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# Listening to Namdapha: Estimating the amphibian species richness of Namdapha Tiger Reserve using passive acoustic monitoring

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# Abstract

Assessment of Amphibians at a large scale is a necessary first step towards conserving the most threatened vertebrate class. Overcoming the challenges of accessibility, manpower, safety and synchronisation, we conduct the first passive acoustic monitoring exercise in Namdapha tiger reserve, India, to estimate the amphibian species richness. We use incidence-based Chao, Jackknife and Bootstrap methods to estimate the amphibian richness. By detecting 19 amphibian species, the acoustic monitoring is fairly accurate in comparison to previously conducted active manual searches. The bias corrected estimate of species richness is marginally higher than the observed value. High species richness is not confined to a single habitat but is found to occur across diverse habitats. The acoustic analysis reveals that the dominant frequency of the amphibians are not different across the different habitats of the Namdapha tiger reserve. Overall the study underscores the accuracy of passive acoustics in monitoring the poorly known amphibians in India's Protected area.


# 1. Introduction

Amphibians are significant for providing regulating, provisioning, supporting and cultural ecosystem services (Hocking and Babbitt 2014). Yet they are the most threatened vertebrate class as per the second Global Amphibian Assessment (GAA2)(Luedtke et al. 2023). A decline in amphibian species is detrimental to the ecosystem which includes threats to other taxa (Whiles et al. 2006). Thus, effective amphibian conservation strategies are required to combat their rapid decline. Amphibian diversity and distribution data over large spatio-temporal scales is a prerequisite to design their conservation strategies. However, the widely used Amphibian assessment and monitoring techniques rely on manual surveys that not only demand manpower but are limited to accessible geographic regions. Reliable occurrence estimates of vocally active species can be obtained using bioacoustics approaches (Blumstein et al. 2011). Passive acoustic monitoring (PAM) offers greater detection ranges, flexibility of prolonged sampling periods and is suitable for sites unsafe for human surveys (Marques et al. 2013; Melo et al. 2021). Greater availability and affordability of PAM devices driven by technological advancements have increased the opportunity to acoustically survey wildlife (Gibb et al. 2018). In terrestrial ecosystems, bats, followed by birds are the most studied using PAM while only 12% of the PAM studies until 2018 focussed on anuran (Sugai et al. 2018).

Northeast India (NEI), comprising eight states viz.,— Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura is a part of the Indo-Burma biodiversity hotspot (Chatterjee et al. 2006). Based on the number of endemic species, species/ area ratio for plants, habitat loss and vertebrates, the Indo-Burma region is one among the eight hottest hotspots of the world (Myers et al. 2000). Among the Northeast Indian states Arunachal Pradesh has the largest forest cover with 21,058 sq. km of very dense forest (VDF) and rich biodiversity (FSI, 2021). Threatened by changing habitat, the need to assess the biodiversity in Arunachal Pradesh has received attention from researchers in the recent past. Annandale (1912) and Smith (1929) are among the earliest to document the amphibian species of the state. While

the effort to survey the region on large spatial scale is in process, inaccessibility and difficult terrain is a major impediment (Ohler et al. 2018).

In this study, we aim to record the amphibian species of Namdapha Tiger Reserve(NTR), Arunachal Pradesh, through passive acoustic monitoring (PAM). Apart from the regional species richness (gamma), we are interested in estimating the amphibian richness per habitat such as lakes, streams, puddles and swamps using multiple species richness estimators. Additionally, the study aims to assess the variation in acoustic characteristics of amphibian calls across habitats.

## 2. Materials and methods

The study was conducted in Namdapha Tiger Reserve which is a 1985 sq. km protected area in Changlang district of Arunachal Pradesh, India (Fig. 1). Namdapha is home to diverse forest types with tropical wet evergreen forests (Champion and Seth 1968) occurring at lower elevations and northern montane wet temperate forest (Champion and Seth 1968) found at higher altitudes. Dense undergrowth and steep hills makes the place hard to human access.. Seven sampling locations were identified for the survey, based on existing literature (Dinesh and Radhakrishnan 2019; Sarkar and Sanyal 1984), previous exploration surveys (conducted in 2022) and the information provided by the local people. Within each location, suitable amphibian habitats were explored and selected by our team of herpetologists. The habitats included were, (1) lake/pond, (2) river stream, (3) road-side water puddle, and (4) swamps within the forest.

Song meter micro by Wildlife Acoustics was used as the passive acoustic monitoring device. Each PAM unit was powered up using three AA alkaline batteries. Silica bags were placed inside each device to prevent moisture accumulation. The digital recordings were stored in microSD cards inserted in the Song meter micro. The device was configured through the Song Meter Configurator mobile App (v 1.5.15). The sampling rate was set to 44.1kHz, Gain was set to 18 dB and individual recordings were saved as 60 minutes files.

## 2.1. Data Sampling

PAM units were deployed during May - June 2023 (46 days), which is in tune with the peak breeding season of amphibians in NTR. PAM units were configured to record from dusk to midnight, i.e., 18:00 to 23:59 hours coinciding with the peak activity period of amphibians. Thus, at every chosen habitat in each location, one PAM unit was installed to record for a duration of six hours. Each device was securely strapped on a tree or plant, ensuring that it is about 1.5 meters from the surface (Walls et al. 2014).

## 2.2. Data Analysis

Raven Pro (v 1.6) was used for analysing the sound files retrieved from PAM units. After discarding corrupt files and those with excessive noise (rain), we obtained 3360 minutes of recordings. The sampling effort was set to 10 min for every hour of recording (Melo et al. 2021; Ribeiro Jr et al. 2018). The 10 min continuous segment within each hour was randomly selected. Thus, for each site a total of 60 minutes (6 (hours) * 10 (min/h)) of call recording was considered as the sample for analysis. Analysis involves

listening to the recordings and visually scanning the spectrogram in Raven Pro with frame length set to 60 seconds (D'Anunciação et al., 2022; Wimmer et al., 2013). The spectrum function was set to Hanning window of 256 samples and DFT size of 1024 samples. Our team of herpetologists (2 members) with adequate knowledge of anuran calls listened to the audio recordings and identified the species specific calls. The identified calls were annotated and the dominant frequency of each call was obtained through peak frequency measurement in Raven's selection spectrum function (Bernardes, De Carvalho, and Giaretta 2015). Dominant frequency is defined as the frequency at which the maximum power is observed in a call selection.

The observed species richness is the lower bound of the true species richness of the sampled area because of undetectability of certain species (Colwell and Coddington 1994). In order to account for the bias in observed species richness, we fit the data to three methods viz., Chao estimator (Chao 1987; Chiu et al. 2014), Jackknife estimator (Smith & Van Belle, 1984) and Bootstrap estimator (Smith and Van Belle 1984). R software (v 4.1.3) and vegan package (Oksanen et al. 2015). were used to run the above estimators and plot the species accumulation curves. Spreadsheet functions were executed using Google Sheets web application.

# 3. Results

## 3.1 Richness

A total of 19 amphibian species belonging to 17 Genera and 6 Family (Frost 2024) were identified from 720 minutes of passive acoustic recordings (Table 1). Only the amphibian species belonging to Raorchestes genus were recorded at all the surveyed habitats. *Fejervarya* sp. ,*Kurixalus naso*, *Rhacophorus rhodopus* and *Polypedates himalayensis* were recorded in 3 out of 4 surveyed habitats. 5 out of the 20 detected amphibian species are endemic to India. We observe that nearly fifty percent of the total species were acoustically detected at a maximum of two sites only. This possibly indicates the presence of a relatively high number of rare amphibian species. Additionally, we observe a maximum number of calling species at 20:00 hours and it decreases as we move away from this time of the day (Fig 2*)*.

The Chao model estimated a species richness of 20.93 (standard error (SE) = 2.36), Jackknife estimate was 23.64 (SE = 2.08) and Bootstrap estimate was 21.57 (SE = 1.29). Thus all the three incidence-based estimators, predict a higher species richness for the sampled region. The species accumulation curve(SAC)(100 permutations) of each of the estimators validates the increase in richness with increase in number of sampled sites (sites with installed passive recorders). While the 95% confidence interval of the estimated species richness fits well for the Jackknife and Bootstrap methods, the Chao estimator has a higher standard deviation (Fig 3). The observed species richness across different habitats are ; 9 in lake/pond, 12 in Road puddle, 4 in streams and 13 in Swamp forest trail. The habitat wise estimated species richness is shown in Table 2.

## 3.2 Acoustic properties

The dominant frequency for the amphibians of NTR ranges between 1386 Hz and 3452 Hz (Mean = 2419 Hz, Standard deviation = 1033Hz, n = 208 calls). The lowest dominant frequency call is of *Limnonectes* sp. (1077 ± 94, n= 12). The highest dominant frequency call is by *Gracixalus patkaiensis* (4651 ± 254,n=4). The variation of dominant frequencies across different habitats is represented through Fig 4. The mean dominant frequencies for the habitats are: lake=2559±1311 (n=41), Road puddle = 2295 ±941 (n=57), Stream 2869±779 (n=23), Swamp forest = 2444±901 (n=87) There is no significant difference in the dominant frequencies of the amphibians in relation to the chosen habitats (Fs = 1.699, p = 0.168, one-way ANOVA). Based on the differences in the dominant frequency of calls of the genus Raorchestes, we identify 3 different cryptic species. The mean dominant frequency of the 3 species are 3553±30 (n=2), 3847±369 (n=8) and 2707±285 (n=13). The variation in the dominant frequency is statistically significant ( Fs= 24.689, $p < 0.05$, one-way ANOVA) for 2 species, while the third species has a different call type compared to the other 2 species. Thus, acoustic properties are used to distinguish and identify species in this study.

# 4. Discussion

In the past, manual amphibian surveys have been conducted in NTR. In 1985, Sarkar and Sanyal conducted an expedition and reported 14 amphibian species (Sarkar and Sanyal 1984). A more recent manual survey conducted in 2009 reported 22 different amphibian species in NTR (Dinesh and Radhakrishnan 2019). Our study reports 19 observed species of Amphibians, proving the effectiveness of passive acoustic monitoring (PAM). However, the estimated species richness by Chao, Jackknife and Bootstrap method are marginally higher than the observed value. All the three non parametric incidence based estimators are effective in minimising the bias in observed species richness (Colwell and Coddington 1994). The species accumulation curves of three estimation methods approach a plateau indicating that they are near asymptote and thus the estimated richness is likely to be accurate (Thompson and Withers 2003). Estimates of species richness are high across different habitats, except in streams. However the estimated low richness in streams can be because of high background noise from stream flow (Buxton et al. 2017; Towsey, Parsons, and Sueur 2014)..

This is one of the first studies in India to estimate the Amphibian richness of a tiger reserve through passive acoustic monitoring. PAM in comparison to active manual surveys has the advantage of covering larger geographical areas with reduced manpower and the ability to simultaneously carry out monitoring at multiple sites (Marques et al. 2013; Melo et al. 2021; Sugai et al. 2018). Thus, PAM offers the twin benefits of reduced efforts in terms of manpower and time. Moreover, technological improvements and open science are bringing affordable and energy efficient passive acoustic monitoring equipment to researchers (Hill et al. 2017).

The acoustic analysis of the recordings revealed the range of amphibian call frequencies. Evidently the amphibian calls fall in the low frequency range (<5000 Hz). Anuran calls are mainly confined to lower frequencies due to a limitation on high frequency transmission in their middle ear (Loftus-Hills and

Johnstone 1970). Thus, PAM devices can be configured to record only the low frequencies which, beyond the reduced background noise, could yield storage and battery benefits in the equipment. Although this study hypothesised a dissimilarity in the mean dominant frequencies of amphibians at different habitat types, no statistically significant distinction was observed. In consonance, earlier studies associate the dissimilarity with differences in body size and phylogenetic relationships and not to the habitat (Zimmerman 1983).

## 5. Conclusions

A reasonably accurate estimate of the amphibian species richness of Namdapha Tiger Reserve was produced by the first passive acoustic monitoring exercise for a large Tiger Reserve in India. High species richness is not restricted to a single habitat of the reserve but observed across diverse habitats. Increasing the sampling effort is likely to provide the most precise estimate of species richness. Configuring the PAM units to record low range frequencies (below 5000 Hz) is sufficient for detecting amphibian calls. Finally our study shows that the dominant frequencies of the amphibian calls are not significantly different across varying habitat types of the study area.

## Declarations

# Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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# Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by N.V.R.. The first draft of the manuscript was written by N.V.R. and A.D. commented on previous versions of the manuscript. All authors read and approved the manuscript.

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# Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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# Tables

**Table 1** Amphibian species identified through passive acoustic monitoring. Occurrence indicates the % of sampled sites where the species was acoustically detected

| Family | Species | Occurrence(%) |
|---|---|---|
| Ceratobatrachidae | *Alcalus fontinalis* [1] | 21.4 |
| Dicroglossidae | *Fejervarya* sp. | 21.4 |
| | *Limnonectes* sp. | 21.4 |
| Megophryidae | *Xenophrys* sp. | 14.2 |
| Microhylidae | *Microhyla eos* [1] | 14.2 |
| Ranidae | *Amolops marmoratus* | 7.1 |
| | *Hydrophylax leptoglossus* | 7.1 |
| | *Nidirana noadihing*[1] | 7.1 |
| Rhacophoridae | *Gracixalus patkaiensis* [1] | 21.4 |
| | *Kurixalus naso* | 50 |
| | *Nasutixalus sp.* | 7.1 |
| | *Polypedates himalayensis* | 42.8 |
| | *Raorchestes* sp.[2] | 71.4 |
| | *Raorchestes* sp.[2] | 42.8 |
| | *Raorchestes* sp.[2] | 7.1 |
| | *Rhacophorus rhodopus* | 57.1 |
| | *Rhacophorus bipunctatus* | 14.2 |
| | *Rohanixalus shyamrupus*[1] | 14.2 |
| | *Zhangixalus smaragdinus* | 14.2 |

[1] *species endemic to India.*
[2] *cryptic species differentiated based on call acoustic characteristics.*

**Table 2** Species richness across different habitat types and associated standard error(SE). S indicates the observed species while Chao, Jackknife and Boot are the estimators. n indicates the number of sites sampled.

| | Species(S) | Chao | Chao(SE) | Jackknife | Jackknife(SE) | Boot | Boot(SE) | n |
|---|---|---|---|---|---|---|---|---|
| lake/pond | 9 | 10.33 | 1.89 | 11.67 | 1.76 | 10.33 | 1.19 | 3 |
| Road puddle | 12 | 21.19 | 9.98 | 17.25 | 3.15 | 14.35 | 1.55 | 4 |
| Stream | 4 | 4.17 | 0.54 | 4.67 | 0.67 | 4.37 | 0.55 | 3 |
| Swamp forest trail | 13 | 21.00 | 7.83 | 19.00 | 3.87 | 15.72 | 2.16 | 4 |

# Figures

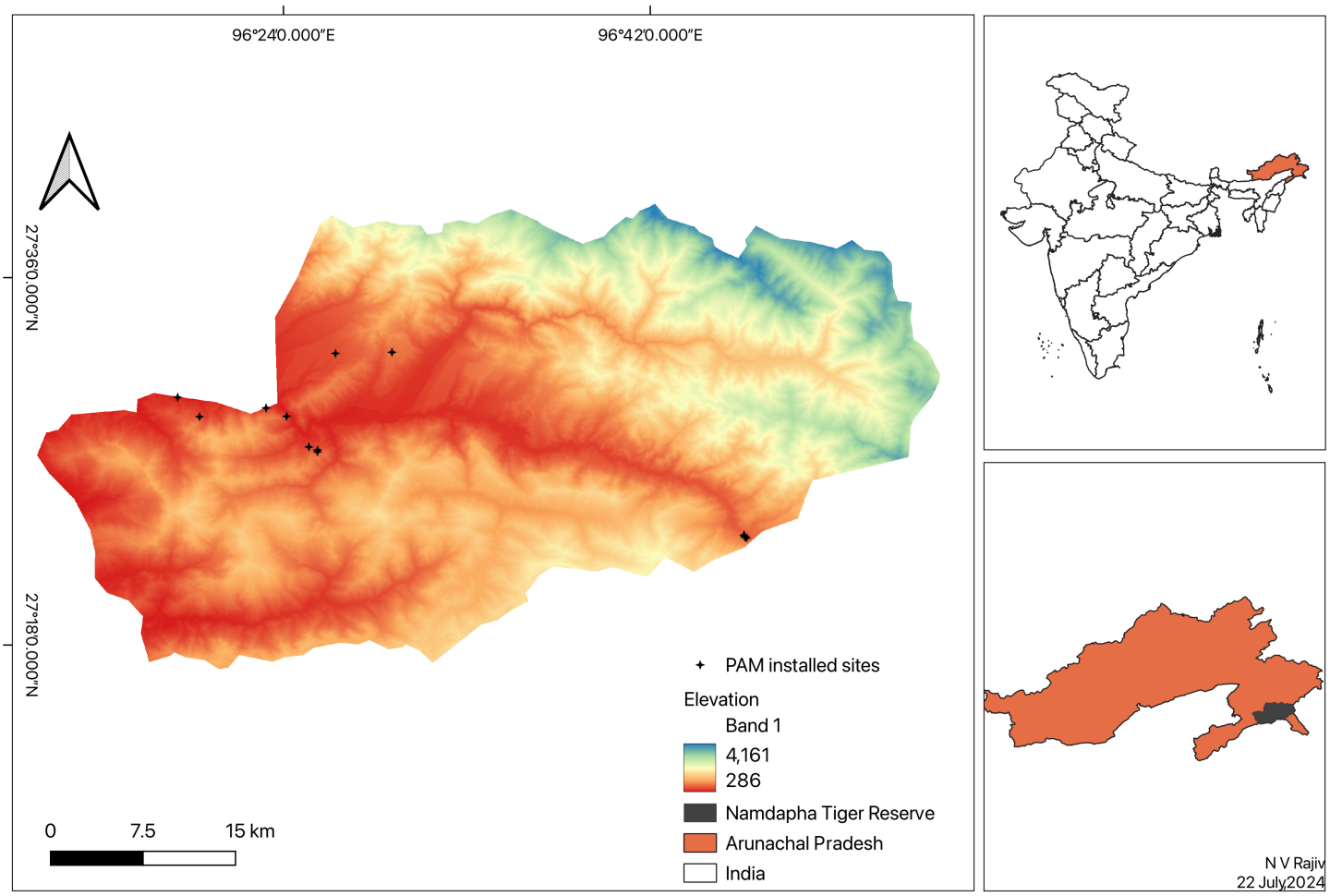


**Figure 1**

Map of Namdapha Tiger Reserve showing the location of passive acoustic monitoring sites

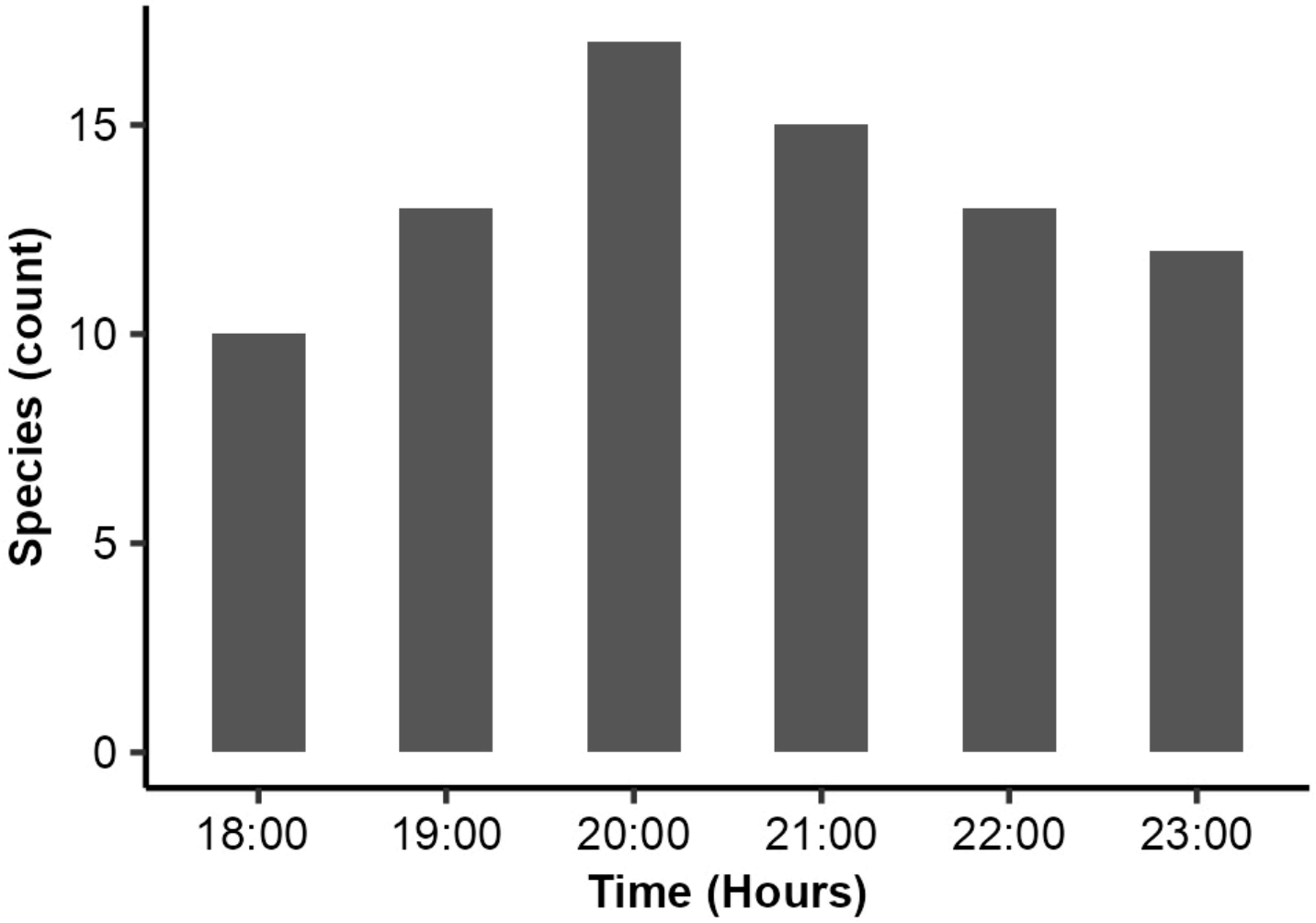


**Figure 2**

The number of active amphibian species between 18 hours and 24 hours

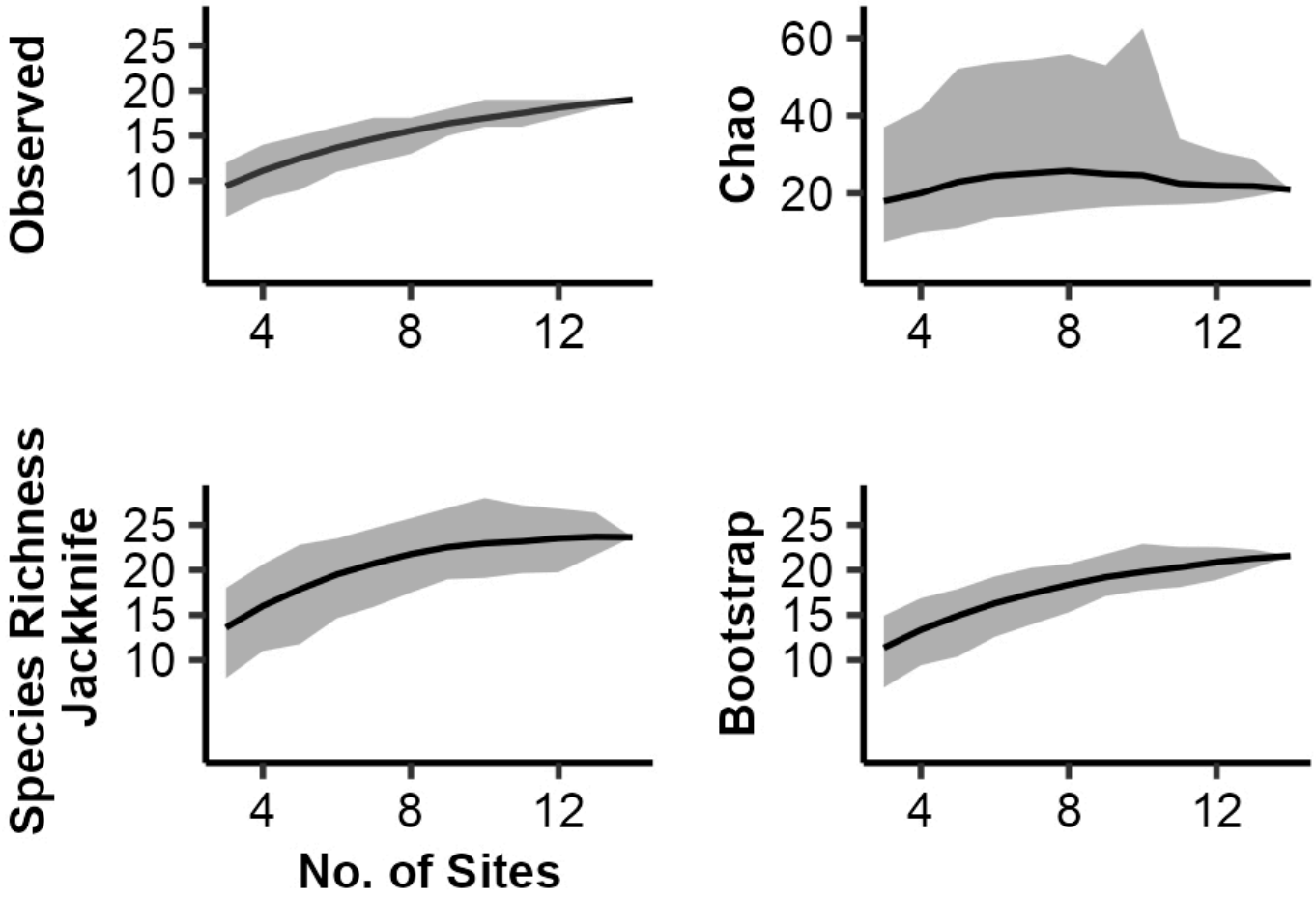


**Figure 3**

Species accumulation curve of species richness with number of sampled sites as effort

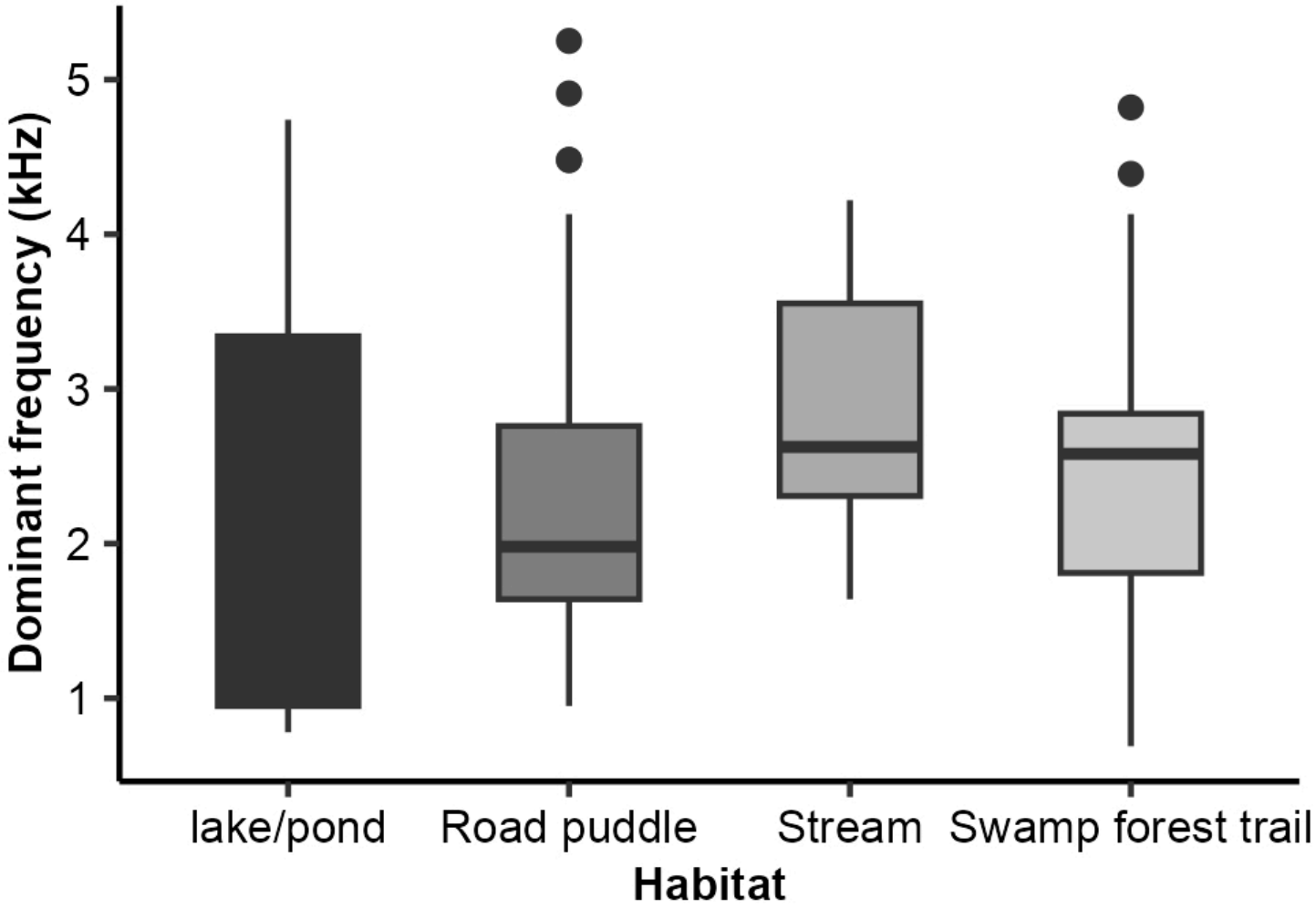


**Figure 4**

The Variation of dominant frequency of amphibians for four habitat types. The horizontal bar marks the median dominant frequency