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# Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection

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**Abstract**

**Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection**. The Neotropics shelter the highest number of frog species on Earth and is also one of the regions where anurans are most threatened. Nonetheless, few data are available to assess the population status of Neotropical anurans. We studied three populations (Tresor, Favard, and Nouragues) of the poison frog, *Dendrobates tinctorius,* in French Guiana and used Capture-Mark-Recapture (CMR) to make robust estimations of the species' density at these three sites. In addition, we assessed the prevalence of the pathogen fungal *Batrachochytrium dendrobatidis* (*Bd*) in two populations (Favard and Nouragues). Based on the CMR protocol, the densities of frogs was 8.43 individuals/100 m² at Favard, 4.28 individuals/100 m² at Nouragues and from 2.30 to 4.67 individuals/100 m² at Tresor (depending on the CMR model used); these data provide a baseline for population densities of *D. tinctorius* in French Guiana, against which future population estimates can be compared. We found that 25 encounter events may be sufficient for stable population estimates, if the captures are concentrated in time. *Bd* was detected at both sites (Favard 7/152; Nouragues 3/18).

**Keywords:** amphibian conservation, *Batrachochytrium dendrobatidis,* CMR, Neotropics, picture-identification.

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**Resumo**

**Estimativas populacionais de *Dendrobates tinctorius* (Anura: Dendrobatidae) em três áreas da Guiana Francesa e primeiro relato de quitridiomicose.** A região Neotropical abriga o maior número de espécies de anuros da Terra e é também uma das regiões em que os anfíbios estão mais ameaçados. No entanto, poucos dados estão atualmente disponíveis para avaliar a situação das populações de anfíbios neotropicais. Estudamos três populações de *Dendrobates tinctorius* na Guiana Francesa (Tresor, Favard e Nouragues) usando o modelo de Captura-Marcação-Recaptura (CMR) para a realização de estimativas robustas da densidade da espécie nesses três locais. Além disso, avaliamos a prevalência do fungo patogênico *Batrachochytrium dendrobatidis* (*Bd*) em duas populações (Favard and Nouragues). O uso do modelo CMR revelou uma densidade de 4,67 indivíduos/100 m² para Tresor, 8,43 indivíduos/100 m² para Favard e 4,28 indivíduos/100 m² para Nouragues, fornecendo estimativas das densidades populacionais de *D. tinctorius* na Guiana Francesa com as quais estimativas populacionais futuras poderão ser comparadas. Constatamos que 25 ocasiões de encontro podem ser suficientes para estimativas de uma população estável se as capturas forem concentradas no tempo. *Bd* foi detectado em ambas as áreas (Favard 7/152, Nouragues 3/18). Propomos, portanto, o início de um acompanhamento de longa-duração dessa espécie em vários locais (dentro e fora de áreas protegidas) da Guiana Francesa, combinando estudos de CMR com ocasiões de encontros concentrados em um curto período de tempo e o monitoramento de *Bd*.

**Palavras-chave:** *Batrachochytrium dendrobatidis,* CMR, conservação de anfíbios, foto-identificação, região Neotropical.

## Introduction

Anurans face an extinction crisis that threatens up to 50% of the 6000 species described to date; this represents the greatest potential loss of biodiversity of any vertebrate class since the mass extinction of the Cretaceous (Stuart *et al.* 2004, Gewin 2008). Threats to lissamphibian diversity are numerous (Collins and Storfer 2003, Fisher *et al.* 2009), and over-exploitation and habitat reduction are thought to have caused many declines. More recently, the importance of an emerging infectious disease, *Batrachochytrium dendrobatidis* (*Bd* hereafter), has been recognized as an important cause of decline (Lötters *et al.* 2009). As monitoring for *Bd* infections has increased, the disease has been discovered to be geographically widespread (Fisher *et al.* 2009). The numerous threats, especially to lissamphibian species of the Neotropics, require urgent initiatives to gather data on population dynamics (Young *et al.* 2001).

The Capture-Mark-Recapture (CMR) technique is the most robust way to estimate population density and follow it through time, but it requires recognizing individuals and this is not always easy to achieve. A variety of techniques is available for individual recognition in anurans. For adults, toe clipping is commonly used; however, this seems to affect the return rate and may affect survival rates (Clarke 1972, McCarthy and Parris 2004). Although Passive Integrated Transponders (PIT) tags have proved to be effective for anurans (Brown 1997), their use is limited to larger anurans (i.e., > 4 cm snout–vent length). The use of color pattern via picture identification (Kenyon 2009) also has been used to identify adults of several tropical anuran species (e.g., Lampo *et al.* 2011). It seems to be particularly well adapted for species harboring a high level of variation with regard to both color and pattern such as dendrobatid frogs (Lötters *et al.* 2007; Wollenberg *et al.* 2008).

*Dendrobates tinctorius* occurs in the eastern part of the Guiana Shield (southeastern Guyana, Suriname, French Guiana, and extreme northeastern Brazil), and is a member of the Neotropical poison frog family Dendrobatidae.

It is terrestrial and diurnal, and is easily recognized by its bright coloration and its individual dorsal and ventral patterns. The distribution of *D. tinctorius* seems to be tightly correlated with tropical forests at elevation more than 100 m above sea level (Noonan and Gaucher 2006). To our knowledge, infection of *D. tinctorius* by the *Bd* fungus has never been recorded in the wild, but infections have been found in captive specimens in Europe (Der Sluijs *et al.* 2011). Moreover, individual *D. tinctorius* experimentally exposed to *Bd* developed chytridiomycosis (Nichols *et al.* 2001). Gross lesions were subtle, usually affected the legs and ventrum, and consisted of mild skin thickening and discoloration. In the U.S., individuals of *D. tinctorius* died 23 and 31 days after being exposed to broth culture of *Bd* (Longcore *et al.* 1999).

We report here results of a study of three distinct populations of *Dendrobates tinctorius* in a tropical forest in French Guiana. Our aim is to (1) estimate the sizes of the three populations to provide baseline data for future long-term studies, and (2) assess the prevalence of *Bd* in wild populations of *D. tinctorius*.

## Materials and Methods

### *Study Sites*

The three sites used in this study are located in three protected area in French Guiana—Favard Mountain in the Kaw-Roura Marshes Natural Reserve (04˚30'30.5" N, 52˚02'58.6" W), Tresor Natural Reserve (04˚34'60.00" N, 52˚16'00" W) and Nouragues Natural Reserve (04˚02'00" N, 52˚41'00" W). In Favard and Nouragues, the study was conducted from April 2009 to April 2010 and in Tresor, the study was conducted in May 2009. Elevations for the three sites range from 100–200 m above sea level.

### *CMR Protocol*

To estimate the size of the population of *Dendrobates tinctorius*, we used a CMR protocol along transects in each site based on Visual Encounter Survey (VES; Rödel and Raffael 2004). A sampling event was defined as one walk across a transect. During surveys, frogs within 1 m on each side of the transect were caught, and images of the ventral, dorsal and lateral patterns were recorded for individual identification.

At Tresor (transect = 1.5 km), surveys were conducted daily for 1 mo ($N$ = 29 encounter events), whereas at Favard (transect length = 1.5 km) and Nouragues (transect length = 8 km), surveys were done once a week for 1 yr ($N$ = 47 encounter events for Nouragues and $N$ = 48 encounter events for Favard).

### *Sampling for* Batrachochytrium dendrobatidis

At Favard and Nouragues, we sampled adult *D. tinctorius* from February 2009–April 2010 for *Bd*. Individuals were caught and their feet and bellies rubbed with sterile swabs (MW 100–100, Medical Wire and Equipment, Bath) to detect *Bd* infection. Swabs were kept in the shadow in the field and stored at 4˚C after return from the field. After swabbing, frogs were released at the point of capture. In total, we obtained 152 samples for the population at Favard and 18 for Nouragues. Swabs were tested for *Bd* using quantitative real-time PCR (qPCR) as described by Hyatt *et al.* (2007). For this purpose, *Bd* DNA was extracted using a PrepMan extraction and run in duplicate. Each plate contained negative controls and included an internal positive control (Internal Positive Control Reagents, Applied Biosystems) to signal amplification inhibition. Standards of known *Bd* quantity are used to construct a quantification curve on each plate to determine *Bd* load (in Genome Equivalent–GE).

### *Statistical Analysis*

Live encounter histories were constructed for each individual at each site based on whether they were detected (1) or remained undetected

(0) during each sampling session. We used the software CloseTest (Stanley and Burnham 1999) to test whether the populations were closed. We also tested that every marked individual in the population had the same the probability of capture and survival as unmarked individuals using the TEST 2 of the Goodness-of-fit (GOF) test implemented in the package RMark of the R software (http://cran.r-project.org/).

To select the best model for population estimates, we used the CAPTURE software included in MARK software (White and Burnham 1999). Models tested are: M(0) Null model with no temporal and individual heterogeneity in capture probability; M(h) Jackknife model with individual heterogeneity in capture probability; M(b) Zippen model with behavioral changes after the first capture; M(t) Darroch model with temporal heterogeneity in capture probability; M(bh) Removal model with behavioral changes and individual heterogeneity in capture probability; M(th) Chao model with temporal and individual heterogeneity in capture probability; and M(tbh) a model taking into account the three factors of variability (temporal, individual and behavioral) in capture probability. Selection of the best model is based on the likelihood criterion.

To test the number of encounter events necessary for a stable estimation of population size, we used the package RMark implemented in the R software (http://cran.r-project.org/). We computed the population size (*N*) and the standard error for *N* for an increasing number of encounter occasions (from 10 to the total number of encounter occasion) using the null model for each site.

## Results

A total of 152 adults was identified at Favard, 166 at Nouragues, and 39 at Tresor. Dorsal pattern together with ventral and lateral patterns allowed for the unequivocal identification of each individual. The results from the program CloseTest suggested that all three populations were closed during the study periods (Stanley and Burnham closure test, Favard F = 55.4, $p = 0.22$; Nouragues F = 36.1, $p = 0.83$, Tresor F = 18.8, $p = 0.88$). The GOF test failed to detect evidence of unequal capture or survival probability between marked and unmarked individuals in all three populations (Favard $x^2 = 13.7$, df = 32; $p = 0.998$, Nouragues $x^2 = 2$, df = 18; $p = 1$; Tresor $x^2 = 0.7$, df =12; $p = 1$). For Favard and Nouragues, the best model to estimate the population size was the Darroch model M(t) with temporal heterogeneity in capture probability (Table 1). With this model, population size for Favard was 253 (standard deviation [SD] = 21) and for Nouragues 684 (SD = 129). For Tresor, the best model was the Jackknife model M(h) with heterogeneity in capture probabilities (Table 1), which led to the estimation of a population of 140 individuals (SD = 35). For Tresor, likelihood of the null model M(0) is close to the likelihood of the jackknife model M(h) (0.96 vs. 1) and it led to an estimation of 69 individuals (SD = 12).

By assuming that individuals were detected in a range of 1 m on each side of the transect, we estimated that the density of individuals was 4.67 individuals/100 m² for Tresor (2.30 individuals/100 m² when using estimation from the null model), 8.43 individuals/100 m² for Favard and 4.28 individuals/100 m² for Nouragues. For Tresor, a stable estimation of the population size was achieved with 25 encounter events with a relatively low error, whereas for Favard and Nouragues, the estimated population sizes were still increasing with more than 25 encounter events (Figure 1).

We detected *Bd*-positive individuals both in Favard (7 *Bd*-positive on 152) and Nouragues (3 *Bd* positive on 18) population. At Favard mean *Bd* load was 147 GE (±200) and at Nouragues 0.9 GE (±0.8). Four of the Favard individuals had a high genome equivalent of *Bd* (>10 genome equivalents), whereas in three cases, *Bd* concentration was at its level of detectability (but double positive).

**Table 1.** Maximum likelihood criteria for model selection of the three populations. M(0) – Null model with no temporal and individual heterogeneity in capture probability. M(h) – Jackknife model with individual heterogeneity in capture probability. M(b) – Zippen model with behavioral changes after the first capture. M(t) – Darroch model with temporal heterogeneity in capture probability. M(bh) – Removal model with behavioral changes and individual heterogeneity in capture probability. M(th) – Chao model with temporal and individual heterogeneity in capture probability. M(tbh) – a model taking into account the three factors of variability (temporal, individual, and behavioral) in capture probability.

| | M(0) | M(h) | M(b) | M(bh) | M(t) | M(th) | M(tb) | M(tbh) |
|---|---|---|---|---|---|---|---|---|
| Favard | 0.15 | 0 | 0.3 | 0.06 | 1 | 0.75 | 0.47 | 0.34 |
| Nouragues | 0.13 | 0 | 0.05 | 0.07 | 1 | 0.63 | 0.28 | 0.18 |
| Tresor | 0.96 | 1 | 0.49 | 0.69 | 0 | 0.55 | 0.41 | 0.76 |

## Discussion

Our estimates of population densities of *Dendrobates tinctorius* results in three populations in the tropical forest of French Guiana provide a baseline for population densities of this species against which future population estimates can be compared. One potential problem with these models is that they assume no losses or gains during sampling events. This is especially true for Nouragues and Favard, where our study was conducted for 1 yr increasing the probability of individual death, immigration, or emigration in the population. Moreover, conducting a survey for such a long period led to a temporal variation in the probability of capture, because the activity patterns of *D. tinctorius* varies seasonally (Born *et al.* 2010). Therefore, results may be biased by the effect of a potential spatial aggregation during the breeding period and probably dispersion outside the breeding season. In addition, estimated population size was more precise and requires fewer encounter events when the study was conducted during a shorter period such as the protocol proposed in Tresor. At this site, a stable and precise estimation of the population size can be achieved with 25 encounter events. For future CMR studies on *D. tinctorius,* we recommend concentrating the encounter events in a smaller period of time, preferably in the rainy season when the activity of this species is greater (Born *et al.* 2010).

The mean estimated density of *Dendrobates tinctorius* is 5.7 individuals/100 m², a density comparable to the one observed in other species of *Dendrobates,* such as *D. pumilio* (2.3 individuals/100m² in primary forest and 6.8 individuals/100 m² in secondary forest; Pröhl 2002). Moreover, we observed a high variation among sites. Differences in resources or microhabitat availability among sites may explain such variations and further studies are needed to determine the parameters that drive *D. tinctorius* distribution. In *D. pumilio,* the abundance of tadpole-rearing sites seems to have a significant effect on population density (Pröhl 2002) and this factor could also be of importance for *D. tinctorius*.

We detected the presence of *Bd* in two wild populations of *Dendrobates tinctorius,* with relatively low infection intensities in most of the individuals. No mortalities were observed. The Guiana Shield has not been highlighted as susceptible to *Bd* infection by models (Rödder *et al.* 2009) and indeed, previous sampling for *Bd* in Suriname failed to detect any infection (Luger *et al.* 2008). *Bd* is classically associated with

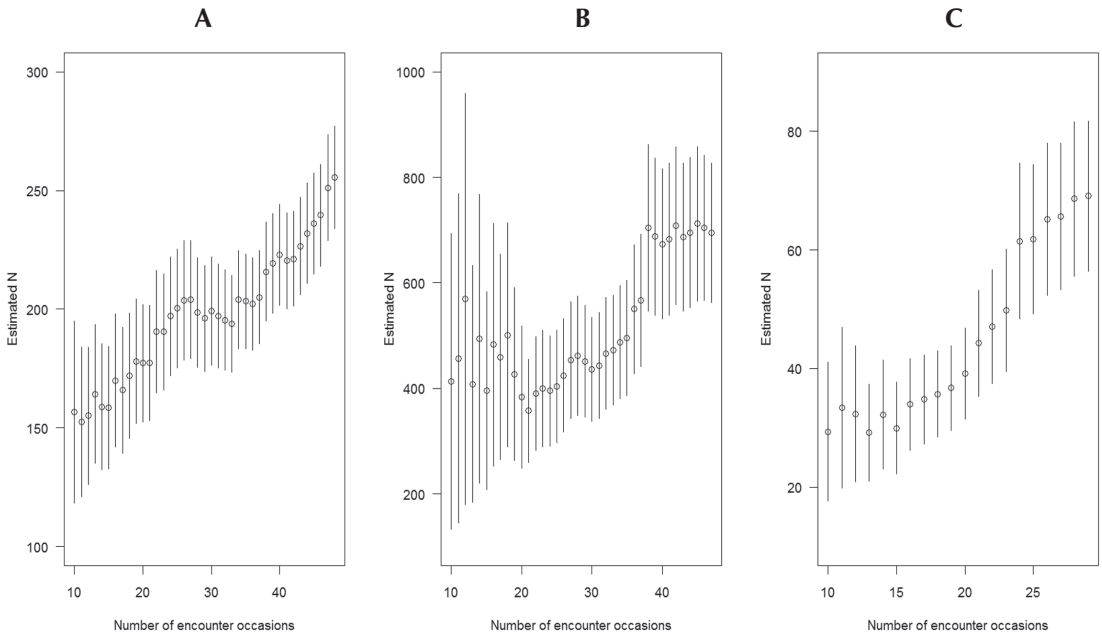


**Figure 1.** Evolution of the estimated *N* (population size) as a function of the number of encounter occasions for (**A**) Favard, (**B**) Nouragues and (**C**) Tresor.

high altitudes and cooler temperatures because it has a growth maximum between 17 and 25˚C (Piotrowski *et al.* 2004), but these temperature thresholds may be dependent on the *Bd* lineage. Mean annual temperature in French Guiana is 27˚C, a temperature that can be compatible with *Bd*-infection (Fisher *et al.* 2009). Sampling efforts for *Bd* in the Guiana Shield remain low and in the light of our results, more efforts should be made to determine *Bd* distribution in the Guiana Shield and its potential impact on anurans occurring in this area.

The origin of the infection is a critical question in need of answer (Fisher *et al.* 2009, Farrer *et al.* 2011). The low prevalence recorded in our study may be linked to an endemic origin of the disease or to a lower susceptibility of *Dendrobates tinctorius* to *Bd* in the wild than in captivity (Longcore *et al.* 1999). It also may indicate a recent introduction of the pathogen in the environment. Therefore, future studies should be conducted to identify which *Bd* strain is present in French Guiana, to determine the distribution of *Bd* at a broader spatial scale, and to determine the origin (anthropogenic or not) of the infection.

No data are available to determine whether amphibians are declining in French Guiana. Therefore, we strongly encourage the start of a long-term survey of *Dendrobates tinctorius* and other anuran species in several localities in French Guiana, including sites located in protected areas (such as the three sites used in this study) and also sites outside the protected areas. We further recommend including standard disease surveillance by sampling 30 individuals per location and year to be able to react early to the arrival and spreading of wildlife diseases and to complement our knowledge of the global distribution pattern of the disease chytridiomycosis, as well as other emerging diseases caused by other pathogens such as ranavirus (Densmore and Green 2007). To mitigate the spread of diseases, it is also important to establish

and follow strict hygiene rules for both fieldwork (Phillott *et al.* 2010) and animal husbandry (Schmeller *et al.* 2011). Our recommendations for amphibian and disease surveillance may contribute to an understanding of amphibian dynamics in the Neotropical rain forests and help to reduce the impact of chytridiomycosis on the highly diverse amphibian fauna.

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