Conservation Letter

Building a Species Conservation Strategy for the brown howler monkey (Alouatta guariba clamitans) in Argentina in the context of yellow fever outbreaks

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Abstract

The brown howler monkey (*Alouatta guariba clamitans*) is endemic to South America's Atlantic Forest, with a small population extending into the northern portion of Misiones province in northeastern Argentina. In 2012, the species was classified as Critically Endangered in Argentina due to its highly restricted distribution, low population density and dramatic declines from recent Yellow Fever outbreaks. In March 2013, we organized an international workshop in Misiones to evaluate population status in Argentina and conduct a threat analysis. We developed population viability models using *Vortex* and *Outbreak* software packages. These tools allowed us to explore how several biological and demographic parameters of brown howlers, as well as factors related to Yellow Fever epidemiology, affect the probability of species extinction. The discussion among diverse specialists and analysis of model results identified Yellow Fever as the main threat to brown howler population persistence in Argentina. Our threat analysis, focused on the dynamics of Yellow Fever outbreaks and their impact on howler populations, led to the identification of gaps in knowledge that helped prioritize objectives and actions for the development of a Species Conservation Strategy in Argentina.

Key words: *Alouatta guariba clamitans*; Population Viability Analysis; Threat Analysis; Yellow Fever.

Resumen

El mono aullador marrón (*Alouatta guariba clamitans*) es endémico del Bosque Atlántico de América del Sur, con una pequeña población que se extiende en la porción norte de la provincia de Misiones en el noreste de Argentina. En el año 2012, debido a su reducida distribución geográfica, su baja densidad poblacional y al dramático impacto de los recientes brotes de Fiebre Amarilla, la especie fue categorizada en Argentina como "en peligro crítico". En el mes de marzo de 2013 organizamos un taller internacional en Misiones con el objetivo de evaluar el estado de la población de esta especie en Argentina y llevar a cabo un análisis de las principales amenazas para su conservación. Para alcanzar estos objetivos construimos modelos de viabilidad poblacional utilizando los programas *Vortex* y *Outbreak*. Los mismos nos permitieron explorar cómo varios parámetros biológicos y demográficos de la especie, así como diversos factores relacionados al impacto de la Fiebre Amarilla, influyen sobre la probabilidad de extinción de la especie. La discusión entre los distintos especialistas y el análisis de los resultados de los modelos identificaron a la Fiebre Amarilla como la principal amenaza para la subsistencia de esta población en Argentina. El análisis de las amenazas se centró en la dinámica de los brotes de Fiebre Amarilla y la severidad de su impacto sobre la población de esta especie, lo que permitió identificar huecos en el conocimiento que permitieron priorizar objetivos y acciones a llevar a cabo para el desarrollo de una estrategia de conservación para esta especie en Argentina.

Palabras clave: *Alouatta guariba clamitans*; Análisis de Viabilidad Poblacional; Análisis de amenazas; Fiebre Amarilla.

Introduction

The brown howler monkey (*Alouatta guariba*) is endemic to the Atlantic Forest, ranging from the Brazilian states of Bahia and Espirito Santo in the north to Rio Grande do Sul and the Argentine province of Misiones in the south [1]. The species has been re-classified globally from "Near Threatened" to "Least Concern" by the IUCN due to its presence in most of the extant conservation units of the Atlantic Forest in Brazil. However, the population trend is still "decreasing" and the future of this species is quite uncertain, since the spatial extent of the Brazilian Atlantic Forest is dramatically reduced and fragmented [2]. In Argentina, the brown howler (*Alouatta guariba* ssp*. clamitans*) is restricted to Misiones and has been recently re-classified from "endangered" to "critically endangered" [3]. Existing data indicate that population density is very low, and its presence is confirmed in only five small protected areas of Misiones [4-5].

In 2005 I. Agostini, I. Holzmann and M. S. Di Bitetti started the first study on the behavioral ecology of brown howlers and the sympatric congener black and gold howler monkeys (*Alouatta caraya*) in one protected area of Misiones, El Piñalito Provincial Park [6-10]. This study was unexpectedly interrupted in January 2008, when a sudden outbreak of Yellow Fever (YF) killed all study group members. The outbreak continued until 2009 and decimated howlers throughout their southern distribution [11-14]. Due to the suspected high impact of these epidemics on the brown howler in Argentina, there is a special concern about the current status of this small population. In March 25-28, 2013, in order to establish conservation priorities and initiate the development of a conservation plan for the species in Argentina, we carried out the First Brown Howler Monkey Conservation Workshop in the Karadya Bio-Reserve near Comandante Andresito and Puerto Iguazú (Misiones, Argentina). A group of nine experts in different fields (primate ecology, eco-epidemiology, mosquito ecology and virology) dedicated themselves to gathering, systematizing and discussing all available data and information on brown howlers and Yellow Fever in the Atlantic Forest. The workshop was facilitated by members of the IUCN/SSC's Conservation Breeding Specialist Group (CBSG), who also provided expertise in the development and use of quantitative risk assessment models used in data assembly and analysis.

The objectives of the workshop were:

1. To provide an updated review of our current knowledge of species status, ecology and population dynamics in Misiones, thereby revealing key information gaps and suggesting new directions for future research.
2. To synthesize our knowledge of the primary factors impacting population dynamics and abundance (i.e., threats analysis).
3. To specify important goals and objectives for species recovery in Argentina.
4. To identify ways to enhance public awareness of the species and its conservation in Misiones, and to specifically create a network of stakeholders committed to work on the next step in brown howler management: a Species Conservation Strategy using the guidelines defined by the IUCN/SSC Species Conservation Planning Handbook [15]. This objective was not the focus of this letter, but will be addressed in another publication.

Status review, vision, challenges and threats analysis

For Objective (1) and part of Objective (2), we carried out a review of current knowledge on brown howlers, especially in Argentina, YF dynamics, the biology and ecology of known and potential vectors for this disease, and its impact on non-human primates. This process helped to reveal how little we know about the small Argentinean brown howler monkey population.

Then, to address Objective (3), we developed the following vision statement about the "ideal future" for brown howler monkeys in Misiones in 100 years:

> *"In 100 years' time, the population of brown howler monkeys in Misiones is viable in terms of demography, genetics and health, and ecologically functional in an environment that maintains the original biodiversity of the region and in a human society committed to its conservation".*

The development of this statement led us to point out the biggest threat to brown howler conservation in Argentina (Objective 2): excessive mortality due to YF outbreaks. Specifically, we identified as major challenges the need to integrate animal and public health, develop a more effective pre- and post- YF epidemic monitoring strategy for brown howlers, acknowledge the complexity of the system including human and non-human primates, other potential hosts, their interface with the landscape, and the dynamics of YF outbreaks.

In order to understand the state of knowledge of YF dynamics and to help achieve Objective (3), we created a system-level diagram of YF vectors, hosts, and the many ways in which those entities interact to influence outbreaks of disease (Fig. 1).This diagram made evident the lack of published data and studies on several critical variables needed to predict what could trigger a YF outbreak (e.g. hosts and vectors densities, dispersal capacity of vectors and connectivity among host sub-populations), and what could determine the impact of a YF outbreak on brown howlers (e.g. genetic resistance to YF, immunological experience, and general immunocompetence).
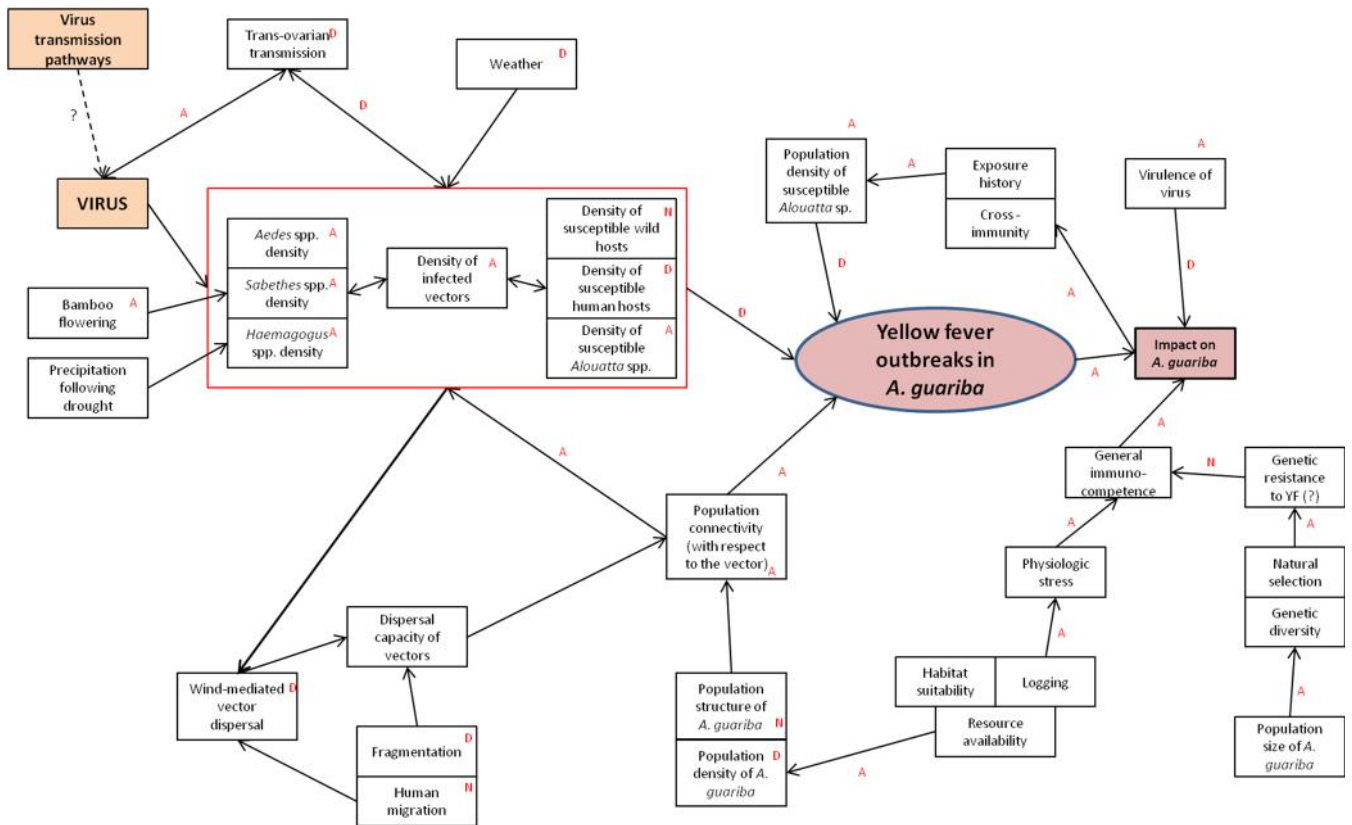
Fig. 1. System-level diagram illustrating links and interactions between factors affecting YF vectors and hosts. Letters in red indicate data quality as determined by workshop participants: D, specific data available to support diagram element; A, validity of diagram element based on assumption; N, no specific data available to support diagram element.
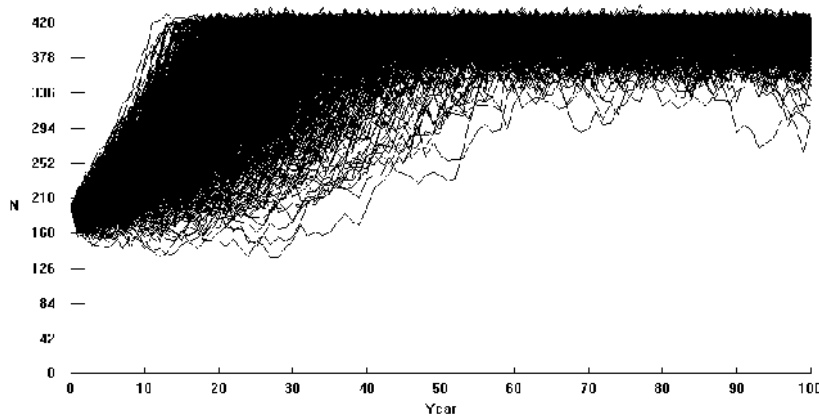
Population modeling

In order to examine the factors that most influence the viability of the brown howler population in Argentina (Objective 2), we examined and estimated input parameters and scenarios for two modeling tools commonly used in CBSG workshop processes: *Vortex* 16] and *Outbreak* [17]. *Vortex* is a Monte Carlo simulation for population viability analysis that examines the effects of deterministic forces as well as demographic, environmental, and stochastic genetic events on wild populations. *Vortex* models population dynamics as discrete sequential events that occur according to defined probabilities. The program is not intended to give absolute answers, because it projects stochastically the interactions of the many parameters used as input to the model, and because of the random processes involved in nature [18-19]. The objective of its use was to identify potential threats by weighing the relative importance of specific factors to predicted future population abundance trajectories.

Vortex models were run by A. Desbiez on the basis of input parameters provided by I. Agostini, I. Holzmann, M. Kowalewski and M. S. Di Bitetti. First, a "baseline" model was created that left out the probability of periodic disease outbreak. A simple form of sensitivity analysis [20] was then used to identify key demographic parameters that contribute significantly to growth of brown howler monkey populations. *Vortex* was then used to examine the relative impact of YF when modeled as a "catastrophe" affecting brown howler monkey survival (Fig. 2 a,b). This approach does not explicitly consider the details of disease ecology and epidemiology that

determine outbreak frequency, but instead simply treats disease outbreaks as generic periodic events that occur according to a constant defined annual probability. Baseline survival values across all age-sex classes are reduced by a specified amount if an "outbreak" occurs within a given year of the simulation. Multiple scenarios were created that explored the relative response of our simulated brown howler population to outbreak catastrophes of different frequency or demographic severity.

Results of simulations indicated that both the severity and the frequency of YF outbreaks have an impact on the rate of extinction of simulated brown howler monkey populations. Survival rates had more impact on model performance than frequency of outbreaks. However this is also because the severity of the outbreak used in the baseline model was high (70% mortality), although it was not an overestimate given the levels of mortality observed at El Piñalito [11]. Measuring the impact of YF outbreaks on survival of individuals from populations of howler monkeys is important for population viability modeling purposes.

a)
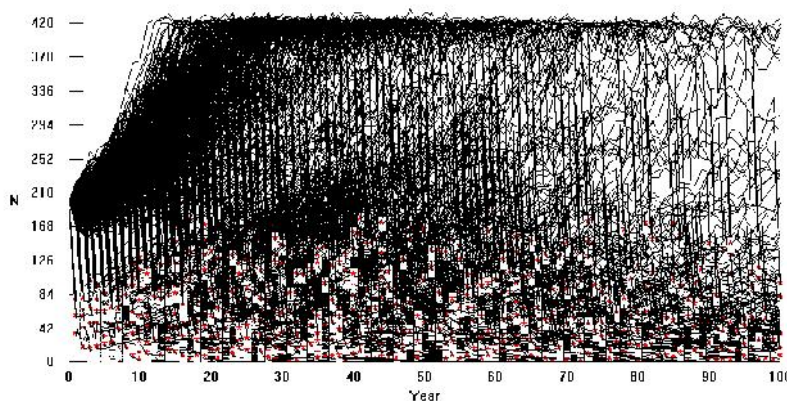
b)

Fig. 2. Abundance trajectories for simulated populations of the brown howler monkey in Misiones, Argentina, in the absence (a, top) and presence (b, bottom) of YF outbreaks. Each panel shows 100 iterations of a demographic model using the *Vortex* software package. Note the severe population declines that occur immediately following the onset of YF outbreaks in the bottom panel.

Finally, we constructed a simple simulated "metapopulation" of multiple brown howler sub-populations (using general knowledge of known remnant groups and their distribution in Misiones) that could exchange individuals at different rates among sub-populations. By adding catastrophic outbreaks to this type of spatially explicit model, we investigated the influence that dispersal can have on the spread of localized disease outbreaks and subsequently, on metapopulation extinction risk. Results indicated that if YF does not impact all sub-populations equally, fragmentation of the brown howler population could actually decrease its probability of extinction. This result is common to many similar and more sophisticated modeling studies (e.g. [21]), but the specific properties of these systems are highly dependent on the spatial arrangement of sub-populations and the degree of connectivity among them.

Disease modeling

A major goal of this workshop was to apply new disease epidemiology simulation modeling tools to the issue of YF epidemiology and transmission dynamics among brown howler monkey populations in northeastern Argentina. We used the software package *Outbreak* [17] to build a simple model of YF dynamics in brown howler monkeys, and to investigate model sensitivity to a set of key epidemiology model input parameters, including rates of contact between individual howlers, contact between howlers and other infected hosts, transmission probabilities, and disease-based mortality rates.

Outbreak models of YF epidemiology were created with the use of input parameters provided by E. Moreno, P. Beldomenico, S. Goenaga, M. Martinez and E. Lestani. Because we have very little data on YF ecology and epidemiology in Misiones region of Argentina, we had to use data from other regions or countries where similar types of YF outbreaks have occurred, e.g., southern Brazil. Although *Outbreak* was not specifically designed to deal with the complexities of transmission dynamics of vector-borne diseases like YF, we created a basic model of YF epidemiology that generated reasonable predictions of disease dynamics in brown howler populations without requiring the explicit consideration of mosquito vector population density or demographic dynamics.

Using new "metamodeling" software technology [22], we physically linked *Vortex* and *Outbreak* models together to create more detailed and informative models of YF dynamics in brown howler populations. This new technology allowed us to utilize the detailed demographic structure of *Vortex* and the more sophisticated disease dynamics of *Outbreak* to more accurately control sources of natural and disease-based mortality, respectively. Using this method, YF outbreaks are not simple catastrophes as defined in *Vortex* but now become emergent events that are a function of the underlying disease epidemiology controlled in *Outbreak*. When epidemics occur in *Outbreak*, animals are randomly selected to die from the disease and this information is then passed to *Vortex,* where the data are used to reduce overall population abundance in the corresponding time step.

Sensitivity analysis featuring this metamodeling approach was used to evaluate the implications of measurement uncertainty in a set of epidemiological input parameters within the *Outbreak* model. Parameters collectively defining the rate of pathogen introduction and transmission into a population were primary drivers of disease outbreak frequency, while other factors such as disease-based mortality rates were important factors that determined the long-term demographic viability of the howler population (Fig. 3). Although the input values of these models have been estimated with considerable uncertainty, the analysis allowed the

researchers to better understand which variables have more relative importance in YF dynamics and the impact of the disease on brown howler monkey population.

With the insights gained from this simple and preliminary analysis, we hope to challenge these tools further to create more realistic models of YF – particularly in spatially structured landscapes – to explore with greater accuracy the fate of brown howler monkeys in Misiones.
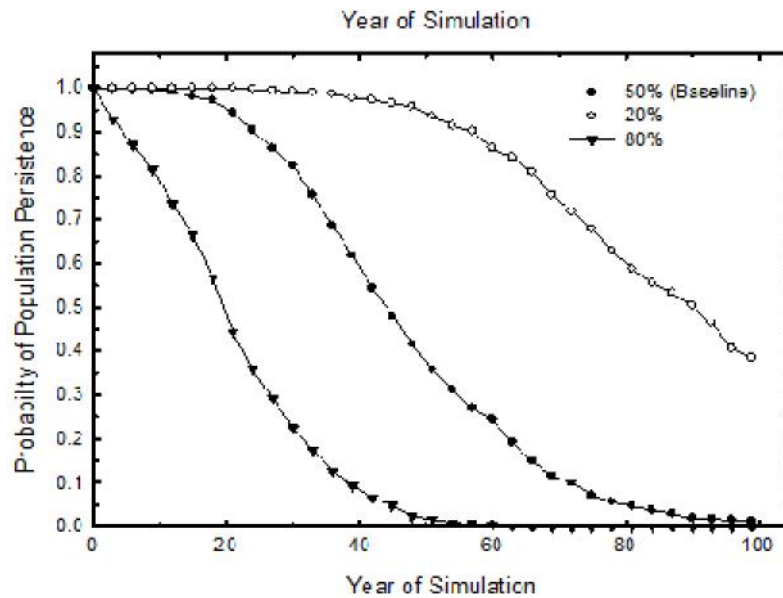
Fig. 3. Extinction risk trajectories for a simulated brown howler monkey population in the presence of ocasional YFoutbreaks. The model structure used in this analysis featured a *Vortex* demographic model linked to a model of YF epidemiology using *Outbreak*, thereby creating a "metamodel" to address more complex population dynamics. Curves feature different levels of disease-based mortality simulated within *Outbreak*, with 50% mortality rate defining the baseline disease model.

Recommendations

Based on the diagramming process and the population and disease modeling, we recommend a list of actions that will address the most important current knowledge gaps. For each action, a focal person of reference, a time frame, important collaborators, potential sources of financial support and degree of difficulty for implementation were specified.

Recommendations and actions in order of priority are the following:

Action 1. Implement a regular surveillance program for alerting local authorities about suspected YF outbreaks in monkeys and/or people.
Action 2. Estimate the population distribution and abundance of brown howler monkeys in Misiones.
Action 3. Conduct health studies of brown howler monkeys in Misiones to evaluate and compare physiological stress, innate and acquired immunity, hematology, etc., across different sub-populations especially before and after YF outbreaks.

Action 4. Isolate YF virus from mosquito adults and larvae.

Action 5. Conduct a thorough literature and archive review of the interactions (environmental and anthropogenic) involved in the maintenance and dynamics of YF outbreaks in South America.

Action 6. Capture adult mosquitoes where monkeys sleep or capture adult mosquitoes through monkey baited capture stations to identify YF vectors and monitor presence of the virus.

Action 7. Define the potential distribution of brown howlers in Argentina.

Action 8. Attempt to isolate or detect the YF virus in suspected vertebrate hosts using virological assays, cell cultures and molecular techniques.

Action 9. Conduct a systematic review of YF virus virulence from different strains in different vertebrate hosts in Misiones and Brazil.

Action 10. Understand the factors that define carrying capacity for brown howler monkeys and their habitat requirements (limiting factors, food, threats).

We hope this letter will stimulate research designed to tackle these actions. Improving knowledge on these issues is vital for understanding and therefore effectively managing the small brown howler population and impact of YF outbreaks in Argentina. Ultimately this will help us develop an effective Species Conservation Strategy for this brown howler population. To ensure that recommendations are effectively implemented, we created the Brown Howler Monkey Conservation group (BHMC group), to which members can be added as they get involved in the actions
(https://www.facebook.com/pages/Brown-Howler-Monkey-Conservation-Group-BHMCG/182361558615174).

Acknowledgements

References

[1]   Kinzey, W. G. 1982. Distribution of primates and forest refuges. In: *Biological Diversification in the Tropics.* Prance, G. T. (Ed.), pp. 455-482. Columbia University Press, New York.

[2]   IUCN/SSC. 2010. *IUCN Red List of Threatened Species.* Version 2010.4. www.iucnredlist.org

[3]   Agostini, I., Aprile, G., Baldovino, M. C., Brividoro, M., Di Bitetti, M., Fantini, L., Fernández, V. A., Fernández-Duque, E., Holzmann, I., Juárez, C. P., Kowalewski, M., Mudry, M. D., Nieves, M., Oklander, L. I., Peker, S. M., Pfoh, R., Raño, M., Steinberg, E. R., Tujague, M. P. and Zunino, G. E. 2012. Orden Primates. In: *Libro Rojo de Mamíferos Amenazados de la Argentina*. Ojeda, R. A., Chillo, V. and Diaz Isenrath, G. B. (Eds.), pp. 81-86. Sociedad Argentina para el Estudio de los Mamíferos (SAREM), Buenos Aires.

[4]   Agostini, I., Pizzio, C. E., De Angelo, C. D. and Di Bitetti, M. S. In prep. Population status of Primates in the Atlantic Forest of Argentina: an assessment through field surveys and inteviews.

[5] Holzmann, I., Agostini, I., De Matteo, K., Areta, J. I., Merino, M. and Di Bitetti, M. S. In prep. Assessing the factors that determine the distribution of two parapatric howler monkey species (*Alouatta* spp.) in South America through habitat distribution modeling.

[6] Agostini, I., Holzmann, I. and Di Bitetti, M. S. 2008. Infant hybrids in a newly formed mixed-species group of howler monkeys (*Alouatta guariba clamitans* and *Alouatta caraya*) in northeastern Argentina. *Primates* 49:304-307.

[7] Agostini, I., Holzmann, I. and Di Bitetti, M. S. 2010. Are howler monkey species ecologically equivalent? Trophic niche overlap in syntopic *Alouatta guariba clamitans* and *Alouatta caraya. American Journal of Primatology* 72:173-186.

[8] Agostini, I., Holzmann, I. and Di Bitetti, M. S. 2010. Ranging patterns of two syntopic howler monkey species (*Alouatta guariba* and *A. caraya*) in Northeastern Argentina. *International Journal of Primatology* 31(3):363-381.

[9] Agostini, I., Holzmann, I. and Di Bitetti, M. S. 2012. Influence of seasonality, group size, and presence of a congener on activity patterns of howler monkeys. *Journal of Mammalogy* 93(3):645-657.

[10] Holzmann, I., Agostini, I. and Di Bitetti, M. S. 2012. Roaring behavior of two syntopic howler monkey species (*Alouatta caraya* and A*. guariba clamitans*): Evidence supports the mate defense hypothesis. *International Journal of Primatology* 33:338-355.

[11] Holzmann, I., Agostini, I., Areta, J. I., Ferreyra, H., Beldomenico, P. and Di Bitetti, M. 2010. Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology* 72: 475-480.

[12] Almeida, M. A. B., Santos, E., Cardoso, J. C., Fonseca, D. F., Noll, C. A., Silveira, V. R., Maeda, A. Y., Souza, R. P., Kanamura, C. and Brasil, R. A. 2012. Yellow fever outbreak affecting *Alouatta* populations in Southern Brazil (Rio Grande do Sul State), 2008–2009. *American Journal of Primatology* 74:68–76.

[13] Bicca-Marques, J. C. and Freitas, D. S. 2010. The role of monkeys, mosquitoes, and humans in the occurrence of a yellow fever outbreak in a fragmented landscape in south Brazil: protecting howler monkeys is a matter of public health. *Tropical Conservation Science* 3:31-42.

[14] Moreno, E. S., Rocco, I. M., Bergo, E. S., Brasil, R. A., Siciliano, M. M., Suzuki, A., Silveira, V. R., Bisordi, I. and Souza, R. P. 2011. Reemergence of yellow fever: detection of transmission in the State of São Paulo, Brazil, 2008. *Revista da Sociedade Brasileira de Medicina Tropical* 44(3):290-296.

[15] IUCN/SSC. 2008. *Strategic Planning for Species Conservation: A Handbook. Version 1.0.* IUCN Species Survival Commission, Gland.

[16] Lacy, R. C. and Pollak, J. P. 2013. *VORTEX: A Stochastic Simulation of the Extinction Process. Version 10.0.* Chicago Zoological Society, Brookfield, Illinois.

[17] Lacy, R. C., Pollak, J. P., Miller P. S., Hungerford, L. and Bright, P. 2012. *Outbreak Version 2.0.* IUCN SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota.

[18] Lacy, R. C. 1993. Vortex: a computer simulation model for population viability analysis. *Wildlife Research* 20:45-65.

[19] Lacy, R. C. 2000. Structure of the Vortex simulation model for population viability analysis. *Ecological Bulletin* 48**:**191-203.

[20] Caswell, H. 2001. *Matrix Population Models*. Sinauer, Sunderland, Massachusetts.

[21] Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. and Dobson, A.P. (Eds.). 2006. *The Ecology of Wildlife Diseases*. Oxford University Press.

[22] Lacy, R. C., Miller, P. S., Nyhus, P. J., Pollak, J. P., Raboy, B. E. and Zeigler, S. L. 2013. Metamodels for transdisciplinary analysis of wildlife population dynamics. *PloS ONE* 8(12):e84211.