

SEXUAL DIMORPHISM AND REPRODUCTIVE BIOLOGY OF A SIKKIM ENDEMIC GRASS LIZARD *TAKYDROMUS SIKKIMENSIS* (SQUAMATA: LACERTIDAE) IN THE EASTERN HIMALAYA, INDIA

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Abstract.—Sexual dimorphism is widespread among lizards and may cause variation in morphology or color between sexes. These variations are determined by ecological, environmental, and genetic factors. In this study, we examined sexual dimorphism in Sikkim Grass Lizards (*Takydromus sikkimensis*) of Sikkim-Darjeeling Himalaya, India. We investigated whether differences exist in morphology and colors between sexes, and we also explored the breeding biology of this lizard in both captivity and under natural conditions. We observed female-biased sexual dimorphism in size and shape with females having greater snout vent length (SVL), forelimb length (FLL), hindlimb length (HLL), length of fourth finger (LFF), and snout to arm length (SAL) than males. Males had larger head length (HL) and head width (HW) compared to females. Our findings showed female-biased sexual dimorphism to fecundity selection, where larger females have higher fecundity than smaller females. Additionally, male-biased sexual dichromatism was observed during the breeding season. The primary habitat of Sikkim Grass Lizard in the study region is agro-ecosystems mostly below 1,000 m elevation. These ecosystems are undergoing rapid changes due to developmental projects, which could jeopardize the long-term survival of this species. Therefore, conservation efforts are needed and should focus on preserving a mosaic of agro-ecosystems, especially in the lower middle hills in Sikkim-Darjeeling region of the Eastern Himalaya.

Key Words.—breeding biology; clutch size; endangered; fecundity selection; Lacertidae; sexual dichromatism

INTRODUCTION

Sexual dimorphism refers to the differences in physical characteristics, behavior, and morphological traits between sexes of the same species (Duellman and Trueb 1994). One of the major examples of sexual dimorphism is sexual size dimorphism (SSD), which is the difference in body size between male and female of a species (Luo et al. 2012). While this phenomenon is common in reptiles, it has been widely examined in other taxonomic groups as well, including mammals (Mitani et al. 1996), birds (Fairbairn and Shine 1993), amphibians (Schäuble 2004), and invertebrates (Foellmer and Moya-Laraño 2007). Understanding sexual dimorphism among lizards is challenging because some are male biased whereas others are female biased. For example, lizards in the families Tropicuridae and Teiidae exhibit male-biased dimorphism (Brandt and Navas 2011) but members of the families such as Pygopodidae and Diplodactylidae display female-biased dimorphism. Conversely, some families, including Anguillidae, Gekkonidae, and Scincidae, do not demonstrate any sexual dimorphism (Cox et al. 2009; Cox and Kahrl 2014). Different selective forces such as sexual selection, fecundity selection, and ecological selection (e.g., niche divergence) act upon sexes

exhibiting a great degree of variation between sexes (Darwin 1871; Olsson et al. 2002; Luo et al. 2012). Sexual selection generally causes male-biased SSD giving males an advantage in intrasexual competition for territorial defense or for attracting females for mating (Endler and Houde 1995). Inversely, female biased SSD is explained by fecundity selection where large females are favored because they tend to make large clutches (Braña1996; Pincheira-Donoso and Hunt 2017). In addition, sexual dimorphism can also be attributed to various environmental and ecological factors such as elevation, latitude, and use and accessibility of resources such as microhabitat and food (Roitberg 2007; Ramírez-Bautista et al. 2014).

Most studies of sexual dimorphism in lizards testing SSD have considered only body weight or snout-to-vent length (SVL), while other body parts are largely overlooked (Kuo et al. 2009; Luo et al. 2012; Ramírez-Bautista et al. 2014). Nevertheless, it would be beneficial to assess morphological characteristics such as head and limb dimensions, as they can provide additional insights into sexual differences (Kuo et al. 2009; Cruz-Elizalde et al. 2020). There is evidence of some lizard species (e.g., anoles, *Anolis* spp.) where males exhibit longer limbs but shorter abdomens than females. Such dimorphism provides benefit to males (due to longer limbs) during

their escape from predators or defending their territory (Butler and Losos 2002) while larger abdomen in females provide more space for accommodating more eggs than females with smaller abdomens (Pincheira-Donoso and Hunt 2017). Consequently, examining both the shape and size of various morphological traits will yield more comprehensive understanding of the ecological significance and evolutionary aspects of sexual dimorphism (Bookstein 1989; Butler and Losos 2002; Fernandez et al. 2021).

Color patterns in the majority of taxa serve as a social signal (Bradbury and Vehrencamp 2011). These color patterns are subject to both ecological and sexual pressure, resulting in different effects on each sex and leading to sexual dichromatism (Badyaev and Hill 2003). Sexual dichromatism, influenced by sexual selection, plays a crucial role in conveying both inter-sexual and intra-sexual information (Fairbairn et al. 2007; Lanuza et al. 2013). In lizards, they aid in mate choice and male-male interaction (Martin et al. 2015). While sexual dichromatism can be observed in some species throughout the year, sexual coloration may become more intense during reproductive season (Martin et al. 2013). In some species, however, sexual dichromatism may be displayed only during breeding season (Borgia 1979). Therefore, sexual dichromatism in an organism conveys information about sexual selection leading to sexual dimorphism.

The general form and magnitude of an organism may have a direct or indirect influence on all aspects of its biology, leading to numerous life-history traits that are associated with the size of an organism (Sibly and Brown 2007). Various life-history strategies such as mortality, age at maturity, size or number of offspring are directly related to the fitness of an organism (Godfray et al. 1991) and are crucial for understanding the evolutionary phenomenon of SSD (Luo et al. 2012). Reproductive output is determined by the product of offspring size and number, which is associated with fitness (Roff 2002). In the majority of organisms, body size and shape are among the most important determinants of reproductive output (Yang et al. 2012).

The Oriental grass lizards (*Takydromus* spp.) belong to the family Lacertidae and are distributed in Oriental and Palearctic regions (Arnold 1997). Globally, 24 species of *Takydromus* are known (Wang et al. 2019). In India, there are four species: Goalpara Grass Lizard (*T. haughtonianus*), Java Grass Lizard (*T. khasiensis*), Asian Grass Lizard (*T. sexlineatus*), and Sikkim Grass Lizard (*T. sikkimensis*), and these species are reported from northeastern part of India. *Takydromus sikkimensis* is endemic to Sikkim-Darjeeling Himalaya (Bhupathy et al. 2009). This rare and unique grass lizard is listed as Endangered in the recent assessment of the International Union for Conservation of Nature

(IUCN) Red List of Threatened Species (IUCN 2021). Because of a lack of comprehensive data, we studied this lizard to: (1) explore sexual dimorphism in size and shape of *T. sikkimensis* of Sikkim-Darjeeling Himalaya; (2) understand its reproductive biology; and (3) examine whether morphological traits are related to reproductive fitness.

MATERIALS AND METHODS

Study area.—We conducted our study in various localities of Sikkim, Darjeeling, and Kalimpong districts of West Bengal (Fig. 1). Sikkim-Darjeeling Himalaya is a part of the Himalaya global biodiversity hotspot (Mittermeier et al. 2011). Sikkim (7,096 km²; 27°5′–28°9′N, 87°59′–88°56′E), Darjeeling District (3,149 km²; 26°27′–27°13′N, 87°59′–88°53′E), and Kalimpong District (1,076 km²; 26°57′–27°10′N, 88°25′–88°52′E) share international boundaries on three sides: (1) the Tibetan plateau in the north; (2) Nepal in the west; and (3) Bhutan in the east. The plains of West Bengal state of India are towards the south. The Sikkim-Darjeeling Himalaya region harbors a rich variety of flora and fauna, owing to its diverse topography and its strategic location at the confluence of biogeographic

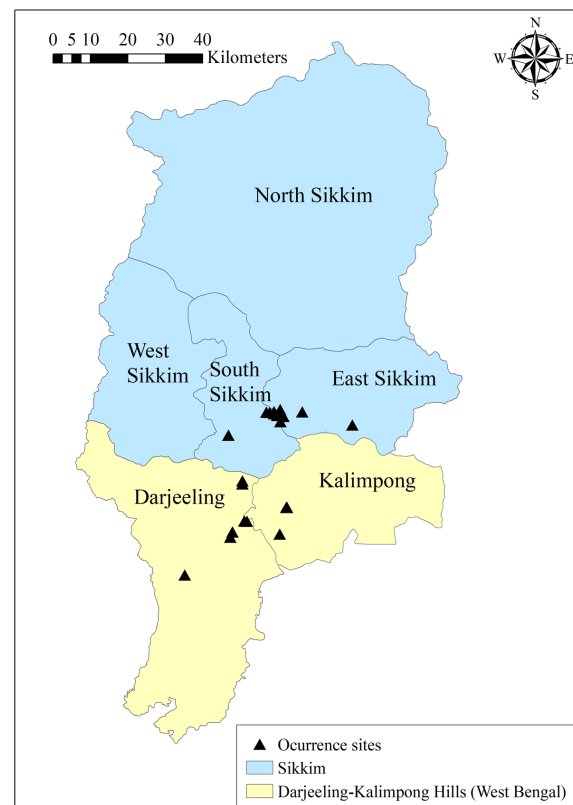


FIGURE 1. Map of Sikkim-Darjeeling Himalaya in Eastern Himalaya, India, showing occurrence sites of the Sikkim Grass Lizard (*Takydromus sikkimensis*).

TABLE 1. Details of the morphological measurements taken for assessing sexual dimorphism in the Sikkim Grass Lizard (*Takydromus sikkimensis*) in Darjeeling-Sikkim Himalaya, India.

Morphological details	Description
Snout vent length (SVL)	from tip of snout to posterior margin of cloaca
Tail length (TL)	from posterior margin of cloaca to tip of tail
Head length (HL)	from tip of snout to gular fold
Head width (HW)	width of the head at its widest point
Head height (HH)	length between gular region to the highest part of the head
Forelimb length (FLL)	from the base of forelimb to tip of longest finger
Hindlimb length (HLL)	from the base of hindlimb to tip of longest toe
Length of fourth finger (LFF)	length of fourth finger excluding claw
Length of fourth toe (LFT)	length of fourth toe excluding claw
Snout arm length (SAL)	from the tip of snout to anterior insertion margin of forelimb
Snout eye length (SEL)	from the tip of snout to anterior margin of eye

realms (Mani 1974). We focused on the lower elevation belt, which is predominantly characterized by a human-modified landscape comprising agro-ecosystems interspersed with tropical semi-deciduous vegetation (Sharma et al. 2019). In total, we conducted surveys at 23 sites scattered throughout the Sikkim-Darjeeling Himalaya region (Supplemental Information Table 1).

Field sampling.—We conducted time-constrained visual encounter survey (VES; Heyer et al. 1994)

in different parts of Sikkim and two hill districts of West Bengal (i.e., Darjeeling and Kalimpong) from November 2019 to August 2021 for a total of 128 d of surveying. Sampling was conducted by two personnel for 3 h a day making six human-hours per day per survey. We collected species by hand and measured 11 morphometric traits (Table 1; Fig 2). We followed Qu et al. (2011) for measuring morphometric traits using digital vernier calipers (± 1 mm). We determined sex in adult and sub-adult males by everting hemipenis, but the same could not be done for juveniles due to their smaller body sizes (SVL < 29 mm). We released lizards in their natural habitat after taking morphometric measurements. Based on field experience and SVL of the smallest gravid individual, we classified *T. sikkimensis* into three classes: (1) individuals with SVL ranging from 40 to 60 mm as adults; (2) SVL 30–39 mm as sub-adults; and (3) < 30 mm as juveniles (Telford 1969). We considered only adults for SSD analysis to avoid variability in size due to differential life stages.

Breeding biology.—To ensure consistency, the breeding study was done in a single location where *T. sikkimensis* were frequently encountered, easily accessible, and thus convenient to observe. Unique agro-ecosystems and rocky outcrops make the area ideal habitat for *T. sikkimensis* (Bhupathy et al. 2009). For this reason, we selected Dalep village (27°14'N, 88°28'E) in South Sikkim as the study site for the breeding study. We captured lizards during the breeding period from April to September, 2019–2020.

We closely monitored the breeding of *T. sikkimensis* in both wild and in captivity. We caught adult females from the field and palpated them to ensure they were gravid before transporting the females to a specially prepared wooden box that measured 60 × 60 × 60 cm. The box was bedded with moist soil, sand, small pebbles, stones, grasses, and leaf litters to provide natural

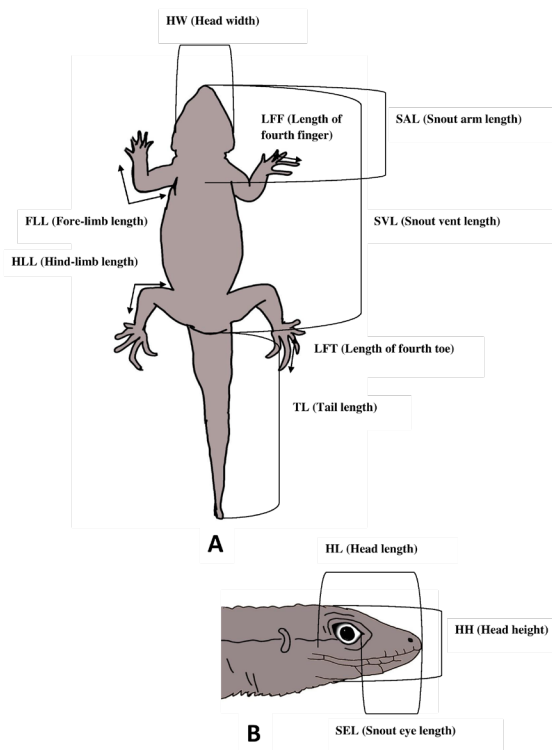


FIGURE 2. Illustration of morphological traits of the Sikkim Grass Lizard (*Takydromus sikkimensis*) measured in this study.

substrate and shelter for the captive lizards. We recorded morphological measurements of the gravid females and marked each individual in the box with paint under the limb joints. Because we observed *T. sikkimensis* feeding upon certain insects in the study area, we daily provided females in captivity with locusts (instar of *Anacridium* spp. with average length of 14.5 mm and body width of 3.3 mm), katydids (juveniles of *Conocephalus* spp. with average length of 15.03 mm and body width of 3.2 mm), spiders (especially juvenile of *Heteropoda* spp. with an average length of 5.93 mm), and incidentally other prey. We provided each lizard with five prey items per day. On an average, we observed that lizards consumed three of the five prey items on a daily basis. We carefully monitored the lizards daily, and we moved eggs laid to another box of same dimension. The second box had a similar artificial microhabitat as that of first box, but with additional moisture to prevent drying of the eggs.

We also monitored eggs in the natural habitat. After locating nests, we laid a 5 × 5 m quadrat at each observation site to determine the vegetation and ground cover of the area. We estimated these variables visually. We used digital vernier calipers (± 0.01 mm) and a digital balance (± 0.01 g) to measure length, breadth, and weight of eggs within an hour of oviposition. In case of oviposition during night, we measured them in the morning.

In both natural habitats and controlled captive environments, we measured eggs weekly until they hatched. Nonetheless, during natural observations, determining the timing of egg-laying was challenging because we did not witness eggs being laid. In captivity, we kept females even after their first oviposition to monitor whether they also produce a second clutch. We measured SVL, tail length (TL), head length (HL), head width (HW), fore-limb length (FLL), and hind-limb length (HLL) of hatchlings but we could not determine their sex as they were small and delicate.

Data analysis.—To examine SSD, we calculated size dimorphism index (SDI) based on Lovich and Gibbons (1992). The index SDI is the size of the larger sex divided by the size of the smaller sex minus one, which is arbitrarily expressed as positive when females are larger and negative when males are larger. This method provides accurate scaling, displays directionality, and revolves around a central value (Lovich and Gibbons 1992). Further, to analyze sexual shape dimorphism (SSHD), we calculated the index of individual size (SIZE) as the 10th root of product of the variables SVL, TL, HL, HW, HH, FLL, HLL, length of fourth finger (LFF), length of fourth toe (LFT), snout to arm length (SAL), and snout to eye length (SEL) to remove the effect of size on each specimen following Mosimann (1970) and Cruz-Elizalde et al. (2020). We then calculated the residual

data from Linear Regression of each morphological variable against SIZE (shape variable). All variables were log-transformed prior to analysis. We assessed the normality of the data using the Kolmogorov-Smirnov test and examined the homogeneity of variance using Levene's test and data met assumptions of parametric tests. We used *t*-tests to analyze the differences in mean values of morphological variables between sexes. To investigate sexual size dimorphism, we used Two-way Analysis of Variance (ANOVA) to examine the impact of sex on SIZE and its interaction. Additionally, we employed Multivariate ANOVA (MANOVA) to explore sexual shape dimorphism and assess the effect of sex on shape variables. Furthermore, we used Tukey's post-hoc tests to identify any significant differences among main effects and interactions.

We computed clutch size as the number of eggs in a clutch and the total mass of eggs in a clutch as the clutch mass. Also, we computed the mean egg mass of a clutch as clutch mass divided by clutch size (Luo et al. 2012). We used Linear Regression to analyze the relationship between female reproductive parameters (clutch size and clutch mass) and female body size (maternal SVL) and also between egg mass at oviposition (EMO) and hatchling morphometry (hatchling mass and hatchling SVL). We considered *P* values ≤ 0.05 to be significant. We used PASW statistics for Windows (version 18.0.0; SPSS Inc, Chicago, Illinois, USA) and R software (version 4.2.1; R Core Team 2021) for data analysis.

RESULTS

Population demography.—We observed 515 individuals of *Takydromus sikkimensis* from various sampling sites of Sikkim and two neighboring hill districts of West Bengal (Darjeeling and Kalimpong) during 2019–2021. The overall encounter rate of lizards was 0.703 lizards/h. Of the total individuals observed, we were able to catch only 361 individuals for morphological measurements. Of the 361 individuals, 243 were adults (139 females and 104 males), 85 were sub-adults (43 females and 42 males), and 33 were juveniles.

Sexual size and shape dimorphism.—Mean SVL of adult female was significantly longer than males (Table 2), indicating female biased sexual size dimorphism in *T. sikkimensis* (Table 3). The SDI was positive with a value of 0.09. LogSIZE differed significantly between sexes ($F_{1,237} = 4.78, P = 0.029$).

There was a significant positive relationship between log of each morphological variables and log SIZE with r^2 values ranging from 0.16–0.54 ($P < 0.001$). Shape variables differed significantly between sexes (Wilk's $\lambda = 0.50, F_{11,227} = 20.43, P < 0.001$). Of the 11 morphological

TABLE 2. Mean values (\pm standard deviation) and *t*-test values of morphometric variables between adult male and female Sikkim Grass Lizards (*Takydromus sikkimensis*) of Darjeeling-Sikkim Himalaya, India. Abbreviations are SVL = snout vent length, TL = tail length, HL = head length, HW = head width, HH = head height, FLL = forelimb length, HLL = hindlimb length, LFT = length of fourth toe, LFF = length of fourth finger, SAL = snout arm length, SEL = snout eye length). An asterisk (*) indicates a significant difference between sexes.

Trait	Females (n=139)	Males (n=104)	<i>t</i>	<i>P</i> -value
SVL	47.7 \pm 4.53 (40.04–57.01)	43.4 \pm 4.03 (40.0–57.0)	7.71	< 0.001*
TL	136.3 \pm 26.45 (89–192)	139.9 \pm 27.55 (80.0–198.4)	-1.05	0.294
HL	9.73 \pm 1.33 (4.80–12.59)	10.2 \pm 1.43 (5.34–14.1)	-2.79	0.006
HW	5.62 \pm 0.66 (4.11–7.16)	5.85 \pm 0.69 (4.66–8.16)	-2.64	0.009*
HH	5.03 \pm 0.61 (4.09–5.98)	5.22 \pm 0.78 (4.00–6.93)	-2.14	0.033*
FLL	9.58 \pm 1.18 (6.82–12.14)	8.96 \pm 1.48 (5.93–13.4)	3.64	< 0.001*
HLL	11.59 \pm 1.75 (7.25–15.64)	10.7 \pm 1.96 (6.08–15.5)	3.95	< 0.001*
LFT	8.41 \pm 1.01 (7.00–11.9)	8.20 \pm 1.11 (7.00–11.9)	1.49	0.137
LFF	6.34 \pm 0.93 (4.56–8.19)	5.89 \pm 1.02 (4.02–7.99)	3.63	< 0.001*
SAL	18.6 \pm 1.94 (14.06–22.37)	17.7 \pm 2.10 (15.2–22.7)	2.83	0.005*
SEL	5.26 \pm 0.56 (4.02–6.74)	5.39 \pm 0.68 (4.02–6.75)	-1.61	0.107

variables, six variables were significantly different between sexes (logSVL: $F_{1,237} = 61.52$, $P < 0.001$; logHL: $F_{1,237} = 6.88$, $P = 0.009$; logHW: $F_{1,237} = 7.04$, $P = 0.008$; logFLL: $F_{1,237} = 10.22$, $P = 0.001$; logHLL: $F_{1,237} = 16.76$, $P < 0.001$; logLFF: $F_{1,237} = 14.01$, $P < 0.001$; logSAL: $F_{1,237} = 8.04$, $P = 0.004$). Variables such as SVL, FLL, HLL, LFF and SAL were significantly larger in females than males (Tukey’s HSD, $P < 0.05$), but males had significantly longer HL and HW than females (Table 2).

Monthly variation in size classes.—Size classes varied monthly showing distinct patterns (Fig. 3). We encountered highest number of adults from March to June while fewer from July to October. We observed only adults and sub-adults from January to April, whereas we observed all size classes from July to December (Fig 3). Numbers of hatchlings and juveniles peaked during July to October with no hatchlings after November. Different size classes showed significant bimonthly variation (adults: $F_{5,181} = 24.08$, $P = 0.001$; sub-adults: $F_{5,181} = 58.84$, $P = 0.001$; juveniles: $F_{5,181} = 87.47$, $P = 0.001$; hatchlings: $F_{5,181} = 55.14$, $P = 0.001$).

TABLE 3. Means of logSIZE and log residuals from Linear Regression of each morphological variable versus SIZE (shape variables) of male and female Sikkim Grass Lizards (*Takydromus sikkimensis*) from Darjeeling-Sikkim Himalaya, India. Results of Tukey’s post-hoc are also provided. Pattern 1 indicates females are significantly larger than males, pattern 2 indicates males are significantly larger than females, and pattern 0 indicates no significant difference between males and females. An asterisk (*) indicates a significant difference between sexes.

Sex	Females	Males	Tukey’s Test	Pattern
logSIZE	1.21	1.19	0.021*	1
Residuals logSVL	0.44	-0.59	< 0.001*	1
Residuals logTL	-0.16	0.21	0.532	0
Residuals logHL	-0.23	0.3	0.009*	2
Residuals logHW	-0.24	0.32	0.008*	2
Residuals logHH	-0.19	0.25	0.213	0
Residuals logFLL	0.12	-0.17	0.001*	1
Residuals logHLL	0.19	-0.25	< 0.001*	1
Residuals logLFT	0.02	-0.03	0.067	0
Residuals logLFF	0.16	-0.22	0.031*	1
Residuals logSAL	0.11	-0.14	0.011*	1
Residuals logSEL	-0.14	0.18	0.058	0

We captured more females than males from March until December but comparatively more males than females in January and February (Fig. 3). The number of males showed significant variation during different months ($F_{5,9} = 4.88$, $P = 0.019$) but the population of females did not vary significantly. Our data revealed an increase in body size of both adults and sub-adults from January to June.

Nest environment.—We found that nest temperatures and relative humidity in the wild ranged from 22.3°–33.0° C and 65.8–94.3%, respectively. In captivity, these measurements ranged between 24.1°–33.0° C for temperature and 77.2–96.3% for relative humidity. We caught 18 gravid females, which laid eggs in captivity with the clutch size ranging from 1–4. We obtained 41 eggs that we monitored in captivity. In addition, we located 13 nests with a total of 31 eggs in the wild during May to August 2019–2020. We found three clutches at the base of Large Cardamom (*Amomum subulatum*) plants, two clutches in between Tiger Grass (*Thysanolaena latifolia*), four clutches on the stumps of Cogon Grass (*Imperata cylindrical*) and four at the base of Common Bamboo (*Bambusa vulgaris*). We found all eggs in a moist environment (average relative humidity = 86.8%), which do not receive direct sunlight. The average ground cover percentage of the wild nest site were represented by barren land (35%), grasses (20%), boulders (18%), stones and pebbles (15%) and dry leaves (12%). The average canopy cover was 53%.

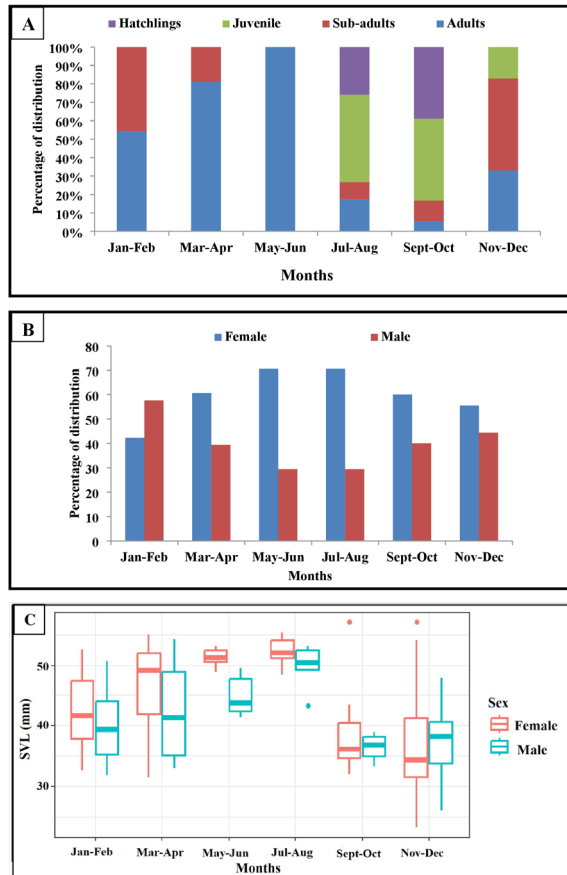


FIGURE 3. Bi-monthly variation of (A) size classes, (B) distribution patterns of males and females, and (C) snout vent length (SVL) between male and female Sikkim Grass Lizards (*Takydromus sikkimensis*) found in Darjeeling-Sikkim Himalaya, India.

Breeding phenology.—During the breeding season, both adult male and female *T. sikkimensis* exhibited light greenish color throughout the belly region (Fig. 4). Males possessed strikingly yellowish-white dorsolateral lines bordered by black above and below (from the posterior margin of last supratemporal extending until the forepart of the tail) exhibiting sexual dichromatism during breeding season, which is absent in females. Non-breeding coloration of the lizards showed slight variation compared to their breeding coloration (Fig. 4).

We observed gravid females of *T. sikkimensis* from April to July with approximately 40% of observation occurring in June (Supplemental Information Fig. S1). We found eggs from early May to mid-August, with the highest proportion (52%) observed in July (Supplemental Information Fig. S1). The size of the eggs increased over the incubation period, which lasted 60–70 d (Supplemental Information Fig. S1), corresponding to embryonic development. Hatching of the eggs commenced in July and continued until September. Of 31 eggs observed in their natural habitat, 28 successfully hatched, while the remaining eggs desiccated. The average length, breadth, and mass of

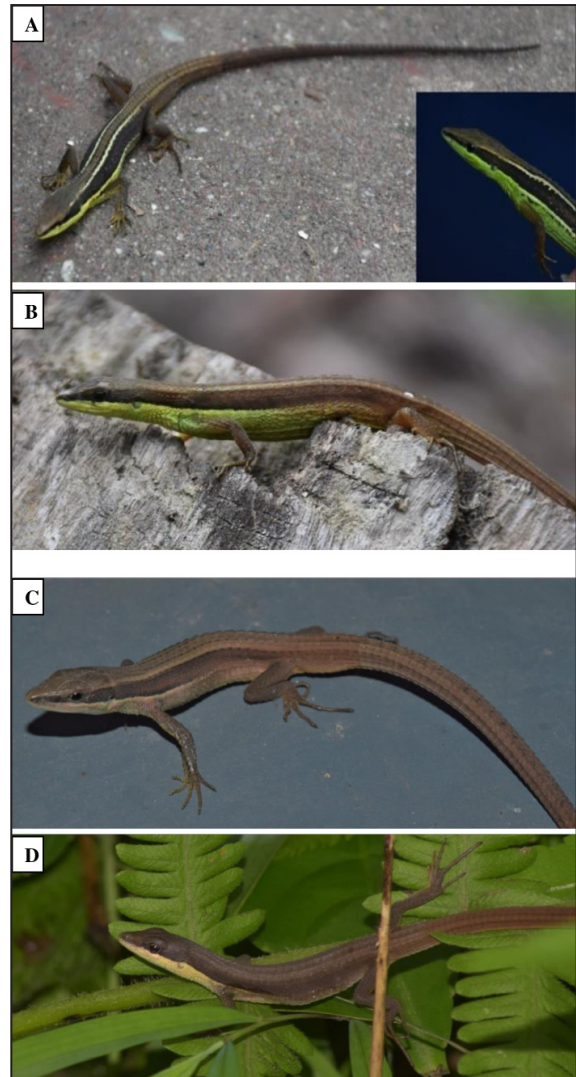


FIGURE 4. Adult reproductive (A) male and (B) female Sikkim Grass Lizards (*Takydromus sikkimensis*) during breeding season and (C) male and (D) female during non-breeding season (inset in panel A showing greenish dorsolateral line in males).

eggs during oviposition were $10.41 \text{ mm} \pm 0.11$ (standard error), $6.5 \text{ mm} \pm 0.06$, and $0.22 \text{ g} \pm 0.003$, respectively. We noted that females only became gravid once a year, both in captivity and in their natural habitat.

The smallest reproducing female was 45.17 mm SVL and the largest was 59.79 mm SVL. Larger females produced larger clutch (four eggs) than the small ones with small clutch (one egg) ($F_{3,14} = 6.14$, $P = 0.007$; Fig. 5). The average clutch mass was 0.525 ± 0.46 and mean egg mass was 0.22 ± 0.004 . Clutch mass also showed a significant relationship with maternal SVL ($r^2 = 0.453$, $F_{1,16} = 31.4$, $P = 0.001$; Fig. 5).

Hatchlings morphometry.—The mean SVL of newly hatched juveniles was 23.77 ± 1.78 and mean TL was 54.1 ± 5.29 . Female *T. sikkimensis* produced one clutch

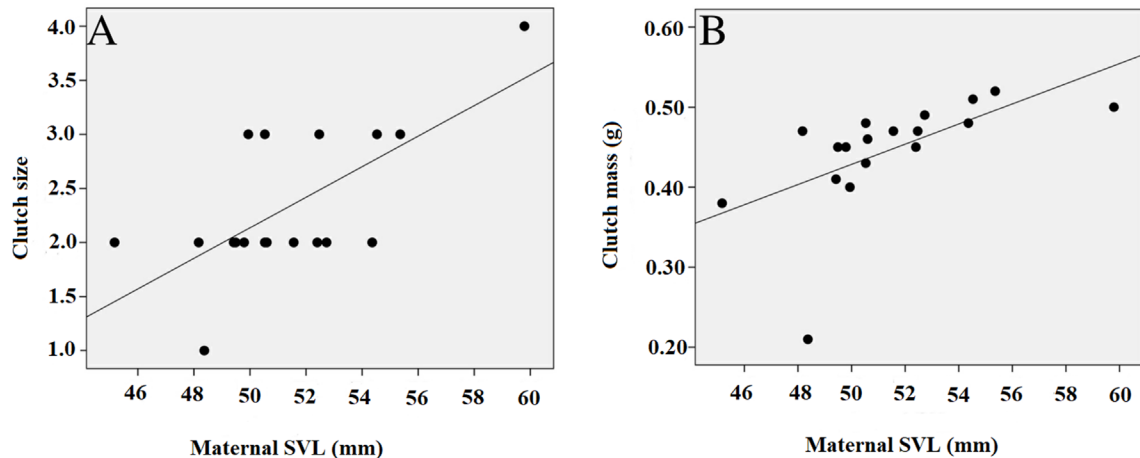


FIGURE 5. Relationship of (A) clutch size (in numbers) and (B) clutch mass (g) to maternal snout-vent length (SVL) of the Sikkim Grass Lizard (*Takydromus sikkimensis*) in Dalep village, South Sikkim, India.

per breeding season. Both hatchling mass ($r^2 = 0.361$, $F_{1,26} = 14.7$, $P = 0.001$) and hatchling SVL ($r^2 = 0.292$, $F_{1,26} = 10.70$, $P = 0.003$) showed a significant positive correlation with egg mass at oviposition (Supplemental Information Fig. S2).

DISCUSSION

Takydromus sikkimensis that we studied displayed sexual dimorphism characterized by females having larger body size, limb lengths (FLL, HLL, and LFF), and snout arm length than males. Larger females have an advantage over smaller females in terms of reproductive success, as they can accommodate a greater number of eggs, which directly influences their reproductive output (Cox et al. 2007; Chettri et al. 2009; Du and Lu 2010). In 1874, Darwin suggested that larger female individuals have greater internal capacity to produce eggs, leading to an increased ability to lay eggs. Consequently, natural selection favors increased fecundity, which is directly related to body size and explains the observed sexual dimorphism in females (Shine, 1988). Supporting this idea, experiments involving the reduction of abdominal size in lizards have shown a decrease in clutch size (Du et al. 2005), providing further evidence of the strong correlation between body size and clutch size (Shine 1991; Cox et al. 2007). Our findings complement those of Cox et al. (2007), who demonstrated a positive association between sexual size dimorphism (SSD) and clutch size, and highlighted the connection between morphological traits and reproductive fitness. Specifically, we found a positive correlation between maternal SVL and clutch size, as well as egg mass at oviposition. Furthermore, egg mass at oviposition showed a positive relationship with hatchling mass and hatchling SVL.

Similar patterns of female-biased sexual size dimorphism (SSD) driven by fecundity selection have been observed in various lizard species, including Indian Forest Skink (*Sphenomorphus indicus*: Ji and Du 2000), Qinghai Toad-headed Agama (*Phrynocephalus vlangalii*: Zhang et al. 2005), Schlegel's Japanese Gecko (*Gekko japonicas*: Ji et al. 1991), Modest Ground Skink (*Scincella modesta*: Yang et al. 2012), and Reeve's Smooth Skink (*Scincella reevesii*: Yang et al. 2012). It should be noted, however, that inferring female-biased SSD solely based on fecundity selection may not always hold true. For instance, the Brown Forest Skink (*Sphenomorphus incognitus*), the Chinese Grass Lizard (*Takydromus septentrionalis*), and the Hsuehshan Grass Lizard (*T. hsuehshanensis*) exhibit sexual monomorphism (Huang 1998; Ji et al. 1998; Zhang and Xiang 2000), despite showing positive association between fecundity and female body size (Luo et al. 2012). The ultimate cause of female-biased SSD may not always be selection favoring fecundity (Shine 1988; Blanckenhorn 2005; Fairbairn 2013). Other potential factors associated with female-biased SSD include differential predation, dispersal patterns, food availability and competition, temperature-dependent development, and social cues (Zhang and Lu 2013). Exploring the effects of these factors could provide insights into the evolutionary mechanism of sexual dimorphism, although these aspects were beyond the scope of the present study.

While female biased SSD can be explained by fecundity selection, sexual selection favoring smaller sized males can be explained by Time and Energy Budget and Agility Advantage hypotheses (Blanckenhorn 2005). The Time and Energy Budget hypothesis explains that smaller individuals can function and sustain themselves with less food, allowing them

to conserve energy and time for increasing their mating and reproductive success, even in situations where food availability is limited (Andersson 1994). Similarly, the Agility Advantage hypothesis anticipates smaller males are expeditious and brisk during courtship, mate hunting, and safeguarding its mating territories, ultimately leading to increased mating and reproductive success (Blanckenhorn 2005). Therefore, in line with the findings in the Mountain Grass Lizard (*T. wolteri*; Luo et al. 2012), the differences in body size between the sexes, the fecundity selection favoring larger females and the combination of better time and agility that smaller males may have compared to larger males likely contributed to the evolution of female-biased SSD in *T. sikkimensis*.

Despite being smaller in overall body size compared to females, male *T. sikkimensis* exhibit significantly larger head dimension (HL and HW) than females. This pattern is commonly observed in lacertid lizards, such as the Sharp-snouted Rock Lizard (*Lacerta oxycephala*; Verwajen et al. 2002), Ordos Racerunner (*Eremias brenchleyi*; Xu and Ji 2003), Multi-ocellated Racerunner (*E. multiocellata*; Li et al. 2006), and other *Takydromus* including the Koshun Grass Lizard (*T. sauteri*; Huang 2006 and *T. septentrionalis*; Ji et al. 1998). The larger head dimensions in males are believed to provide an advantage during male-male combat by enabling wider jaw opening (Verwajen et al. 2002; Gvozdik and Van Damme 2003; Lailvaux et al. 2004; Huyghe et al. 2005). In a similar manner to *T. wolteri*, the larger head size in male *T. sikkimensis* may also play a role in successfully grasping the abdomen of a female during mating (Luo et al. 2012).

During the breeding season, the evolution of sexual dichromatism may have occurred for greater reproductive success. As males actively compete for mates during breeding season (Cox and Kahrl 2014), females exhibit preference for brightly colored males thus offering greater social dominance, and therefore, have differential mating advantages (Darwin 1871; Andersson 1994; Sundberg 1995; Kwiatkowski and Sullivan 2002). This phenomenon, referred to as sexual dichromatism, may also be linked to male-male combat and warning of rivals (Healey and Olsson 2008; Huyghe et al. 2007). In *T. sikkimensis*, sexually dichromatic males were observed only during the breeding season, suggesting that this phenomenon may be associated with advertisement for mate attraction. In some Agamids and Lacertids, however, sexual dichromatism appears to be positively associated with sexual size dimorphism, indicating that color divergence is directed by sexual selection (Stuart et al. 2004; Chen et al. 2012; Lanuza et al. 2013). Hence, exhibition of coloration in an organism has a pivotal role in delivering sexual information to individuals of same and opposite sex (Rossi et al. 2019).

Similar breeding coloration was observed in closely related species *Takydromus khasiensis* in Assam by Islam and Saikia (2013). In many other species such as *Anolis carolinensis* (Sigmund 1983), Red Headed Rock Agamas (*Agama agama*; Harris 1964) and Broad Headed Skinks (*Eumeces laticeps*; Cooper and Vitt 1988), bright coloration has been found to be important in sexual behavior.

In our study, the adult population of *T. sikkimensis* exhibited an increase in its population from January to June, indicating onset of sexual maturity for the breeding season. This demographic pattern observed in *T. sikkimensis* throughout different seasons of the year suggests that sexual maturity occurs within a year. This finding aligns with the research conducted by Jackson and Telford (1975) on the Japanese Grass Lizard (*Takydromus tachydromoides*), which demonstrated that these lizards reach maturity in May, approximately 8 mo after hatching. Nevertheless, when examining the bi-monthly distribution pattern, we found that there were higher numbers of males recorded between January and February. This observation suggests an increase in male activity, potentially driven by their search for receptive females and preparations for the breeding season. The breeding season for *T. sikkimensis* begins in April and concludes in September.

Species inhabiting seasonal environments exhibit cyclic reproduction so that they can escape the harsh climatic extremes, whereas species inhabiting tropical regions (without much climatic fluctuation) may be able to reproduce throughout the year (Pianka and Vitt 2003). *Takydromus formosanus*, which inhabits at an elevation up to 1,500 m, produces one clutch per season (Norval et al. 2016). Similar to *T. formosanus*, *T. sikkimensis* also produces one clutch per year; however, multiple clutches of eggs in a year has also been reported from various species of *Takydromus*: *T. tachydromoides* (Telford 1969); *T. hsuehshanensis* (Huang 1998); Kuhne's Grass Lizard (*T. kuehnei*; Norval and Mao 2008); Luye Grass Lizard (*T. lueanus*; Lue and Lin 2008); *T. sauteri* (Huang 2006); the China Grass Lizard (*T. septentrionalis*; Ji et al. 2007; Luo et al. 2010); Stejneger's Grass Lizard (*T. stejnegeri*; Lin et al. 2004); Green-spotted Grass Lizard (*T. viridipunctatus*; Lue and Lin 2008); and the Mountain Grass Lizard (*T. wolteri*; Luo et al. 2012). Hence, this trait (producing multiple clutches) seems common in the genus *Takydromus*, but most of these species producing multiple clutches inhabit warm tropical areas (Huang 2006). Temperature also plays an important role in altering incubation period and the fitness of hatchlings (Du et al. 2006). Though we did not explore thermal influence on incubation period and fitness of hatchlings in *T. sikkimensis*; by constantly monitoring temperature both in wild and captivity, we infer that the optimum temperature for the incubation of

T. sikkimensis eggs was 22.3° to 33° C. Further research on the impact of temperature on their breeding aspect will help us to understand the impact of climate change in future.

Takydromus sikkimensis is a unique species that is a single representative in the family Lacertidae in the Sikkim Himalaya. A previous study reported the species from a single area of the Teesta Valley in South Sikkim, India (Bhupathy et al. 2009) and recently from Gorumara, West Bengal (Mothey et al. 2022). Due to intense sampling, we were able to record this species from different sites of Darjeeling and Sikkim Himalaya with many new locality records. We recorded this species from an elevational range of 250 to 1,200 m. The lower elevation belt of Sikkim and Darjeeling Himalaya is now undergoing dramatic change in land-use pattern due to many developmental projects in Teesta River Valley and adjoining areas. These projects may affect the habitat of this restricted-range species posing threats to its future survivability. Therefore, extensive study on its habitat, distribution range, and other ecological features are crucial for the conservation of this rare species. The conservation of traditional mosaic agro-ecosystems in the lower belt of Darjeeling-Sikkim Hills also is crucial for the retention of suitable habitat for this grass lizard in particular and the entire herpetofauna in general.

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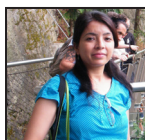
Supplemental Information: http://www.herpconbio.org/Volume_18/Issue_3/Rai_etal_2023_Suppl



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SUPPLEMENTAL INFORMATION

**SEXUAL DIMORPHISM AND REPRODUCTIVE BIOLOGY OF
SIKKIM ENDEMIC GRASS LIZARD *TAKYDROMUS SIKKIMENSIS*
(SQUAMATA: LACERTIDAE) IN THE EASTERN HIMALAYA, INDIA**

ANANTA RAI, RABINA MOTHEY, AND BASUNDHARA CHETTRI¹

The following material is provided by the authors and was not subject to review or editing by *Herpetological Conservation and Biology*.

Table S1: Details of the study sites in Darjeeling-Sikkim Himalaya, India.

Sl no.	Site	Area	Coordinates	Elevation (m)	Habitat types
1	Rekep	Sikkim	27° 13' 5.63" N 88° 29' 0.06" E	420	Agro-ecosystem along with tropical semi-deciduous forest
2	Pabong Tanak	Sikkim	27° 14' 28.8" N 88° 26' 58.1" E	570	Agro-ecosystem along with dominant cardamom plantation
3	Lower Namphing	Sikkim	27° 14' 12.4" N 88° 28' 37.01" E	590	Agro-ecosystem along with patches of bamboo clumps
4	Dalep	Sikkim	27° 14' 32.1" N 88° 28' 04.9" E	630	Agro-ecosystem along with rocky outcrops surrounded by remnant patches of sub-tropical forest
5	Reshep	Sikkim	27° 14' 0.35" N 88° 28' 33.78" E	770	Agro-ecosystem along with rocky outcrops surrounded by remnant patches of sub-tropical forest
6	Tokal Bermoik	Sikkim	27° 14' 13.9" N 88° 28' 5.5" E	820	Farm based agro-forestry system interspersed with sub-tropical forest
7	Daring	Sikkim	27° 14' 24.1" N 88° 27' 27.2" E	850	Agro-ecosystem along with sub-tropical forest
8	Kamrang	Sikkim	27° 11' 08.5" N 88° 21' 30.8" E	1080	Mixed crop agro-ecosystem with dense human habitation
9	Adarshgoan	Sikkim	27° 13' 51.84" N 88° 29' 27.12" E	300	Agro-ecosystem with dominant bamboo plantations along with sub-tropical forest

10	Sirwani	Sikkim	27° 14' 51.79" N 88° 28' 58.8" E	600	Mixed crop agro-ecosystem with dense human habitation
11	Bering	Sikkim	27° 12' 37.32" N 88° 28' 33.78" E	700	Mixed crop agro-ecosystem with dense human habitation
12	Sumin Sangkhola	Sikkim	27° 14' 30.3" N 88° 32' 08.1" E	780	Agro-ecosystem with steep rocky slopes, covered with thick leaf litter
13	Kharbari Peshok	Darjeeling	27° 04' 35" N 88° 23' 32" E	820	Mixed crop agro-ecosystem predominated by tiger grass
14	Peshok, Dong Busty	Darjeeling	27° 04' 07.3" N 88° 23' 32.0" E	1080	Agro-ecosystem predominated by cardamom cultivation
15	Simlay	Darjeeling	26° 58' 47.6" N 88° 23' 47.8" E	690	Mixed crop agro-ecosystem with dense human habitation
16	Labdah	Darjeeling	26° 57' 13.3" N 88° 22' 03.9" E	850	Agro-ecosystem dominated by Cinchona plantation and Epicacnursery
17	Bhalukhop Simlay	Darjeeling	26° 58' 45.3" N 88° 24' 09.6" E	490	Mixed crop agro-ecosystem with patches of sub-tropical forests and sparse human habitation
18	Sittong II	Darjeeling	26° 56' 27.4" N 88° 21' 45.4" E	640	Mixed crop agro-ecosystem along with tiger grass and turmeric cultivation
19	Makaibari	Darjeeling	26° 51' 01.7" N 88° 15' 12.1" E	830	Tea cultivation along with steep slopes and sparse human habitation
20	Sinji	Kalimpong	27° 00' 58.1" N 88° 29' 58.1" E	1130	Mixed crop agro-ecosystem predominated by tiger grass along with

					sub-tropical forest
21	Bara Suruk	Kalimpong	26° 56' 54.5" N 88° 28' 54.5" E	720	Agro-ecosystem with patches of bamboo clumps and tiger grass
22	Taneng	Kalimpong	27° 01' 49.5" N 88° 00' 49.5" E	1120	Mixed crop agro-ecosystem with tropical broad-leaved forest
23	Chedung	Kalimpong	27° 00' 45.8" N 88° 29' 50.6" E	1150	Mixed crop agro-ecosystem with degraded sub-tropical forest

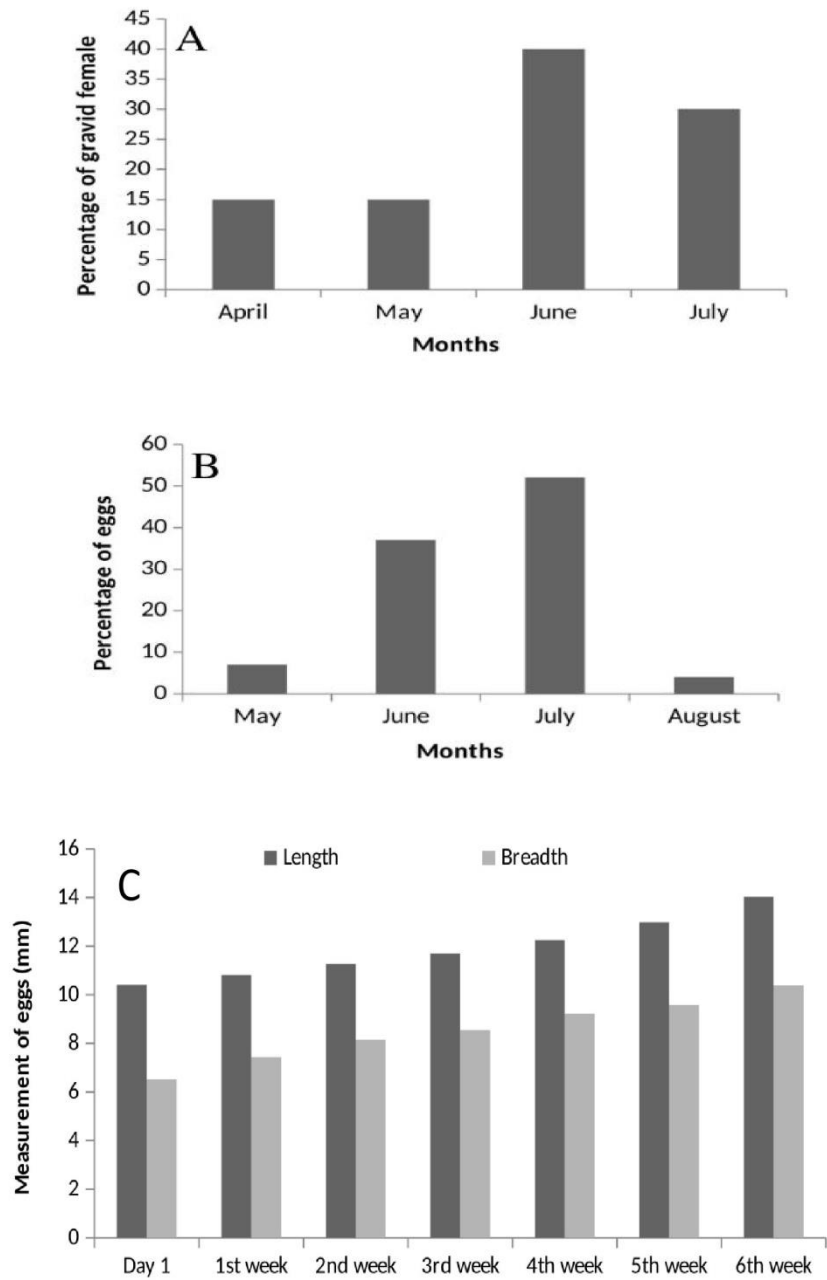


Figure S1. Monthly distribution of (A) Gravid females and (B) Eggs of *T. sikkimensis* during breeding season; (C) Trends in development of egg size of *T. sikkimensis* over the period of six weeks after oviposition in Dalep village, South Sikkim, India.

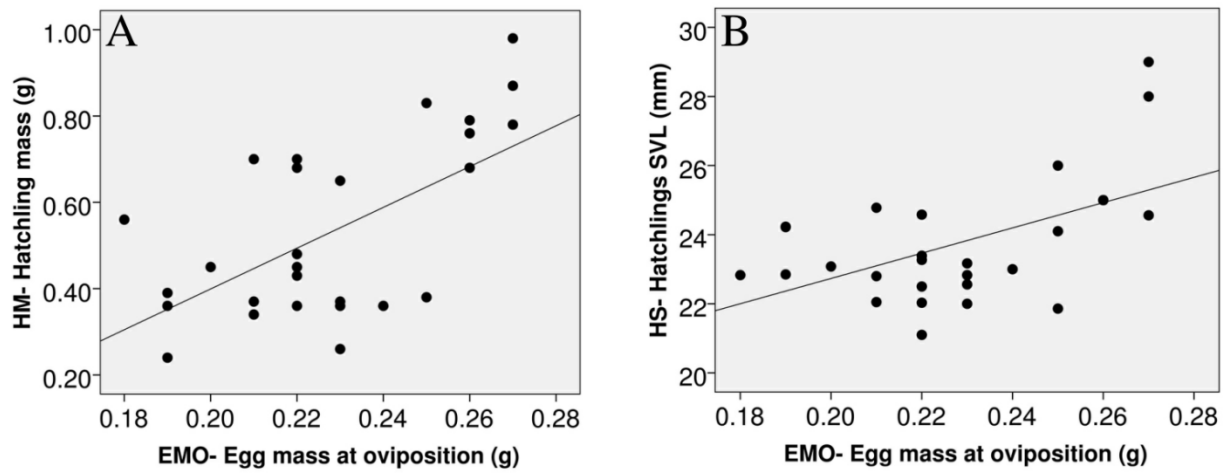


Figure S2. Scatter-plot of egg mass at oviposition (EMO) with that of (A) hatchling mass (HM) and (B) hatchling SVL (HS) of *T. sikkimensis* in Dalep village, South Sikkim, India.