No evidence for precipitous declines of harlequin frogs (Atelopus) in the Guyanas

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The species-rich Neotropical harlequin frogs (Atelopus) have experienced drastic population reductions that some herald as potential extinction at the genus level. Principal causes for this included an emerging infectious disease, chytridiomycosis, caused by the chytrid fungus, and climate change. Responses by Atelopus species typically involve rapid “population crashes”. We here report on two populations of Atelopus hoogmoedi from Suriname and Guyana which show densities similar to other members in the genus before “population crashes”. Further, tests for the chytrid fungus proved negative, suggesting that it may currently be absent at these two locations. Our findings indicate that A. hoogmoedi may currently be escaping extinction processes plaguing the genus elsewhere. Nevertheless, this may change rapidly, as has been shown in other Atelopus. For this reason, we strongly encourage proactive conservation measures before the pattern observed in so many congenerics is played out again.

Keywords: amphibian decline; Batrachochytrium dendrobatidis; extinction; population monitoring

Introduction

The Neotropical harlequin frogs (Atelopus) are bufonid lineage including a known 113 species (some of which are undescribed; La Marca et al. 2005), many of which are known from one to a few localities only. These diurnal, slow-moving and often colorful animals were partly common in the past (e.g. Ron et al. 2003; Rueda-Almonacid et al. 2005). However, within the last decade populations of many species have rapidly and drastically declined, including some located in relatively pristine areas (e.g. La Marca et al. 2005). These on-going extinction processes render survival of harlequin frogs questionable (e.g. La Marca et al. 2005; Löters et al. 2005a; Rueda-Almonacid et al. 2005; Löters 2007). During the 2002-2004 IUCN/Conservation International/NatureServe Global Amphibian Assessment (GAA: www.globalamphibians.org; Stuart et al. 2004), 77 Atelopus described species were allocated to IUCN Red List categories: of these, three were categorized as “Extinct” and a further 62 were categorized as “Critically Endangered” and possibly extinct (Löters 2007). La Marca et al. (2005) reported that in at least 42 of 113 Atelopus species, population sizes have decreased and at least 30 species have not been recorded since the end of the 1990s. Causes of Atelopus extinction trends are currently under discussion and possible drivers include an aggressively invasive chytrid fungus (Batrachochytrium dendrobatidis = Bd) and global warming (Ron et al. 2003; La Marca et al. 2005; Pounds et al. 2006; Lips et al. 2008).

Are there still Atelopus species that have not experienced rapid and catastrophic reductions in numbers? The GAA classified three species as “Data Deficient” and nine were ranked below “Critically Endangered” (Table 1). The latter group includes some species which appear to exhibit relatively large distributions, an exception for most Atelopus species. As small population size and reduced area of occupancy are both recognized as indicators of risk of extinction (IUCN 1994), large species distribution should buffer against any processes that have caused sharp declines in less broadly distributed Atelopus species. However, the taxonomy of these nominal species and their actual distributions remain largely unresolved (Table 1). Among the least understood Atelopus are those from the Amazon basin and the Guyanas (Boistel et al. 2005; Löters et al. 2002; Noonan & Gaucher 2005), including Atelopus spumarius which the GAA treated as a less-threatened and widespread species (Table 1). This harlequin frog is actually a species complex, taxa of which show allopatric distributions in the upper and lower Amazon basin and the Guyanas (Löters et al. 2002; Noonan & Gaucher 2005; S. Löters, unpublished data from DNA barcoding). One of

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Table 1. Atelopus species which have been categorized as "Endangered" (EN) or "Vulnerable" (VU) during the 2002-2004 GAA (www.globalamphibians.org; Rueda-Almonacid et al. 2005) and information on the geographical ranges encompassed by them (i.e. "small" or "large", using 2500 km² as calibration; source: GAA, for A. limosus: R. Ibáñez, personal communication) and whether their taxonomic status can be considered sufficiently resolved according to Lötters (1996), Lötters et al. (2002, 2005b), Boistel et al. (2005), Noonan & Gaucher (2005), R. Ibáñez (personal communication) and S. Lötters (unpublished data).

<table>
<thead>
<tr>
<th>Species</th>
<th>IUCN Red List status</th>
<th>Distribution</th>
<th>Country</th>
<th>Size</th>
<th>Taxonomy</th>
</tr>
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<tbody>
<tr>
<td>A. certus</td>
<td>EN</td>
<td></td>
<td>Panama</td>
<td>Small</td>
<td>Resolved</td>
</tr>
<tr>
<td>A. dimorphus</td>
<td>EN</td>
<td></td>
<td>Peru</td>
<td>Small</td>
<td>Unresolved</td>
</tr>
<tr>
<td>A. flavicans</td>
<td>VU</td>
<td></td>
<td>French</td>
<td>Large</td>
<td>Unresolved</td>
</tr>
<tr>
<td>A. franciscus</td>
<td>EN</td>
<td></td>
<td>Guyana</td>
<td>Large</td>
<td>Resolved</td>
</tr>
<tr>
<td>A. limosus</td>
<td>EN</td>
<td></td>
<td>Colombia</td>
<td>Large</td>
<td>Resolved</td>
</tr>
<tr>
<td>A. longibrachius</td>
<td>EN</td>
<td></td>
<td>Amazon</td>
<td>Large</td>
<td>Unresolved</td>
</tr>
<tr>
<td>A. spanarius</td>
<td>VU</td>
<td></td>
<td>Guyana</td>
<td>Large</td>
<td>Resolved</td>
</tr>
<tr>
<td>A. spurrelli</td>
<td>VU</td>
<td></td>
<td>Colombia</td>
<td>Large</td>
<td>Unresolved</td>
</tr>
<tr>
<td>A. tricolor</td>
<td>VU</td>
<td></td>
<td>Bolivia</td>
<td>Large</td>
<td>Resolved</td>
</tr>
</tbody>
</table>

these is Atelopus hoogmoedi, native to portions of French Guyana, Suriname, Guyana and adjacent Brazil (Noonan & Gaucher 2005; Lötters et al. 2005b). Detailed population information is not available for this species, as is the case for all harlequin frogs listed in Table 1. Here we provide the first data on apparently intact and probably Bd-free populations of A. hoogmoedi from Brownsberg Nature Park (BNP), Suriname, and Mabura Hill Forest Reserve (MHFR), Guyana.

Materials and methods
At Koemboe Creek, BNP (4°56’39.8”N, 55°11’47.7”W; ca. 370 m above sea level), population surveys were undertaken from 19 April to 18 August 2004. A 1000 m² plot in primary (riparian) forest was surveyed every second to fourth day. All Atelopus hoogmoedi specimens found were individually recorded through photographing of the dorsal color pattern and their locations were marked on the ground with labeled flags. Field data for MHFR (5°09.322”N, 58°41.983”W; ca. 60 m above sea level) were acquired between 10 November 2002 and 8 September 2004, covering both the dry and wet seasons. Wet season data were collected for a period of 8 months; dry season data covered 4 months. We established 11 transects, six in primary forest and five in exploited forest, respectively. Each rectangular transect had a total length of 600 m. Transects were subdivided into 25 m subunits (SU; i.e. 24 per transect). Standardized visual and acoustic transect sampling (SVTS and SATS) were applied on a weekly basis throughout the entire study period. All individuals encountered during SVTS were marked, and recaptures consequently excluded from time-based density calculations. Detailed descriptions and discussion of the transect design, data acquisition routine, tests for spatial autocorrelation and an evaluation of various methods have been published earlier (Rödel & Ernst 2004; Ernst & Rödel 2005; Ernst et al. 2005).

During the survey at the BNP, toe tips of 19 individuals were clipped and stored in 98% ethanol for screening for the presence of Bd. Ten samples collected in 2003 at the same locality and five from MHFR were also available for testing. Samples were screened for Bd using quantitative real-time polymerase chain reaction of the ITS-1/5.8S ribosomal DNA region of Bd (Boyle et al. 2004). We used replication, four concentrations of standards and negative controls, and followed the same criteria for exclusion used by Garner et al. (2006).

Results
Our BNP surveys revealed a total of 57 specimens (19 males, one female, 37 juveniles) at a mean density of 0.007 ± 0.003 individuals/m² (range 0.001-0.013; n = 37 observation days). In Guyana (MHFR) we recorded a total of 202 Atelopus hoogmoedi individuals (all males) during 393.5 h of SVTS and SATS, equalling 0.513 individuals per transect hour (th). Most of the individuals (184 or 0.641 individuals/th) were encountered during the wet season. Only 18 (0.169 individuals/th) were registered during the dry season. Time-based density measures as given above translate into area-based density measures as follows: total = 0.042 individuals/m²; wet season = 0.038 individuals/m²; dry season = 0.004 individuals/m². In addition to the males recorded during transect walks, two amplexant pairs were encountered during the entire study period. The first pair was found on a laterite slope in Mixed Greenheart (Chlorocardium rodiei) forest on 12 December 2002 and the second on a lateritic ascend in the vicinity of a black water creek in Mora excelsa forest. However, reproduction was not confirmed. All attempts to amplify Bd were unsuccessful.

Discussion
The relative rarity of females in Atelopus hoogmoedi, which we attribute to spatial partitioning of the sexes outside the breeding season as sexual segregation
during non-reproductive periods, is also known for other Atelopus species (Lötters 1996). At the very least, the presence of frogs at BNP suggest that reproduction had taken place shortly before the survey was carried out since larval development in Atelopus lasts about 2 months (e.g. Lötters et al. 2002).

Because our monitoring results are “snap-shots” in time, no long-term population trends could be estimated. However, A. hoogmoedi in this study was found in numbers similar to those recorded for other Atelopus species before catastrophic declines and far greater than those recorded after “population crashes” in numerous species of the genus. La Marca et al. (2005) provided population data for nine Atelopus species from 10 localities which were abundant in the past but have not been traced in recent years despite extensive efforts. Our findings from two different sites in the Guyanas lead us to conclude that in contrast to other harlequin frogs, A. hoogmoedi – at least locally – has not experienced the same rate of rapid and drastic decline exhibited by Atelopus species categorized as “Critically Endangered”.

Using the formula from DiGiacomo and Koepsell (1986), a sample size of 19 specimens analyzed for the presence of Bd (i.e. BNP individuals sampled in 2004) would be sufficient to detect infection with 95% confidence if the actual prevalence in the population was 15%, with 80% confidence if prevalence was 8%, and with 65% confidence if prevalence was 5%. By comparison, this sample size was sufficient to directly detect infection in declining Atelopus spp. discussed in La Marca et al. (2005). To the best of our knowledge Bd has never been detected in amphibians in the Guyanas, so we preliminary conclude that Bd is absent from the A. hoogmoedi population at BNP but await further sampling before we conclude the same for the MHFR.

Although we find no evidence that A. hoogmoedi at two localities in Suriname and Guyana has experienced “population crashes”, we still believe that immediate and substantial conservation effort is urgently required to ensure the species’ survival. Climate change occurs at a global scale, and processes described by Pounds et al. (2006) and Bosch et al. (2007) are expected to influence A. hoogmoedi habitat. Moreover, although the species’ range is relatively large within the Guyanas, it is patchily distributed (see Noonan & Gaucher 2005) as are many amphibian species in this region (cf. Wynn & Heyer 2001). Range in this case is a rather misleading estimator of species density and population size. Human impacts on habitat in the region include forest over-exploitation (Ernst et al. 2005, 2006; Ernst & Rödel 2008) and the increasing effects of small-scale gold mining along forest streams (Peterson & Heemskerk, 2001) which are utilized by A. hoogmoedi for breeding (M. Luger, unpublished data). Further, Bd has the ability to emerge and cause substantial declines in biomass and diversity in a matter of months (Lips et al. 2006, 2008) and there are no standards in place to prevent the introduction of Bd into the region: the possibility exists that the pathogen is already in place and has simply not yet emerged in this species. It is essential that quarantined captive breeding colonies be established for this species, and ideally for all the species listed in Table 1 and that in situ habitat monitoring and disease screening be implemented.

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References


