



'On the rocks': reproductive biology of the endemic toad *Xanthophryne* (Anura: Bufonidae) from the Western Ghats, India

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ABSTRACT

Xanthophryne is a toad genus endemic to the northern Western Ghats of India, with two extant sister species – *Xanthophryne koy-nayensis* and *Xanthophryne tigerina*. Both species are local endemics and endangered. We studied reproductive biology of these toads and found that they are specialized to the lateritic rocky outcrops at mid-elevations in high rainfall areas. *Xanthophryne* toads have sporadic, multiple spawning bouts lasting 2–4 days during early monsoon. In this explosive breeding behaviour, we observed male toads to engage in 'pelvic thrusts', a unique and novel behaviour among anurans. Females oviposit in shallow pools in depressions of lateritic boulders where their tadpoles metamorphose. These ephemeral rocky pools have limited resources and they desiccate rapidly with a break in the rains. To mitigate the stochastic risk of desiccation and subsequent large-scale egg/tadpole mortality, females may disperse their reproductive investment spatially and temporally in multiple clutches, and tadpoles metamorphose rapidly. Here, we describe the amplexus, spawning and male advertisement call, and provide a comparative account of the life history traits of the two *Xanthophryne* species. These toads face numerous threats and are in need of urgent conservation action. These toads, seemingly well adapted to the isolated rocky outcrops, offer an excellent opportunity to understand endemism, mating systems, anuran ecology and behaviour.

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Introduction

The tropical semi-evergreen forests of the Western Ghats biodiversity hotspot have a rich assemblage of herpetofauna, especially amphibians (Daniels 1992; Bossuyt et al. 2004; Gunawardene et al. 2007). Although the ecology and behaviour of most anurans from the Western Ghats is largely unknown, many unique and extraordinary behaviours and adaptations in amphibians parallel this high amphibian diversity (Kunte 2004; Preininger et al. 2013; Gaitonde and Giri 2014; Crump 2015; Seshadri et al. 2015; Senevirathne et al. 2016). The Western Ghats region is tremendously diverse across its

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length in climate, rainfall pattern, elevation, geology, topography, biota and landscape features (Prasad et al. 2009; Watve 2013). Many endemic anurans (frogs and toads) of the Western Ghats have narrow distributions and some are point endemics due to geographical barriers, specific niche requirements and habitat specialization (Vasudevan et al. 2001; Naniwadekar and Vasudevan 2006; Gunawardene et al. 2007; Van Bocxlaer et al. 2012). Toads are widely distributed and have attained a nearly cosmopolitan distribution (Pramuk et al. 2008; Van Bocxlaer et al. 2010). Their characteristic features, such as the parotoid glands, exotrophic tadpoles, large clutch size and ability to reproduce mostly throughout the year even in degraded habitats, have contributed to their success and enabled them to disperse, resulting in wide distributions (Saidapur 2001; Van Bocxlaer et al. 2010). True toads (Bufonidae) arrived in India after the drifting Indian plate collided with Asia, facilitating biotic exchange with Laurasia (Van Bocxlaer et al. 2009). The lineages that dispersed into the Indian subcontinent diversified and gave rise to numerous endemic taxa (Biju et al. 2009; Van Bocxlaer et al. 2009). The northern Western Ghats turned out to be an excellent opportunity for diversification, as the volcanic eruptions that formed the Deccan traps during the Late Cretaceous wiped out the then contemporary biota, creating new habitats open for colonization (Widdowson and Cox 1996; Prasad et al. 2009; Watve 2013). One of the specialized genera that arose from the diversification of ancient lineages is the recently described genus *Xanthophryne* Biju, Van Bocxlaer, Giri, Loader and Bossuyt, 2009 (Biju et al. 2009). It is endemic to the northern Western Ghats and contains the sister species, *Xanthophryne koynayensis* (Soman, 1963) from Koyana and *Xanthophryne tigerina* Biju, Van Bocxlaer, Giri, Loader and Bossuyt, 2009 from Amboli, Maharashtra, India (Biju et al. 2009). Both species specialize on natural rocky outcrops, which are large expanses of exposed lateritic rock that host unique and fragile ecosystems with a large proportion of endemic flora and fauna (Watve 2013). Hence, they are integral landscapes that substantially contribute to the high levels of endemism in the Western Ghats biodiversity hotspot (Watve 2013). The rocky outcrops are patchily distributed and have cliffs at the edges and/or are surrounded by forests and have been aptly described as 'terrestrial habitat islands' (Watve 2013). The distinct microhabitat on rocky outcrops and their isolation exposes organisms to novel pressures that may lead to unique adaptations. An understanding of the reproduction of these toads will be useful – how they are specialized to a highly seasonal environment and the strategies they employ to overcome numerous biotic and abiotic challenges.

Amphibians have manifested several morphological, physiological and behavioural traits, especially with regard to reproduction, that make them interesting not only from a natural history perspective but also from ecological and evolutionary standpoints (Wells 2007; Crump 2015). Anurans exhibit maximum diversity in reproductive modes among tetrapod vertebrates and exhibit aquatic and terrestrial eggs, aquatic as well as semi-terrestrial larvae, viviparity and even direct development (Haddad and Prado 2005; Wells 2007; Crump 2015). Understanding the breeding biology of amphibians is crucial for successful conservation of species and their habitats. This view has especially gained traction in the contemporary scenario where climate change, habitat loss, infectious disease and pollution are threatening amphibians worldwide (Stuart 2004). The two toads, *X. koynayensis* and *X. tigerina* are endemic, restricted to a narrow altitudinal range (700–1000 m above sea level) and appear to have specific microhabitat requirements (Biju et al. 2009). Very little is known about the natural history and ecology of the two

toads. Here, we describe amplexus, spawning and male advertisement calls, and give a comparative account of life history traits of *X. koynayensis* and *X. tigerina*.

Both the toads are found on rocky, lateritic outcrops that are neglected, lack protection, show increased rates of degradation and are even classified as 'wastelands', perhaps because they lack tree cover and appear barren outside the monsoon season (Wastelands Atlas of India 2011; Watve 2013). During the monsoon months, however, the outcrops become hospitable and are transformed into areas sustaining rich biodiversity. The thousands of small ephemeral pools that are formed serve as breeding habitats for many organisms, especially amphibians. Most rocky outcrops are located outside protected areas and are rapidly deteriorating (Wastelands Atlas of India 2011; Watve 2013). Such habitat degradation and loss will push endemic flora and fauna further towards extinction.

Materials and methods

The study was conducted from June to September of 2014 and 2015 on several rocky outcrops in Koyna and Amboli, Maharashtra, India – the type localities of *X. koynayensis* and *X. tigerina* (Figure 1). Field work was carried out daily from 1900 up to 0100 hours during the entire month of June and July of 2014–15. We marked a 50 × 5 m line-transect

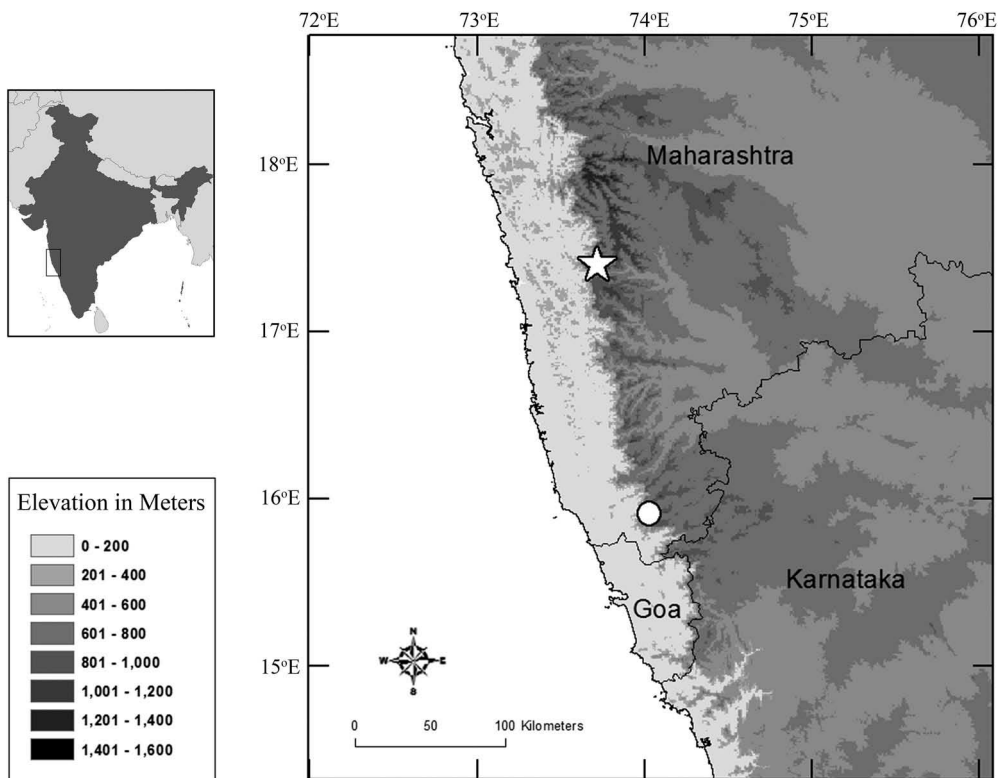


Figure 1. Map showing type localities and study sites of *Xanthophryne koynayensis* (white star) at Koyna and *Xanthophryne tigerina* (white circle) at Amboli, Maharashtra, India.

that crossed the breeding site of both species and conducted intensive searches on three consecutive nights. We counted total number of calling males, pairs in amplexus and single females, if any, to estimate chorus size and sex ratio for a particular night. Data for the three nights were pooled and the average sex ratio was estimated. Amplexus was recorded using a Sony digital handycam PJ670 and a Canon 1100D digital SLR camera. Vocalization of ten *X. koynayensis* and *X. tigerina* individual males was recorded between 1900 and 2100 hours on 10 June and 8 July 2015, respectively. Recordings were made using a solid-state Marantz PMD 671 professional field recorder and a hand-held Sennheiser ME67 unidirectional microphone that was held pointed towards the vocal sac of the focal male at a distance of 30–35 cm. All recordings were made in .wav (uncompressed) format and later analysed using Raven Pro version 1.4 (Charif et al. 2010). We measured the peak frequency and five temporal call properties such as call duration, call interval, pulse duration, pulse interval and number of pulses per call. Body sizes of 25 calling males and 25 amplexant pairs of *X. tigerina* were measured using a digital caliper to the nearest 0.01 mm. Relative humidity and air temperature were recorded using a thermo-hygrometer (608 H1, Testo Ltd). Three amplexant pairs of each species were confined overnight before they started spawning on the first day of the first breeding bout in plastic tubs partially filled with water and covered with a shade cloth in natural conditions at the breeding site to determine clutch size and egg parameters. We opportunistically encountered road-kill females – one *X. koynayensis* and eight *X. tigerina* – throughout the duration of the study and dissected them to determine egg loads. Larval duration of tadpoles was calculated by checking three small marked pools daily from the day eggs were laid until tadpoles metamorphosed and left the larval habitat. Opportunistic observations were undertaken to understand their larval predators and general ecology. All statistical analyses were performed in the statistical software PAST 3.0 (Hammer et al. 2001). Videos of male advertisement call (video 1), amplexus and oviposition (video 2) and mating ball (video 3) can be accessed as Supplemental material. No animals were harmed, collected, killed or experimented upon. Necessary permits were obtained from the Forest department, Maharashtra State; all methods performed were approved as part of the permit.

Results

Breeding period

The breeding season of these toads starts with the onset of the monsoon and lasts for approximately 2 weeks. In 2014, we observed two bouts of breeding activity, first on 12 June with the early rains that lasted for 5 days and the second on 20 August comprising 7 days when the rains resumed, after a dry spell of almost a month. This pattern was repeated in 2015 with the first bout beginning on 10 June and the second around 7 July.

Male vocalization

The males started aggregating on and around boulders or on exposed rock sheets and commenced calling by late evening around 1900 hours and continued until approximately 0030 hours. Males have a single whitish subgular vocal sac (Figure 2(b)). The



Figure 2. *Xanthophryne tigerina* breeding sequence. (a) A typical breeding habitat; (b) a calling male perched on a boulder; (c) a mating ball; (d) a pair in amplexus. Size bar = 5 mm.

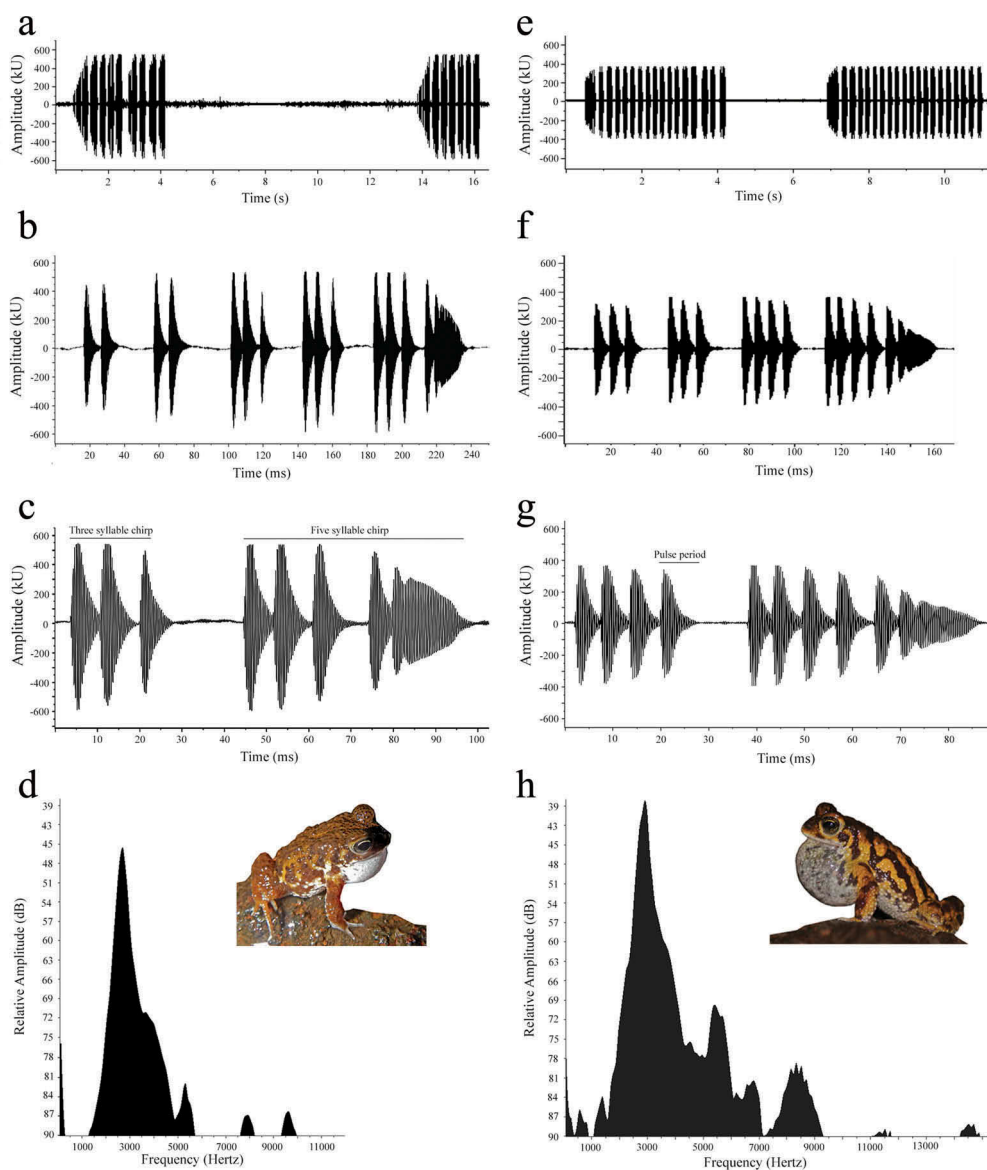


Figure 3. *Xanthophryne* male advertisement calls. Macrotemporal structure of advertisement call of *Xanthophryne koynayensis* (a) and *Xanthophryne tigerina* (e). Microtemporal call structure of *X. koynayensis* (b, c) and *X. tigerina* (f, g) of a single advertisement call. Power spectrum showing the distribution of energy in a call of *X. koynayensis* (d) and *X. tigerina* (h).

males call intermittently and the advertisement call of these species is a short continuous chirp (Figure 3(a,f); Supplemental material Video 1). Male *X. koynayensis* had an advertisement call with five notes. The first four notes were of two or three pulses followed by a final five-pulse note (Figure 3(b)). The peak frequency of *X. koynayensis* was 2687 ± 118 Hz. (Figure 3(d) and Table 1). The advertisement call of *X. tigerina* also had five notes. The first three notes comprised two to three pulses followed by the final

Table 1. Temporal and spectral properties of *Xanthophryne tigerina* and *Xanthophryne koynayensis* male advertisement call.

Call parameter	<i>X. koynayensis</i> (n = 10)	<i>X. tigerina</i> (n = 10)
Temporal parameters		
Call duration (ms)	206 ± 27.15	142.5 ± 26.54
Call interval (ms)	108.5 ± 60.14	81.5 ± 17.67
Pulse duration (ms)	7 ± 1.32	8 ± 0.82
Pulse interval (ms)	3 ± 0.48	2 ± 0.52
Number of pulses /call	15.5 ± 3.03	10 ± 0.99
Spectral property		
Peak frequency (Hz)	2687 ± 118	2844 ± 172.08

Parameter values depicted as median ± standard deviation.

five-pulse note (Figure 3(g)). The peak frequency of the *X. tigerina* call was 2844 ± 172.08 Hz (Figure 3(i) and Table 1). Both species have similar peak frequency and pulse structure (Figure 3 and Table 1). The temperature during the recording of *X. tigerina* calls was 24°C but was 19°C when recording the *X. koynayensis* calls. The relative humidity was constant at 99.9% at both localities.

Sexual size dimorphism

Xanthophryne tigerina males had an average body size (snout–vent length (SVL)) of 31.62 ± 2.06 mm, $n = 25$; females had an average SVL of 32.23 ± 2.41 mm, $n = 25$. There is a lack of sexual size dimorphism in this species ($n = 25$, $p = 0.366$). There was no significant difference in the body size (SVL) of successful (amplectant) and calling males ($n = 22$, $t = -1.836$, $p = 0.069$). The body size of amplectant males and females was not correlated ($R^2 = 0.011$, $p = 0.648$). Male *X. koynayensis* had an average body size of 29.83 ± 1.34 mm, $n = 7$ and the female body size was 30.84 ± 1.12 mm, $n = 7$.

Reproductive strategy and mating system

Xanthophryne has an explosive type of mating system with multiple breeding bouts. With the onset of the monsoon, males aggregated near boulders and chorused to attract females. There was intense male competition and males outnumbered females as much as 7 : 1 for *X. koynayensis* and 9 : 1 for *X. tigerina*. Intense male competition led to ‘mating balls’ early during the breeding period, (Figure 2(c)). A mating ball was formed when two or more males tried to amplex a single female or tried to dislodge the male from an amplectant pair. The male in amplexus kicked vigorously to ward off the intruding males (Supplemental material Video 3). Pairs hopped away from the chorus in search of suitable oviposition sites. Unsuccessful males aggregated near oviposition sites and tried to dislodge amplectant males from pairs approaching to lay eggs.

Amplexus and oviposition

Here we describe the amplexus and oviposition of 27 pairs of *X. tigerina* and 18 pairs of *X. koynayensis*. Females approaching the chorus were quickly mounted by males into amplexus. The males scouted for females within the chorus and jumped onto any

moving object, most often onto other males. The mismatched pairs quickly disengaged after the subordinate male gave a short release call. Once a male successfully paired with a female they left the chorus in search of vernal water pools to lay eggs. These species exhibit axillary amplexus and the male clasps the female at the armpits (Figure 2(d)). The amplexus is long and lasted several hours (up to 9 hours, $n = 3$). The female carried the male around as she searched for suitable oviposition sites. Once the female halted at particular spot of her choice, she cleared gravel at the oviposition site and slightly spread her hind limbs; simultaneously raised her head so that her vent touched the small pool of water and deposited two to four eggs. Immediately after the female assumed this position, the male extended his hind limbs and made a few pelvic thrusts and presumably ejaculated to fertilize the eggs (Supplemental material Video 2). *Xanthophryne koynayensis* had two to three pelvic thrusts whereas four to five thrusts were seen in *X. tigerina* (Supplemental material Video 2). The pelvic thrusts by the male occurred immediately after the eggs had been deposited and did not seem to assist in expelling the eggs. Female *X. tigerina* visited four or five pools in a 2-hour period ($n = 4$) and laid seven or eight eggs in each pool. The average distance between the pools was 12.6 m ($n = 5$, range 7–20 m).

Clutch and egg characteristics

The semi-terrestrial eggs of *Xanthophryne* were deposited in small depressions on lateritic rocks or on wet rocky surfaces. The eight *X. tigerina* pairs that were brought into captivity laid 630 eggs; each female laid an average of 78 eggs (Table 2). The six *X. koynayensis* pairs laid 960 eggs. We counted the eggs from six road-killed gravid *X. tigerina* females (Figure 6). Four females had ova in a single ovary whereas the other two had ova in both. A single ovary had an average of 59 ± 10.61 ova (range 51–74); whereas both ovaries combined contained 142 ± 6 ova (range 136–148, $n = 6$). We found only one *X. koynayensis* gravid female road kill and she had 85 and 89 ova in her ovaries. *Xanthophryne tigerina* eggs had a diameter of 3.27 ± 0.5 mm with jelly and 2.28 ± 0.3 mm without the jelly coat ($n = 21$) whereas diameters of *X. koynayensis* eggs with and without jelly were 2.60 ± 0.30 mm and 1.72 ± 0.26 mm, respectively. The ratio of the egg to the outer jelly coat was 1.43 in *X. tigerina* and 1.51 in *X. koynayensis*. Eggs developed fast and hatched on the 4th day after they were laid ($n = 68$). All the eggs laid in captivity were fertilized and hatched successfully.

Table 2. Life history traits of *Xanthophryne koynayensis* and *Xanthophryne tigerina*.

Trait	<i>X. koynayensis</i>	<i>X. tigerina</i>
Body size (mm)	Small (30–33)	Small (30–33)
Mating system	Multiple short explosive bouts	Multiple short explosive bouts
Parotoid glands	Present (small)	Present (small)
Clutch size (total number of ova)	Small (150–180)	Small (150–180)
Egg size (mm)	Large (2–3)	Large (2–3)
Clutch morphology	Single detached eggs	Single detached eggs
Oviposition site preference	Strict	Strict
Hatching duration (days)	Short (3–4)	Short (3–4)
Larval duration (days)	Short (30–35)	Short (30–35)
Mode of nutrition of tadpoles	Exotrophic	Exotrophic

Larval habitat and development

The shallow pools where the tadpoles developed measured 6.83 ± 0.48 mm in depth and were 60–180 mm wide ($n = 11$, [Figure 4\(a\)](#)). The average temperature during the entire larval development was 25.7°C (min 23.9°C and max 27.2°C) with average relative humidity at $90\% \pm 5\%$. The tadpoles were exotrophic and fed on algae growing in and around the small pools. They lived in high densities with as many as 100–120 tadpoles in a pool measuring 150 mm wide and 6.7 mm deep ([Figure 4\(a\)](#)). They ventured out of the water and fed on the algae growing on the moist rock when they ran out of food within the pool. Mosquito larvae and



Figure 4. Larval development of *Xanthophryne tigerina*. (a) A shallow pool in a depression on a boulder serves as the larval habitat; (b) eggs laid in a pool on a lateritic boulder; (c) hatchlings; (d) growing tadpoles scraping on the surface of an egg laid during the second breeding bout; (e) tadpoles at different developmental stages; (f) a metamorphosed toadlet.



Figure 5. Desiccated pool and stranded tadpoles of *Xanthophryne tigerina*.

occasionally aquatic beetles inhabited these pools but no other anuran larvae or other organisms were observed. Tadpoles metamorphosed in 39 days from three pools we monitored in the wild ($n = 17$). No predators were observed feeding on *X. tigerina* eggs or tadpoles but we observed a terrestrial crab and a water scorpion (*Ranatra* sp.) feeding on the freshly hatched immobile tadpoles of *X. koynayensis* from a pool at the edge of the rocky outcrop. During the break in the rainfall in 2014 and 2015 we observed stranded tadpoles in desiccated pools. *Xanthophryne* tadpoles were found to be surprisingly tolerant to desiccation as they were alive 5 hours after the pool had completely dried (Figure 5).

Discussion

After more than a century of research on the breeding biology of amphibians, we continue to discover diverse novel strategies that they exhibit to overcome various environmental challenges to successfully reproduce (Crump 2015). The underappreciated anuran diversity in the Western Ghats biodiversity hotspot has recently received much deserved attention with reports of several new species, novel reproductive behaviours and modes (Kunte 2004; Abraham et al. 2013; Gaitonde and Giri 2014; Gururaja et al. 2014; Seshadri et al. 2015; Senevirathne et al. 2016). The reproductive ecology of most Western Ghats endemics is unknown and many novel strategies and patterns are yet to be discovered. One such example is the recently evolved genus *Xanthophryne* that has an explosive breeding system, exclusively using rocky outcrops as its breeding and larval habitats.

Xanthophryne breed with the onset of the monsoon and heavy rain was a probable cue for the toads to come out of aestivation. They have a temporal pattern to their breeding system where short bouts of breeding for 2–3 days were observed during the first week of the monsoon. The synchronous arrival of males and females at breeding sites and a male-biased sex ratio led to mating balls, typically observed in explosive breeders (Wells 2007). Similar to species with intense male competition, these toads have a developed and pointed prepollex (nuptial spine at the tip of the thumb) that they used to securely grasp the females, and vigorously kicked off intruding males (Wells 2007; Biju et al. 2009). Occasionally, there were dire consequences of such mating balls as males in combat ripped each other apart or the female in the centre of the ball suffocated to death. Usually the pair hopped away in search of suitable oviposition sites.

Xanthophryne exhibits axillary amplexus, which is a derived character exhibited by most anurans (Gomez-Mestre et al. 2012). One hypothesis for the switch from the ancestral inguinal amplexus to axillary amplexus is that it facilitates efficient fertilization by juxtaposing the male and female cloacae and synchronizing egg deposition with sperm release (Wells 2007). In anurans exhibiting axillary amplexus, eggs are usually deposited as one large mass in a single convulsive expulsion and are immediately fertilized. Many Microhylids, Hylids and Ranids lay eggs in a single cluster as a globular mass or a floating jelly raft (Wells 2007). Most Bufonids typically lay eggs in a jelly tube that are deposited as a single mass and later pulled into a string-like structure. Fertilization of a single egg mass is relatively simple as males deposit sperm mixed with dilute urine onto the egg mass (Wells 2007). The pelvic thrusts of *Xanthophryne* males may potentially be an adaptation that increases successful fertilization of a small number of single eggs in a semi-aquatic environment and minimizes wastage of gametes and energy. The pelvic thrusts may help the male to reach the deposited eggs and release the sperm on them accurately, leading to efficient fertilization. The pelvic thrusts may also serve as a tactile cue for the female indicating that ejaculation has occurred before she moves forward en route to the next pool. In the absence of communication between the pair, there are chances of the eggs remaining unfertilized and both the male and the female would entail high fitness costs. Contrary to the previous belief that amplexus is a static position that a pair assumed during oviposition and fertilization (Wells 2007), in these toads amplexus was dynamic and may be multi-functional. Interestingly, a consistent difference was observed in the number of pelvic thrusts between the sister species pair. *Xanthophryne koynayensis* exhibited two or three ($n = 9$) thrusts per fertilization event whereas *X. tigerina* had four or five ($n = 15$) thrusts per event (Supplemental material Video 2, amplexus and oviposition). This indicates that axillary amplexus and pelvic thrusts could be ecologically relevant and not merely ancestrally retained characters. Other species where pelvic-thrust like actions have been observed are *Aschapus truei* Stejneger, 1899 and *Fritziana goeldii* (Boulenger, 1895) (Stephenson and Verrell 2003; Duellman et al. 2011). In *Aschapus truei* the fertilization occurs internally and it has an erectile intermittent organ called the 'tail'. Thrusts were observed when it was being inserted into the cloaca of the female during copulo-amplexus (Stephenson and Verrell 2003). In *Fritziana goeldii*, the male beats a foam matrix from the mucus secreted by the female and places fertilized eggs into it. Pelvic thrusts were observed when the smaller male tried to bring forward the mucus and the egg extruded from the female's cloaca onto her back. The thrusts reduced in

intensity as egg deposition neared completion (Duellman et al. 2011). In *Xanthophryne* the intensity and the number of thrusts remained constant across multiple oviposition events and they are functionally different compared with the above examples. An alternative hypothesis for the functional significance of amplexus is to stimulate the female to lay eggs. Many male frogs show undulating abdominal movements in amplexus that are assumed to stimulate the female to oviposit (Wells 2007). However, in these toads the majority of the movements by the rather stationary males are just after the female began oviposition.

The eggs deposited in the small rocky pools hatched within 3–4 days. The pools provide refuge for *Xanthophryne* tadpoles with no competitors and few predators but are prone to desiccation. The climate of the Indian subcontinent, especially the monsoon, is influenced by the rise and fall in sea-surface temperatures and the prevailing conditions of a deficit rainfall/drought or adequate monsoon corresponding to a cold or a warm event known as El Niño and La Niña (Gadgil 2014). Due to El Niño conditions in 2014 and 2015, the onset of the monsoon was advanced and an abrupt dry spell of almost a month disrupted the breeding of many anurans (NG personal observations; Gadgil 2014). *Xanthophryne* started breeding with the first rains and initially laid many eggs, but with the cessation of intermittent rain and a subsequent drop in humidity most rocky pools dried up and there was high mortality (Figure 5). The Indian monsoon is highly variable and its short-term pattern is highly unpredictable; organisms dependent on the seasonal rains have to cope with its uncertainty and irregularity (Gadgil 2003; Touchon and Warkentin 2009). Organisms facing unpredictably varying environments evolve to spread out their risks or ‘hedge their bets/investments’ (Philippi and Seger 1989; Hopper 1999; Fox and Rauter 2003). In fluctuating environments, natural selection favours genotypes that have a low variance in fitness over those with high variance in fitness at the cost of low arithmetic mean fitness (Hopper 1999; Fox and Rauter 2003). The small ephemeral pools that serve as oviposition sites and larval habitats depend on the vagaries of intermittent rain and desiccate with cessation of rainfall. *Xanthophryne* females seem to counter the unpredictability of the larval habitat by spreading out eggs across both space and time. These toads breed in multiple explosive bouts and the presence of ova in either ovary at various developmental stages suggests that *Xanthophryne* females mate and oviposit multiple times. The single eggs they lay are exceptionally large in comparison to other Bufonids or frogs of similar size (see Wells 2007; Han and Fu 2013; Pruvost et al. 2013; Liedtke et al. 2014). Both *Xanthophryne* species make heavy investments per egg and distributing their clutch into numerous pools may be adaptive. This strategy increases the chance of some offspring surviving even if most of the pools dry up, and avoids reproductive failure. The other advantage of spreading out their clutch may be mitigating inter-specific competition. The limited number of small rocky pools results in larval crowding and limitation of resources. By spreading out the clutch, females may improve the likelihood of some offspring concluding development in sparsely populated pools and metamorphosing as larger and heavier toadlets, enhancing their quality.

Both *X. koynayensis* and *X. tigerina* lack sexual size dimorphism, and their small clutch with large eggs indicates a trade-off between egg and clutch size (Crump 1974). Body size is strongly correlated to egg size across anuran species and fecundity selection has been attributed for the evolution of sexual size dimorphism, where females are larger

than males (Han and Fu 2013). *Xanthophryne* may have evolved large eggs as a consequence of the resource-poor habitats of its tadpoles at a cost of a reduced clutch size; a typical pattern observed in species with terrestrial/semi-terrestrial eggs. The transient nature of larval habitat, limited resources and high competition may have contributed to the evolution of large egg size helping the tadpoles grow rapidly and metamorphose successfully before the ephemeral pool desiccates.

The most intriguing aspect of these toads is the male advertisement call. One expects advertisement calls to have diverged during speciation and be a contributing factor in reproductive isolation (Heyer et al. 2003; Lemmon and Lemmon 2010). Surprisingly, *X. tigerina* and *X. koynayensis* have similar advertisement calls. Both species have a similar peak frequency (2569–3016 Hz) and similar pulse structure (Figure 3(d,i); Table 2). A similar case has been reported in the *Leptodactylus pentadactylus* (Laurenti 1768) species complex where two morphologically and genetically distinct sister species have identical calls (Heyer et al. 2003). Factors such as an explosive mating system with little scope for females to exert strict choice, recent differentiation due to vicariance and retention of the ancestral advertisement call may have led to the similarity in advertisement calls of the two species. This observation needs to be analysed quantitatively and is being further investigated.

Both *Xanthophryne* species are locally abundant but have extremely restricted distributions and are endangered (Biju et al. 2004; IUCN SSC Amphibian Specialist Group 2013). The breeding sites of *X. koynayensis* are legally protected as they fall under the Sahyadri Tiger Reserve and are relatively undisturbed. However, rocky outcrops at Amboli where *X. tigerina* occurs are subjected to severe pressure due to infrastructure development projects, tourism and changes in land use (Watve 2013). One of the threats that these toads face is depletion of loose rock due to its local use as construction material (VG and NG personal observations). During the day these toads take refuge



Figure 6. Road kill of a gravid *Xanthophryne tigerina* female.

under loose rocks and scarcity of refuge sites may adversely affect their survival. The increased road network in and around Amboli may threaten *X. tigerina* because they often mistake a patch of road for an exposed lateritic rock sheet and aggregate to form choruses. In one instance we observed approximately 32 individual road kills in a span of 10 m with the passing of just one vehicle (Figure 6). The threat perception to these two known species of *Xanthophryne* is based on our preliminary observations and a detailed study is immediately warranted.

The evolutionary trajectory of *Xanthophryne* with regard to its reproductive biology appears to be heavily influenced by the unique environment on the rocky outcrops. The diversifying toad lineage that occupied these open habitats evolved several novel modifications in life history traits aiding their specialization (Van Bocxlaer et al. 2010). These toads seemingly well adapted to the rocky outcrops offer an excellent opportunity to better understand endemism, mating systems, anuran ecology and behaviour.

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