Adaptive plasticity in anuran metamorphosis: response of tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae) to pond drying

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**Abstract**

Adaptive plasticity in anuran metamorphosis: response of tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae) to pond drying. The influence of desiccation on metamorphic traits (larval duration and size at emergence) was studied in *Polypedates maculatus* under laboratory conditions. Gosner Stage 23 tadpoles were exposed to decreasing water levels (gradual or rapid) until the beginning of metamorphic climax (Stage 42). A control group was reared in constant water levels. Tadpoles reared in decreasing water levels reached the metamorphic climax earlier and metamorphosed at a smaller size than those reared in constant water levels. Further, tadpoles experiencing rapid depletion of water reached the metamorphic climax earlier and metamorphosed at a smaller size than those experiencing gradual depletion of water levels. Tadpoles of *P. maculatus* showed adaptive plasticity in metamorphosis to pond drying. Survival of tadpoles in treatments and the control was 100%. The study revealed that tadpoles of *P. maculatus* have plastic development in response to water levels; the trade-off between growth and development favors development, which results in early metamorphosis at a small size.

**Keywords:** Desiccation, Metamorphic traits, Phenotypic plasticity, Tadpoles, Tree frog.

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**Resumo**

Plasticidade adaptativa na metamorfose de anuros: resposta dos girinos de *Polypedates maculatus* (Anura: Rhacophoridae) ao dessecamento do tanque. A influência do dessecação nas características metamórficas (duração do estágio larval e tamanho na emergência) foi estudada em *Polypedates maculatus* sob condições de laboratório. Girinos no Estágio 23 de Gosner foram expostos a níveis de água decrescentes (graduais ou rápidos) até o início do climax metamórfico (Estágio 42). Um grupo-controle foi criado em níveis de água constantes. Os girinos criados em níveis de água decrescentes atingiram o climax metamórfico mais cedo e se metamorfosearam em um tamanho menor do que aqueles criados em níveis de água constantes. Além disso, os girinos que sofreram um rápido esgotamento da água atingiram o climax metamórfico mais cedo e se metamorfosearam em um tamanho menor do que aqueles que sofreram um esgotamento gradual dos níveis de água. Os girinos de *P. maculatus* mostraram plasticidade adaptativa na metamorfose em
relação ao dessecamento do tanque. A sobrevivência dos girinos nos tratamentos e no grupo-controle foi de 100%. O estudo revelou que os girinos de *P. maculatus* têm um desenvolvimento plástico em resposta aos níveis d’água; a compensação entre crescimento e desenvolvimento favorece o desenvolvimento, o que resulta em metamorfose precoce em um tamanho pequeno.

**Palavras-chave:** Características metamórficas, Dessecamento, Girinos, Pererecas, Plasticidade fenotípica.

### Introduction


In temporary water bodies, desiccation is a threat, and completion of metamorphosis before drying of aquatic habitats is necessary. Slow growth rates and/or prolonged larval periods in unpredictable hydroperiods decrease the chances of tadpoles completing metamorphosis before habitats dry (Altwegg and Reyer 2003). In contrast, faster larval development can lower larval mortality, but it is invariably at the cost of growth, resulting in a smaller size at metamorphosis that may have consequences in later survival and reproductive success (Reques and Tejedo 1997, Morey and Reznick 2000, Altwegg and Reyer 2003). When larval mortality risk increases because of pond desiccation, early metamorphosis may be favored despite the costs associated with smaller size. Phenotypic plasticity involving a trade-off among certain life history traits (e.g., larval growth, length of the larval period, size at transformation) is a useful strategy. The original Wilbur and Collins’ model of amphibian metamorphosis predicts that in an aquatic environment when conditions are favorable for larval growth (i.e., in permanent or slowly desiccating ponds), tadpoles should delay metamorphosis and transform at a larger size. But when conditions of temporary ponds become precarious, a strategy to adjust developmental processes to favor early metamorphosis and emergence from the aquatic habitat is useful (Wilbur and Collins 1973). A developmental strategy of phenotypic plasticity can decrease exposure to risky conditions and thereby increase survival rate.

In the city of Dharwad in Southern India, many anuran species reproduce in rain-filled ephemeral habitats formed during the southwestern monsoons. Tadpoles living in such ponds face a perennial threat of desiccation because of intermittent rains (Mogali et al. 2011a, 2017). *Polypedates maculatus* (Gray, 1830) is widely distributed in India. In Southern India, populations breed only during the rainy season. Females deposit eggs in foam nests. Nests are attached to vegetation, underneath stones above a water body, in bushes over rain-filled puddles, or to walls of cement cisterns.
Early embryonic development (up to Stage 23 of Gosner 1960) occurs inside the foam nests, after which tadpoles drop into the water where they undergo further development and metamorphosis. Tadpoles of *P. maculatus* occur in both ephemeral and permanent ponds, thus providing an excellent model to study developmental plasticity in response to varying degrees of evaporation of pond water. The present study was designed to determine, in a laboratory, the influence of gradual or rapid water depletion on the two major metamorphic traits, the larval period and size at emergence. We hypothesized that tadpoles with depleted water levels (either low or high rates of desiccation) would metamorphose earlier and at a smaller size than those developing in constant water levels. We also hypothesized that tadpoles facing rapidly depleting water would metamorphose earlier and at a smaller size than those developing in gradually declining water levels. The experimental design permitted us to exclude the influence of confounding factors such as food scarcity and predator pressure that generally interfere with growth and development of tadpoles in nature.

**Materials and Methods**

Three foam nests of *P. maculatus* were collected in July 2015 from temporary ponds on the Karnataka University Campus (15.440407° N, 74.985246° E) Dharwad, Karnataka State, India. They were transported to the laboratory and each nest was placed in a separate plastic tub (32 cm diameter and 14 cm deep) with 1 L of water and with substratum collected from the same pond. Tadpoles stages are according to Gosner (1960). The tadpoles hatched from the foam nests almost synchronously after five days at Stage 23. Tadpoles from all three nests were then mixed to normalize genetic difference among the groups. Tadpoles (Stage 23) were selected randomly and were reared in the plastic tubs (32 cm diameter and 14 cm deep) with 3–0.6 L of aged tap water until the onset of metamorphic climax stage (Stage 42). Fifteen tubs with 10 tadpoles each were maintained (in total 150 tadpoles). The experimental groups of 50 tadpoles were as follows:

- **Group I. Constant water:** Tadpoles were reared in constant water levels (3 L).
- **Group II. Gradual desiccation:** Tadpoles were reared in 3 L of water for the first 4 days and then subjected to 0.2 L decrease in water at 4 day intervals.
- **Group III. Rapid desiccation:** Tadpoles were reared in 3 L of water for a day and from the second day onward 0.1 L of water was reduced each day.

In the two groups with receding water, when the water reached 0.6 L (day 49 in Group II; day 25 in Group III) no further reductions were made. Groups II and III thus provided low and high desiccation risks. All tadpoles were fed boiled spinach ad libitum. Water was changed on alternate days and fresh food was provided. The rearing tubs were placed on a flat surface in a room with natural photoperiod and temperature. The positions of tubs were randomized on alternate days to avoid possible effects of position. Water temperature (°C) in tubs was recorded twice daily at 10:00 and 15:00 h. Following the onset of metamorphic climax (MC, emergence of forelimbs, Stage 42), subjects were transferred to small plastic tubs (19 cm diameter and 7 cm deep) with a small amount of water, covered with fine nylon mesh, and placed on an incline to provide a semi-terrestrial environment to facilitate emergence. The days to reach MC were noted for each individual. After completion of metamorphosis (Stage 46), snout–vent length (SVL in mm; measured using a digital caliper, accuracy 0.01 mm) and body mass (in mg; measured using an electronic balance, accuracy 0.001 g) were recorded. No tadpoles died during the course of the experiment. After completion of the experiments, the froglets were released near natural water bodies. We used the mean values of each variable (days to reach MC, SVL, body mass of froglets, and water temperature) within each tub for analysis in
order to avoid pseudo replication. Data were analyzed by one-way ANOVA using mean tub days to reach MC, SVL, and body size at metamorphosis as the response variables in separate tests, and treatment (water level: constant, gradual desiccation, rapid desiccation) as the effects, followed by Tukey’s post-hoc tests. Data for each parameter were organized into frequency distributions to determine the percentage of individuals falling within a particular dataset.

**Results**

Time taken to reach MC and size at metamorphosis (SVL and body mass) differed significantly between treatment groups ($p < 0.001$, Table 1). Tadpoles reared in declining water levels (Groups II and III) reached MC earlier ($p < 0.001$) and metamorphosed at a smaller size ($p < 0.001$) than those reared in constant water levels (Group I). Tadpoles experiencing rapid depletion of water (Group III) reached MC earlier ($p < 0.001$) and metamorphosed at smaller sizes ($p < 0.001$) than those experiencing gradual depletion in water levels (Group II).

The daily water temperature of various tubs fluctuated between 22–23°C and did not differ significantly throughout the course of the experiments (morning hours: $F = 6.125$, $df = 2, 12$, $p = 0.224$; afternoon hours: $F = 0.452$, $df = 2, 12$, $p = 0.647$). The effects of water temperature, if any, were uniform across the control and experimental groups.

The frequency distribution data showed that all individuals (100%) from the rapid desiccation group and 22% of individuals from the gradual desiccation group metamorphosed (Stage 46) at < 14.00 mm SVL, but none of the individuals subjected to constant water levels metamorphosed at comparable SVLs (Figure 1A). All individuals (100%) from the rapid desiccation group and only 14% of individuals in the gradual desiccation group metamorphosed at a smaller body mass (< 275 mg), but none of the individuals subjected to constant water levels metamorphosed at a comparable body mass (Figure 1B). The data on days to onset of MC showed that all individuals (100%) reared in the rapid desiccation group and 12% of individuals from the gradual desiccation group took < 63 days, but no individuals subjected to constant water levels initiated MC by this same time (Figure 1C).

**Discussion**

Environmental heterogeneity plays a key role in the evolution of biological phenotypic plasticity (Sultan and Spencer 2002). Natural selection favors organisms with the most suitable phenotype for interacting with their environment, and variations in phenotype and the corresponding genotype exist to adapt to a specific environment.

<table>
<thead>
<tr>
<th>Rearing groups</th>
<th>SVL (mm)</th>
<th>Body mass (mg)</th>
<th>Onset of MC (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Constant water</td>
<td>17.34 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>466.76 ± 7.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.84 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II. Gradual desiccation</td>
<td>14.60 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>315.30 ± 4.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.54 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III. Rapid desiccation</td>
<td>13.44 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>229.04 ± 2.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.16 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>$F_{2,12}$</td>
<td>1120.0</td>
<td>817.863</td>
<td>1411.0</td>
</tr>
<tr>
<td>$p$ value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
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(Whitlock 1996). Indeed, the ephemeral ponds many anuran amphibians use for egg deposition and those with growing tadpoles are typical examples of environmental heterogeneity.

Anuran amphibians are characterized by phenotypic plasticity both in time to metamorphosis and in size at metamorphosis, especially for species that breed and develop in temporary ponds or highly unpredictable environments (Crump 1989, Newman 1992, 1994, Denver et al. 1998, Loman 1999, Mogali et al. 2011a, Richter-Boix et al. 2011).

Our study demonstrated that tadpoles of *P. maculatus* have phenotypic plasticity and that timing of metamorphosis responds to the availability of water. Tadpoles raised in constant water levels metamorphosed later and at a larger size than tadpoles from decreasing water levels (gradual or rapid). Tadpoles raised in gradually depleted water levels metamorphosed later and at a larger size than tadpoles from rapidly depleting water levels. Tadpoles that experienced a severe desiccation threat (rapidly depleting water levels) metamorphosed earlier and at smaller sizes. Hence, tadpoles of *P. maculatus* showed adaptive plasticity in metamorphosis to pond drying. Our results are in accordance with Wilbur and Collins’ model (1973) for amphibian metamorphosis, which assumes that tadpoles encountering favorable conditions postpone

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**Figure 1.** Percent metamorphs of *Polypedates maculatus* (A) per snout–vent length class (mm) and (B) per body mass class (mg) in different groups. (C) Percent tadpoles of *P. maculatus* per class (days) to reach metamorphic climax (MC, Gosner Stage 42) in different groups. Rearing groups: constant water (blue bars), gradual desiccation (red bars), rapid desiccation (green bars).
metamorphosis, capitalizing on the opportunity for additional growth, while tadpoles exposed to hostile conditions (rapid desiccation threat) develop quicker and leave the aquatic habitat earlier. Our results support previous research (Newman 1989, Denver et al. 1998, Loman 1999, Mogali et al. 2011a, 2017, Székely et al. 2017).

The mechanisms proposed to explain the acceleration of metamorphosis in anuran tadpoles facing the risk of desiccation differ. Elevated temperature (Newman 1992, Tejedo and Reques 1994) or a decrease in food (Alford and Harris 1988, Newman 1994) has been attributed to lowered growth rate with accelerated developmental rate. Other studies indicated that temperature has no influence on developmental rate (Loman 1999, Laurila and Kujasalo 1999, Márquez-García et al. 2009, Mogali et al. 2017). In the present study, water temperature of the rearing containers did not vary, and excess food was provided to all groups. The main factor influencing accelerated metamorphosis of tadpoles of *P. maculatus* in this study was the desiccation threat rather than temperature or shortage of food availability.

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