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Influence of Water Desiccation and Predator on Metamorphic Traits in the Indian Common Toad, "*Bufo Melanostictus*"

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Abstract: ***ANURAN amphibians have a complex life history strategy with an aquatic and a terrestrial stage (Wilbur 1980). The larval stage is critical as the larvae have to complete development and attain a threshold size before metamorphosis before emergence on land. The size at metamorphosis and duration of larval period are two important metamorphic traits in anuran amphibians (Wilbur and Collins 1973; Wilbur 1980). Both biotic and abiotic factors are known to influence the age and size at metamorphosis; these in turn have strong ecological implications with respect to long term fitness involving juvenile survival, age and size at first reproduction, and adult fecundity (Smith 1987; Semlitsch et al. 1988; Berven 1990).***

Keywords: Metamorphosis, Gosner stage, depletion, desiccation, cues, climax stage

1. Introduction

Breeding frogs / toads lay their eggs mostly in temporary ponds that are sporadically filled by rain during early monsoon period. The anuran larvae face many challenges such as crowding effect (either from conspecifics or heterospecifics), competition for food and space (either from intra or interspecific members), predator pressure (generally aquatic insect larvae, aquatic beetles, water scorpion and carnivorous anuran tadpoles etc.) and more importantly, pond desiccation. All of these necessitate evolution of appropriate strategies for successful completion of metamorphosis and emergence on land (Newman 1992).

Earlier studies have shown that under predation pressure, the prey animals alter their foraging, morphology and metamorphic traits, the larval duration and size at metamorphosis (Skelly 1992; Petranka and Heyes 1998; Kraft et al. 2005; Mogali et al. 2011 a). Ephemeral ponds are shallow and often do not last for a fortnight in the absence of intermittent showers. Hence tadpoles have to face severe risk both from predators and habitat desiccation. In the present study we investigated the phenotypic plasticity of *B. melanostictus* tadpoles exposed to receding water levels at different rate and in the presence of predators.

2. Materials and Methods

Eggs of the Indian common toad, *Bufo* were collected from a temporary pond in Athani, Dt:Belgaum. Immediately after collection they were transported to laboratory and placed in a plastic tub (42 cm dia; 16 cm H) containing 10L aged (dechlorinated) tap water. Eggs hatched on the next day of collection at Gosner stage, 19 (Gosner 1960). When tadpoles reached feeding stage, 600 tadpoles were arbitrarily picked up and used in the experiment. The tadpoles were provided with boiled spinach as food.

Egg masses of *Hoplobatrachus tigerinus* were collected from the pond. Eggs were maintained in plastic tub (42 cm dia; 16 cm H) containing 10 L aged tap water. Eggs hatched on the 2nd day of collection. After reaching feeding stage, 15 tadpoles of *H. tigerinus* were reared in a plastic tub (32 cm dia; 14 cm H) containing 5L of aged tap water. Tadpoles of *H. tigerinus* were fed with tadpoles of *Bufo*. Tadpoles of *H. tigerinus* of stage, 28-30 were used as predators in the experiment.

3. Experimental Design

The experiment was conducted to test the influence of 3 different water levels viz constant, moderate depletion and rapid depletion in presence and in absence of predators (3 × 2 factorial design.

Tadpoles of *Bufo* (n = 20) were reared in each plastic container (32 cm dia; 14 cm H) with aged tap water. A small plastic container (6.5 cm dia; 14 cm H) wrapped with cheese cloth (either empty or housed with the predatory, *H. tigerinus* (n = 1) tadpole) was placed in the center of these rearing tubs. Thus prey tadpoles had no direct contact with predators but they were continuously exposed to chemical cues emanating from the predator. The details of the experimental groups are given below.

Group-I: Rearing in constant water volume with or without predator

Bufo tadpoles were reared in 4L of water in tubs without predator (Group-I A; or with predator (Group-I B) until they reach metamorphic climax.

Group-II: Rearing in moderate depletion of water with or without predator

Bufo tadpoles were initially reared in 4L of water in tubs withou predator (Group-II A) or with predator (Group-II B). The water from each tub was then reduced by 0.5 L on every 4th day. From the day when the wate volume in the tub reached 0.5L; no further removal of water from there tubs was done since it would leave these tadpoles without water.

Group-III: Rearing in Rapid depletion of water with or without predator

Bufo tadpoles were initially reared in 4L of water (day 1 only) in tubs without predator (Group-III A) or with predator (Group-III B) and from 2nd day onwards 0.25L of water was reduced daily. However, when water in the tub reached 0.5L of water, no more water was removed from tubs until the tadpoles reach metamorphic climax.

Each group had five replicates of 20 tadpoles in each tub. All tubs housing tadpoles were placed in an area with a roof and wall on two sides. The position of tubs was interchanged on every 2nd day to avoid positional effect, if any.

Caged predators, *H. tigerinus* were fed daily with 10 *Bufo* tadpoles (Gosner stage, 26-28). The tadpoles of *Bufo* were fed with boiled spinach *ad libitum*. Water temperature (°C) in the tubs of all groups was recorded twice daily in morning (1000 h) and afternoon (1500 h). The experiment was terminated when *Bufo* tadpoles from all the groups were metamorphosed. As and when the tadpoles of a group reached metamorphic climax (Gosner stage, 42), the date was recorded and they were placed in small plastic tub (14 cm dia; 7 cm H) is covered with fine nylon mesh and kept inclined with a little water to provide semi-terrestrial area to facilitate metamorphosis. At metamorphosis (Gosner stage, 46), the various body parameters like body mass (mg), snout-vent length (SVL in mm), fore-limb length (mm) and hind-limb length (mm) of toadlets were recorded. Also, day of reaching metamorphosis for each toadlets was recorded. Immediately after completion of measurements, toadlets were released near water ponds in the University Campus.

Statistical Analysis

The recorded data were tested for normality by using Kolmogorov-Smirnov test and met the assumptions. Multivariate analysis of variance (MANOVA) was employed to test the overall effects of water volume, predator and their interaction on metamorphic traits of toad. Two-way analysis of variance was performed for each response variable such as SVL, body mass, fore-limb length, hind-limb length and days required to reach metamorphic climax to determine influence of each factor on response variable independently and both together. To assess differences between the groups for each response variables at different water volume levels in presence and absence of predator the ANOVA was employed followed by Scheffe's post-hoc test. The water temperatures (morning and afternoon) in the different treatments were analyzed by ANOVA followed by Scheffe's post-hoc test to determine whether there is any significant variation in water temperature among the tubs among the groups. In order to obtain biological meaning of the data, we compared the data for all parameters of all the groups with respect to median value for each parameter.

4. Results

The SVL, body mass, fore-limb and hind-limb lengths at metamorphosis and days required to reach metamorphic climax were significantly influenced by both water levels and presence or absence of predator (Table 1). Decreasing water levels are found to influence SVL ($F_{2, 295}$ = 166.14; p < 0.001), body mass ($F_{2, 295}$ = 289.28; p < 0.001), fore-limb length ($F_{2, 295}$ = 96.65; p < 0.001) and hind-limb length ($F_{2, 295}$ = 145.74; p < 0.001) of metamorphs among different groups (Table 2). SVL, body mass, fore-limb length and hind-limb length of toadlets of the rapid water depletion treatment were significantly lower than those in moderate water depletion group and constant water level group. Further, SVL, body mass, fore-limb length and hind-limb length of toadlets from the moderate water depletion treatment were also significantly lower than in the control but significantly higher than the toadlets of rapid water depletion group. The time taken to reach metamorphic climax (MC) ($F_{2, 295}$ = 54.98; p < 0.001) also varied significantly among the individuals of the different groups (Table 2). The tadpoles of moderate and rapid water depletion group reached MC earlier than the tadpoles of constant water volume group. However, days to reach MC were comparable in moderate and rapid depletion groups (Table 2).

Decreasing water levels with predators has also a significant influence on SVL ($F_{2, 286}$ = 14.12; p < 0.001), body mass ($F_{2, 286}$ = 22.45; p < 0.001), fore-limb length ($F_{2, 286}$ = 19.64; p < 0.001) and hind-limb length ($F_{2, 286}$ = 8.92; p < 0.001) of metamorphs among different groups (Table 3). SVL, body mass, fore-limb length and hind-limb length of toadlets reared in the rapid depletion with predator treatment were significantly lower than that of other two groups. However SVL, body mass, fore-limb length and hind-limb lengths were comparable in constant water and moderate depletion with predator groups (Table 3). The time taken to reach MC ($F_{2, 286}$ = 95.21; p < 0.001) also varied significantly among the individuals of different groups (Table 3). The tadpoles of groups, moderate water depletion and rapid water depletion with predator reached MC earlier than that of tadpoles reared under constant water with predator (Table 3).

Percentage data of all parameter of all the treatment in relation to median values metamorphs of constant water level group (control) is presented in Table 4. It is observed that percent individuals metamorphosing at ≥ 10.88 mm SVL were 53% in constant water level group, 7% in constant water level with predator, 3% in moderate water level, 7% in moderate water level with predator, 1% in both the rapid water depletion groups with or without predator. 51% individuals in constant water level group metamorphosing at ≥ 118 mg. However, only 5% individuals in constant water level with predator, 2% in both the moderate depletion of water groups with or without predator. Interestingly all individuals in rapid water depletion groups with or without predator weighed < 118 mg. The percentage data on MC shows that percent individuals attain MC in ≤ 33 days were 90% in rapid depletion of water with predator, 84% in rapid depletion of water without predator, 74% in moderate depletion of water, 66% in moderate depletion of water with predator, 35% in constant water without predator but only 28% in constant water with predator.

Table 5 shows the daily recording water temperatures (°C) at two different intervals in a day. There was no significant variation in the water temperature, both in morning ($F_{5,\,456}$ = 1.097; p = 0.361) and afternoon ($F_{5,\,456}$ = 1.934; p = 0.087) among different treatment tubs.

Table 1. Shows the results of MANOVA for overall effects of water volume and predator and their interaction, and two-way ANOVAs of each response variables within each main effect. Response variables are SVL (mm), mean mass (mg), fore-limb length (mm), hind-limb length (mm) at metamorphosis and mean days required to reach metamorphic climax

A. MANOVA for overall effects

Source	Wilk's Lambda	*F*	*p*
Water volume	0.436	49.4	0.000
Predator	0.859	15.76	0.000
Water volume × Predator	0.651	23.02	

B. Two-way ANOVA for snout-vent length (SVL) at metamorphosis

Source	df	*MS*	*F*	*p*
Water volume	2	60.23	124.41	0.000
Predator	1	10.50	21.69	0.000
Water volume × Predator	2	26.15	54.00	0.000
Error	581	0.48		

C. Two-way ANOVA for mean body mass at metamorphosis

Source	df	*MS*	*F*	*p*
Water volume	2	48068.80	216.97	0.000
Predator	1	8666.87	39.12	0.000
Water volume × Predator	2	17924.98	80.91	0.000
Error	581	221.54		

D. Two-way ANOVA for fore-limb length at metamorphosis

Source	df	*MS*	*F*	*p*
Water volume	2	41.70	92.29	0.000
Predator	1	0.85	1.84	0.175
Water volume × Predator	2	8.64	19.12	0.000
Error	581	0.45		

E. Two-way ANOVA for hind-limb length at metamorphosis

Source	df	*MS*	*F*	*p*
Water volume	2	88.25	102.44	0.000
Predator	1	7.48	8.68	0.003
Water volume × Predator	2	36.22	42.04	0.000
Error	581	0.86		

F. Two-way ANOVA for days required to reach metamorphic climax

Source	df	*MS*	*F*	*p*
Water volume	2	613.06	151.32	0.000
Predator	1	72.90	17.99	0.000
Water volume × Predator	2	81.38	20.09	0.000
Error	581	4.05		

Table 2: Effect of changing water levels on metamorphic traits of *Bufo melanostictus*

Rearing protocol	Metamorphic traits (mean ± SE)				
	SVL (mm)	Body mass (mg)	Fore-limb length (mm)	Hind-limb length (mm)	Days to reach metamorphic climax
I A. Constant volume of water with predator (n = 99)	10.97[a] ± 0.07	118.72[a] ±1.74	6.87[a] ± 0.07	11.86[a] ± 0.09	33.22[a] ±0.18
II A. Moderate depletion of water with predator (n = 99)	9.87[a] ± 0.66	87.86[a] ± 1.28	6.07[a] ±0.06	10.56[a] ±0.08	31.53[b]± 0.14
III A. Rapid depletion of water with predator (n = 100)	9.18[b] ± 0.08	69.51[b] ± 1.33	5.60[b]± 0.06	9.70[b]± 1.00	31.09[b] ± 0.13
F value	166.14	289.28	96.65	145.74	54.98
p value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Dissimilar letters indicate the significant difference between the groups; *n* = number of tadpoles

Table 3: Effect of changing water levels and caged predators on metamorphic traits of *Bufo melanostictus*

Rearing protocol	Metamorphic traits (mean ± SE)				
	SVL	Body mass Fore-limb (mm)	Hind-limb (mg)	Days to reach length (mm) length (mm) metamorphic climax	Metamorphic traits (mean ± SE)
I A. Constant volume of water with predator (n = 97)	9.87[a] ± 0.08	89.28[a] ±2.11	6.31[a] ± 0.08	10.67[a] ± 0.11	35.40[a] ±0.37
II A. Moderate depletion of water with predator (n = 95)	9.92[a] ± 0.07	87.82[a] ± 1.28	6.25[a] ±0.07	10.63[a] ±0.1	31.67[b]± 0.16
III A. Rapid depletion of water with predator (n = 97)	9.43[b] ± 0.06	75.93[b] ± 1.01	5.75[b]± 0.06	10.14[b]± 0.08	30.88[b] ± 0.13
F value	14.12	22.45	19.64	8.92	95.91
p value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Dissimilar letters indicate the significant difference between the groups; *n* = number of tadpoles

Table 4: Percentage metamorphos of *Bufo melanostictus* reared in different water level regimes and with or without predator

Rearing protocol	SVL (mm ≥ 10.88)	Body mass (mg ≥ 118)	Days to reach MC (≤ 33)
I A. Constant volume of water without predator	53	51	35
I B. Constant volume of water with predator	7	5	28
II A. Moderate depletion of water without predator	3	2	74
II B. Moderate depletion of water with predator	7	2	66
III A. Rapid depletion of water without predator	1	0	84
III B. Rapid depletion of water with predator	1	0	90

Table 5: Temperature (°C) of water in different treatment tubs

Treatments	Maximum water temperature (°C)	
	Morning (1000 h)	Afternoon (1500 h)
I A. Constant volume of water without predator	23.08 ± 0.02	23.53 ± 0.05
I B. Constant volume of water with predator	23.15 ± 0.03	23.55 ± 0.05
II A. Moderate depletion of water without predator	23.15 ± 0.04	23.49 ± 0.06
II B. Moderate depletion of water with predator	23.19 ± 0.04	23.41 ± 0.06
III A. Rapid depletion of water without predator	23.12 ± 0.03	23.33 ± 0.06
III B. Rapid depletion of water with predator	23.18 ± 0.03	23.40 ± 0.05
F value	1.097	1.934
p value	$p > 0.05$	$p > 0.05$

5. Discussion

The present study shows that *B. melanostictus* tadpoles reared under water depletion regimes metamorphosed earlier at a smaller size than those reared in constant water level group. Further the tadpoles reared under rapid water depletion regime metamorphosed earliest and at smallest size. Thus there is a positive association between size and duration of reaching metamorphic climax. Whereas, under similar ecological conditions, tadpoles of *Bufo bufo*, *R. temporaria* and *Bufo calamita* metamorphose at a smaller size, but the timing of metamorphosis is unaffected (Brady and Griffiths 2000). However, Loman (1999) reported that *R. temporaria* tadpoles accelerate their development in response to habitat desiccation, reaching metamorphosis at an earlier age and at a similar size compared to those exposed to constant water. In desert ponds also for anuran tadpoles desiccation is the major cause of mortality and depends upon their density. In low-density ponds, tadpoles of *Scaphiopus couchii* develop quickly and metamorphose, whereas in high-density ponds under desiccation they rarely complete their larval development (Newman 1987). Thus depending upon the species, we come across an early or late metamorphosis accompanied by normal, small or large body mass at emergence. Thus it is obvious that when the larval period is short, the size at metamorphosis is usually smaller and a larger size at metamorphosis is associated with late metamorphosis with a few exceptions.

The present study shows that both size and age at metamorphosis in common toad, *B. melanostictus* is influenced by presence of the predator. Toad tadpoles reared with predator in constant water levels delayed metamorphosis and emerged at smaller size. These results are similar to that reported in *Rana dalmatina* and *B. melanostictus* (Lardner 2000; Mogali et al. 2011 a). They also showed that in presence of predatory cues, *B. melanostictus* tadpoles feed for shorter duration and remain stationary for longer periods and thus their growth rate is affected.

In the present experiment, when *Bufo* tadpoles are experiencing to predator as well as water depletion of their habitat, we observed diverse responses in metamorphic traits in tadpoles among the groups. The tadpoles in constant water level with predator delayed metamorphosis and metamorphosed at a smaller size than that predator-free environment. However, the tadpoles with receding water levels with predator metamorphosed early at a smaller size. These observations throw light on very interesting strategy adopted by tadpoles of *B. melanostictus*. When tadpoles face predator pressure but have no fear of pond desiccation, the metamorphosis is delayed perhaps they spend less time in feeding similar to that reported by Mogali et al (2011 a). But present study also showed that under pond desiccation fear and presence of predator, toad tadpoles metamorphosed early at smaller size in order to escape out from drying ponds. Thus, pond desiccation or pond drying has greater stress on toad tadpoles, though presence of predator does influence their metamorphic traits to a lesser extent and in a different fashion. *Bufo* tadpoles are shown exhibit antipredator strategies such as less movements, less time in feeding and high sprint speed to escape from getting predated (Saidapur et al. 2009; Mogali et al. 2011 a) as a result they metamorphose late at smaller size but yet they survive if escape from predation. But under pressure of pond desiccation, they do not have any option but to metamorphose at earliest to avoid mortality. Hence under pond desiccation condition, early metamorphosis is at premium.

The present study thus shows that *B. melanostictus* tadpoles perceive rapid depletion of water levels (pond drying) as a real threat compared to the presence of predator, and in anticipation of pond drying and to avoid imminent mortality due to desiccation, metamorphose early at a smaller size.

References

[1] Alford RA, Harris RN (1988) Effects of larval growth history on anuran metamorphosis. Animal Nature 131:91–106

[2] Brady LD, Griffiths RA (2000) Developmental responses to pond desiccation in tadpoles of the British anuran amphibians (*Bufo bufo*, *B. calamita* and *Rana temporaria*). Journal of Zoology 252:61–69

[3] Chivers DP, Wisenden BD, Smith RJF (1996) Damselfly larvae learn to recognize predators from

chemical cues in the predator's diet. Animal Behavior 52:315–320

[4] Fraker ME (2008) The effect of hunger on the strength and duration of the antipredator behavioral response of green frog (*Rana clamitans*) tadpoles. Behavioral Ecology and Sociobiology 62:1201–1205

[5] Gervasi SS, Foufopoulos J (2008) Costs of plasticity: responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. Functional Ecology 22:100–108

[6] Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

[7] Hickman CR, Stone MD, Mathis A (2004) Priority use of chemical over visual cues for detection of predators by Graybelly Salamanders, *Eurycea multiplicata griseogaster*. Herpetologica 60:203–210

[8] Hoff KVS, Blaustein AR, McDiarmid RW, Altig R (1999) Behavior: interactions and their consequences. Pp. 215–239. In McDirmid RW and Altig R (Eds.). Tadpoles: Biology of Anuran Larvae, University of Chicago Press, Chicago, USA

[9] Kraft PG, Wilson RS, Franklin CE (2005) Predator-mediated phenotypic plasticity in tadpoles of the striped marsh frog, *Limnodynastes peronii*. Austral Ecology 30:558– 563

[10] Loman J (1999) Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. Amphibia Reptilia 20:421–430

[11] Maciel TA, Junca FA (2009) Effects of temperature and volume of water on the growth and development of tadpoles of *Pleurodema diplolister* and *Rhinella granulosa* (Amphibia: Anura). Zoologia 26:413–418

[12] Marquez-Garcia M, Correa-Solis M, Mendez A (2010) Life-history trait variation in tadpoles of the warty toad in response to pond drying. Journal of Zoology 281:105– 111

[13] Mogali SM, Saidapur SK, Shanbhag BA (2011 b) Receding water levels hasten metamorphosis in the frog, *Sphaerotheca breviceps* (Schneider, 1799): a laboratory study. Current Science 101:1219–1222

[14] Newman RA (1994) Effects of changing density and food level on metamorphosis of a desert amphibian, *Scaphiopus couchii*. Ecology 75:1085–1096

[15] Richter J, Martin L, Beachy C (2009) Increased larval density induces accelerated metamorphosis independently of growth rate in the frog *Rana sphenocephala*. Journal of Herpetology 43:551–554