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Cover: A female Javan Leopard *Panthera pardus melas* in rehabilitation phase at Cikananga Wildlife Center. © Yayasan Cikananga Konservasi Terpadu.



## INTRODUCTION

Amphibians throughout the world are in decline, with 41% of the world's 6,638 known amphibian species threatened with extinction (Walls et al. 2017; Grant et al. 2020). A global decline in amphibians was first recognized in 1989 (Vitt et al. 1998; Wake 1991), and the situation has not improved since then. According to IUCN (2010a), towards the end of 1998, 124 species of amphibians were categorized as threatened; but by 2010 the number had increased more than 15 times to 1898 threatened species (Hamer & Parris 2011). This decline represents 29% of the total number of amphibian species described in the IUCN Red List (IUCN 2010a), more than any other category of animals. The decline is likely to have implications on the ecosystem services provided by amphibians such as biological pest controlling and bioturbation (Valencia-Aguilar et al. 2013).

*Afrixalus sylvaticus* Schiøtz, 1974 is a species of frog in the family Hyperoliidae (Frost, 2022). The species is categorised as Vulnerable in the IUCN Red List (IUCN SCC Amphibian Specialist Group 2016), and the distribution range is limited to Shimba Hills (Kenya), through the East Usambara foothills in northeastern Tanzania. The species occurs only very patchily within the mapped range due to limited suitable habitat. Its natural habitats are dry forests, moist lowland forests, intermittent freshwater marshes, plantations, and degraded forest.

The primary threat to this species is habitat loss through drainage of wetlands and afforestation (Bwong et al. 2017). In several areas of prime habitat, the planting of exotic trees such as *Eucalyptus* sp. and *Casuarina* sp. forests has lowered the water table to such a degree that many ponds within the coastal dune forest have entirely disappeared (Martin et al. 2004). Along the gradient of Shimba Hills National Reserve, there are wide-ranging human activities that extend to the protected area since the reserve policy in Kenya allows human activities within the national reserves (The Wildlife Act Chapter 376 of 2010). These activities include resins collection, fuel wood collection, and herbs collection. Shimba Hills is under category "B" together with Arabuko Sokoke Forest Reserve, Ndere Island and Tana Primate Reserve. Along the gradient of the reserve boundaries, there were human settlement, agro-ecosystems, infrastructure such as roads, making it an "ecological island."

Beebee & Griffiths (2005) noted that the global amphibian decline phenomenon is associated with population decrease and extinctions in many regions of the world. The need for ecological studies of amphibians, especially those addressing population dynamics, is a

priority (Houlahan et al. 2000). The main objective of this research was to assess the breeding ecology and population dynamics of the species in the protected area and community lands.

## MATERIALS AND METHODS

### Study area

We carried out the study in Shimba Hills National Reserve and its hinterlands (4.2572°S, 39.3877°E) in Kwale County, Kenya (Figure 1). The area was selected as it is the only remaining habitat that *Afrixalus sylvaticus* is found after the cultivation of rice schemes in former habitats (Kaloleni areas). The Shimba Hills National Reserve is part of the larger Shimba Hills ecosystem, which also comprises the Mwaluganje Elephant Sanctuary, the Mwaluganje Forest Reserve, the Mkongani North Forest Reserve and the Mkongani West Forest Reserve and covers a total area of 250 km<sup>2</sup> (Bwong et al. 2017).

### Methods

Ecological surveys were conducted for 60 days in September–December 2016 (dry season) and another 60 days in April–June 2017 (wet season). The sampling was done along six transects distributed equally both in protected and unprotected areas. The unprotected parts were characterized by human activities ranging from agro-ecosystems to settlements and infrastructure. During the fieldwork, visual encounter surveys (VES) (Crump & Scott 1994; Peek et al. 2017) were used in all potential microhabitats of the species. The survey was along the river transect (1,000 m) but the sampling protocol was adjusted in some areas due to difficult terrain or at pools of water where line transect was limiting. The number of individuals encountered within 10m of each side of the transect line was captured, marked, morphometric/biometric and population attributes recorded. Marking was by using toe clipping as adopted by (Donnelly et al. 1994).

Morphometric parameters measured were; snout-vent length (SVL) in mm  $\pm 0.01$ mm, weight (g), head width (HW), number of resting lines (RL) and population attributes were sex, age, and other visible traits such as skin colour or visible eggs. The maturity levels were classified using the presence of resting lines (that are found usually only in adults). Additionally, we determined the sex of the individuals using coarseness of the dorsum. Males have a coarse dorsum, while females have smooth dorsum (Tillack et al. 2021). Additionally, males develop nuptial pads on the underside of limbs

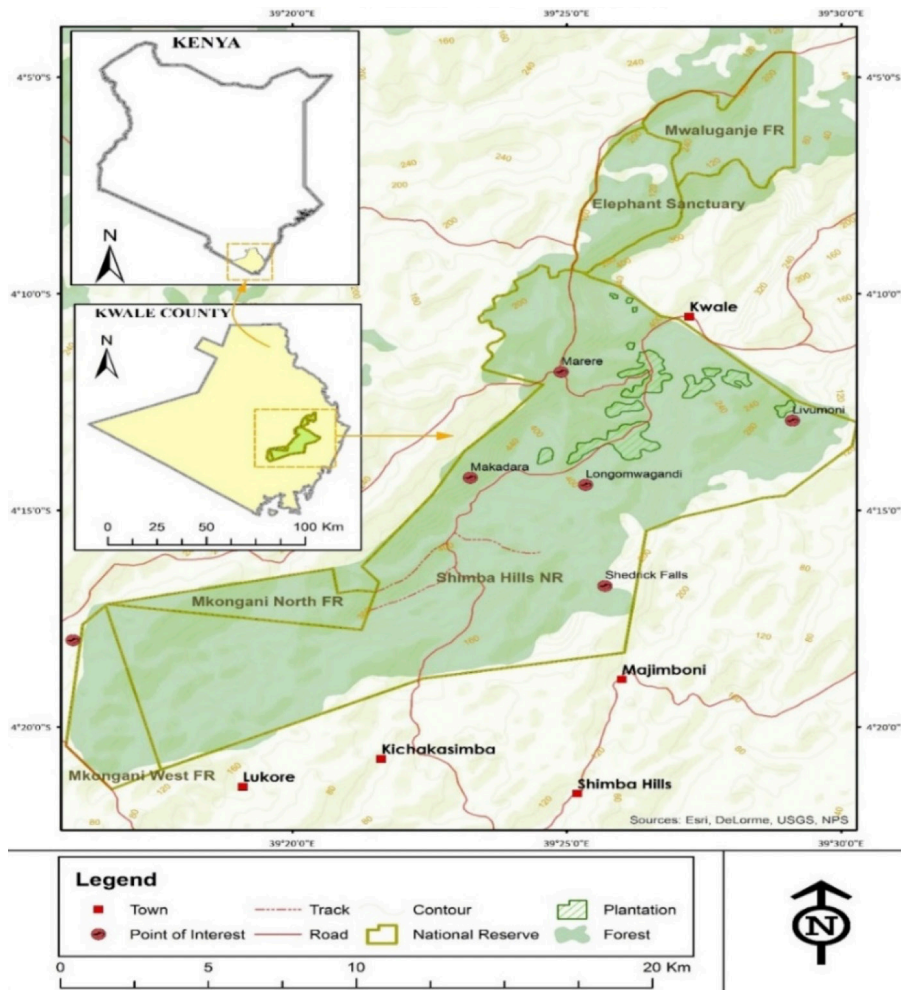


Figure 1. Study area—Shimba Hills National Reserve (Source: Modified data from ESRI, 2016).

and toes which facilitate amplexus. These are patches of darkened skin which are rough to touch. The parameters recorded during sampling include: seasons, land tenure system, time of the surveys and weather. The population density within the study areas was determined using Peterson method (Krebs, 1989) comparing the observed individuals between sample periods once every month. Chi-square test, ANOVA, and t-tests were used for comparisons of variables.

Population estimate was determined using repeat mark-recapture technique

$$N = \frac{M_2 C_2}{R_2} + \frac{M_3 C_3}{R_3} + \frac{M_3 C_3}{R_4} \dots \dots \dots (1)$$

Where:

N-Estimated population size

M-Number Marked in first catch

C-Number caught in second catch in total

R-Number Recaptured in second catch

The two sampling periods were treated as separate marking exercises, as there were no recaptures in wet period that were marked in dry period.  $M_3$  was total of captures in second month and first month minus the recaptures in second round of sampling and  $C_3$  was total of captures in 3<sup>rd</sup> month in each sampling period while  $R_3$  was total recaptures in 3<sup>rd</sup> month. Population density was estimated using Canonical density estimator since in some situations, the transect layout was non-uniform (not linear for instance in water pool).

$$D = \frac{n}{a} \dots \dots \dots (2)$$

Where:

D-Density of the species

N-Total number of animals counted

A-Total area located at random (transect)

## RESULTS

### Species occurrences in sampling sites

Within the protected area, Shimba lodge marshes were densely populated (Wet season: 13 and Dry season: 6). It represents 19% of the entire sampling sites. Other sites in the protected area were; Manolo and Mwele catchment (Table 1). In the community lands, Mwandabara represents the highest population density zone (seven individuals in each season).

### Population size and related parameters

The total population estimate of the species was 192 individuals (95% confidence interval) with a density of 0.98 individuals/km<sup>2</sup>. Generally, the species was more abundant during the wet season than dry (58% and 42%, respectively). This represents a significant difference (t-value = -3.38, p-value = 0.002, df = 30) in the individuals recorded in two different seasons. Land tenure types in this area are private and public land ownership. The distribution was almost equal in both land tenure types (51% in protected and 49% in community land). Regarding the time of the survey, more individuals were encountered during morning sampling than other sampling periods though there was no significant difference (F-value = 0.25, df = 2, p-value = 0.781). Weather played a role in the species capture success as, during the cool and rainy period we captured the highest number of individuals (47%) as compared to the cool and dry period (8%) where only five individuals were captured. The results are shown in Table 2.

### Morphology

The mean snout-vent length (SVL) for males was 15.12 mm, while mean SVL for females was 15.96 mm. The maximum and minimum SVL of females was 24mm and 4mm respectively, while that of males was 22 mm and 5 mm, respectively. The SVL for females was higher than that of males, but there was no significant difference (t-test = 0.87, p = 0.390, df = 39). ANOVA, however, shows that there was a statistical difference between the mean of males, females, and juveniles SVL (F-test = 11.00, df = 2, p-value = 0.000). Consequently, the mean weight of females was higher than males (6.05 g and 4.82 g, respectively). This shows females are significantly heavier than males (t-test = 3.50, p-value = 0.001, df = 39), a demonstration that sexual dimorphism is evident in the species. The other morphometric characteristics taken include head width (HW) and resting lines (RL). On average, females had higher HW than males (4.3 mm and 3.5 mm). The resting lines were more related to both

**Table 1. Number of *Afrixalus sylvaticus* captured in each transect during wet and dry seasons.**

	Dry season	(%)	Wet season	(%)	Total
Kivumoni	4	44	5	56	9
Marere	2	50	2	50	4
Mwandabara	7	50	7	50	14
Manolo	3	38	5	63	8
Mwele catchment	3	60	2	40	5
Shimba lodge	6	32	13	68	19
Total	25	42	34	58	59

males and females but were missing in juveniles. On average both male and female had 1.59 and 1.2 resting lines respectively whereas juveniles had none (Table 3).

### Population structure and sex ratio

There were more individuals captured during the wet period (57.4%) as compared to the dry period (42.3%). In terms of population structure, the largest proportion are females (29) and juveniles the smallest (10). There was no significant difference across all categories (Pearson chi-square = 2.068, df = 2, p-value = 0.356). Sexual dimorphism in young individuals was difficult to point out, and we classified them as juveniles (Table 4).

The sex ratio was relatively stable throughout the sampling period, but females were more than male, an indicator of the population's reproductive potential (Yildiz & Göcmen 2012). The sex ratio was 1:1 in the year 2016, but the following year it was 1:2 in favor of females.

### Activity period and breeding ecology

#### Activity periods

The activity of the amphibians is associated with calls. We recorded a number of calls of the species and other species in each habitat for five hours every sampling period, a reflection of the activity period (Table 6). The mean calls show there was more activity in the wet than the dry season. On maximum, four calls/min were recorded in dry at 2000 h. This was the time with maximum activity ( $x = 0.875$  calls/min) as the number of calls peaked (Figure 2). In wet season, however, the maximum activity period was at 1900 h. In both periods, the number of calls declined from 2000 h and was lowest at 2200 h.

In comparison with other species in each habitat, the *A. sylvaticus* calls represents a small proportion. There average calls of other species combined were above 4.5 calls, and the maximum was 7.7 calls/min (Figure 3).

**Table 2. Population size and related parameters.**

Parameter	Category	N	Mean	SE (±)	SD (±)	Min	Max	Percent
Season	Dry	25	1.042	0.0417	0.204	1	2	42
	Wet	34	1.417	0.103	0.504	1	2	<b>58</b>
Land tenure type	Protected area	30	1.364	0.064	0.326	1	2	<b>51</b>
	Community area	29	1.115	0.105	0.492	1	2	49
Time of survey	Evening	18	1.286	0.125	0.469	1	2	31
	Morning	21	1.235	0.106	0.437	1	2	36
	Night	20	1.177	0.095	0.393	1	2	34
Weather	Cool and dry	5	1.667	0.333	0.577	1	2	8
	Cool and rainy	28	1.167	0.078	0.381	1	2	<b>47</b>
	Hot and dry	8	1.000	0.000	0.000	1	1	14
	Warm and dry	18	1.385	0.140	0.506	1	2	31

**Table 3. Morphometric characteristics of *Afrivalus sylvaticus*.**

Parameter	Sex	Mean	SE	SD	Var	Min	Max
SVL(mm)	Female	<b>15.96</b>	1.22	5.73	32.86	4	<b>24</b>
	Juvenile	5	1.98	5.23	27.33	0	13
	Male	<b>15.12</b>	1.11	4.83	23.37	5	<b>22</b>
Wg(g)	Female	<b>6.05</b>	0.228	1.069	1.143	3.4	7.2
	Juvenile	0.9429	0.0481	0.1272	0.0162	0.8	1.1
	Male	<b>4.821</b>	0.229	0.996	0.993	2.9	6
Hw(mm)	Female	<b>4.318</b>	0.363	1.701	2.894	3	5
	Juvenile	1.143	0.634	1.676	2.81	0.2	2
	Male	<b>3.579</b>	0.377	1.644	2.702	1.2	4
RL(Count)	Female	1.591	0.17	0.796	0.634	0	3
	Juvenile	0	0	0	0	0	0
	Male	1.211	0.164	0.713	0.509	0	3

Like the *A. sylvaticus*, the mean number of calls of other species also declined after 2000 h and was at lowest as 2200 h.

### Breeding ecology

During the activity periods, some solitary and mating pairs of *A. sylvaticus* were seen in the reeds and water lilies in waterlogged habitats (Image 1a,b). We captured one pregnant female notable from a swollen abdomen. After two days, we found the spawn deposited at the place (Image 2a). In some situations, spawn were also deposited in leaf foldings. The breeding of the species was predominantly on reeds and water lilies (Image 1a,b and 2b). The breeding period was confined to the months

of April and May, during which the highest number of calls were recorded (Figure 4).

### Influence of human activities on species population attributes

Although wetlands such as marshes and water pools were abundant in human occupied lands including Mwandabara and Kivumoni, human activities such as bush burning, livestock grazing, drainage, and plantation of exotic tree species (*Eucalyptus* sp.) has converted most wetlands to terrestrial habitats and limit the species occupancy. However, there was no significant difference in species population between protected area and community area (At 95% CI, P-Value = 0.795, DF = 36).

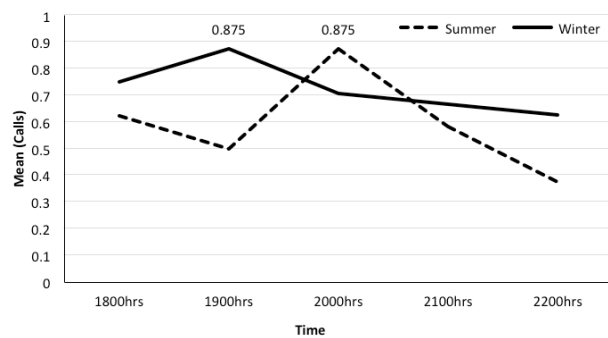


Figure 2. Activity trends in dry and wet seasons

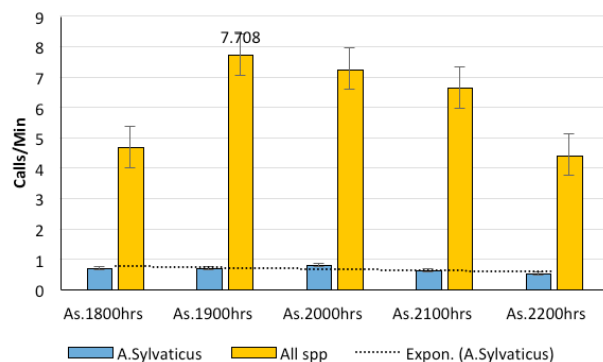


Figure 3. Number of calls/min of *Afraxalus sylvaticus* and other species.

## DISCUSSION

### Population Size, Structure and Dynamics

The abundance of the species during the wet season demonstrated the synchrony of breeding with season and availability of breeding habitats such as water pools and moist reeds. The species deposit the spawn masses in the aquatic plants. While there was no previous study outlining the population status of the species, the IUCN Red List indicates the species is declining. In the study sites, numerous human activities such as wetland drainage, livestock grazing in wetlands and bush burning are attributed to the decline of the species population. Habitat loss has received much attention as a driving factor in amphibian declines and is widely considered one of the leading causes (Gallant et al. 2007; Gardner et al. 2007).

During dry weather, most sightings were largely of adults, as compared to the wet season, when there were more sightings of juveniles. This difference can be attributed to the influence of temperatures and favorable breeding weather. Temperature and moisture influence amphibian ecology, physiology, and behavior because

Table 4. Population structure.

	Dry season	(%)	Wet season	(%)	Total
Female	10	34.48	19	65.52	29
Juvenile	4	40	6	60	10
Male	9	45	11	55	20
<b>Total</b>	<b>25</b>	<b>42.37</b>	<b>34</b>	<b>57.63</b>	<b>59</b>

Table 5. Sex Ratio

Year	Sampling 1	Sampling 2	Sampling 3	Annual
2016	1:2	1.3:1	2:1	1:1
2017	1:1	4:1	1.3:1	2:1

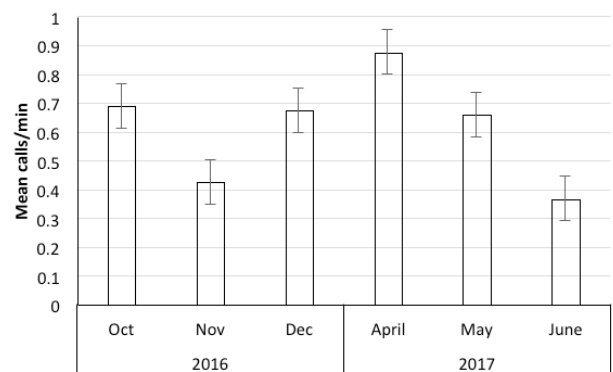


Figure 4. Activity periods per month.

amphibians must maintain moist skin for oxygen and ionic exchange and temperature influences metabolic rates (Burrowes et al. 2004; Blaustein et al. 2010). Additionally, apart from particulate dynamics, tadpoles may be influenced by dissolved nutrients in streams (Hamer & Parris 2011), especially in the dry season when densities are high and flow is relatively low.

The species is not very mobile as it is endemic to Shimba Hills (Spawls et al. 2019) and we hold the assumption that the marking did not affect their movement or catchability. The population size and density of this species are insignificant compared to other amphibians in the area (192 individuals and density of 0.98/km<sup>2</sup>). Many factors are significant in influencing the small species population. In the previous field surveys, the species was sighted in Kivumoni Gate Swamp, Marere headworks, Sheldrick Falls, and Shimba Lodge Swamp (Bwong et al. 2017). However, we made a transect sampling in some of these sites, but there was no capture for the entire survey (both in the dry and wet season). This suggest a potential shift in species

Table 6. Activity periods

Variable	Season	Mean	SE	SD	Var	Min	Max
1800 h	Dry	0.625	0.189	0.924	0.853	0.000	3.000
	Wet	0.750	0.150	0.737	0.543	0.000	2.000
1900 h	Dry	0.500	0.147	0.722	0.522	0.000	2.000
	Wet	<b>0.875</b>	0.193	0.947	0.897	0.000	3.000
2000	Dry	<b>0.875</b>	0.220	1.076	1.158	0.000	<b>4.000</b>
	Wet	0.708	0.175	0.859	0.737	0.000	3.000
2100 h	Dry	0.583	0.190	0.929	0.862	0.000	3.000
	Wet	0.667	0.155	0.761	0.580	0.000	2.000
2200 h	Dry	<b>0.375</b>	0.132	0.647	0.418	0.000	2.000
	Wet	0.625	0.145	0.711	0.505	0.000	2.000

The figures in bold represent the highest and lowest mean number of calls.

habitat or decline in the species population. Stuart et al. (2004) projected that the loss of habitat is relevant to all regions and is also linked to habitat fragmentation and degradation.

In other studies, conducted elsewhere, Hamer & Parris (2011) noted that in tropical regions, the roles of amphibians in both aquatic and terrestrial habitats are more consistent through time because of their more stable seasonal abundance patterns, high species richness, and diversity of reproductive modes. In this species, however, there was an unstable age structure, more females, few juveniles, and few males.

Schmidt et al. (2002) noted that the estimation of basic demographic parameters and the identification of their determinants are essential to improve the understanding of population dynamics and life histories of amphibians. The general observation from the survey shows declining habitats, as some wetlands are converted to farmlands, livestock grazing fields, and tree plantations (Image 1a and b).

### Morphology

*Afrixalus* is a sub-Saharan genus containing about 35 taxa (Pickersgill 2005). Most of the members in this genus are referred to as 'dwarf' because of their small body length and weight. The term 'dwarf' was loosely applied to species which did not exceed 25 mm in length (Pickersgill 2005). This species averaged less than 24 mm in SVL and weighs about 6.05 g.

In *A. sylvaticus* recorded in Tanzania, the males' length was between 15.2–21.1 mm (mean 18.1 mm, N = 28), females 17.2–25.0 mm (mean 20.3 mm, N = 41) (Pickersgill 2005). This morphometrics features confirms the relationships of the species in different habitats and the sexual dimorphism where female is slightly larger in

size than males. It was also evident that males are fewer than females, but the reason for the variation is still not clear. Schiøtz (1999) records a snout-vent length of up to 24 mm in females with dorsal asperities fine and weak, confined to head or at least not extending to tibiae and feet; gular disc with or without fine asperities; no ventral asperities. Additionally, Schiøtz recorded the length of males from 18.0–20.8 mm (mean 19.4 mm, N = 17), females 20.6–22.8 mm (mean 21.8 mm, N = 3).

In most anurans, females are bigger than males, but males are bigger in some species in which males have physical competition for mating (Shine 1979). The principal diagnostic characters in *Afrixalus* are mostly secondary sexual characters present only in male frogs (Pickersgill 1984). For this reason, outside the species descriptions, all morphological analyses of frogs are based on sexually mature males. *Afrixalus* are often known as Spiny Reed Frogs due to the presence of minute spinules, or asperities, in the skin of most species. We followed the detection technique previously used (Pickersgill 1984). The primary means of detection was through a 10X or 20X hand lens, with the wet specimen held at an angle to a light source so that as the fluid evaporated from the skin, the asperities pierced the surface film. In most mature individuals we captured, the color of the dorsum was silverish to greenish-yellow, usually with scattered dark spots and with up to three darker transversely oriented bands which we labeled resting lines.

### Breeding ecology and activity periods

According to Pickersgill (2005), this species is commonly referred to as 'Leaf folding Frogs' because of the typical mode of oviposition, they occupy a range of habitats from seasonally moist open grassland and savanna to rain forest, and from sea level to altitudes



Image 1. a—*Afrixalus sylvaticus* in reeds at 2000 h | b—*Afrixalus sylvaticus* and *Hyperoleous rebrovermicalatus* at 0700 h. © Authors, 2019.



Image 2. a—Spawn of *Afrixalus sylvaticus* | b—*Afrixalus sylvaticus* in water lilies. © Authors, 2019.

above 2,000 m. Though it was not found in savanna, there was evidence that it occupies the moist grassland during the dry period, as was the case in Mwandabara and Marere. However, the species is commonly found in reeds and wetland vegetation in water lilies and reeds.

Forest species produce some of the shortest and simplest *Afrixalus* calls, and voice evolution in the closely related species *A. stuhlmanni* complex, may reflect a transition between forest and savanna habitation (Pickersgill 2005). An analysis of voices in the genus

*Afrixalus* shows a gradual increase in the number of pulses per call, from *A. sylvaticus* with 2–5 pulses to the dry savanna (Martin et al. 2004; Pickersgill 2005). While we also noted the variation in habitats, we did not compare the wetlands to savanna. It was only evident that the calls vary significantly between the dry and wet season. This is attributed to the activity of breeding influenced by a rainy period. Pickersgill (2005) also found the variation in the number of calls in different temperature conditions and concluded that there is a negative correlation between

number of calls and temperature.

We found more activity during the wet period than dry, and consequently, more captures were done during the cold and rainy weather. The activity was high in the month of April, with heavy rains, but it declined in the subsequent months of May and June (2017). This confirms the breeding period peaks in wet season and not common in the dry season.

## CONCLUSION

The distribution of the species is patchy and limited to some regions within the study area. There was evidence that the species is shifting sites or declining in their numbers as some of the previous sites no longer support the species. Some sites are shrinking in size and habitat quality, such as Kivumoni and Mwandabara. There is an imbalance in sex ratio, and more synchronized population dynamic studies are needed to highlight the driver(s) behind the male decline. Moreover, efforts to safeguard the species habitats, especially in community lands, are essential to enhance the species niches.

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## Articles

**The Javan Leopard *Panthera pardus melas* (Cuvier, 1809) (Mammalia: Carnivora: Felidae) in West Java, Indonesia: estimating population density and occupancy**

– Anton Ario, Senjaya Mercusiana, Ayi Rustiadi, Robi Gumilang, I Gede Gelgel Darma Putra Wirawan & Toni Ahmad Slamet, Pp. 21331–21346

**Breeding phenology and population dynamics of the endangered Forest Spiny Reed Frog *Afrixalus sylvaticus* Schiøtz, 1974 in Shimba Hills, Kenya**

– Alfayo Koskei, George Eshiamwata, Bernard Kirui & Phylus K. Cheruiyot, Pp. 21347–21355

**Ichthyofaunal diversity of Senkhi stream, Itanagar, Arunachal Pradesh: a comparative status between 2004–05 and 2018–19**

– Koj Taro, Lakpa Tamang & D.N. Das, Pp. 21356–21367

**First record of *Proceratium* Roger, 1863, *Zasphinctus* Wheeler, 1918, and *Vollenhovia* Mayr, 1865 (Hymenoptera: Formicidae) from the Western Ghats of peninsular India, description of three new species, and implications for Indian biogeography**

– Kalesh Sadasivan & Manoj Kripakaran, Pp. 21368–21387

## Communications

**New queen? Evidence of a long-living Jaguar *Panthera onca* (Mammalia: Carnivora: Felidae) in Tikal National Park, Guatemala**

– Carlos A. Gaitán, Manolo J. García, M. André Sandoval-Lemus, Vivian R. González-Castillo, Gerber D. Guzmán-Flores & Cristel M. Pineda, Pp. 21388–21395

**First camera trap record of Striped Hyena *Hyaena hyaena* (Linnaeus, 1758) (Mammalia: Carnivora: Hyaenidae) in Parsa National Park, Nepal**

– Pramod Raj Regmi, Madhu Chetri, Haribhadra Acharya, Prakash Sigdel, Dipendra Adhikari, Naresh Subedi & Babu Ram Lamichhane, Pp. 21396–21401

**Range extension and new ecoregion records of the Crocodile Monitor *Varanus salvadorii* (Peters & Doria, 1878) (Reptilia: Varanidae) in Papua New Guinea**

– Borja Reh & Jim Thomas, Pp. 21402–21408

**A checklist of fish and shellfishes of the Poonthura estuary, southwestern coast of India**

– Kiranya Bella, Pramila Sahadevan, Giri Bhavan Sreekanth & Rajeev Raghavan, Pp. 21409–21420

**A new species of *Protosticta* Selys, 1885 (Odonata: Zygoptera: Platystictidae) from Western Ghats, India**

– Kalesh Sadasivan, Vinayan P. Nair & K. Abraham Samuel, Pp. 21421–21431

**A case study on utilization and conservation of threatened plants in Sechu Tuan Nalla Wildlife Sanctuary, western Himalaya, India**

– Puneet Kumar, Harminder Singh & Sushil Kumar Singh, Pp. 21432–21441

**A survey of ethno-medicinally important tree species in Nauradehi Wildlife Sanctuary, central India**

– Tinku Kumar, Akash Kumar, Amit Jugnu Bishwas & Pramod Kumar Khare, Pp. 21442–21448

## Short Communications

**Effects of a Bengal Slow Loris *Nycticebus bengalensis* (Primates: Lorisidae) bite: a case study from Murlen National Park, Mizoram, India**

– Amit Kumar Bal, Anthony J. Giordano & Sushanto Gouda, Pp. 21449–21452

**First record of *Garra birostris* Nebeshwar & Vishwanath, 2013 (Cypriniformes: Cyprinidae) from Doyang and Dikhu rivers of Brahmaputra drainage, Nagaland, India**

– Sophiya Ezung, Metevinu Kechu & Pranay Punj Pankaj, Pp. 21453–21457

**Two new records of Lilac Silverline *Apharitis lilacinus* (Lepidoptera: Lycaenidae) from northeastern India**

– Monsoon Jyoti Gogoi, Ngulcholal Khongsai, Biswajit Chakdar & Girish Jathar, Pp. 21458–21461

**Illustrated description of the mantis *Mesopteryx platycephala* (Mantodea: Mantidae) collected from West Bengal, India**

– Gauri Sathaye, Sachin Ranade & Hemant Ghate, Pp. 21462–21466

***Cetrelia isidiata* (Asahina) W.L. Culb. & C.F. Culb. (Parmeliaceae) – an addition to the Indian lichen biota**

– Gaurav K. Mishra, Pooja Maurya & Dalip K. Upreti, Pp. 21467–21469

## Notes

**A new southern distribution record for Pacific Marten *Martes caurina***

– Maximilian L. Allen, Brianne Kenny, Benjamin Crawford & Morgan J. Farmer, Pp. 21470–21472

**First Asian record of Light-mantled Albatross *Phoebastria palpebrata* (Foster, 1785) from Rameswaram Island, Tamil Nadu, India**

– H. Byju & N. Raveendran, Pp. 21473–21475

***Salvia misella* Kunth (Lamiaceae) – a new record for Eastern Ghats of India**

– Prabhat Kumar Das, Pradeep Kumar Kamila & Pratap Chandra Panda, Pp. 21576–21579

***Salsola oppositifolia* Desf. in Great Rann of Kachchh, Gujarat – a new record for India**

– Rakesh Gujar, Vinesh Gamit, Ketan Tatu & R.K. Sugoor, Pp. 21580–21483

**Extended distribution of *Impatiens scapiflora* (Balsaminaceae) to the flora of Eastern Ghats, India**

– T.S. Saravanan, S. Kaliamoorthy, M.Y. Kamble & M.U. Sharief, Pp. 21484–21486

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