarid (UNESCO, 1977). The region is characterized by the presence of two distinct seasons; a cool and rainy season from December to February (the temperature ranges from 9.6 to 14.6°C with a mean of 11.9°C in January, the mean annual precipitation is 150 mm), and a hot dry season from June to August (the temperature ranges from 27.8 to 35.8°C with a mean of 31.7°C in July). The relative humidity appears to be without marked

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fluctuations as it ranges from 60 to 75%. Although occasional short rainstorms occur in winter, most of days are sunny and mild. According to FAO (1970), the topography of the area have different structural habitats such as rocky ridges, depressions and eroded land. The soils of this region are essentially alluvial or silt derived from the Mariut inland plateau and composed essentially of limestone alternating with strata of limestone and shale, so the primary cause of water loss after rainfall is evaporation.

The green toad, Bufo viridis Amphibia, Salientia, Bufonidael was chosen as the study organism because it is abundant in the study site. Their tadpoles exhibit a rapid developmental time and have strong responses to environmental variation. Ties toad is active throughout the whole year except in winter, where it hibernates. It is a nocturnal animal and observed lumping after sunset around scattered ponds and water remains. The general colour of the back is grayish olive with green spots but it is very changeable with the surrounding medium. The breeding season of this species starting in early spring essentially in temporary ponds that are sporadically filled by rain and then dry at different rates. The breeding activity were monitored both by listening for mating calls which looks like the slow creaking of a door and observing amplexus. After the short breeding period, the spawning occur in March and than larval development and metamorphosis takes place (Degani et al., 1984; Hussein, 1995).

Field observations and experimental design: The season of toads activity, mating behaviour and oviposition were detected until larvae were free swimming. After tadpoles had become mobile, the natural ponds were seized at regular intervals until metamorphosis occurred and the young toads left then, the ponds were visited daily until they dried. The data collected for each pond included maximum depth, water temperature, larval duration until the newly metamorphs left. Water temperature was measured 1 cm below the surface and 1 cm above the bottom at maximal depth. The natural ponds were daily inspected for metamorphs (individuals with at least one forelimb exposed, stage 58, Gosner, 1960). Metamorphs were easily observed and caught as they spent much of their time at the surface near the edges of ponds. Body length (snout vent length) of all collected metamorphous were measured to the nearest 0.1 mm and the length of larval period for all metamorphs were also estimated as the median time from oviposition within a pond to the metamorphosis as soon as forelimbs appeared. Total number of metamorphs depended on the initial density of hatchlings that could not be determined by visual inspection and were not included in the observations of natural ponds.

The effects of pond drying on growth history were estimated by raising larvae in a factorial experiment manipulating food level, larval density and pond duration under field conditions. Eight experimental ponds were established in the field near the natural ponds. Ponds were roughly oval with surface dimensions of 0.9 x 1.4 m tapering 0.5 x 0.8 m at the bottom. The substrate of the study site is silt, so the primary cause of water loss is evaporation and pond duration, therefore, depends on initial water depth which is an indirect way of controlling pond duration. Ponds either 35-65 cm deep were made resembling the minimal and maximal initial depth of the natural ponds respectively, the advantage is that these experimental ponds dry exactly as do natural ponds, All the experimental ponds were filled with rainwater from a neighboring pool.

The experiment employed a factorial design with two levels of population density (100 or 200 individual), two levels of food (15 or 30 g of boiled spinach day) and two levels of pond duration with initial depth either 35 or 65 cm (Fig. 2). In the first 4 treatments (shallow pondweitinitial depth - 35 cm). Two ponds were allocated with 100 B. viridis tadpoles (low density) received daily either 15 g food supplement (low level) or 30 g food supplement (high level) and either two allocated with 200 B. virides tadpoles (high density) received daily either low food level or high food level. Other four treatments were similar to the above cited condition, of food level and population density but deep ponds (initial depth - 65 cm were used. The tadpoles for the above cited experiment were collected from same natural ponds after three days of oviposition and distributed an the same day, when all tadpoles were at stage 44 in which the beginning of feeding takes place. This stage of development is exactly as in the natural ponds. The experimental ponds were visited daily to measure the changes in maximum depth, water temperature (as in natural ponds) and to record when each pond dried. Metamorphosing individuals in different experiments were collected from the ponds as soon as fore legs appeared. These were transferred to separate plastic boxes lined with damp tissue paper to complete metamorphosis. When the tail was fully disappeared, the snout-vent length were measured. Survival and percent metamorphosis was also determined. Larval survival is the proportion of the original stock of tadpoles that remained alive or had metamorphosed at the time of pond drying. Percent metamorphosis is the proportion of the original stalk that metamorphosed.

Analysis of variance of body length of metamorphs and length of larval period together with pond duration were performed by two-way ANOVA according to Green (1978). The effects of population density, food level, and pond duration and interactions between them on larval period and size at metamorphosis were also performed by F-ratio test according to Sokal and Rohlf (1981). If significant changes were indicated, levels of significance were inferred at p<0.01. The study were conducted from December, 1999 to the end of April, 2000.

Results

Field observations indicated that the amounts of monthly precipitation were net equally throughout the rainy cold season. Most of the rainfall occurs during January and February. The ponds were refilled completely after each rainfall as soon as adults emerged from hibernation in late winter. This was shortly followed by short breeding period and spawning until larvae were free swimming.

The developmental duration in natural ponds depended, in part, on pond duration and the variation in pond duration was primarily depended on initial depth. The data presented in Table 1, reveals that the pond duration ranged from 22 to 34 day. Tadpoles in shorter duration ponds metamorphosed earlier, on average, than tadpoles in longer duration ponds. The first appearance of metamorphs was also earlier in shorter duration ponds. In natural ponds, tadpoles were able to metamorphose generally between 9.8 to 11.8 mm with the larval period ranged from 21.1 to 31.8 day (Table 1).

The correlation between size of tadpoles at metamorphosis and larval period was positive correlation but not significant (p > 0.05). On the other hand, water temperature (surface and bottom) of natural ponds was increased as ponds dries (Fig. 3).
accelerated development could be explained by an elevation monitored throughout the experiment to determine if the ponds. The bottom and surface water temperatures were constant for natural and experimental ponds. This pattern was the same for natural and experimental ponds. Food level also affected length of larval period. The tadpoles in high food, low density taking shorter time to develop than those in low food, low density. For a given pond duration, larvae in high food ponds metamorphosed earlier than did larvae in low food. Also, the larvae grew much better under conditions of low population density than high one. The correlation between density level and larval period was positive but not significant (p>0.01, Table 3). The Statistical test of linearity isoslopes of separate regressions) for the interaction between food level and pond duration reveals that the length of larval period on pond duration for each food level were significantly different (0.01>>0.001, Table 2), indicating that the effect of pond duration on timing of metamorphosis was strongly affected by food level. In long-duration ponds, metamorphosis occurred later in either low or high food treatments (Fig. 4). However, the effect of the interaction between pond duration and food was small compared to the overall effect of pond duration (p<0001, Table 3). Tadpoles at constant high food in long pond duration metamorphosed at the largest sizes. At low food level, tadpoles metamorphosed at Small sizes, but varied in the time required to reach that size according to pond duration and treatment (Fig. 4). Short pond duration accelerated metamorphosis at a smaller size. The correlation between size and metamorphosis in response in habitat desiccation. Low food function to alter developmental duration and timing of metamorphosis in response in habitat desiccation. Low food and high population density conditions lowered growth and development, regardless of pond duration. Higher food level.
**Fig. 1:** A location map of the study site.

**Fig. 2:** The factorial field experimental design manipulating food level, larval density and pond duration used by the tadpoles of the green toad, Bufo viridis.

**Fig. 3:** Average surface and bottom water temperatures of the natural experimental ponds. Each point represents the mean temperature at each depth and vertical lines represent ± SE in the natural ponds.

**Fig. 4:** Developmental duration of Bufo viridis, tadpoles in different pond durations: (a) short pond duration, (b) long pond duration. Each point represents the average of three replicate of one pond per treatment. Numbers are the sizes at metamorphosis (mm).

**Fig. 5:** Larval survival and percent metamorphosis in experimental ponds at different levels of population density, food and pond duration.
or lower tadpole density resulted in higher proportion of larval survival, higher percent metamorphosis, faster development and larger size at metamorphosis. Shorter pond duration resulted in an earlier developmental duration (earlier metamorphosis) and smaller size at metamorphosis. Thus, the results presented here are consistent with models that predict smaller size at metamorphosis when the growth opportunity in aquatic habitat is diminishing and earlier metamorphosis when growth opportunity decreases during the larval period (Wilbur and Collins, 1973; Werner, 1986; Ludwig and Rowe, 1990; Rowe and Ludwig, 1991). Timing of metamorphosis and pond duration were correlated in both natural and experimental ponds. The similar relations between pond drying and metamorphosis in experimental ponds and natural ponds indicate that developmental plasticity may play an important role in the ecology of this population. Percent metamorphosis in the experimental ponds depends on larval survivorship, and larval survivorship may be related to metamorph size (Smith, 1987; Semlitsch et al., 1988).

However, the effect of pond duration on timing of metamorphosis, size at metamorphosis and survival depended on food level, so tadpoles in high food ponds attained a larger size and metamorphosed somewhat earlier for a given pond duration than did tadpoles in low food ponds. Additionally, pond drying may correlate with increasing population density and water temperature which also affect amphibian development (Stewart, 1956; Wilbur and Collins, 1973; Smith-Gill and Berven, 1979; Alford and Harris, 1988).

As ponds dried and its depth decreased, the larval density greatly increased, so the amount of food, added to experimental ponds did not decrease as pondA dried, but decreased foraging area and increased crowding may have diminished growth and survival (Gromko et al., 1973). Temperature may also influence the rate of development through a general effect on the rate of physiological processes or through the growth rate of the tissue production of hormones that promote development (Etkin, 1964; Dodd and Dodd, 1976). Higher temperature may cause faster larval development and slower growth (Smith-Gill and Berven, 1979), all of which were seen in short duration ponds. Temperature effects, therefore, appear to be consistent with the effect of pond drying.

Increased crowding as short duration ponds dried may have caused slower growth. Wilbur and Collins (1973) proposed that decreased growth rate may cause initiation of metamorphosis. It is even possible that different effects of pond drying such as decreased growth rate and accelerated development may have different causes (increased crowding and increased temperature). However, even of the pond drying affect is not the result of changes in growth rate, the interaction between food levels and food level suggests that growth or size also influences timing of metamorphosis. The present experiments with different treatments demonstrated that pond drying times and chance physiological differences among individuals may be one important component of the within - group variation. In the populations of short duration ponds, larger numbers of individuals metamorphosed earlier at smaller sizes and the populations tend to dominate the smaller individuals due to behavioural interactions as a result of crowding. This process generate an increase in variance as the larval period proceeds. Griffiths and Foster (1998) have argued that behavioural interactions can account for the crowding effect that Gromko et al. (1973) and others have attributed to art interference mechanism of competition. These previous experimental studies have not controlled food levels (as in The present study) but have attempted to keep food constantly available. The space per individual decrease and individuals interactions increase With increasing population density, elowiaver, increased interaction between individuals, resulted in increased variation in size. so. the average growth rate was also affected which may lead to smaller size at metamorphosis. Phenotypic plasticity in larval duration and size at metamorphosis is found in all amphibian families (Wilbur and Collins, 1973; Werner, 1986; Newman, 1992). Several species from diverse families have been shown to be capable of accelerating metamorphosis as their pond dries (Byte americaniS, Wilbur (1987); Atystotna talpoideum, Semlitsch and Wilbur (1988); Hyla Aseidopvma, Crump (1989); Scaphlopus couchli, Morey and Janes (1994). In general, these species land auto viniliis; this study) breed in temporary ponds and exhibited plasticity in developmental duration in response to a changing in water level, and this response varied in direct relation to the magnitude of the environmental signal. Metamorphosis in response to pond drying may be as much an adaptation to the temporary desert ponds as is the rapid development achieved by desert species.

References
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