Year-round activity patterns in a hyperdiverse community of rainforest amphibians in Madagascar

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Madagascar hosts a high diversity of amphibians estimated at over 500 species, nearly all of them endemic. Surprisingly few data are available on the activity cycles of this fauna, despite its importance for ecological, evolutionary and conservation research. Here we report the results of a year-round survey of amphibians along a transect bordering the Analamazaotra forest near Andasibe in central eastern Madagascar. During 120 transect walks evenly spaced through the year, a total of 2530 individuals of 40 species of anurans was observed. Abundance was higher during the warm/rainy season (December to April) and peaked in February. Of the five climatic predictors measured, only mean temperature and relative humidity showed high importance values, and multi-model averages indicate that these two variables have a strong effect on amphibian abundance along the transect. Species richness showed no evident peak during the study period and was best explained by a model including average temperature and rainfall. Canonical correspondence analysis indicates that \textit{Boophis sibilans}, \textit{B. tephraeomystax}, \textit{B. boehmei} and \textit{Plethodontohyla notosticta} were more frequently encountered along the transect on cold and humid days while \textit{Plethodontohyla mihanika}, \textit{Gephyromantis boulengeri} and \textit{Spinomantis aglavei} were distinctly more abundant on cold and dry days, and \textit{Paradoxophyla palmata} on warm and dry days. The results of our study flag a number of species as suitable candidates for future monitoring initiatives and suggest that a simple combination of visual and acoustic surveys can estimate amphibian activity with high sample sizes in Madagascar’s rainforests.

Keywords: amphibia; mantellidae; hyperoliidae; microhylidae; monitoring; phenology; Analamazaotra forest

Introduction

Madagascar is a biogeographic region characterized by a high diversity of amphibians. Current estimates based on DNA barcoding suggest that over 500 species might occur on the island (Perl et al. 2014). All of these frogs belong to five distinct phylogenetic clades (Crottini et al. 2012), and except for two introduced species, all are endemic to Madagascar. Species richness is unevenly distributed and mainly centered in the rainforest band stretching along Madagascar’s east coast. Particularly rich amphibian communities are known from mid-elevation rainforest sites (approximately 700–1200 m above sea level) in the central eastern part of the
island. Over 100 species are known from two sites (Vieites et al. 2009): around Ranomafana National Park; and around the village of Andasibe (comprising Analamazaotra forest and Andasibe-Mantadia National Park). Andasibe has also been highlighted as the area of highest amphibian richness in spatial analyses (Lees 1996) and served to develop the hypotheses of a geographic mid-domain effect influencing spatial richness patterns (Lees et al. 1999).

Despite the alarming rate of habitat destruction in Madagascar (Green and Sussman 1990), which is one of the most important hotspots for biodiversity conservation worldwide (Myers et al. 2000; Ganzhorn et al. 2001), its amphibian fauna remains less heavily affected by decline than in other parts of the world. According to Red List assessments (Andreone et al. 2005; Andreone, Cox, et al. 2008), only 68 of 238 species assessed were included in one of the threatened categories, and only six species qualified as Critically Endangered. Massive die-offs and declines due to pathogens have not yet been reported from Madagascar; at many sites, frogs occur in high population densities, all known species have been recently confirmed to persist in the wild (Andreone, Carpenter, et al. 2008) and until recently, the amphibian chytrid fungus was not reported from the island (Vredenburg et al. 2012).

However, as anticipated by Andreone, Cox, et al. (2008), this situation might be about to change and the island’s unique and spectacular amphibians might be heading towards a dire future. Recent studies have recorded the amphibian chytrid fungus in exported pet trade amphibians (Kolby 2014), as well as in wild Malagasy amphibian populations (Rabemananjara et al. 2011, Bletz et al. 2015 Forthcoming), reported on invasive species of crayfish (Jones et al. 2009) and toad (Kolby 2014). This together with the possible vulnerability of montane amphibians to climate change (Raxworthy et al. 2008) and the detrimental effects of habitat fragmentation (Vallan 2000, 2002; Lehtinen et al. 2003; Vallan et al. 2004; Lehtinen and Ramanamanjato 2006), coupled with continued habitat destruction (Kremen et al. 2008), suggest that massive amphibian population declines and extinctions might occur at any time. To detect such negative population trends, careful monitoring of the native amphibian species is of utmost importance.

Understanding population trends and the suitability of various monitoring techniques for different species requires a basic understanding of their natural history and, in particular, of their activity patterns. Seasonality in anurans is to a major degree influenced by variation in their breeding activity cycles and can strongly differ between and within species. One important factor along which species can be arranged is the duration of breeding activity, with prolonged breeders on one end of the continuum, and explosive breeders reproducing only on a few days every year on the other end (Wells 1977). Another factor is the timing of the breeding activity which can occur either early versus late in spring, or in summer versus winter. A third important factor is whether the breeding season of a species is mainly triggered by intrinsic or extrinsic factors. In the tropics, this factor might partly correspond to the categories of Duellman and Trueb (1994) for an Amazonian anuran community, with continuous breeders reproducing largely independent from climatic conditions, opportunistic breeders that are prepared for breeding all year round as soon as favorable environmental conditions arise, and sporadic breeders that breed only at specific times. The start of breeding activity in opportunistic and sporadic breeders can be triggered by a variety of climatic variables (Saenz et al. 2006; Wells 2007). Among the most important variables limiting breeding activity of tropical frog
species are rainfall (Donnelly and Guyer 1994; Bevier 1997; Gottsberger and Gruber 2004), temperature (Bertoluci and Rodrigues 2002; Saenz et al. 2006), or the availability of suitable sites for breeding (Sullivan 1982).

Knowledge of phenology is important when establishing monitoring protocols but also to understand the ecological and evolutionary processes regulating the assembly and dynamics of species-rich amphibian communities. Given this importance it is surprising that few data are available on activity and reproductive cycles of the herpetofauna of Madagascar. Glaw and Vences (1996) summarized the knowledge of reproductive cycles in Malagasy reptiles which were centered largely in the warm and rainy season, but with some montane species surprisingly reproducing in the cold season. Vences et al. (2004) also reported continuous reproductive activity in a small lizard from arid western Madagascar. For amphibians, most observations of calling and mating have been made in the warm/rainy season (Glaw and Vences 2007), but very few data from the cold/dry season exist at all, and the only systematic monitoring study is that of Segev et al. (2012) on the breeding phenology of the tomato frog (Dyscophus antongilii).

Here, we report on the first year-round survey of activity patterns in a species-rich amphibian community in Madagascar, based on observations along a transect established at the edge of the Analamazaotra forest near Andasibe. We apply a multivariate approach informed by information theory model selection to statistically assess the main factors triggering diversity and abundance patterns, and to classify the 40 observed anuran species based on their activity patterns.

Materials and methods

Study site and survey method

The Analamazaotra forest is located in central Madagascar south of the village of Andasibe at 930–980 m altitude (18°56.288’ S, 048°24.851’ E). The name is here used mainly to refer to a section of rainforest delimited as forest station (Dolch 2003), bordering but not included in the Andasibe-Mantadia National Park. The various forests surrounding Andasibe are known for their high biodiversity, especially in amphibians with more than 80 species of frogs reported in a 20 km radius of the village (Glaw and Vences 2007) and over 100 in a somewhat wider area (Vieites et al. 2009). For this study we selected a 2.2 km transect including sections of 1.3 km along a road and 0.9 km through Analamazaotra forest (Figure S1). The vegetation consisted mainly of secondary growth of Malagasy tree species with patches of primary forest that might have been selectively logged in the past. The forest trail included two small slow-flowing streams and several temporary ponds that completely dried during the cold/dry season. We added the forest transect to be able to also survey activity patterns of riparian and leaf-litter specialized frogs. This habitat heterogeneity along the transect will not lead to statistical biases as, on each observation day, the same transect was surveyed and only seasonal differences, not differences among habitats, are the purpose of this study. Visual encounter surveys were conducted at dusk, by observers walking along the transect for 2.5–4 hours. Frogs were searched for on the ground and in the vegetation within 2–4 m from the transect using flashlights and headlamps. A total of 120 surveys was carried out from 20 August 2011 to 6 August 2012. Representative individuals were captured by hand,
identified to species based on Glaw and Vences (2007), and released in situ. Transect walks were carried out by a team of 2–3 observers (a single observer only on six days), and in almost all cases (again, all but six days), the same observer (J.H.) lead the transect walk to avoid biases in observer capacity and search intensity. The second and sometimes third team member was mostly involved in assisting with recording and photographing specimens rather than actively searching for amphibians, so that observation intensity was largely similar among transect walks. Variation in transect walk duration was caused by increased handling time on nights with many frog encounters (i.e. recording, identifying, measuring and photographing selected frog specimens) while every effort was made to keep survey effort (i.e. walk speed and search intensity) comparable along each transect walk.

We registered climatic data with a programmed Freetec PX1117 weather station placed 4 m above the ground in an open area near the forest at 964 m altitude. Measurements of air temperature, relative humidity, barometric pressure, wind speed and precipitation were taken at intervals of 60 minutes.

**Statistical analysis**

Field count data were cross-tabulated by species and days and later processed in R (R Core Team 2014) using the package Biodiversity.R (Kindt and Coe 2005). For each transect-day we calculated the total amphibian abundance and species richness. In order to classify the temporal activity patterns, we calculated the total monthly counts per species and expressed it as observation frequencies (proportions of the species total). We then calculated the variance of the monthly abundance per species, which in this case represent the temporal dispersion of observations ($p$) (Bell 2003). On the basis of these values, we classified the activity of the species as: continuous ($p < $ first quartile); prolonged ($first > p < $ second quartile); and explosive ($p > $ second quartile). Rare species (observed only once, see Results) were excluded from these calculations. An overall species accumulation curve, describing the relationship between sampling effort and richness, was constructed from the transect-day data using a permutation procedure.

The raw climatic measurements were used to calculate daily maxima, minima and average values for each of the variables, except for precipitation. Two precipitation variables were calculated: the daily rainfall accumulates and the weekly rainfall accumulates (sum of the seven days preceding each survey). To avoid co-linearity problems we used principal component analysis to identify the less correlated and easier to interpret environmental variables. After this exploratory analysis, four variables were kept for subsequent analyses: mean daily temperature, mean daily relative humidity, mean daily wind speed, and daily accumulated rain (Table S1, Figure S2).

In addition to weather variables, lunar cycles could potentially influence amphibian behaviour and population counts although relatively few studies have addressed this effect (Grant et al. 2009, 2013). We evaluated this effect at the study site by including an additional predictor describing the illuminated fraction of the moon, calculated for each of the sampling dates at midnight [Universal Time (GMT)] using the R package ‘oce’ (Kelley 2014).

We used an information theory approach to analyze the relationship between the four environmental variables (predictors) and amphibian abundance and richness
Information theory methods rank the data-based evidence of several plausible hypotheses and thus provide quantitative measures of the strength of evidence that outperform null hypotheses testing; especially in ecological and behaviour studies (Burnham and Anderson 2002; Burnham et al. 2011).

As none of the preliminary information allowed us to discard the effect of any of the four ecological predictors and their combinations, we included all the models relating the four predictors with the observed abundance of amphibians (32 models) and, in an additional analysis, with species richness. We used the R package ‘glmulti’ which automatically generates all possible models relating predictors and the response variable and then conducts an automated model selection by ranking the models by a chosen information criterion (Calcagno and de Mazancourt 2010). We fitted generalized linear regression models with negative binomial function (nb.glm) using R package MASS (Venables and Ripley 2002). Negative binomial distribution fitted our count (discrete) data better than Poisson distribution, as evaluated by the deviance values of the most complex models. Models were ranked by their respective second-order Akaike Information Criteria (AICc) and their Akaike weights were computed. We also calculated for each model ΔAICc and evidence ratios as a measure of selection uncertainty (Mazerolle 2006). We used a multi-model inference approach, as implemented in glmulti, to estimate the relative importance of predictors (ratio of the cumulative weights of all models including the variable to those not including the variable) and the regression coefficients (β), and their associated 95% confidence intervals (CI). We considered as important those predictors with importance values above an 80% importance threshold, a level which minimizes the probability of type I and II errors in simulations with sample sizes comparable with ours (Calcagno and de Mazancourt 2010).

In order to relate species abundances with environmental predictors, we also conducted a multivariate ordination of observations. We used canonical correspondence analysis (CCA), an algorithm that produces axes that represent maximum correlations with linear combinations of the environmental variables but at the same time maximizes the correlation with additional variables (species abundances in our case) (Ter Braak 1986; Quinn and Keough 2002). CCA was conducted using Chi-squared distances between species, as implemented in Biodiversity.R after excluding from this analysis the data from the rare species (observed only on one day).

To evaluate potential differences in the importance of variables influencing abundance and richness in the climatic seasons of Madagascar, warm/rainy season (November–April) and cold/dry season (May–October), we repeated the model selection routine separately for each period. Additionally, to illustrate cyclical oscillations of abundance, we performed an autocorrelation analysis following Dornelas et al. (2012). This analysis calculates correlations of a series with itself at different time lags, which allows the estimation of how long the historical effect is felt, as well as the identification of cycles in the time series.

Results

A total of 2530 individuals of 40 species of anurans was observed during the study period (Table 1). A tendency for higher abundance was observed from December to April, with an abrupt peak in February where 152 individuals were observed on a single day (Figure 1). During this specific survey, a large breeding aggregation of
Table 1. Diversity of amphibians observed after 120 visual encounter surveys along the study transect near Andasibe, Madagascar, ordered by numbers of total observations. For each species, the abbreviation, total number of individuals observed, number of nights observed, the temporal dispersion of the observations ($p$), and the temporal activity pattern classification are listed.

| Species                        | Abbreviation | Total observations | Nights observed | Activity     | $p$  |
|--------------------------------|--------------|--------------------|-----------------|--------------|------|
| Boophis pyrrhus                | BP           | 1093               | 54              | prolonged    | 0.017|
| Boophis viridis                | BV           | 207                | 72              | continuous   | 0.003|
| Heterixalus punctatus          | HP           | 170                | 69              | continuous   | 0.003|
| Heterixalus betsileo           | HB           | 137                | 60              | prolonged    | 0.010|
| Paradoxophyla palmata          | PP           | 129                | 11              | explosive    | 0.055|
| Mantidactylus grandidieri      | MG           | 121                | 78              | continuous   | 0.003|
| Aglyptodactylus madagascariensis | AM         | 121                | 52              | prolonged    | 0.007|
| Blommersia blommersae         | SB           | 112                | 41              | continuous   | 0.005|
| Boophis madagascariensis       | BM           | 82                 | 50              | continuous   | 0.004|
| Boophis bottae                 | BB           | 47                 | 22              | prolonged    | 0.011|
| Mantidactylus femoralis        | MF           | 34                 | 28              | continuous   | 0.003|
| Spinomantis aglavei            | OA           | 31                 | 24              | continuous   | 0.010|
| Guibemantis liber              | GL           | 28                 | 25              | continuous   | 0.004|
| Mantidactylus opiparis         | MO           | 27                 | 21              | prolonged    | 0.007|
| Boophis pauliani               | BN           | 26                 | 19              | prolonged    | 0.009|
| Platypelis barbouri            | YP           | 25                 | 22              | continuous   | 0.006|
| Boophis guibei                 | BG           | 21                 | 18              | continuous   | 0.005|
| Boophis idae                   | BI           | 15                 | 11              | prolonged    | 0.008|
| Guibemantis tornieri           | GT           | 11                 | 9               | prolonged    | 0.010|
| Boophis rappiodes              | BR           | 11                 | 11              | prolonged    | 0.011|
| Boophis sibilans               | BS           | 10                 | 1               | explosive    | 0.083|
| Mantidactylus mocquardi        | MM           | 9                  | 7               | prolonged    | 0.018|
| Guibemantis sp. [Ca3]          | GV           | 8                  | 8               | prolonged    | 0.012|
| Ptychadena mascareniensis      | TM           | 8                  | 7               | explosive    | 0.027|
| Boophis luteus                 | BL           | 7                  | 7               | prolonged    | 0.017|
| Blommersia grandisonae         | SG           | 7                  | 2               | explosive    | 0.039|
| Scaphiophryne marmorata        | SC           | 6                  | 6               | explosive    | 0.028|
| Gehyromantis boulengeri        | RB           | 5                  | 5               | prolonged    | 0.011|
| Boophis boehmei                | BO           | 5                  | 5               | prolonged    | 0.018|
| Plethodontohyla notosticta     | LN           | 5                  | 4               | prolonged    | 0.018|
| Boophis picturatus             | BC           | 4                  | 3               | explosive    | 0.083|
| Boophis tasymena               | BY           | 3                  | 3               | explosive    | 0.043|
| Plethodontohyla mihanika       | LM           | 3                  | 3               | explosive    | 0.083|
| Boophis tephraeomystax         | BT           | 2                  | 2               | explosive    | 0.038|
| Boophis albilabris             | BA           | 1                  | 1               | rare         | 0.083|
| Boophis feonyala               | BF           | 1                  | 1               | rare         | 0.083|
| Boophis goudoti                | BU           | 1                  | 1               | rare         | 0.083|
| Anodontyla politcaris          | DM           | 1                  | 1               | rare         | 0.083|
| Guibemantis pulcher            | GP           | 1                  | 1               | rare         | 0.083|
| Mantidactylus melanopleura     | MA           | 1                  | 1               | rare         | 0.083|
Paradoxophyla palmata was observed along the trail; the number of individuals was estimated as 100, as no precise count was possible. An extreme value of mean daily temperature was registered on this particular day (the maximum of all sampling

Figure 1. Variation in amphibian diversity and environmental conditions from August 2011 to August 2012 along the study transect in Andasibe, Madagascar. (A) variation in amphibian abundance and species richness throughout 120 surveys; (B) variation in the four less correlated and most meaningful climatic variables during the same period: Temp = daily mean temperature, Humid = daily mean relative humidity, Wind = daily mean wind speed, Rain = daily accumulated rainfall.

*Paradoxophyla palmata* was observed along the trail; the number of individuals was estimated as 100, as no precise count was possible. An extreme value of mean daily temperature was registered on this particular day (the maximum of all sampling
days), coupled with a minimum in precipitation. In the next few days heavy rains occurred and the overall rainfall maximum was registered 12 days after what we interpret as a massive breeding event of this species, which is known to be an explosive breeder (Glaw and Vences 2007).

Species richness showed no evident peaks during the study period and oscillated around a median of six (range: 1–15 species). The maximum value of species richness matched one of the precipitation peaks registered in February, and earlier in the same month, a minimum of richness coincided with a precipitation minimum (Figure 1). Despite the considerable sampling effort, the species accumulation curve did not show signs of plateau and species richness continued to increase even after 100 samplings of the transect (Figure S3).

Overall, the most abundant species was *Boophis pyrrhus* of which 1093 individuals were observed (43% of the observations) followed by *Boophis viridis* (207 individuals), and *Heterixalus punctatus* (170 individuals) (Table 1). Based on the temporal dispersion of the observations in the transect, the following nine species can be classified as continuously active throughout the year: *Boophis viridis* (Figure 2), *Heterixalus punctatus*, *Mantidactylus grandidieri*, *Blommersia blommersae*, *Boophis madagascariensis*, *Mantidactylus femoralis*, *Guibemantis liber*, *Platypelis barbouri* and *Boophis guibei*. A group of 16 species can be considered as having a prolonged annual activity, e.g. *Boophis pyrrhus* (Figure 2). On the other end of the annual activity spectrum, nine

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**Figure 2.** Annual variation of relative abundance in eight species with more than 100 individual observations along the study transect. Note different activity patterns displayed by the species, some active year round (*M. grandidieri*), some restricted to the warm/rainy season (*A. madagascariensis, B. pyrrhus*), some active year round but with a clear peak in the late cold/dry season (*B. viridis*) or early dry season (*Heterixalus spp.*), and *P. palmata* showing an extremely seasonal, explosive breeding activity. The asterisk in the plot for *P. palmata* indicates the massive breeding of the species when the number of individuals was uncountable and estimated as 100.
species can be classified as explosive (with marked abundance peak in one or two
months): *Paradoxophyla palmata* (Figure 2), *Boophis sibilans, Ptychadena mastreadiensis, Blommersia grandisonae, Scaphiophryne marmorata, Boophis picturatus, Boophis tasymena, Plethodontohyla mihanika* and *Boophis tephraemystax*. Six species were observed only once during the study period and are considered as rare for the study transect: *Boophis albilabris, Boophis feonnyala, Boophis goudoti, Anodonthyla pollicaris, Guibemantis pulcher and Mantidactylus melanopleura*.

Four models relating amphibian abundance with the daily means of temperature, relative humidity, moonlight and wind ranked best among the candidate model set showing similar AICc values (Table 2). Of the five predictors, only mean daily temperature and relative humidity showed importance values above the 80% importance threshold (Figure 3). Multi-model averages indicate that both mean temperature (β = 0.170, 95% CI: 0.126–0.214) and relative humidity (β = 0.025, 95% CI: 0.001–0.048) have a strong effect on amphibian abundance in the transect (Table 3). The effects of moonlight, wind speed and rainfall can all be considered weak as their respective 95% confidence intervals of the multi-model averaged estimates included zero (Table 3).

Species richness was best explained by a model including mean temperature and rainfall which outperformed competing models (Table 2, Figure 3). Importance values of these two predictors were above 80% and were distinctly higher than those of the other three predictors (Figure 2). Multi-model averages indicate that the effect of mean temperature on species richness (β = 0.046, 95% CI: 0.020–0.073) can be considered strong, but the effect of rainfall (β = 0.013, 95% CI: −0.001–0.027) was relatively weak. The effects of relative humidity, wind speed and moonlight can all be considered weak (results not shown).

Identical model selection analyses, including only the data from the warm/rainy season (November–April), showed a similar pattern to the overall analysis with mean temperature and relative humidity being main predictors for abundance, and rainfall being the main predictor of species richness. On the contrary, for the cold/dry season (May–October) none of the predictors had a strong effect on either abundance or richness, suggesting that the overall pattern in our data set is mainly caused by the pattern in the data from the warm/rainy season. The autocorrelation analysis (Figure S6; relative to initial September values) revealed strong positive correlations in time lags of 1–50 days; this indicates that any two transects conducted within this time span will show a high similarity in amphibian abundance. A shift to negative autocorrelation values was observed around 80–200 days, indicating strong variations in abundance over longer time spans representing a seasonal trend every three to seven months.

Five canonical correspondence axes were derived; the first two captured 82% of the variation in the overall data. The correlations between CCA axes and the four environmental variables indicate that the first axis mainly represents changes in average temperature while the second axis mainly represents the changes in average relative humidity (Table 4). Inspection of the CCA biplot (Figure 4) shows that *Boophis sibilans, Boophis tephraemystax, Boophis boehmei and Plethodontohyla notosticta* were more frequent in cold and humid days while *Plethodontohyla mihanika, Gephyromantis boulengeri, and Spinomantis aglavei* were distinctly more abundant on cold and dry days. *Paradoxophyla palmata* was distinctly more
Table 2. Model selection results after information theory analysis of the relationship between four environmental variables and amphibian abundance and species richness in 120 surveys of the same transect along the study transect near Andasibe, Madagascar. Models are ranked by their corrected Akaike Information Criteria (AICc), indicating also the differences between a given model and the best fitting model (ΔAICc), the model weight, and the evidence ratio (ER) which is the ratio between the weights of the best model and competing models. Only the models with AICc weight > 0.01 are shown. Environmental predictors are abbreviated as follows: Temp = daily mean temperature, Humid = daily mean relative humidity, Wind = daily mean wind speed, Rain = daily accumulated rainfall, and Moon = lunar fraction illuminated.

| Rank | Model                                      | AICc  | ΔAICc | Weight | ER   |
|------|--------------------------------------------|-------|-------|--------|------|
|      | Abundance                                  |       |       |        |      |
| 1    | Abundance ~ 1 + Humid + Temp + Moon        | 920.67| 0.00  | 0.23   | 1.00 |
| 2    | Abundance ~ 1 + Humid + Temp + Wind + Moon| 921.29| 0.61  | 0.17   | 1.36 |
| 3    | Abundance ~ 1 + Humid + Temp + Wind        | 921.55| 0.87  | 0.15   | 1.55 |
| 4    | Abundance ~ 1 + Humid + Temp               | 921.73| 1.05  | 0.13   | 1.69 |
| 5    | Abundance ~ 1 + Humid + Temp + Rain + Moon | 922.87| 2.19  | 0.08   | 2.99 |
| 6    | Abundance ~ 1 + Humid + Temp + Rain        | 923.54| 2.87  | 0.05   | 4.19 |
| 7    | Abundance ~ 1 + Humid + Temp + Wind + Rain + Moon | 923.73| 3.06  | 0.05   | 4.62 |
| 8    | Abundance ~ 1 + Humid + Temp + Wind + Rain | 923.79| 3.11  | 0.05   | 4.74 |
| 9    | Abundance ~ 1 + Temp + Rain                | 925.58| 4.91  | 0.02   | 11.63|
| 10   | Abundance ~ 1 + Temp + Rain + Moon         | 925.70| 5.02  | 0.02   | 12.32|
| 11   | Abundance ~ 1 + Temp + Wind + Rain         | 925.76| 5.09  | 0.02   | 12.73|
|      | Richness                                   |       |       |        |      |
| 1    | Richness ~ 1 + Temp + Rain                 | 577.59| 0.00  | 0.30   | 1.00 |
| 2    | Richness ~ 1 + Humid + Temp + Rain         | 578.88| 1.29  | 0.16   | 1.91 |
| 3    | Richness ~ 1 + Temp + Rain + Moon          | 579.39| 1.80  | 0.12   | 2.46 |
| 4    | Richness ~ 1 + Temp + Wind + Rain          | 579.76| 2.18  | 0.10   | 2.97 |
| 5    | Richness ~ 1 + Humid + Temp + Rain + Moon  | 580.60| 3.02  | 0.07   | 4.52 |
| 6    | Richness ~ 1 + Humid + Temp + Wind + Rain  | 581.09| 3.51  | 0.05   | 5.77 |
| 7    | Richness ~ 1 + Temp + Wind + Rain + Moon   | 581.60| 4.02  | 0.04   | 7.45 |
| 8    | Richness ~ 1 + Humid + Temp                | 582.04| 4.46  | 0.03   | 9.29 |
| 9    | Richness ~ 1 + Temp                        | 582.11| 4.52  | 0.03   | 9.59 |
| 10   | Richness ~ 1 + Humid + Temp + Wind + Rain + Moon | 582.86| 5.27  | 0.02   | 13.97|
| 11   | Richness ~ 1 + Humid + Temp + Moon         | 583.43| 5.84  | 0.02   | 18.58|
abundant on warmer and dryer days (corresponding to the species’ single activity peak, see above).

**Discussion**

*Seasonal activity patterns*

Our study provides the first quantitative assessment of anuran activity patterns from a renowned center of species richness of these organisms in Madagascar (Lees 1996; Vieites et al. 2009). The data confirm a generally higher activity in the warm/rainy season (December to March, corresponding to the austral summer) assumed from previous anecdotal observations (Glaw and Vences 2007). Surprisingly, several species strongly deviate from this pattern and appear to be more active during the austral winter. The two *Heterixalus* had highest individual counts in June–July, and the peak activity of *Boophis viridis* was in September and October.

The relationship between air temperature, relative humidity and rainfall with the abundance and richness of amphibians along the transect is in close concordance with the
Table 3. Multimodel-averaged effect estimates of the five predictors related to the abundance
and richness of amphibians in 120 surveys from August 2011 to August 2012 along a transect in
Andasibe, Madagascar. Environmental predictors are abbreviated as in Table 2 and terms
showing strong effects (95% CI excluding zero) are highlighted in bold.

|             | Estimate | Unconditional variance | Nb models | Importance ± (α = 0.05) |
|-------------|----------|------------------------|-----------|-------------------------|
| Abundance   |          |                        |           |                         |
| (Intercept) | −2.435   | 1.539                  | 32        | 1.00 2.457              |
| Temp        | 0.170    | 0.000                  | 16        | 1.000 0.044             |
| Humid       | 0.025    | 0.000                  | 16        | 0.898 0.023             |
| Moon        | −0.177   | 0.042                  | 16        | 0.570 0.406             |
| Wind        | −0.034   | 0.002                  | 16        | 0.461 0.096             |
| Rain        | 0.001    | 0.000                  | 16        | 0.259 0.007             |
| Richness    |          |                        |           |                         |
| (Intercept) | 0.703    | 0.265                  | 32        | 1.000 1.020             |
| Temp        | 0.046    | 0.000                  | 16        | 0.993 0.026             |
| Rain        | 0.013    | 0.000                  | 16        | 0.872 0.014             |
| Humid       | 0.002    | 0.000                  | 16        | 0.366 0.008             |
| Moon        | −0.022   | 0.003                  | 16        | 0.295 0.100             |
| Wind        | 0.000    | 0.000                  | 16        | 0.251 0.016             |

Table 4. Scores of the canonical correspondence variables (CCA) relating environmental
predictors with amphibian species abundances in 120 surveys along the study transect near
Andasibe, Madagascar. Environmental predictors are abbreviated as in Table 2 and the most
important loadings are highlighted in bold.

|            | CCA1  | CCA2  | CCA3  | CCA4  | CCA5  |
|------------|-------|-------|-------|-------|-------|
| Humid      | −0.134| −0.951| −0.086| 0.144 | 0.226 |
| Temp       | 0.967 | 0.219 | −0.114| −0.010| −0.102|
| Wind       | −0.196| 0.211 | −0.834| 0.465 | −0.063|
| Rain       | 0.118 | −0.379| 0.329 | 0.476 | −0.713|
| Moon       | −0.239| −0.239| −0.501| −0.648| −0.460|
| Eigenvalue | 0.387 | 0.071 | 0.041 | 0.032 | 0.025 |
| Proportion explained | 0.695 | 0.128 | 0.074 | 0.058 | 0.045 |
| Cumulative inertia (variance) | 0.695 | 0.823 | 0.897 | 0.955 | 1.00  |

Preponderant effects that these bioclimatic factors have on the ecology and behaviour of
amphibians (Wells 2007). Although an increase of relative humidity is to be expected
with lower temperature due to the interdependence of these two variables, it still is worth
mentioning that mean relative air humidity was not distinctly lower in much of the cold/
dry season compared with the warm/rainy season, despite a strongly reduced amount of
rainfall. For instance, taking into account our observation days only, the mean relative
humidity was 87% in February, 93% in May and 80% in September, whereas absolute
humidity decreased from 0.018 kg/m³ in February to 0.015 kg/m³ in May, and 0.012 kg/
m³ in September. However, our autocorrelation analysis (Figure S6) suggests a rather
clear distinction of abundance data between two seasons, roughly corresponding to the
warm/rainy season (November–April) and cold/dry season (May–October). The
comparatively high relative humidity across seasons would doubtless allow for continued activity of most amphibian species, and as a testable hypothesis, we suggest that the decrease in frog individual abundance during the austral winter might be mainly attributed to low temperatures rather than to increased dryness.

Typically, reports of amphibians being affected by the lunar cycle are qualitative and many have not been statistically validated (Grant et al. 2013). Concerning the activity or abundance (number of individuals encountered per night) the published data are ambiguous, with some species showing greater abundances in full moon and others at new moon (Grant et al. 2013). Our results, obtained from statistical analysis of year-round community data, indicate a rather limited lunar effect on amphibian abundance and richness. Probably the moon effects on forest amphibians is weak, as compared with open habitat species, or is restricted to some aspects of behaviour (like calling, arrival to breeding sites or mating) which require a higher temporal resolution sampling to be properly quantified (Grant et al. 2009).
Considerations on survey techniques

A major caveat of our data is that they reflect the number of specimens observed but do not discern between breeding and other activity. For instance, the relatively constant activity observed for *Aglyptodactylus madagascariensis* most probably reflects migration and feeding, while breeding in this species is known to be explosive (Glaw and Vences 2007), probably similar to the secretive *Paradoxophyla palmata* where the bout of activity on one day was due to a large number of calling males. Also, our data do not allow us to ascertain whether the large number of *Heterixalus* in June and July are due to increased breeding or migration of specimens away from breeding waters. For *Boophis viridis*, however, it is obvious that this species calls and breeds preferentially at the end of the cold season before the onset of major rainfalls, as only at this time can larger numbers of calling males be observed (Glaw and Vences 2007). Seasonal activity patterns reported in this study should therefore not be considered as objectively defined species traits, but are clearly specific to the study region and methodology applied. It is likely that some species herein considered rare could be continuously active throughout the year (i.e. common) in other areas of Madagascar or vice versa. Furthermore, even in this same transect populational fluctuations could change the species temporal abundance pattern in future years. As temporal patterns of breeding appear to be relatively flexible in anurans and known to vary even among populations of the same species (Wells 2007), more studies will be necessary to characterize the variation in seasonality exhibited by the species studied.

A further question is how representative the chosen transect is for rainforest frog communities in Madagascar. The transect ran largely along a paved road and mostly through degraded edges of rainforest. Such disturbed forests in Madagascar are known to differ in community composition from pristine rainforest (Vallan et al. 2004) and edge effects are in general well known in tropical amphibians, although their spatial variability is often unforeseeable (Laurance et al. 2007). However, our study focused on comparing relative activity patterns rather than estimating absolute abundances, and therefore a higher species and individual abundance caused by edge effects along our transect would not be of concern since it would even allow us to better quantify activity patterns and within-year fluctuations in relative abundance.

A related consideration is that any long-term comparison in an unstable environment such as that surrounding our transect needs to carefully take habitat changes into consideration. Obviously, forest regeneration as well as further degradation might take place between years, and changes in community composition might be caused by these habitat changes rather than by any other factors that the monitoring effort would aim to capture, such as climate change or pathogen effects.

Implications for future long-term monitoring programs in Madagascar

Monitoring megadiverse areas poses a challenge for field ecology as the sampling effort necessary to achieve the desired statistical power is often impossible or economically prohibitive (Feinsinger 2001). In our study, with a substantial sampling effort, the species accumulation curve indicates that unrecorded species can be observed along the very same transect that has already been inspected a hundred
times. This suggests that additional sampling units or alternative survey methods will be required if the goal is to fully describe the extraordinary complex dynamics of this amphibian community. In a survey targeting the Betampona low-elevation rainforest, located not far from Andasibe, a saturated species accumulation course was reached in less than one year (Rosa et al. 2012) but was based on an intense survey effort combining night and day searches of a full array of available habitat, pitfall trapping and direct searches of calling frog specimens. This suggests that such integrated surveys achieve full species inventories more effectively than standardized transect monitoring studies like the one reported herein.

However, a simplified method is required for standardized monitoring activities to detect possible amphibian declines and die-offs in Madagascar (Carpenter et al. 2008; Vences et al. 2008; Andreone et al. 2012). Such monitoring needs to be established as a long-term measure, based on a sufficient ecological and phenological knowledge of the species concerned (e.g. Skelly et al. 2003; Duellman 1995), and should be based on species that are easily seen and identified, and continuously active. At Andasibe, and probably at similar sites of mid-altitude rainforest in central eastern Madagascar, according to our results this applies to Boophis viridis, B. pyrrhus, Aglyptodactylus madagascariensis (only in the warm/rainy season) and Mantidactylus grandidieri (only if calm sections of streams are included in the monitoring transects). Other species are clearly not recommendable. For highly seasonal, occasional breeders such as Paradoxophyla palmata, observations will be highly erratic and, depending on field work, coinciding with the reproductive peak. The two Heterixalus species are typical for disturbed landscapes and require transects extending to stagnant waters outside dense forest.

Numerous other amphibian survey techniques exist besides the visual encounter surveys applied herein. Especially a combination of visual encounter surveys and auditory monitoring can provide reliable estimates of site-specific amphibian biodiversity (Rödel and Ernst 2004; Dorcas et al. 2009). In Madagascar’s rainforest frog communities, surveying tadpoles was the most successful method to detect a maximum of species richness but, as a drawback, relied on molecular identification of these larvae, and bioacoustic monitoring was successful especially in arboreal and nidicolous frogs (Vences et al. 2008). Based on our anecdotal experience and the results of Vences et al. (2008), several such species in the Andasibe area emit loud and characteristic calls relatively continuously, at least in the warm/rainy season: Boophis luteus, B. bottae, Spinomantis aglavei, Gephyromantis boulengeri, Anodonthyla pollicaris and Plethodontohyla mihanika. We hypothesize these species could be easily recorded by automated sound loggers and detected, in the recordings, by bioacoustic software. Importantly, these species are complementary to those recommended herein for visual encounter surveys, suggesting that a combination of the two methods holds great promise for a consistent evaluation of population trends of 10–15 species of rainforest frogs in this part of Madagascar.

It is encouraging that the last several years have seen first steps taken in this direction. In Andasibe, local monitoring agents from Association Mitsinjo, initially trained for the study described herein, have continued conducting regular surveys to monitor their local amphibian populations in Analamazaotra forest. In the eastern lowland forest of Betampona, a regular amphibian monitoring program has also been established by the Madagascar Flora and Fauna Group as a part of a larger biodiversity monitoring and inventory project. Both of these newly created initiatives
rely on Malagasy staff to implement them, whereby local people with specialized
knowledge of their native amphibian diversity serve as para-ecologists who contribute
to the broader scientific community while also sustainably benefiting from living
within an area of extreme biodiversity. Should sufficient support and training be
available, and the basic activity patterns of local anurans understood, it seems
feasible to establish additional long-term programs in other priority areas of the
country to monitor for declines or extinctions, thereby allowing conservation mea-
sures to swiftly be enacted if needed.

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Supplemental material

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