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Evaluation of the Amphibian Fauna of the Semi-arid and Arid Grassland Habitats in and around the Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries in Maharashtra, India

Nikhil Dandekar[1], Varun Kher[1], Chaitanya Risbud[1], Mayuresh Kulkarni[1], Makarand Ketkar[1], Srushti Bhave[1], Pushkar Pansalkar[2], Shauri Sulakhe[1], Sunil Limaye[3], and Anand Padhye[1,4]

[1]Institute of Natural History, Education and Research (INHER), c-26/9, Ketan Heights, Kothrud, Pune, Maharashtra-411038, India
[2]A/2, Ajinkyanagari, Karvenagar, Pune, Maharashtra-411052, India
[3]APCCF (WL) West, Mumbai, (formerly CCF–WL Pune Division), Maharashtra State Forest Department, Near Old MHB Colony, L.T. Road, Borivali (West), Mumbai, Maharashtra-400091, India
[4]Department of Zoology, MES Abasaheb Garware College, Karve Road, Pune, Maharashtra-411004, India (anand.padhye@mesagc.org)

Abstract.—Few studies document amphibian species composition from arid and semiarid environments. Scattered bodies of water in the semi-arid and arid landscape around Pune, Maharashtra, India, could be viable amphibian habitats; however; information on amphibians using these bodies of water is not available. In a preliminary study, we assessed the amphibian diversity and population status in three wildlife sanctuaries and surrounding areas near Pune. We also compared the use of artificial and natural ponds by amphibians and surveyed the amphibians for the presence of the chytrid fungus.

Few studies address amphibian species in tropical arid and semi-arid habitats (Sullivan 1989; Dayton and Fitzgerald 2001) or in protected areas (Dayton et al. 2004; Boeing et al. 2014). Complete reproductive failure is not unusual among amphibians that breed in temporary ponds, especially in arid climates (Semlitsch et al. 1996), and prolonged droughts have been suggested as one of the many possible causes for declines and local extinctions of amphibian populations, which can result from either a failure to reproduce in several successive years or drought-induced mortality of juveniles and adults (Corn and Fogelman 1984; Kagarise Sherman 1993; Grafe et. al. 2004). In addition, diseases like chytridomycosis, caused by chytrid fungus (*Batrachochytrium dendrobatidis*), are major stressors (Skerratt et al. 2008).

Most of the amphibian species in Maharashtra, India, are found in the Western Ghats, which receive heavy rains (Padhye et al. 2012). Western and central Maharashtra, on the other hand, receive little rain and it falls erratically, leading to semi-arid conditions. Surveys for amphibians in Maharashtra have been largely restricted to the Western Ghats and very little information is available for the semi-arid and arid dry regions of the state (Dahanukar et al. 2005). Grasslands and savannahs traditionally have been treated as wastelands in Maharashtra and have undergone large-scale land-use changes in the past few decades (Jayanthi 2015).

A network of protected areas in this region includes the Nannaj, Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries. All four sanctuaries and surrounding areas are typical of this landscape, with a mosaic of grassland fragments interspersed among human-dominated areas of agriculture, grazing lands, and housing. Although widespread in their extent of occurrence, these grasslands are shrinking at an alarming rate and are becoming more threatened as time progresses (IUCN 2014).

We conducted surveys of potential amphibian habitats in the Mayureshwar Wildlife Sanctuary (MWLS), Rehekuri Wildlife Sanctuary (RWLS), and Karmala Wildlife Sanctuary (KWLS). We herein provide abundance estimates of amphibian species at selected bodies of water in the MWLS, preliminary measure of amphibian diversity in the RWLS and KWLS, the status of chytrid fungus infection in amphibian species found in these areas, and managerial recommendations that might aid in the conservation of amphibians in this landscape.

Methods

Study Area (Fig. 1).— Mayureshwar Wildlife Sanctuary (Figs. 2 & 3) is near Supe Village in Taluka Baramati, of Pune District (18.3444°N and 74.3648°E). It falls in biogeographic province 6B (Deccan Plateau). The 5.14-km^2 sanctuary contains dry deciduous scrub forest with interspersed grasslands (Ben et. al. 2013). The principal mammalian species protected in the sanctuary is the Indian Gazelle (*Gazella benetti*). Rehekuri Wildlife Sanctuary (Fig. 3) is in Karjat Taluka in Ahmednagar District (18.6012°N, 74.9724°E) and covers 2.17 km^2. The forest type is dry deciduous southern tropical thorny forest. The Blackbuck (*Antilope cervicapra*) is the main attraction in this sanctuary (Kharat and Mokat 2018). The 157.29-km^2 Karmala Bird Sanctuary (Fig. 2) is in Solapur District (18.5009°N, 75.2111°E) and was previously part of the Great Indian Bustard Sanctuary (Varghase et. al. 2010).

Data collection.—We conducted this study in two phases in 2015 and 2016, respectively. In the first phase during the monsoon of 2015, we searched around bodies of water and collected chytrid samples. In the second phase, we conducted bimonthly amphibian-diversity surveys using point counts lasting 15 min around selected bodies of water and recorded the numbers of individuals of each species. In addition, we conducted 4-km-long road-transect surveys in and near the sanctuaries to account for species that were not encountered in point-count surveys. As in Dahanukar and Padhye (2005), we tabulated the relative abundance of species as very common (VC), common (C), occasional (O), and not recorded (NR).

For more detailed studies in and around the MWLS, we selected two natural and one artificial body of water. Data collected included water and air temperatures, dissolved oxygen (DO), pH, salinity, and relative humidity.

Data analysis.—Data were analyzed using PAST 3.25 (Hammer et al. 2010). We used Canonical Correspondence Analysis (CCA) to analyze the effects of environmental parameters on seasonal occurrence and abundance of different species and the species accumulation curve was drawn using EstimateS Win910 software (Robert Colwell 2013) to estimate the maximum number of species that could be supported by the habitat.

Chytrid strain infection study.—For the detection of *Batrachochytrium dendrobatidis* (*Bd*) fungal spores, we used a non-invasive protocol to collect 72 skin swab samples (Table 1) following Skerratt et al. (2008) and Dahanukar et al. (2013). We extracted potential fungal DNA from swabs following the procedures in Goka et al. (2009) with modifications outlined by Dahanukar et al. (2013), and checked for the presence of *Bd* using quantitative PCR based on the SYBR Green method using the primer pair ITS1-3 Chytr and 5.8S Chytr (Boyle et al. 2004) following the protocol described by Dahanukar et al. (2013) while using serially diluted DNA

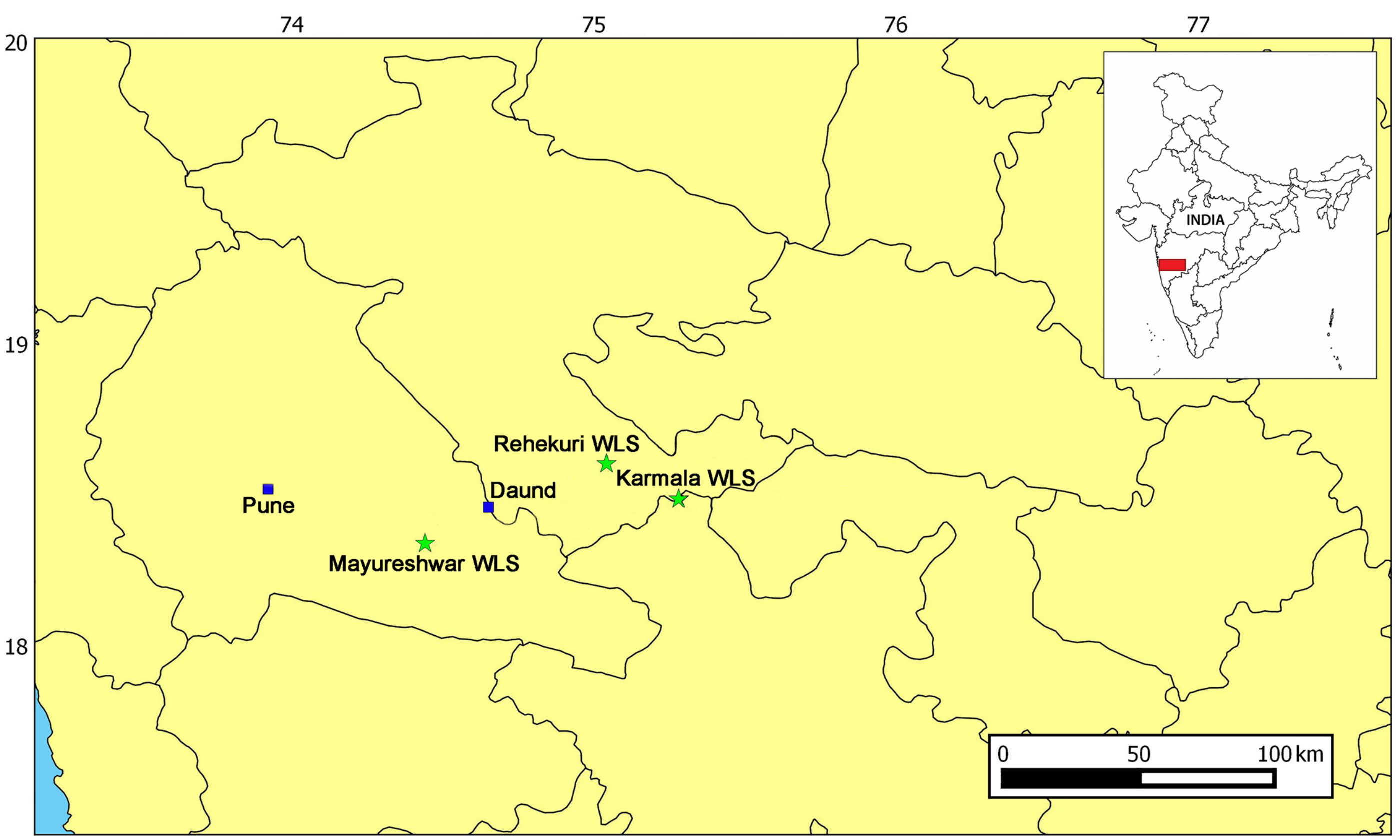

Fig. 1. Map showing the location of the Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries in Maharashtra, India.

Fig. 2. Natural ponds in the Mayureshwar (left) and Karmala (right) Wildlife Sanctuaries in Maharashtra, India. Photographs by Chaitanya Risbud (left) and Nikhil Dandekar (right).

Fig. 3. Artificial bodies of water in the Mayureshwar (left) and Rehekuri (right) Wildlife Sanctuaries in Maharashtra, India. Photographs by Nikhil Dandekar.

Table 1. Number of chytrid samples tested per species per sanctuary.

Species	Mayureshwar Wildlife Sanctuary	Rehekuri Wildlife Sanctuary	Karmala Wildlife Sanctuary
Common Asian Toad (*Duttaphrynus melanostictus*)	3	4	2
Marbled Toad (*Duttaphrynus stomaticus*)	2	4	0
Skittering Frog (*Euphlyctis cyanophlyctis*)	9	0	8
Indian Bullfrog (*Hoplobatrachus tigerinus*)	1	1	0
Western Burrowing Frog (*Sphaerotheca pashchima*)	2	0	4
Syhadra Frog (*Minervarya syhadrensis*)	13	0	0
Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*)	17	0	0
Marbled Globular Frog (*Uperodon systoma*)	0	2	0

extracted from 10^6 *Bd* zoospores as a standard. All sample runs were duplicated and those with genomic equivalents of more than one were considered positive for chytrid.

Results

Patterns of species diversity.—We recorded eight anuran species (Figs. 4 & 5) in seven genera and three families. Based on the percent relative abundance calculated from the point-count surveys (Table 2), the Skittering Frog (*Euphlyctis cyanophlyctis*) was the most abundant species in the MWLS and KWLS, whereas the Common Asian Toad (*Duttaphrynus melanostictus*) was the most abundant species in the RWLS. The least frequently encountered species were the Marbled Globular Frog (*Uperodon systoma*) in MWLS, the Skittering Frog in RWLS, and the Western Burrowing Frog (*Sphaerotheca pashchima*) in KWLS. The Marbled Toad (*Duttaphrynus sto-*

maticus), Syhadra Frog (*Minervarya syhadrensis*), Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*), and the Marbled Globular Frog were not encountered in any body of water in the RWLS during the point-count surveys. However,

Fig. 4. Frogs from the Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries in Maharashtra, India: (A) Common Asian Toad (*Duttaphrynus melanostictus*), (B) Indian Bullfrog (*Hoplobatrachus tigerinus*), (C) Marbled Toad (*Duttaphrynus stomaticus*), and (D) Syhadra Frog (*Minervarya syhadrensis*). Photographs by Nikhil Dandekar.

Fig. 5. Frogs from the Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries in Maharashtra, India: (A) Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*), (B) Marbled Globular Frog (*Uperodon systoma*), (C) Skittering Frog (*Euphlyctis cyanophlyctis*), and (D) Western Burrowing Frog (*Sphaerotheca pashchima*). Photographs by Varun Kher (A), Shauri Sulakhe (B), Nikhil Dandekar (C), and Mayuresh Kulkarni (D).

Table 2. Percent relative abundance of anuran species recorded during point counts in three wildlife sanctuaries in Maharashtra, India.

Species	Mayureshwar Wildlife Sanctuary	Rehekuri Wildlife Sanctuary	Karmala Wildlife Sanctuary
Common Asian Toad (*Duttaphrynus melanostictus*)	1.32	50.00	3.17
Marbled Toad (*Duttaphrynus stomaticus*)	2.15	0.00	4.06
Skittering Frog (*Euphlyctis cyanophlyctis*)	38.08	9.09	45.69
Indian Bullfrog (*Hoplobatrachus tigerinus*)	3.15	13.64	6.73
Western Burrowing Frog (*Sphaerotheca pashchima*)	7.78	27.27	2.92
Syhadra Frog (*Minervarya syhadrensis*)	27.32	0.00	20.18
Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*)	19.70	0.00	17.26
Marbled Globular Frog (*Uperodon systoma*)	0.50	0.00	0.00

Table 3. Relative abundance of anuran species recorded during road transects. VC = very common, C = common, O = Occasional, NR = not recorded.

Species	Mayureshwar Wildlife Sanctuary	Rehekuri Wildlife Sanctuary	Karmala Wildlife Sanctuary
Common Asian Toad (*Duttaphrynus melanostictus*)	VC	O	O
Marbled Toad (*Duttaphrynus stomaticus*)	VC	O	VC
Skittering Frog (*Euphlyctis cyanophlyctis*)	NR	NR	NR
Indian Bullfrog (*Hoplobatrachus tigerinus*)	O	NR	NR
Western Burrowing Frog (*Sphaerotheca pashchima*)	C	O	C
Syhadra Frog (*Minervarya syhadrensis*)	C	O	O
Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*)	C	C	NR
Marbled Globular Frog (*Uperodon systoma*)	NR	NR	NR

the first three species were encountered in the RWLS during road transects (Table 3). In the KWLS, Marbled Globular Frogs were recorded neither during point counts nor road transects. Healthy populations of Skittering Frogs, Nilphamari Narrow-mouthed Frogs, and Syhadra Frogs were observed throughout the monsoon both inside and outside the protected areas. A healthy population of Western Burrowing Frogs (*Sphaerotheca pashchima*) was observed only during the early monsoon. A few Marbled Globular Frogs were encountered only around bodies of water and never during road-transect surveys.

All eight species of frogs were present in the MWLS, where the estimated species richness calculated by EstimateS was also eight, suggesting that our sampling was adequate (Fig. 6). More species were associated with natural ponds than artificial bodies of water (Table 4). Skittering Frogs, Syhadra Frogs, Nilphamari Narrow-mouthed Frogs, and Western Burrowing Frogs were encountered regularly at the natural ponds (Wadhane, Korti, and Karmala Lakes), whereas the artificial waterholes (e.g., Sloping Tank in the MWLS) were used solely by the more aquatic species such as Skittering Frogs and Indian Bullfrogs (*Hoplobatrachus tigerinus*). We recorded the lowest number of species in the artificial saucers in the RWLS.

The rank-abundance curve clearly showed that species were more evenly distributed in natural ponds, whereas the relative abundance of Skittering Frogs (72.5%) in the artificial waterhole resulted in considerable unevenness (Fig. 7). Also, we

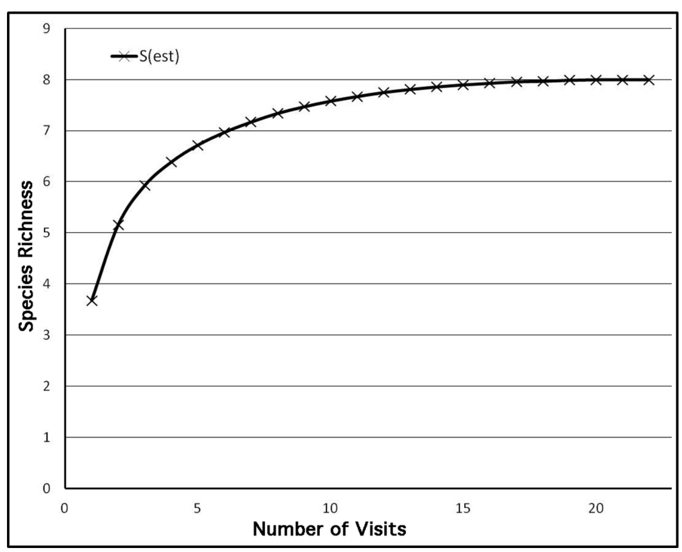

Fig. 6. Species-richness curve for the amphibians of the Mayureshwar Wildlife Sanctuary, Maharashtra, India.

Table 4. Percent relative abundance in natural versus artificial bodies of water in the Mayureshwar Wildlife Sanctuary.

Species	Natural Bodies of Water	Artificial Bodies of Water
Common Asian Toad (*Duttaphrynus melanostictus*)	6.20	0.00
Marbled Toad (*Duttaphrynus stomaticus*)	9.30	1.10
Skittering Frog (*Euphlyctis cyanophlyctis*)	23.26	72.53
Indian Bullfrog (*Hoplobatrachus tigerinus*)	3.10	4.40
Western Burrowing Frog (*Sphaerotheca pashchima*)	10.08	13.19
Syhadra Frog (*Minervarya syhadrensis*)	31.78	1.10
Nilphamari Narrow-mouthed Frog (*Microhyla nilphamariensis*)	13.95	7.69
Marbled Globular Frog (*Uperodon systoma*)	2.33	0.00

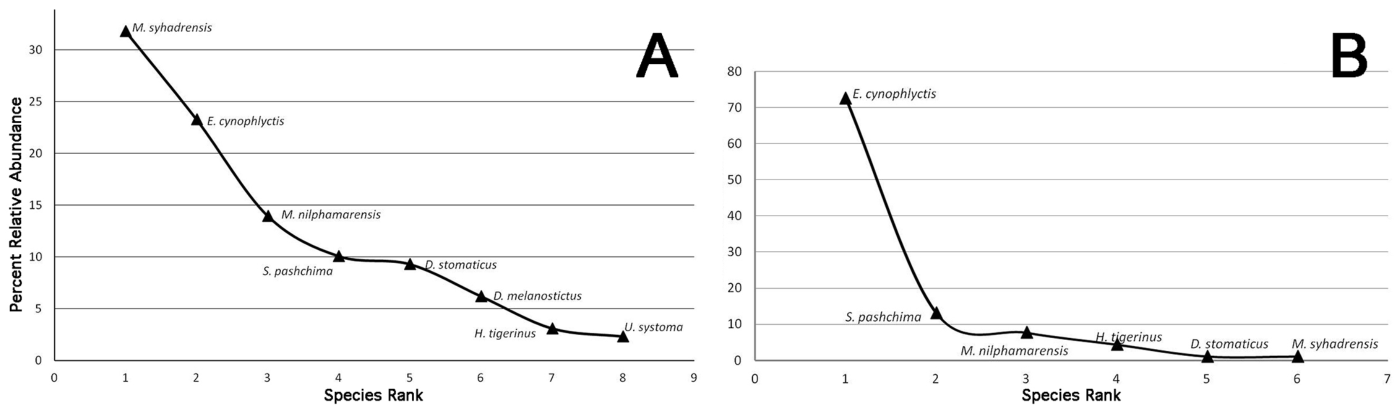

Fig. 7. Rank-abundance curves for the amphibians of the Mayureshwar Wildlife Sanctuary in a natural pond (A) and an artificial waterhole (B).

found no Common Asian Toads or Marbled Globular Frogs at artificial bodies of water, and Marbled Toads and Syhadra Frogs showed only a 1.1% relative abundance in artificial waterholes compared to 9.3% and 31.8%, respectively, in natural ponds.

The CCA (Fig. 8) showed that Western Burrowing Frogs and Common Asian Toads were heavily dependent on the

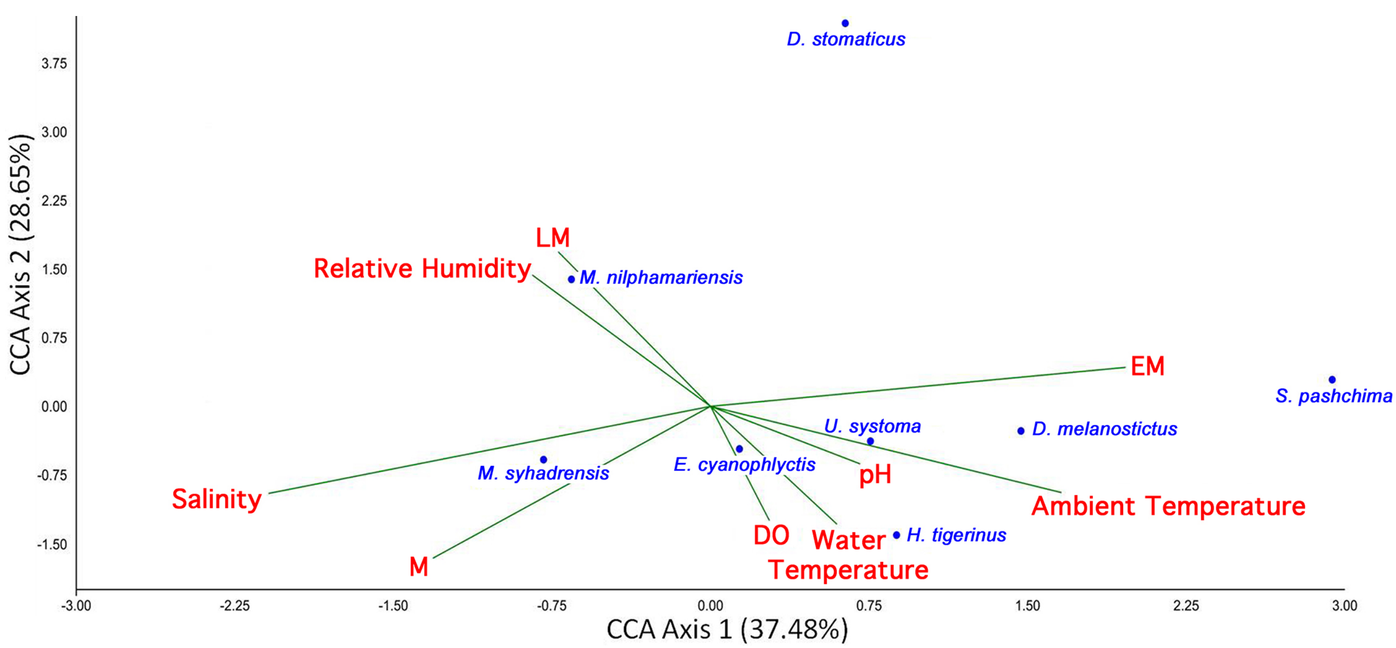

Fig. 8. Canonical Correspondence Analysis (CCA) plot showing the relative effects of various environmental factors on the presence of amphibians in the Mayureshwar, Rehekuri, and Karmala Wildlife Sanctuaries in Maharashtra, India. M = monsoon, EM = late monsoon, EM = early monsoon, DO = dissolved oxygen (mg/L). Units: temperatures (°C), relative humidity (%), salinity (psu).

early monsoon rains, whereas Nilphamari Narrow-mouthed Frogs and Marbled Toads were more frequently associated with late monsoon showers. Skittering Frogs and Syhadra Frogs were observed throughout the monsoon. Relative humidity appeared to affect the activity of Nilphamari Narrow-mouthed Frogs while ambient temperature appears to have a greater effect on the activity of Common Asian Toads and Western Burrowing Frogs.

Chytrid strain infection study.—All 72 skin swab samples tested in the current study (Table 1) were negative for chytrid infection. We also failed to observe any visible skin sloughing or other symptoms of disease in any of the populations.

Discussion

Only those amphibian species that have adapted to arid conditions occur in these arid and semi-arid areas. The amphibian species diversity is almost the same in all three sanctuaries, but the relative and temporal abundance varies. Although all of the sanctuaries are in the rain shadow of the Western Ghats, the MWLS is closer to the mountains and thus receives comparatively more rainfall and has a slightly longer wet period. Also, in the MWLS, one natural pond is adjacent to a blacktop road whereas the other is surrounded by agricultural fields. The one near the agricultural fields dries out completely after November, while the roadside pond and an artificial pond are not subjected to drying as the state forest department refills them at regular intervals as a conservation management practice.

We observed drying of temporary ponds during early monsoon months, leading to considerable mortality of tadpoles of many species. Although most frogs in the region were confined to the immediate vicinity of water, we observed road-killed individuals that must have been travelling to or from various bodies of water.

Diseases affecting amphibians are increasing globally and the high virulence of some pathogens combined with a broad host range and the presence of biotic or abiotic pathogen reservoirs can lead to population-level effects, including extinction (e.g., Fisher et al. 2012; McCallum 2012; Telfer and Brown 2012). The only previous surveys for the chytrid fungus in Maharashtra (Molur et al. 2015; Dahanukar et al. 2013) were conducted in the Western Ghats. Fortunately, we did not find any populations of any species in the studied sanctuaries infected by *Batrachochytridium dendrobatidis*.

Semi-arid grassland habitats usually are managed primarily for large mammals or critically endangered species of birds (e.g., the Great Indian Bustard, *Ardeotis nigriceps*, in the RWLS and KWLS). Bodies of water in the sanctuaries also are constructed with a focus on the conservation of flagship species, although those bodies of water also are used by less charismatic fauna such as amphibians. However, our results show that, compared to the natural pond, an artificial waterhole supported fewer species with an uneven number of individuals per species. This coupled with the low absolute abundance of amphibians in the artificial waterhole highlights the importance of having natural ponds to sustain amphibians in a semi-arid landscape.We recommend the construction of additional semi-natural ponds. Shallow depressions with black cotton soil beds that harvest rainwater appear to be more effectively used by amphibians than saucer-shaped artificial waterholes. Also, sloping tanks are better than saucers for avoiding frequent drying due to evaporative loss and thus provide a longer-lasting habitat for macroinvertebrates and at least some species of amphibians.

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