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ACTIVITY PATTERNS AND HABITAT USE OF *ANSONIA HANITSCHI* ON GUNUNG KINABALU, SABAH, MALAYSIA

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Abstract.—*Ansonia hanitschi* is a small, stream-breeding toad endemic to Borneo. Little is known about its ecology or behavior. We documented diurnal activity patterns and habitat use, nocturnal habitat use, and body sizes of *A. hanitschi* near streams and in forests on Gunung Kinabalu, Sabah, Malaysia, on the island of Borneo. We identified 12 unique diurnal behaviors in this species. *Ansonia hanitschi* are sit-and-wait predators that spend > 75% of their time during the day, on average, sitting inactive in shady areas within 1.2 m of the stream. Observed toads spent 6% of their time jumping, 4% of their time crawling, and 4% of their time engaging in an arm waving behavior. During the day, individuals were more commonly found on smaller sand and cobble substrates. At night, individuals rested on the leaves of slender-stemmed plants within 2 m of the ground surface and at a mean distance of 2.3 m from the stream edge. Female snout-vent length averaged 32 mm, and gravid females ranged from 28 to 35 mm in length. We found that 88% of females near the stream during the day were gravid, compared with 80% of females away from streams at night. Our observations indicate that this species is diurnal and retreats to the leaves of small plants at night, possibly to avoid predation.

Key Words.—behavior; body size; Borneo; Bufonidae; diurnal; waving; nocturnal; sexual maturity

INTRODUCTION

Borneo, located within the Sundaland biodiversity hotspot (Myers et al. 2000), harbors extremely high amphibian diversity, including a large number of endemic species. To date, 179 species of frogs have been described from Borneo (Haas, A., I. Das, and S. Hertwig. 2016. Frogs of Borneo - The frogs of East Malaysia and their larval forms: an online photographic guide. Available from <http://www.frogsfoborneo.org> [Accessed 27 January 2017]). Twelve species of *Ansonia*, comprising small stream-breeding frogs in the family Bufonidae, occur on Borneo (Hertwig et al. 2014), including *Ansonia hanitschi* Inger 1960. *Ansonia hanitschi* is endemic to the island and known from only a few mountain ranges in northern Sarawak and western Sabah, Malaysia. Considered a highland stream species, it occurs from 750 to 1,600 m above sea level (Inger, R.F., I. Das, R. Stuebing, M. Lakim, and P. Yambun. 2009. *Ansonia hanitschi*. IUCN Red List of Threatened Species. Available from <http://www.iucnredlist.org> [Accessed 27 January 2017]). During the day, *A. hanitschi* forage along stream edges and males

can be heard calling during the daytime when breeding. Males defend territories by calling but will also engage in physical combat (Malkmus et al. 2002). At night, both sexes move onto leaves of plants up to 2 m above the ground where they are largely inactive, although foraging has been observed (Malkmus et al. 2002). The species is sexually dimorphic with males measuring up to 32 mm in length and females measuring up to 37 mm in length (Malkmus et al. 2002). The International Union for the Conservation of Nature (IUCN) lists the species as Near Threatened (Inger, R.F., I. Das, R. Stuebing, M. Lakim, and P. Yambun. 2009. op. cit.) because of its small range and need for undisturbed streams and intact adjacent forest areas. The ecology of *A. hanitschi* has not been well documented beyond brief descriptions. Our objectives were to describe diurnal activity patterns and diurnal and nocturnal habitat use of *A. hanitschi* on Gunung Kinabalu, Sabah, Malaysia.

MATERIALS AND METHODS

Study area.—In June and July 2010, we studied *A. hanitschi* on Sungai Liwagu (Liwagu Stream; UTM



FIGURE 1. Section of reach surveyed for *Ansonia hanitschi* along Sungai Liwagu, Gunung Kinabalu Park, Sabah, Malaysia. (Photographed by Nancy E. Karraker).

Zone 50Q 664160N; 449128E; elevation 1,512 m above sea level at the center of study area) on Gunung Kinabalu (Mount Kinabalu) in Gunung Kinabalu Park, Sabah, Malaysia. Sungai Liwagu is a perennial stream, with flatter reaches bearing broad banks of smaller substrates (sand, pebble, gravel, and cobble) and punctuated by shorter, higher gradient sections with larger boulder and bedrock substrates. The stream flows through mature tropical rainforest.

Diurnal focal observations.—Diurnal focal observations of toad behaviors were made between 1100 and 1500 along a 150-m study reach. Prior to data collection, we compiled a list of behaviors (Table 1) and defined each behavior during pilot observations of toads. If new behaviors were subsequently observed during data collection, these were defined by the observer, added to the list, and explained to the daytime observers on the team. When more than one of us was working at the same time, we remained at least 20 m apart to avoid disturbing toads being observed. After completing data collection on a particular toad, a researcher would move upstream to avoid observing the same toad twice. On subsequent days of data collection, we began searches

for toads 20 m upstream of the location of the last toad observed on the previous day to ensure that each toad was observed only once.

Individual observers walked slowly back and forth along the stream from the wetted edge to the stream bank, which ranged from about 1–5 m in width, moving upstream, and located individual toads by scanning as far ahead as possible. When a toad was sighted, the observer carefully approached and sat down about 3 m away from it and waited for 5 min before beginning to record observations. At 30-s intervals, we recorded behavior the toad was engaged in from those in our standard list, and we took additional notes on any details of each behavior. At each 30-s interval, we also recorded if the position of the toad was located in the direct sunlight or in the shade or if the sky was overcast, and we estimated distance from toad to the wetted edge of the stream. We observed each toad for 30 min, and at the end of the observation period, we captured the toad and measured its snout-vent length (SVL) to the nearest mm. All observers measured toads in the same way by setting the toad on a flat surface or holding the toad gently and laying a ruler along its back. Because we did not document presence of nuptial pads in males, we were only able to definitively identify females as those frogs ≥ 33 mm in SVL (Malkmus et al. 2002) and frogs that were gravid. Eggs are easily observed in the body cavity of gravid females through the translucent abdominal skin (Malkmus et al. 2002). All other frogs were recorded as of unknown sex.

To describe microhabitats used by toads, we established a 1-m² quadrat where the toad spent the majority of its observation period. Within the quadrat, we visually estimated percentage of each substrate type (silt, sand, pebble, gravel, cobble, boulder, or bedrock, following Wentworth 1922) and area covered by leaf litter or woody debris. We measured stream velocity at the location closest to the quadrat with a flowmeter. Using a digital camera resting on the ground in the center of the quadrat and aimed skyward, we took a photograph for estimation of canopy closure. We established a random point quadrat for each toad observed and collected the same data as above. We determined locations of random quadrats using a random distance (in m) upstream from the start of our study reach and within the bounds of our study reach, a random distance (in cm) from the wetted edge of the stream toward the stream bank (bounded by the distance from the stream in which toads were observed), and on a randomly selected (right or left) side of the stream.

At the start (approximately 1100) and end (approximately 1500) of the time period over which we were collecting data, we recorded air temperature and relative humidity, percentage cloud cover, and water temperature and pH. To characterize stream habitat



FIGURE 2. Typical diurnal microhabitat of *Ansonia hanitschi* along edge of Sungai Liwagu, Gunung Kinabalu Park, Sabah, Malaysia. (Photographed by Nancy E. Karraker).

in which we observed toads, we set 10 equally spaced transects with a meter tape across the stream along the 150-m reach. Transects extended across the width of the stream channel. We averaged values for each variable measured across transects. We measured stream channel width (15 ± 1 m, range 11–18 m) and wetted width (6 ± 1 m, range 2–13 m) and recorded the substrate type at 1-m intervals along each transect within the wetted width, and determined which type was dominant across each transect. Cobble-sized substrates were dominant on 60% of transects and boulder-sized substrates on 40% of transects. We quantified canopy closure by taking a digital image of the canopy, as described above, from the center of the stream along each transect. We analyzed images in Adobe Photoshop Pro (Adobe Systems, Inc., San Jose, California, USA) following the methods of Silva-Rodriguez et al. (2010) to determine percentage of canopy closure from each image. We measured canopy closure at 10 points along the stream. Stream gradient was measured at the center of each transect to a point 15 m downstream using a clinometer. We determined stream aspect facing downstream in transects 1, 5, and 10.

We determined the frequency with which each identified behavior was engaged by individuals, proportion of time spent in the sun or shade, and average distance from water at which individuals were located during focal periods. Using this information, we calculated the mean values of these variables to characterize activity patterns for females and all other individuals. We determined mean SVL for females and individuals of unknown sex, and we documented the proportion of gravid females found near the stream. We used *t*-tests to compare canopy cover, mean water

flow, area covered by woody debris, and area covered by leaf litter at toad locations and random points. We used *G*-tests to compare proportion of area covered by different surface substrate types (sand, pebble/gravel, cobble, boulder, bedrock) between habitats used and those available to toads. For all tests, $\alpha = 0.05$. We determined captures per person-hour for diurnal searches, from which we subtracted observation time of located individuals.

Nocturnal transect searches.—We conducted searches of four transects from 1930–2130 along Sungai Liwagu and two small tributaries, whose confluences were located approximately 900 m upstream and 300 m downstream of the daytime search area, to document nocturnal habitat use of *A. hanitschi*. We searched each transect one time with researchers walking slowly along the edges of streams or forest trails adjacent to streams. When toads were observed, we recorded time, substrate on which toad was sitting, height of toad above ground, and estimated distance of toad to the wetted edge of the stream (in 50 cm increments). We captured each toad and measured SVL, determined sex, and documented if females were gravid. For reasons explained above, we classified individuals carrying eggs and individuals ≥ 32 mm (Malkmus et al. 2002) as females and all other individuals as unknown sex. We determined proportions of females and individuals of unknown sex observed on transects at night and proportion of gravid females. We characterized substrate type used and compared height above ground and distance from water by females and all frogs observed. We documented search time to determine captures per unit effort.



FIGURE 3. *Ansonia hanitschi* moving along the edge of Sungai Liwagu during the day in Gunung Kinabalu Park, Sabah, Malaysia. (Photographed by Nancy E. Karraker).

RESULTS

Diurnal activity patterns and habitat use.—During daytime, we made focal observations of 30 *A. hanitschi*, including 19 females and 11 individuals of unknown sex. We searched for a total of 1,846 min to locate 30 individuals, for a search effort of 61.5 min per toad. We documented 12 behaviors in which *A. hanitschi* engaged during the day (Table 1). On average, females spent $79 \pm 6\%$ (SD) of time sitting. Each other behavior engaged in by females made up $< 5\%$ of time (Table 1). During our timed focal observation periods, we did not observe amplexant pairs along the stream.

Females ($n = 19$) spent $73 \pm 10\%$ of their time in shade and $3 \pm 2\%$ of their time in direct sunlight. When

we observed these individuals the sky was overcast $25 \pm 10\%$ of the time. Toads of unknown sex ($n = 11$) spent $62 \pm 2\%$ in shade and $3 \pm 3\%$ in direct sunlight; the sky was overcast $35 \pm 15\%$ of the time. Distances from the wetted stream edge at which we found females averaged 27.3 ± 6.6 cm (0 to 90.3 cm), and distances for toads of unknown sex averaged 31.3 ± 14.9 cm (0 to 120.0). Toads appeared to be solitary in their use of habitats along the stream. During focal observations, we did not observe a single instance of another toad approaching the focal toad.

Canopy closure was similar ($t = 1.23$, $df = 58$, $P = 0.224$) between toad locations ($82 \pm 2\%$, range 59–97%) and random points ($85 \pm 1\%$, range 69–94%). Mean water flow did not differ significantly ($t = 0.08$, df

TABLE 1. Mean percentage of time (\pm SE) *Ansonia hanitschi* spent engaging in each behavior during the day along Sungai Liwagu, Gunung Kinabalu Park, Sabah, Malaysia.

Behavior	Description	Females (n = 21)	All others (n = 9)
Sitting	Alert but motionless	79 ± 6	93 ± 3
Jumping	Short hops with all four feet off the ground	4 ± 1	1 ± 1
Crawling	Movements low to the ground with at least two feet touching the ground at a time	3 ± 1	1 ± 0
Feeding	Tongue flicks at substrate	2 ± 1	1 ± 1
Waving	Raising forearm up and over head from front to back	2 ± 1	1 ± 1
Head tilting	Sudden change in angle of head upward or downward	2 ± 1	1 ± 1
Turning	Turning body position left or right $\leq 90^\circ$	2 ± 1	< 1
Kicking	Backward motion of hind legs from sitting position	< 1	1 ± 1
Blinking	Blinking of either eye	< 1	< 1
Repositioning	Turning body position left or right $> 90^\circ$	< 1	< 1
Scanning	Slow movement of head horizontally	< 1	< 1
Swimming	Short movements in water along the stream edge	< 1	< 1



FIGURE 4. *Ansonia hanitschi* resting on leaf at night near Sungai Liwagu, Gunung Kinabalu Park, Sabah, Malaysia. (Photographed by Nancy E. Karraker).

= 58, $P = 0.933$) between toad locations (0.04 ± 0.04 m-s, range 0–0.9 m-s) and random points (0.04 ± 0.02 m-s, range 0–0.4 m-s). Quantities of woody debris ($t = 0.62$, $df = 54$, $P = 0.538$) and leaf litter ($t = 1.41$, $df = 54$, $P = 0.164$) overlying rocky substrates did not differ significantly between used and available locations. Mean proportions of surface substrate composition differed between toad locations and random points ($G = 19.00$, $df = 1$, $P < 0.001$), with toads using areas with proportionately higher amounts of sand- and cobble-sized substrates relative to their availability. During focal surveys, air temperature averaged $18.5 \pm 0.7^\circ\text{C}$ and relative humidity averaged $94 \pm 3\%$. Mean cloud cover was $57 \pm 9\%$. Water temperature averaged $18.3 \pm 0.2^\circ\text{C}$, and water pH ranged from 6.85 to 7.31. Canopy closure along the stream averaged $79 \pm 5\%$ (40–93%). Mean stream gradient was $8 \pm 2\%$ (1–17%), and mean stream aspect was 22° .

Nocturnal habitat use.—During nighttime searches of forest and stream edge transects, we observed 81 individuals (20 females and 61 individuals of unknown sex). We found all individuals resting on leaves at an average height of 62.9 ± 5.2 cm (10–200 cm) above ground, except for one individual, which we found on a stone at a height of 30 cm above the ground. All frogs we observed were motionless, and we observed no individuals moving or foraging. We heard no males calling at night. We documented *A. hanitschi* at an average distance of 2.3 ± 0.2 m (0 to 15 m) from the wetted edge. We searched three forest and one stream transect(s) for 1 h each on four different nights, totaling 16 person-h of search time. We found one *A. hanitschi* for every 11.9 min of searching.

Body size and sexual maturity.—We observed 111 *A. hanitschi*, including individuals from daytime focal observations and nighttime searches, of which one female and one individual of unknown sex were not

measured. Of 38 females (those with body sizes ≥ 33 mm or gravid), SVL averaged 32.1 ± 0.2 mm (28 to 35 mm). Overall, 84% of females we encountered were gravid. Gravid females averaged 31.8 ± 0.3 mm SVL (range 28–35 mm). We found that 89% of females observed along the stream during the day were gravid, and 80% of females observed resting above the ground at night were gravid.

DISCUSSION

Ansonia hanitschi are diurnal frogs, but our observations along the stream indicate that they spend $> 75\%$ of their active period remaining relatively motionless. This suggests that individuals using habitats adjacent to streams are sit-and-wait predators. Males may be feeding opportunistically as they defend territories and wait for females (Malkmus et al. 2002). Toads are solitary, and although most females observed along the stream during the day were gravid, we did not observe any amplexant pairs or interactions between females and males. No focal toads called during our daytime focal observations (11 of 30 toads were of unknown sex), but we often heard males calling during other research activities along the stream during the study period.

We observed *A. hanitschi* engaging in a behavior that we called Waving, in which individuals raised a forearm above their head and moved it quickly from front to back. This behavior was noted in observations of one-third of frogs, including seven females and three males. Frogs engaging in this behavior may be trying to rid themselves of biting mosquitoes or midges (Borkent and Grafe 2012), communicating with other frogs on the stream (Hartmann et al. 2005), or using this motion for some other purpose. We believe that the behavior we observed is not associated with communication, as we never observed another frog in the direct line of sight of our focal toads.

During daytime surveys, we found females within 0.9 m of the stream and all individuals were found within 1.2 m of the stream. Although not particularly strong swimmers, *A. hanitschi* are known to dive into the stream when threatened (Malkmus et al. 2002), and thus activity centers closer to the stream may aid this species in avoiding predation, as well as reducing energy requirements for males searching for mates and accessing breeding sites. The small size of these frogs, the rough texture of their skin, and skin coloration, which resembles that of streamside substrates, makes them difficult to detect during the day, and individuals are found most often on sand or cobble, rather than on boulders or bedrock where they may be more obvious to predators. This created challenges for us to detect them during the day versus at night when they are sitting on

the green leaves of plants. Focal toads are distributed along both lower-velocity and higher-velocity sections of the stream, suggesting that stream velocity may not influence selection of territories, calling sites, or oviposition sites. Similarly, stream velocity at oviposition sites of *Atelopus zeteki*, an ecologically-similar neotropical toad, does not differ from random sites (Karraker et al. 2006).

Except for one individual, we found all frogs on leaves of plants above the ground at night. Plants harboring frogs were almost exclusively narrow-stemmed and non-woody. *Ansonia vidua* has been documented resting above the ground on leaves at night (Hertwig et al. 2014), and we observed *Ansonia longidigita* at night near the study area engaging in the same behavior. It is likely that plants with this structure permit resting frogs to detect approaching snakes or small mammals by vibrations made by climbing such plants. In a few instances, when we disturbed a plant bearing a resting frog, the frog quickly leaped from the plant leaf to the ground and attempted to escape. Many diurnal frogs that may be vulnerable to nocturnal predators, including snakes, seek resting sites on small plants at night (Lindquist et al. 2007). In the neotropical genus *Atelopus*, an ecologically-similar group, adults move up onto these perches at night and generally rest on leaves or at the tips of fern fronds, but are only seldom found at the axils of leaves or branches (Lindquist et al. 2007). Our observations and those of others (Malkmus et al. 2002; Wood et al. 2008; Hertwig et al. 2014) suggest that some *Ansonia* species behave similarly.

Ansonia hanitschi have been described as sexually dimorphic by body size and by the development of nuptial pads in males (Inger 1966, Malkmus et al. 2002). Because we did not record presence of nuptial pads and thus sizes of males, we cannot contribute new information regarding body sizes of males. However, we noted the presence of eggs in females and found that sexually mature females ranged in SVL from 28 to 35 mm. Our data largely corroborate the maximum size for females of 37 mm summarized in Malkmus et al. (2002).

Our research provides new information on habitat use and activity patterns of *A. hanitschi*, but this species remains relatively understudied. We recommend that future research includes an investigation of the hand/forearm-waving behavior we observed to determine its purpose. Of 22 species of *Ansonia* that have been assessed by the IUCN, 14% are classified as Endangered, 27% as Vulnerable, 23% including *A. hanitschi* as Near Threatened, one species as Least Concern, and 32% as Data Deficient (IUCN Red List of Threatened Species. 2016. Available from <http://www.iucnredlist.org> [Accessed 27 January 2017]). Thus, 64% of species in this genus are of conservation concern, yet the status

of populations of another 32% could not be assessed because of lack of data. Although the genus is poorly studied overall, *Ansonia* frogs appear to be ecologically similar to frogs in the genus *Atelopus* in Latin America. *Atelopus* are small, slender, diurnal toads that breed in mid- to high-elevation streams (Karraker et al. 2006) and occupy forest habitats during and outside of the breeding season (Luger et al. 2009). More than 95 species of *Atelopus* frogs have been described (AmphibiaWeb. 2016. *Atelopus*. Available from <http://www.amphibiaweb.org> [Accessed 27 January 2017]). Populations of most species have undergone dramatic declines and most of these declines are associated with the fungal disease chytridiomycosis (La Marca et al. 2005; Lötters et al. 2009), to which they seem particularly susceptible. Population declines associated with the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) that have largely occurred in the Neotropics (La Marca et al. 2005), western North America (Fellers et al. 2011), and Australia (Woodhams and Alford 2005; Kriger and Hero 2008) appear to be rare thus far in tropical East and Southeast Asia (but see Kusriani et al. 2008; Vörös et al. 2012). However, researchers screening for this pathogen in Asia should focus on species like those in the genus *Ansonia* that may be ecologically similar to species that are susceptible to the amphibian chytrid fungus in other regions.

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