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Short Communication

The moult process in *Bufo Melanostictus* (Schn.) (Anura; Bufonidae)

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Abstract

The process of moulting in the common Indian toad, *Bufo melanostictus* has been described. Staging of the moulting cycle, which occurs once in 5 to 6 days, has been done based on the behaviour of the toads and visual morphology of the skin. These stages have been compared with those of a temperate species.

Key words: Common Indian toad, moult process, moult-staging, gular movements

1. Introduction

Moulting in vertebrates is known to be generally associated with life on land and as such the process is known to be absent in fishes and aquatic stages of amphibians[1]. All amphibians appear to undergo regular moulting throughout their life, following metamorphosis[2]. A survey of literature provides evidence to the occurrence of regular moulting cycles in 22 urodele and 24 anuran species. Most information on moulting in amphibians is, however, derived from studies on bufonids. For several reasons, species of *Bufo* are considered as ideal animals for studies on amphibian moulting[3]. So far, information on the moult process is drawn only from the work on temperate species of bufonids. The present paper is the first attempt at describing the moult process in a tropical bufonid, the common Indian toad, *Bufo melanostictus*.

2. Material and methods

For the present studies, individuals of *Bufo melanostictus* were collected in and around Bangalore. They were maintained in large cement cisterns ($120 \times 280 \times 125$ cm) with free access to water. Stock individuals were fed twice a week with live earthworms (*Pentascolex* sp.), offered in exclusive feeding cups.

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For experimental studies, active toads of either sex were individually maintained in earthen pots (24 × 12 cm). The pots were covered with mosquito netting cloth through which adequate light could penetrate. Each pot was filled with a known quantity of fresh water and placed slightly at an angle, so that it could provide the required amphibious situation. The water inside the pots was changed every day. The average air and water temperatures in the laboratory during the period of study were 24.1 ± 1.7°C and 22.7 ± 2.0°C, respectively. The relative humidity was 59.3 ± 0.9%. The experimental individuals were force-fed every day with a known quantity (8% of initial body weight) of minced beef.

Since moulting is a frequent and regular phenomenon and the sloughed-off skin is usually eaten by the toads, it was necessary to mark the keratinized layer in order to obtain a reliable record of the incidence of moulting. During the present study, following the standard proceedure[4-6], marking of the toads was done by smearing lipstick on the back of the individuals, in the region between the two parotid glands. Disappearance of this mark was taken as an index for the occurrence of a moult.

Based on the morphological characteristics outlined in Table I, adult individuals of *B. melanosticius* were separated into males and females, each of two different size classes. They were maintained individually and the incidence of moulting was recorded.

In order to gain an insight into the histomorphological changes that occur in the skin in relation to moulting, it is necessary to distinguish the different phases of the moulting cycle. This also helps in recognising the stage in which the individual toads are at the beginning of an experiment. Such a differentiation can be achieved by visual observations of the external morphology (macroscopic appearence) of the skin, which is believed to reflect the successive stages in a normal moult cycle[6]. The moult cycle of *B. melanostictus* was categorised into three stages based on the visual variations in the external morphology of the skin. These stages have been compared with the moult staging reported for *B. bufo*[6].

Table I
Morphological differences between males and females of *Bufo melanostictus*

Characters	Males	Females
Body colour	Brown with a greenish tinge on the dorsal side Pale white ventrally	Brown on the dorsal side Pale white ventrally
Colour of the throat	Reddish orange with black margins	Always greyish white
Vocal sacs	A black, pigmented, sub-vocal sac opening into the pharynx usually by a single slit	Absent
First inner finger (Thumb)	Swollen, thickly pigmented to form a thumb pad	Not different from the remaining fingers

3. Results and discussion

Present observations indicate that the general pattern of moulting in *B. melanostictus* is similar to that of other bufonids studied[4-9]. Individuals of *B. melanostictus* undergo a regular moulting which begins immediately after metamorphosis. Each moult cycle involves a periodic shedding of the outermost keratinized layer of the skin. Table II presents the inter-moult duration (IMD)* in males and females. It is evident from the table that moulting occurs once in 5 to 6 days. There was no apparent difference in the IMD in relation to the two sizes selected. However, a slight, but statistically significant difference ($P < 0.05$) was noticed in the IMD of males and females. Among individuals of a similar size, males appear to moult more frequently than the females.

Table III presents the staging of the moult cycle of *B. melanostictus*, based on the visual morphological changes in the skin. From the table, it is clear that in the moulting cycle of *B. melanostictus* three stages can be distinguished, namely, I: Post-moult stage; II: Inter-moult stage and III: Pre-moult stage.

The post-moult stage marks the beginning of a new moulting cycle since the individuals would have just then completed the process of previous moulting cycle, by shedding the keratinised layer. The toads in this stage are sluggish. The skin appears pale in colour and the cranial ridges are whitish (fig. 1a). Body is slimy and glistening. The individuals exhibit occassional retraction of the eye balls and gaping of the mouth. This stage of the moulting cycle of the *B. melanostictus* can be compared with the 'differentiation phase' reported for *B. bufo*[6].

The inter-moult stage spans between day 2 and day 3 after the previous moult. During this stage, the individuals are active. The skin is slightly darker and keratinization is

Table II
***Bufo melanostictus*: Inter-moult duration (IMD) in relation to size and sex (Mean ± S.D.)**

Sex	Series	Body size (g)	Inter-moult number					Mean ± S.D.
			1	2	3	4	5	
Males	I	22.32 ± 4 48	5.33 ± 0.74	4.83 ± 0.37	5 20 ± 0 40	5 00	5.20 ± 0 40	5.11 ± 0 18
	II	36.78 ± 4 59	4.83 ± 0 90	5 00	5 20 ± 0 40	5.60 ± 0.49	5 25 ± 0.43	5.18 ± 0.26
Females	III	27.82 ± 3.88	5.66 ± 0.94	5.33 ± 0 47	5 50 ± 0 50	5.00	5.50 ± 0 50	5.40 ± 0.22
	IV	46.24 ± 8.24	5.16 ± 0.90	5.40 ± 0.49	5 40 ± 0.47	5.33	5 66 ± 0.47	5.39 ± 0.16

Note: Each value represents the mean obtained from 50 toads observed for a period of 30 days

*IMD, the time interval between two successive sheddings of the keratinised layer

Table III

***Bufo melanostictus*: Staging of the moult cycle based on external morphology and behaviour of the toads**

Stage of the moulting cycle		Remarks	Comparable moult stage as given by Budtz and Larsen[6] for *Bufo bufo*
I. Post-moult		Skin pale in colour, cranial ridges whitish. Body slimy and glistening. Buccal movements very fast. Occasional retraction of eye balls observed. Individuals sluggish (just moulted) (fig. 1a)	Differentiation phase
II. Inter-moult		Skin slightly darker than in stage I, keratinization visible at the warts, digits and in the region of the cranial ridges. Individuals active (two to three days after the previous moult (fig. 1b))	Inter-moult phase (preparation phase)
III. Pre-moult	A) Early	Skin darker than in stage II, keratinization more pronounced. Individuals active (few hours to a day prior to a moult (fig. 1c)).	Preparation phase
	B) Late	Skin darker than in III-A, Slimy. Presence of a middorsal slit in the keratinized layer. Buccal movements slower. Individuals sluggish. Adoption of moulting posture (few minutes before shedding)	Shedding phase

evident in the warts, digits and cranial ridges (fig. 1b). This stage can be compared to the early 'preparation phase' of *B. bufo*[6].

The pre-moult stage can be further distinguished into an early (stage III A) and a late

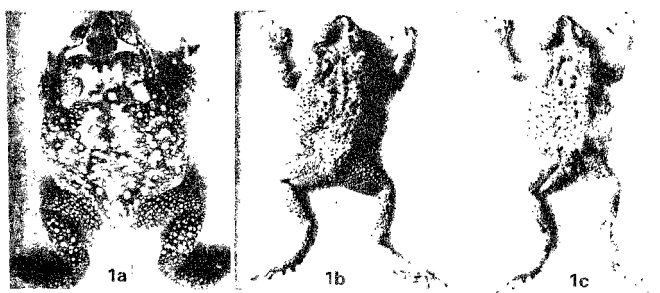

FIG. 1. (a) *Bufo melanostictus*. An adult female immediately after shedding (post-moult stage). Note the blanched cranial ridges, warts and tips of digits. (b) An adult female in the inter-moult stage. Note the keratinized cranial ridges, warts and tips of the digits as compared to fig. 1a. (c) An adult female in the pre-moult stage. Note the highly keratinized cranial ridges, warts and tips of digits as compared to (a) and (b).

pre-moult (III B) stages, based on the behaviour of the toads. In the early pre-moult stage the individuals are active and exhibit a darker skin than in stage II. Keratinization is more pronounced in the warts, digits and cranial ridges (fig. 1c). As the shedding of the keratinized layer approaches, the individuals enter the late pre-moult stage. The beginning of this stage is marked by the individuals becoming sluggish and adopting a characteristic 'moulting posture'. During this, the limbs are stretched and the belly is lifted above the ground. A mid-dorsal slit appears in the skin marking the onset of shedding. This stage is comparable to the 'shedding phase' of *B. bufo*[6].

The metabolic activity of the individual toads varies in different stages of the moult cycle. The frequency of gular movements is known to be directly related to the metabolic rate of the individual since breathing rate is dependent on metabolism[10]. Hence, the frequency of gular movements was taken as an index of the metabolic rate. While many

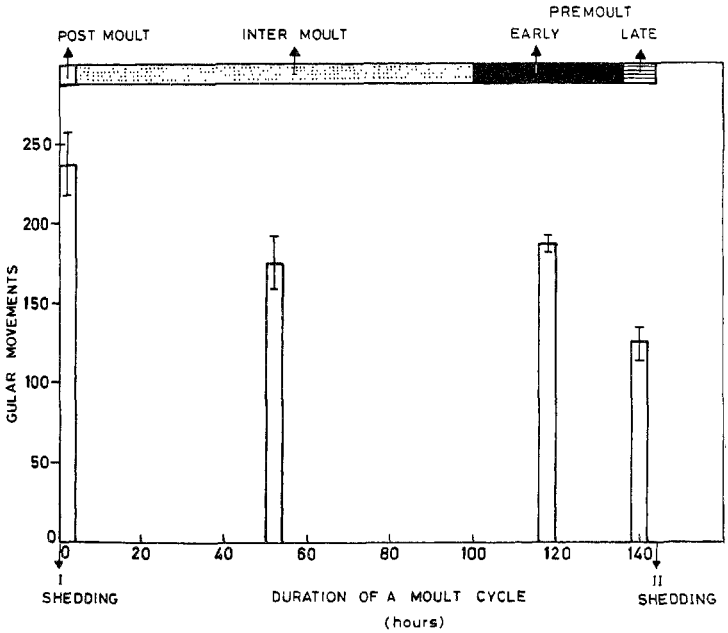

FIG. 2 *Bufo melanostictus*. Frequency of gular movements in relation to different stages of moulting (Mean ± S.D.)

other factors may also determine the frequency of gular movements in amphibians, at more or less constant (undisturbed) laboratory conditions of rearing, employed during the present studies, it was interesting to note that the rate of gular movements in *B. melanostictus* varied in different stages of moulting, perhaps indicating corresponding variations in the rate of respiration. Figure 2 is a graphic representation of the frequency of gular movements in *B. melanostictus*, in different stages of the moult cycle. It is evident from the figure that the frequency of gular movements is very high during the post-moult stage, immediately after shedding. Thereafter, the frequency decreases and remains more or less constant throughout the inter-moult stage. There is a slight increase in the frequency of gular movements during the early pre-moult stage, but the frequency is reduced markedly in the late-pre-moult stage just when shedding is initiated. This corresponds to the period in which the animal is sluggish and before it adopts the typical 'moulting posture'.

The present observations indicate that the moulting frequency and IMD of *B. melanostictus* are more or less similar to those of the temperate zone bufonid *B. bufo*[4–6].

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