Reproductive phenology of the tomato frog, *Dyscophus antongili,* in an urban pond of Madagascar's east coast

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Abstract. Based on daily monitoring around an urban pond in the coastal town of Maroantsetra, from 2003-2011, we provide an analysis of the yearly reproductive activity of the tomato frog (*Dyscophus antongilii*), a large-sized and prominent red-coloured microhylid frog from north-eastern Madagascar. Frogs were observed all year round but despite the limited climatic seasonality in the region it was possible to identify a high activity period between January-May and a lower activity period between June-December. Freshly laid eggs were found in all months except November, and with highest incidence between January and May, while calling was heard in all months. We found a positive correlation between daily adult counts and minimum air temperature. On the contrary rainfall did not significantly predict activity, although boosts of calling and egg-laying especially in the austral winter were observed after heavy rainfall events. We define *D. antongilii* in Maroantsetra as a sporadic wet season breeder that reproduces at irregular intervals following heavy rain events.

Keywords. Amphibia, Anura, Microhylidae, Maroantsetra, sporadic wet season breeder.

Anurans are characterized by a high variability in breeding seasonality among species, populations, and years. One end of the breeding phenology continuum constitutes of explosive breeders and the other of prolonged breeders (Wells, 1977). Environmental conditions or abiotic factors have been suggested as the determinants for anuran temporal breeding patterns (Saenz et al., 2006; Wells, 2007). Duration and timing of breeding may be limited by 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). Temporally short breeding bouts were also suggested as an adaptation to reduce predation pressures (Woodward and Mitchell, 1990; Lucas et al., 1996), cannibalism (Petranka and Thomas, 1995), and the energetic costs of reproduction (McCauley et al., 2000). In temperate and subtropical regions that are char-

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acterized by distinct dry and wet seasons, the reproduction of pond-breeding anurans occurs typically during the wet season while in those tropical and subtropical regions with little climatic variation throughout the year, more species demonstrate largely aseasonal breeding phenologies (Crump, 1974). Duellman and Trueb (1994) divided an Amazonian anuran community that reproduces all year round into *continuous* breeders that potentially breed every night, *opportunistic* breeders that breed regularly after heavy rains, and *sporadic* wet and dry breeders that breed at irregular intervals after heavy rains or throughout dry spells, respectively. They suggest that the availability of breeding sites dictates inter-species variation in breeding phenology.

The true tomato frog or northern tomato frog, *Dyscophus antongilii* is a representative of the Madagascar-endemic microhylid subfamily Dyscophinae that is related to Asian microhylids (Van Bocxlaer et al., 2006; Van der Meijden et al., 2007). *Dyscophus* is the sole genus in the subfamily, and it contains three species: *D. insularis* occurring in dry areas of Madagascar's west coast, *D. antongilii*, living in a small area of the north-east and east coast, and *D. guineti,* the false tomato frog, living in eastern mid-altitude rainforest areas (Glaw and Vences, 2007). *Dyscophus insularis* is a medium sized (40-50 mm snout-vent length) and cryptically coloured species that breeds explosively in temporary as well as permanent ponds and has small tadpoles that complete their metamorphosis very fast (Glos, 2003; Grosjean et al., 2007; J. Glos, pers. comm.), the two closely related eastern species *D. antongilii* and *D. guineti* are larger (60-101 mm SVL), bright red-orange coloured, breed in temporary as well as larger permanent ponds and have large-sized tadpoles that, as far as known, require a longer time to complete metamorphosis (Pintak, 1987; Glaw and Vences, 1994, 2007). While *D. guineti* is regularly exported from Madagascar for the exotic pat-trade and assessed within the Least Concern threat category, *D. antongilii* is considered to be Near Threatened and is listed in the Appendix I of the Convention on the International Trade in Endangered Species (CITES) (Raxworthy and Nussbaum, 2000) which largely inhibits legal exports for the pet trade taking place from Madagascar (Andreone et al., 2005, 2008). Due to the prominence of *D. antongilii* which in the area of the town of Maroantsetra and the nearby Masoala National Park is considered as an important flagship species for conservation, several studies have addressed aspects of its genetic structure (Chiari et al., 2006) and ecology (Tessa et al., 2007, 2011). Basic information on its activity, activity cycles, reproduction, and habitat choice is however still missing. Here we contribute to closing this gap in knowledge and provide an analysis on the yearly activity of a population of *D. antongilii* located in an urban area within Maroantsetra.

This study was carried out in a pond within the urban area of Maroantsetra, a small coastal town located in north-eastern Madagascar that is located within an area of very high yearly rainfall. The town consists mainly of small buildings of one or two storeys built on sandy ground with a network of open ditches running throughout the town to drain rainwater and partly sewage water. Most houses have gardens and numerous semi-abandoned parcels are scattered throughout the town. *D. antongilii* occurs at many parts within the town, breeding both in ditches and in small and large ponds.

The study pond (located at 15°25'47"S, 49°44'23"E) had a diameter of 6-7 m and was surrounded by fences and garden areas, very close to several huts and houses, partial-ly surrounded by a fence, and by some vegetation at the edges and in the water (Fig. 1), and populated by domestic ducks. This anthropogenic habitat is in the property of a local nature guide in Maroantsetra living in an adjacent house. The *Dyscophus* population found in and around the study pond was relatively large. For instance, from 12-15 February 2003 we performed intensive searches and collected 78 individuals, and on 24 February 2004 we found six males (snout-vent length 62-67 mm, hereafter: SVL) and 10 females (84-101 mm SVL) of which 3-5 were toe-clipped recaptures from the previous year. During a total of nine years (2003-2011) the local nature guide in whose property the *Dyscophus* study pond was located regularly undertook a daily monitoring of the tomato frog population in and around the pond, noting (1) the number of adult frogs

Fig. 1. Photographs of the tomato frog, *Dyscophus antongilii* and its habitat. (A) Study pond with partial view of the garden. (B) Adult male. (C) Adult female. (D) Study pond and surrounding vegetation.

observed active on the ground and in the water, (2) presence or absence of egg clutches which float on the water surface, and (3) emission of calls by the frogs. Because *Dyscophus* males often call from concealed positions and the design of our study was so to obtain daily data by untrained observers, calling intensity was estimated in four relatively rough categories: 0 = no calls heard in the respective afternoon/evening, 1 = few isolated calls heard, 2 = regular calling, 3 = large choruses. A distinction between calls of different individuals was not possible, but the calling in large choruses certainly referred to multiple individuals calling simultaneously. Presence of freshly laid eggs was scored as 0 = no eggs, 1 = few eggs, and 2 = large quantity of eggs corresponding to multiple clutches. When egg-laying took place on consecutive days, a distinction of newly laid eggs from those laid previously probably was not always possible for the untrained observers, but because the eggs hatch after 36 hours (Pintak, 1987) this possible error is limited to a maximum period of two days. Due to the limitations concerning the call and egg data, most of our statistical analyses are based on the counts of adults. Although call, egg and adult count data is available for every year from 2003 to 2011, due to logistic reasons in several years there are gaps of several months in the dataset (e.g., no complete data for January-February 2003).

For the period January 2003 to January 2004, data on daily minimum and maximum temperature (°C) as well as daily accumulated precipitation (mm) and daily mean relative humidity (%) were obtained from the meteorological station of Maroantsetra, at a distance of less than 1 km from the study site; unfortunately, these meteorological data were not available for subsequent years. We used a non-parametric test, Kruskal-Wallis, to test for difference in adult numbers between years and between months. For the correlation analysis of 2003 climatic data with the phenological data we used Pearson pairwise correlation for the adult counts and Spearman rank correlation for the categorical eggs and calls. Statistical analysis was carried out using the statistical software JMP IN version 5.0 by SAS Institute, Inc. and SigmaPlot version 10.0 by Systat software, Inc. for the exponential curve-fitting of the non-linear regression analysis. A table with all original data has been deposited in the Dryad data repository (http://dx.doi.org/10.5061/dryad.5h52h).

Our long term survey based on the data from 2003-2011 provides evidence that *D. antongilii* in Maroantsetra breed almost all year round as reflected in the temporal distribution of adults, egg clutches, and calls (Figs 2-3). The mean rank of the number of adults observed differed between months (Kruskal-Wallis; $H = 20.87$; $df = 11$; $P > 0.05$) and between years (Kruskal-Wallis; $H = 16.26$; $df = 8$; $P > 0.05$), with counts being particularly high through 2003 and particularly low through 2007 (Fig. 4). Despite the lack of apparent climatic seasonality in the region the breeding of *D. antongilii* can be roughly divided according to its activity level into a high activity period between January-May and a lower activity period between June-December (Fig. 2).

Freshly laid eggs were found in all months except November, and with highest incidence between April and May, while calling was heard in all months (Fig. 3) during both mornings and evenings. The number of adults observed was relatively stable over the years, with no trend of declining values (Fig. 4). The maximum number of adult individuals observed per day during the transect walks around the pond were 12 in 2004, 20 in 2005, 17 in 2006, 20 in 2007, 35 in 2008, 27 in 2009, 30 in 2010, and 35 in 2011.

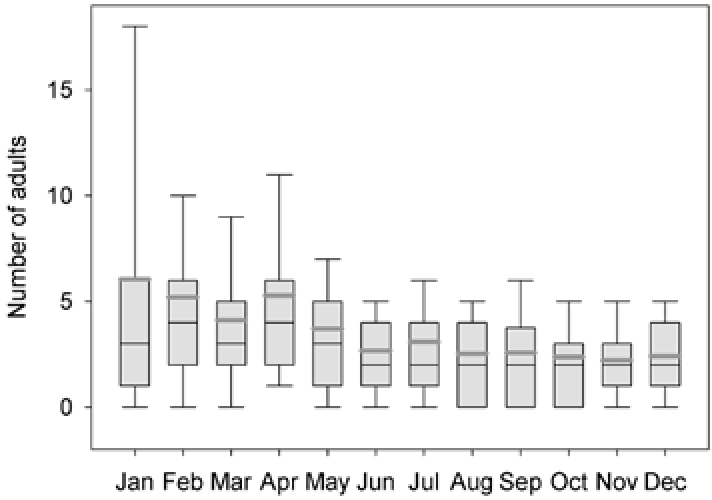

Fig. 2. Daily number of adults per month during 2003-2011. Box boundaries indicate 25^{th}-75^{th} percentiles, the black line in the box indicates the median, the grey line indicates the mean, and the error bars indicate 10^{th}-90^{th} percentiles.

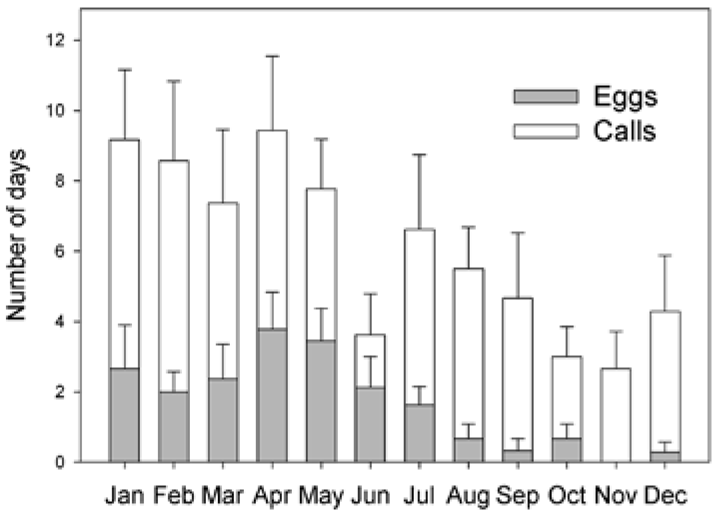

Fig. 3. Mean number of days per month (± SE) when eggs and calls were recorded, data from 2003-2011. Note that bars are not cumulative but the number of days on which calls were heard also starts from zero.

We examined how *D. antongilii* breeding phenology co-varies with climatic variables recorded at Maroantsetra meteorological station during 2003. Interestingly, in 2003, weekly rainfall in Maroantsetra was highest during some weeks of the austral winter, in June

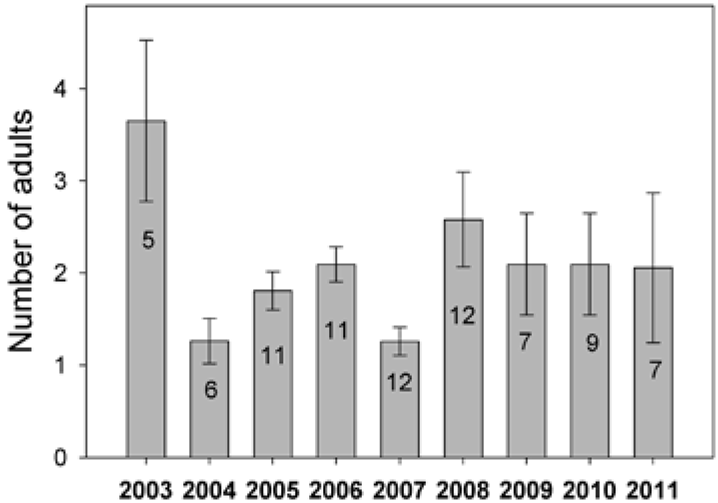

Fig. 4. Inter-year (2003-2011) variation in mean daily number (± SE) of adults per month. Numbers in bars indicate months per year included in the calculation.

Table 1. Pearson pairwise correlation of daily mean adult counts, and Spearman's rank correlation of egg clutches, and calls recorded during 2003 with air temperature (daily maximum and minimum), daily rainfall, and daily mean relative humidity recorded at Maroantsetra during 2003 (n = 10 months with frog data available, i.e., March-December).

	Mean	SD		Correlation coefficient	p
Maximum air temperature (°C)	28.92	2.51	Adults	0.44	0.204
			Eggs	0.265	0.459
			Calls	-0.182	0.614
Minimum air temperature (°C)	21.7	2.13	Adults	0.657	0.039
			Eggs	0.524	0.120
			Calls	0.024	0.947
Rainfall (mm)	17.14	10.91	Adults	-0.016	0.966
			Eggs	0.265	0.459
			Calls	0.194	0.590
Humidity (%)	82.1	2.29	Adults	0.059	0.870
			Eggs	0.226	0.530
			Calls	0.085	0.815

and July. This period of intense rainfall was then followed by breeding activity, with calls and eggs recorded. However, the more continuous breeding activity at constant high levels was observed in the austral summer, between March and May. Consequently, we found a positive correlation between mean adult counts, and minimum air temperature (Fig. 5). Following this correlation analysis we performed a regression of daily mean adult counts versus daily minimum air temperature during 2003. Adult activity increased nonlinear-

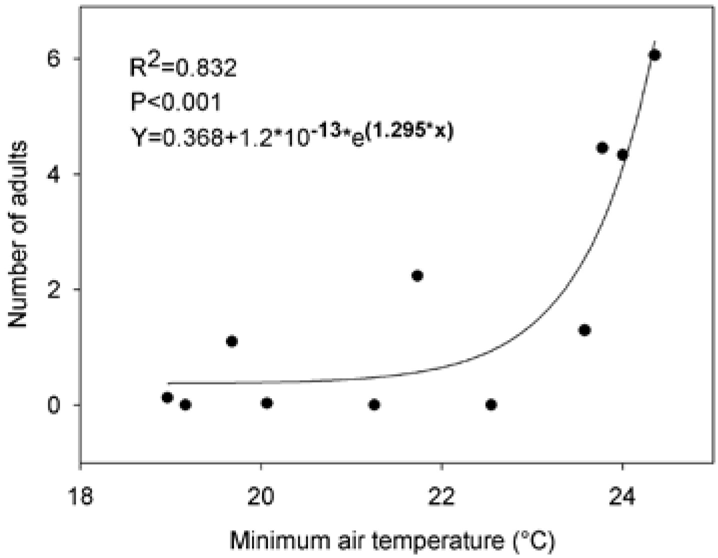

Fig. 5. Relationship between daily mean number of adults per month (n = 11) versus minimum daily air temperature (°C) recorded at Maroantsetra during 2003.

ly with minimum temperature, and above 23°C there was an exponential increase in the mean daily number of adults (Fig. 5).

Northern Madagascar has two seasons, a hot, wet summer that lasts from November to April and a cooler, dry winter that lasts from May to October. The island climate is dominated by the south-eastern trade winds originating from the Indian Ocean. The eastern coast and particularly the bay of Antongil area are exposed to almost constant blowing of these winds which carry hot humid air and heavy rains. Despite the high research intensity recently targeting Madagascar's amphibian fauna the phenology of these animals has been little studied, with most of the breeding information collected during the wet season. Malagasy reptiles reproduce typically during the wet season (Glaw and Vences, 1996) but breeding can also take place during the dry season, both along the west coast and on higher elevation mountains (Vences et al., 2004). In areas of higher elevations, e.g. around 900 m above sea level where the highest species diversity of frogs occurs, low temperatures in the austral summer may be an important limiting factor for breeding activity. At low-elevation coastal areas, temperature is high year-round, but we nevertheless found that adult counts in Maroantsetra co-varied with minimum air temperature, and breeding activity was not limited only to the wet season as evidenced by records of freshly laid eggs and calling individuals in all seasons (Fig. 3). The bay of Antongil area is featured by tropical wet and warm winter with no real dry season, mean annual temperatures of 24 °C and annual rainfall above 3000 mm. The combination of high temperatures and high rainfall during the entire year probably enables year-round reproduction for *D. antongilii*. Adopting the reproduction mode division of Duellman and Trueb (1994) we thus define *D. antongilii* in Maroantsetra as a sporadic wet season breeder that breeds at irregular intervals.

A better knowledge of the phenology of *Dyscophus antongilii* may have conservation implications beyond the species biology. Understanding the interactions between climatic conditions, biological parameters (such as calling, mating, egg-laying), and their respective seasonality can serve to fine tune conditions for captive breeding, in case the species needs to be kept in captivity. One suggested factor driving the global decline of amphibian populations is the chytrid fungus (*Batrachochytrium dendrobatidis*) (Lips et al., 2006). Global warming and changes in the thermoregulatory regimes of species might facilitate the global spread of the disease, with possible severe consequences for Madagascar's amphibians. In fact, the tomato frog has been shown to be susceptible to chytrid infection in captivity (von Oevermann et al., 2005), and we can thus foresee that it might be one of the Malagasy species affected by the pathogen in case of chytrid introduction to Madagascar (Andreone et al., 2012). Human disturbance, as in our highly anthropogenic study site, does in principle not have negative consequences for *D. antongilii* conservation as indicated by the absence of a distinct decline in individual counts over the years (Fig. 4). In Maroantsetra, just close to the current study site, a small urban protected area has been purchased and enriched with artificial ponds (Andreone, 2008) specifically for tomato frog conservation. Our data suggest that it will probably be rather easy to ensure continuous reproduction of tomato frogs at such artificially created breeding sites. A crucial subject of future studies will be to obtain information how the development and survival of eggs, tadpoles and juveniles might be influenced by the disturbances prevailing in these urban environments (such as presence of ducks, sewage waters, or usage of fertilizers), in order to be able to boost the reproduction of this species under controlled conditions.

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