BROWN HOWLER MONKEY CONSERVATION WORKSHOP

Status Review and Population Viability Assessment (PVA): A first step in building a Species Conservation Strategy

Photo: Ilaria Agostini

Misiones, Argentina, March 25-28, 2013

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Population Viability Assessment (PVA)

Iguazú, Misiones, Argentina, March 25-28, 2013

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Instituto de Biología Subtropical – sede Iguazú, CONICET - UNAM

INSTITUTIONAL SUPPORT:

IUCN/SSC Conservation Breeding Specialist Group (CBSG) - Brazil Network
Royal Zoological Society of Scotland (RZSS)

FINANCIAL SUPPORT:

IUCN/SSC Species Conservation Planning Sub-committee (SCPSC);
Conservation International –Margot Marsh Biodiversity Foundation – Primate Action Fund;
Banham Zoo of England.

LOGISTIC SUPPORT:

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Agostini I, Desbiez ALJ & Miller P (Editors). 2013. Brown Howler Monkey Conservation Workshop

IUCN/SSC Conservation Breeding Specialist Group (CBSG), Brasil.

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I. Executive Summary

Brown howlers (*Alouatta guariba*) are one of the endemic primate species of 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 (Kinzey 1982). In Argentina, the brown howler (*Alouatta guariba* ssp. *clamitans*) has been re-classified from "endangered" to "critically endangered" (Agostini et al. 2012) and included in the national list of the most threatened mammal species compiled by the Argentine Society for the Study of Mammals (SAREM) and by the National Authority in Fauna and Flora of Argentina. The province of Misiones has declared this species by law a Provincial Natural Monument.

During three years (2005-2007), Ilaria Agostini, Ingrid Holzmann, and Mario Di Bitetti, carried out a comparative study on the behavioral ecology of brown howlers living in sympatry with the congener black and gold howler monkeys (*Alouatta caraya*) in one protected area of Misiones, El Piñalito Provincial Park. Then, in 2008 and 2009, yellow fever outbreaks killed all study groups and dramatically decimated howlers throughout its southern distribution (Almeida et al. 2012; Bicca-Marques et al. 2010; Holzmann et al. 2010). Due to the suspected high impact of these epidemics, there is a special concern about the current status of the brown howler, which is the rarest monkey species in Argentina, only restricted to Eastern Misiones.

This situation makes conservation action urgent. In order to establish conservation priorities for this species and its habitat in Argentina, an assessment of the current brown howler population status and the main threats has become critical. This step is necessary to develop and implement effective conservation and management plans that will increase the probability of persistence of this population in the medium-long term.

The first BROWN HOWLER MONKEY CONSERVATION WORKSHOP Population Viability Assessment (PVA) was held in Iguazú, Misiones, Argentina, March 25-28, 2013 with the objective of establishing conservation priorities for this primate species in Argentina.

During this three-day meeting 11 specialists (primatologists, epidemiologists, mosquito ecologists), examined the current knowledge and situation of brown howlers in Argentina and nearby areas of Brazil. To guide the work, a vision of what the group wanted to achieve was drafted: "*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 society committed with its conservation*".

Participants then proceeded to a threat analysis and concluded that the two biggest challenges to brown howler monkey conservation in Misiones were: lack of public awareness of the species and yellow fever outbreaks. To take advantage of the participants' areas of expertise, this workshop focused mainly on all aspects of yellow fever outbreaks. A flow chart was constructed to represent the factors (and the interactions between them) that influence the probability of occurrence of a yellow fever outbreak (e.g. virus virulence, mosquito species demographic dynamics, etc.) and its impact on brown howler population (population structure and connectivity, general health status, genetic resistance, etc.). Through this diagram, the most important gaps in knowledge were identified and a list of prioritized objectives and actions to be implemented was created.

Flow chart created by workshop participants

The first 10 actions recommended by the participants in order of priority are:

Action 1. Implement a regular surveillance system for alerting suspected Yellow Fever outbreaks in monkeys and people.

Action 2. Estimate the population abundance of brown howler monkeys in Misiones.

Action 3. Health studies of all brown howler monkey populations in Misiones to evaluate parameters such as physiological stress, innate and acquired immunity, hematology, etc., to be able to evaluate and compare different populations especially before and after Yellow Fever outbreaks.

Action 4. Isolate Yellow Fever virus from adult and larvae of mosquitoes.

Action 5. Conduct a thorough literature and archive review to enhance our understanding of the interactions (environmental and anthropogenic) involved in the maintenance and dynamics of Yellow Fever outbreaks in South America.

Action 6. Capture adult mosquitoes where monkeys sleep or capture adult mosquitoes through monkey baited capture stations.

Action 7. Refine the current and potential distribution of brown howler monkeys in Argentina.

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

Action 9. Conduct a Systematic review about the virulence of the Yellow Fever virus from different strains in different vertebrate hosts in Misiones and Brazil.

Action 10. Understand what defines the carrying capacity of brown howler monkeys and their habitat requirements (limiting factors, food, threats).

During the workshop both software *Vortex* and *Outbreak* were used to examine multiple scenarios and create hypotheses. The *Vortex* model demonstrated the probability of brown howler extinction depending on severity and frequency of Yellow Fever outbreaks. Most interestingly, the modeling showed that if habitat fragmentation meant that not all populations of Misiones were impacted at the same time by Yellow Fever, then fragmentation of populations could actually help increase the probability of survival of brown howlers in Misiones. The *Outbreak* model demonstrated the influence of resistant individuals, and how this may explain the cycles of the outbreaks.

When the workshop was finished, all participants gathered in a conference room in Puerto Iguazú to present results of the workshop to representatives of most governmental authorities involved in conservation in Misiones province and Argentina, as well as other NGOs, local stakeholders, some local media and anyone interested in conservation. This enabled the group to share first-hand conclusions and knowledge acquired during the workshop.

An implementation strategy for workshop actions and recommendations was agreed upon and an agenda including timeframes, focal persons and collaborators was set for every action.

II. Workshop Background

Brown howlers (*Alouatta guariba*) are one of the endemic primate species of 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 (Kinzey 1982). Brown howlers have been recently re-classified globally from Near Threatened to Least Concern by the IUCN due to the presence of the species 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 Brazilian Atlantic Forest is dramatically reduced and fragmented (IUCN 2010). Furthermore, the species is listed on CITES Appendix II. In Argentina, the brown howler (*Alouatta guariba* ssp. *clamitans*) has been re-classified from “endangered” to “critically endangered” (Agostini et al. 2012) and included in the national list of the most threatened mammals species compiled by the Argentine Society for the Study of Mammals (SAREM) and by the National Authority in Fauna and Flora of Argentina. The province of Misiones has declared this species by law a Provincial Natural Monument (Law N. 3455). For the small Argentinean portion of the brown howler’s range, density estimates are limited but generally very low, and its presence has been confirmed in only five small protected areas of the province.

During three years (2005-2007), Ilaria Agostini, Ingrid Holzmann, and Mario Di Bitetti, carried out a comparative study on the behavioral ecology of brown howlers living in sympatry with the congener black and gold howler monkeys (*Alouatta caraya*) in one protected area of Misiones, El Piñalito Provincial Park. This was the first long-term study on these two sympatric species of howler monkeys in the region. Part of the results of this study have already been published in peer-reviewed journals (see the Appendix I), while others are still in preparation. We found that both howler species share a high trophic and spatial niche overlap and could potentially compete for resources in sympatry (Agostini et al. 2010a,b). Also, we reported direct evidence of hybridization between the two species occurring within the area of sympatry (Agostini et al. 2008). Then, in 2008 and 2009, Yellow Fever outbreaks killed all study groups and dramatically decimated howlers throughout its southern distribution (Almeida et al. 2012; Bicca-Marques et al. 2010; Holzmann et al. 2010; Moreno et al. 2011).

Due to the suspected high impact of these epidemics, there is a special concern about the current status of the brown howler, which is the rarest monkey species in Argentina, only restricted to Eastern Misiones. Given its initial small size, the remnant brown howler population is now considered to be seriously endangered, and at risk of disappearing from Misiones in the next few decades due to the increasing habitat loss and recurrent Yellow Fever outbreaks. This situation makes conservation action urgent. In order to establish conservation priorities for this species and its habitat in Argentina, an assessment of the current brown howler population status and the main threats affecting this population has become critical. This step is necessary to develop and implement effective conservation and management plans that will increase the probability of persistence of this population in the medium-long term.

III. Workshop Objectives

The main goal of this first Brown Howler Monkey Workshop was to conduct a status review of the brown howler population inhabiting the Atlantic Forest of Misiones Province in Argentina, providing the summary of all the factors relevant to the population’s conservation status and incorporating an analysis of the primary threats affecting its persistence. In particular, the workshop participants have worked on gathering, systematizing and discussing all available data and information on brown howlers in the Atlantic Forest of Misiones (population demographic parameters – e.g. age structure, birth rates, mortality, dispersal, other biological data, the species historical and current status and distribution, available habitat, and ongoing and potential threats to survival) and used this information for planning specific objectives of research, conservation and management for the species in the region.

The specific objectives of this process were (1) an updated review of our current knowledge on population status, ecology and dynamics of brown howlers in Misiones, followed by an identification of key information gaps and a consequent re-direction of future research efforts; (2) a synthesis of the currently or potentially interactive factors affecting the trends in population behavior and the identification of the factors that most influence the viability of the brown howler population (i.e. threat analysis); and (3) a statement of target recovery objectives and goals that have to be reached. Finally, another specific objective of this workshop was (4) the enhancement of public awareness about the conservation status of brown howlers in Misiones, and the creation of a network of stakeholders committed to progress in the further step: planning a future Species Conservation Strategy according to the guidelines provided by the IUCN/SSC Species Conservation Planning handbook.

IV. Status Review

For the Objective (1) and part of Objective (2), participants made presentations about threats and challenges for the conservation of brown howlers in Argentina. In particular, participants carried out a review of the current knowledge about brown howlers in Argentina, Yellow Fever dynamics, the biology and ecology of known and potential vectors for this disease, and its impact on non-human primates. This status review made by experts from each field - primate ecology, eco-epidemiology, mosquito ecology, virology - was key in bringing all participants up to date with the latest information and starting to integrate the available data. What follows are the abstracts of the presentations made by the participants of this workshop.

Threats and challenges for the conservation of brown howler monkeys

(*Alouatta guariba clamitans*) in Argentina

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The Brown Howler Monkey (*Alouatta guariba clamitans*) is an endemic species of the Atlantic Forest of Brazil and Argentina. In Argentina the species survives as a small population. Its presence has been confirmed in five strictly protected areas and overall it occupies a total area of <10.000 km^2 in the central-eastern portion of Misiones Province, where it persists at extremely low densities. The objective of this contribution is to analyze the main threats that affect the brown howlers in Misiones and recommend some necessary steps for the development of a conservation strategy for this population.

The brown howlers are being affected by progressive and severe habitat loss in Misiones and nearby areas of Brazil, given that the Atlantic Forest has been reduced to 8-12% of its original distribution and is still undergoing a process of degradation and fragmentation throughout its extension. Brown howlers, as all members of their genus, are particularly susceptible to epidemic diseases such as yellow fever. During the recent epidemics of 2008-2009, we found 59 dead howlers in Misiones and we suspect that this outbreak has decimated the species in this region. Thus, yellow fever susceptibility is thought to be one of the most important threats to the persistence of this small population. In addition, brown howlers have shown to overlap extensively in their trophic, spatial and temporal niche with black and gold howler monkeys (*A. caraya*) in the contact zone where both species coexist in Misiones. This ecological overlap could lead the two species to compete for the same resources whenever resources are limited. Finally, where species are syntopic, as occurring in Misiones, there are records of mixed groups and hybridization, which could represent a further threat to the species conservation. Overall, we consider that habitat loss and recurrent epidemics of yellow fever, added to the hybridization and interspecific competition, are progressively driving brown howlers to extinction in Argentina.

In order to establish priorities for the conservation of this population and its habitat, we are organizing the first workshop of brown howler conservation for Argentina, where we will bring together primatologists and epidemiologists. Through the use of Population Viability Analysis (PVA) models, we expect to highlight important gaps in our knowledge of the species and evaluate the most important threats for its persistence in Misiones. The PVA will serve to select the most effective management alternatives and define the conservation goals and targets. During the workshop we will also develop together communication strategies to raise public awareness about the situation of brown howlers in the region. This workshop will hopefully lead to a broader and participative process for the development of a brown howler conservation strategy in Argentina.

Ecological and Anthropogenic Influences on Patterns of Parasitism in Free-Ranging *Alouatta*

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Parasites play a central role in ecosystems, affecting the ecology and evolution of species interactions, host population growth and regulation, and community biodiversity. Infectious diseases caused by pathogens are now recognized as one of the most important threats for primate conservation. The fact that howler monkeys (*Alouatta* spp.) are widely distributed from Southern Mexico to Northern Argentina, inhabit a diverse array of habitats, and are considered "colonizers" particularly adapted to exploit marginal habitats, provides an opportunity to explore general trends of parasitism and evaluate the dynamics of infectious diseases in this Genus. I present the results of two meta-analyses, one run with howler species across South America, and the second one considering Central American howlers too. Thus we take a meta-analysis approach to examine the effect of ecological and environmental variables on parasitic infection using data from eight howler monkey species (*Alouatta palliata*, *A. pigra*, *A. macconnelli*, *A. sara*, *A. seniculus, A. belzebul, A. guariba,* and *A. caraya*), at more than 35 sites throughout their distribution. The analysis run across South and Central America indicated that different factors including precipitation, latitude, altitude, and human proximity may influence parasite infection according to parasite type: nematodes, trematodes, cestodes, amoebae. We also considered specifically the effect of these variables on *Trypanoxiuris* sp., *Giardia* sp. and *Plasmodium* sp. due to their presence across study sites, finding equivocal results. We also found that parasites infecting howler monkeys followed a right-skewed distribution suggesting that only few individuals harbor infections. This highlights the importance of collecting large sample sizes when developing these studies (or to find accurate results). The analyses run for only South American species indicated that type of human contact (degree) affected the prevalence of different parasites. Our general analysis suggests that the prevalence of parasites did not vary across fragmented and continuous forests. Logistic regression models suggested latitude and altitude were mediators of the likelihood of having high or low parasitic prevalence (either higher or lower than 20%). The relationship between gastrointestinal parasite diversity at a study site and average annual precipitation was positive and significant ($r = 0.72$, $P < 0.05$). In general we found that almost 86% of gastrointestinal parasites, and 100% of blood-borne parasites found in howlers are found in humans. We also present data on a preliminary analysis of gastrointestinal parasite prevalence on *A. guariba* and *A. caraya* living in sympatry. Our data suggest that these 2 species share their parasites although prevalence seems to be higher for *A. guariba*.

Then, what do we need to include when interpreting patterns of parasitism? (1) A general quantitative assessment of habitat disturbance such as an index of logging extraction, and size and shape of howler habitats; (2) exposure rates of individuals to the matrix; (3) human and domestic animal proximity; (4) quantitative data of microclimatic variation (e.g., humidity, temperature, rainfall); (5) correct identification of parasite taxa hosted by howler monkeys and then assessment of disease risk; (6) to focus on studies on different population of primates that live under different degree of habitat alteration; (7) to include complete annual cycles of parasite profiles; after profiles most of the studies are "snap-shots" and hence limited in terms of providing sufficient information on parasite profiles and on host-parasite temporal dynamics and phenology (interannual comparisons); (8) to include in the analysis shared water and food sources, and finally (9) to standardize data collection both in research design and methodology. Finally, we suggest that future studies should focus to obtain or interpret fine-grained estimations of ecological and microclimate change to provide better insights into the proximate factors that promote parasitism.

Note: Most of the analyses were done in collaboration with Rodolfo Martinez-Mota (Department of Anthropology, University of Illinois at Urbana-Champaign) and Thomas Gillespie (Departments of Environmental Studies & Environmental Health, Emory University).

Yellow fever in Argentina: studies of possible vectors

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Yellow fever (YF) is an important re-emerging arboviral disease and a cause of severe illness and death in South America and Africa. The agent of the disease is yellow fever virus (YFV), the prototype member of the family Flaviviridae. The enzootic transmission cycle involves two components: the mosquitoes and the nonhuman primates. In Africa the vector involved in this enzootic cycle is a mosquito from the genus *Aedes*, whereas in South America the vectors associated with the transmission cycle are mosquitoes from the genera *Haemagogus* and *Sabethes*. Specifically in Argentina, the last YFV outbreak was detected in Corrientes and Misiones provinces in 1966, in which a total of 53 human cases were notified: 41 in Misiones province and 12 in Corrientes province. However, only five cases were confirmed by means of virological and/or histopathological studies. Nevertheless, the role of mosquitoes as potential vectors in the transmission cycle of this epizootic is still unknown. During the summer of 2007-2008, a sylvatic outbreak of YFV affected monkeys and humans, after 50 years without viral activity detected in Argentina.
In December 2008/January 2009, a high mortality rate of monkeys close to Posadas city (Misiones province) was observed. Therefore, the main goal of this study was to investigate mosquito species that could play a role in the sylvatic transmission of YFV in Argentina. Field studies at the subtropical rain forest surrounding Posadas city were conducted in January 2009. Mosquitoes were captured from human bites and by using CDC traps. Insects were kept in liquid nitrogen until transported to the laboratory. Specimens were sorted according to method of collection, location, date of capture and genus. Supernatant of mosquitoes pool homogenates was inoculated into Vero and C6/36 cells for virus isolation and RT-PCR for flavivirus studies. Virus isolates were identified by imnumoflouescence using monoclonal antibodies and by RT-PCR. Out of 506 mosquitoes captured, 51 belonged to the species *Sabethes albiprivus*. The 51 captured specimens of *Sa. albiprivus* were sorted into 20 pools, and one of them was positive for YFV by RT-PCR and by imnumoflouescence assays. The YFV strain was isolated in Vero C/76 and C6/36 cell cultures.

The phylogenetic analyses were carried out by sequence data based on the nucleotide sequences of the prM/E region, and NS5/3`NCR. They were analyzed with the use of algorithms for parsimony method, for distance method, and for Bayesian analysis. The phylogenetic trees generated by these analyses had the same topology. The phylogenetic analysis showed that the strain of YFV isolated from *Sa. albiprivus* is positioned together with other Argentinean YFV strains isolated from humans and monkeys, forming a well-supported clade. Remarkably, in the same period, several YF cases in humans and monkeys were also diagnosed in Brazil and Paraguay.

Our results reveal that the YFV strain isolated from *Sa. albiprivus* from Argentina is placed with other strains from the last YF outbreak that occurred in Brazil in 2008, within the genotype I clade. This genotype was first reported in Brazil, suggesting that the outbreak in Argentina may be a spillover from Brazil.

In summary, the isolation of a YFV strain from *Sabethes albiprivus* from Argentina reported in this study is the first case of YFV isolation from mosquitoes in this country. Furthermore, it is the first time that a YFV strain has been isolated from *Sabethes albiprivus*. In general, the entomologic vigilance is principally focused on mosquitoes from genus *Haemagogus*; our results indicate the necessity of studying the population of species from genus *Sabethes*, including *Sa. albiprivus*, during YF outbreaks. These findings indicate that *Sabethes albiprivus* is a putative vector of sylvatic YF, at least in Argentina. It is possible that *Sa. albiprivus* plays an important role in the maintenance of YFV, although further studies are necessary to determine its effectiveness as a YFV vector in the study area.

Infection, susceptibility and population dynamics

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Recent research suggests that host susceptibility should be considered carefully if we are to understand how parasite dynamics influence host dynamics and vice versa. Studies in insects, fish, amphibians and rodents show that infection occurrence and intensity are more likely and more severe in individuals with an underlying poor condition. Moreover, infection itself results in further deterioration of the host and a ‘vicious circle’ is created (Beldomenico & Begon 2010). This potential synergy between host susceptibility and infection should be more widely acknowledged in disease ecology research.

It is now well recognized that infectious diseases represent a considerable threat contributing to biodiversity loss (Pedersen et al. 2008; Smith et al. 2009). While it has been posited that pathogens might not be able to drive their hosts to extinction because they would ‘fade out’ when host density is below a threshold that is critical for disease persistence (McCallum et al. 2001) (except for cases where transmission is frequency-dependent), we should note that pathogens are not independent entities but are part of a rich parasite community. Hence, host populations that survive the impact of specialist pathogens are then left vulnerable to density-independent generalists and opportunistic environmental pathogens. The fate of a wild animal population, then, might depend on the proportion of individuals that are prone to developing vicious circles. This may have important consequences for the effects of other factors on the population (e.g. resource shortage or predation). Environmental stress (caused by habitat destruction, pollution, climate change, etc.) might cause a large proportion of the population to be vulnerable, and thus (otherwise tolerated) native parasites could become a health threat for wildlife. The vicious circle might become a vicious spiral, down towards population extinction.

Reference List

Beldomenico PM, Begon M (2010). Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol. Evol. 25, 21-27.

McCallum A, Barlow N, Hone J (2001). How should pathogen transmission be modelled? Trends Ecol. Evol. 16, 295-300.

McCallum H (2008). Tasmanian devil facial tumour disease: lessons for conservation biology. Trends Ecol. Evol. 23, 631-637.

Pedersen AB, Jones K, Nunn C, Altizer S (2008). Infectious diseases and extinction risk in wild mammals. Cons. Biol. 21, 1269-1279.

Smith K, Acevedo-Whitehouse K, Pedersen AB (2009). The role of infectious diseases in biological conservation. Anim. Cons. 12, 1-12.

Yellow fever dynamics and the impacts on non-human primate surveillance system in Brazil

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A Febre Amarela é uma doença viral transmitida por vetores. São considerados dois ciclos da doença: urbano e silvestre. No ciclo urbano a doença é transmitida por mosquitos da espécie *Aedes aegypti* diretamente ao homem, considerado o único hospedeiro vertebrado. Este ciclo não é identificado no Brasil desde 1942. No ciclo silvestre a doença tem como principais espécies vetoras mosquitos do gênero *Haemagogus spp.* e *Sabhetes spp.,* e como principais hospedeiros primatas não-humanos. Porém, os primatas, incluindo o homem, são considerados hospedeiros terminais da doença, pela reduzida duração da viremia, de forma que, as espécies consideradas vetoras são também consideradas reservatório deste vírus, dada sua capacidade de transmissão vertical.

As espécies de primatas apresentam diferentes níveis de susceptibilidade a doença. O gênero *Sapajus* spp. quando infectado, não apresenta sintomas ou apresenta poucos sintomas, níveis baixos de viremia, sendo esta de pouca duração. Inquéritos sorológicos realizados com esta espécie demonstram alta freqüência de indivíduos com anticorpos contra febre amarela. No gênero *Ateles* spp. sintomas parecem ser freqüentes, porém de pouca gravidade. A viremia nestes animais costuma ser alta, e com duração que pode superar 10 dias, sendo que na natureza são facilmente identificados portadores de anticorpos conta a Febre Amarela. Para outras espécies como do gênero *Challithrix* spp., *Saimiri* spp., *Aotus* spp., e *Alouatta* spp. a infecção pelo vírus da febre Amarela costuma ser muito grave, apresentando altas taxas de letalidade. Apesar da alta viremia apresentada por estas espécies, sua duração costuma ser muito pouco duradoura, uma vez que os animais freqüentemente vão a óbito. Asism, poucos são os indivíduos apresentando anticorpos para o vírus em animais de vida-livre. O gênero *Aotus spp.*, apesar da grande susceptibilidade apresentam pouca exposição as espécies de mosquitos vetoras, dado seu hábito noturno.

Dentre todas estas espécies, nenhuma parece ser tão susceptível a doença quanto as do gênero *Alouatta* spp., com registros históricos de grandes epizootias em toda América do Sul.

Estudos de série histórica consideram intervalos inter-epidemicos que variam de 7 (regiões sudeste, sul, centro-oeste e nordeste do Brasil) a 14 anos (Amazônia). Segundo alguns autores, este seria o tempo necessário para a renovação da população de primatas susceptíveis a doença.

Entendendo a suscetibilidade dos primatas não-humanos neo-tropicais a Febre Amarela, o Ministério da Saúde do Brasil, criou em 1999, o Sistema de Vigilância de Epizootias em primatas não-humanos, vinculado ao Programa Nacional de Controle da Febre Amarela. Assim, este sistema tem como principais objetivos identificar precocemente a circulação do vírus, de forma a prevenir casos em humanos. Desde então, o sistema vem sendo implementado no Brasil, através da sensibilização da população para identificação e notificação de primatas não-humanos encontrados mortos, assim como, a capacitação de profissionais de saúde pública para a coleta de amostras biológicas, de forma que estas sejam enviadas a laboratórios de referência para identificação do vírus amarílico.

Entre o período de 2007 a 2009, mesmo período de circulação do vírus da Febre Amarela na Argentina, foram identificadas no Brasil 1971 epizootias, totalizando 3602 animais. A média de animais acometidos por epizootia foi de 1,8 animais, variando de 1 a 20 animais. Destes, 88% puderam ser identificados até o gênero taxonômico, sendo 64,4% *Alouatta* spp, 29% *Challithrix spp.* e 6,6% *Sapajus* spp. Foram coletadas amostras de 22% (191/437) dos animais, de forma que 209 epizootias puderam ser confirmadas para Febre Amarela. Destas, 96,7% das epizootias foram causadas em espécies do gênero *Alouatta* spp.

Somente 01 estudo, realizado no estado do Rio Grande do Sul - Brasil identificou até espécie os indivíduos encontrados mortos, sendo 58% *A. g. clamitans,* e 42% *A. caraya.* Porém não se tem informações do tamanho populacional destas espécies na área para calcular a taxa de mortalidade específica para cada uma. A distribuição dos eventos epizoóticos de acordo com o tempo evidenciam a presença de picos de ocorrência e taxas básicas de mortalidade.

V. Vision for Brown Howler Monkey Conservation

To reach Objective (3) participants were asked to develop a vision of how they would like to see brown howler monkey populations in Misiones in 100 years, considering that all the conservation measures were successful. Participants brainstormed individually, then in pairs and then in groups of three until the following vision the whole group agreed upon was formulated.

"That 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 society committed with its conservation"

VI. Conservation Challenges – Threats Analysis

Participants were then asked to individually list what they perceived as the most important challenges and biggest threats to brown howler monkey conservation. Threats and challenges were grouped into the following list (which is NOT in order of priority):

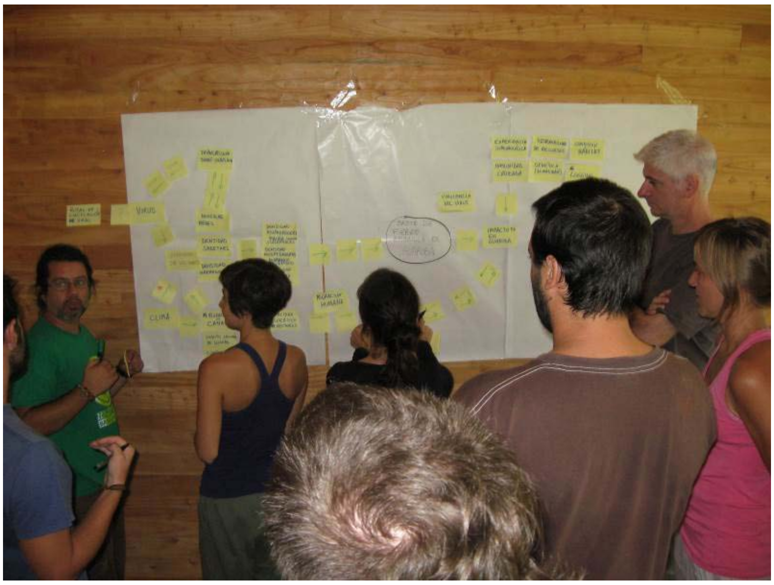
1. Low public awareness. The species is not a priority among authorities. Although it is critically endangered in Argentina, it receives little attention compared to other local critically endangered species such as jaguars.
2. Managing disease epidemics/Disease management. This could be a key factor to ensure the persistence of brown howlers in the future.
3. Integrating animal health and public health. Getting the health authorities engaged and communicating with the wildlife health authorities.
4. Involving the local community in the conservation of the species and raising their awareness about the disease.
5. Getting authorities to become more involved in land management strategies so that both humans and howlers can live side by side. Communicating importance of conservation to politicians.
6. Managing howlers to minimize problems generated by humans entering howlers' habitat (e.g., hunters, loggers). Need to minimize contact between humans and howlers.
7. Developing a more effective pre- and post- Yellow Fever epidemic monitoring strategy for brown howler populations.
8. Acknowledging the complexity of the system. The system including human and non-human primates, their interface with the landscape, and the dynamics of Yellow Fever outbreaks is very complex and makes predictions very challenging.

Participants examined this list (Objective 2) and stated that currently the two biggest challenges to brown howler monkey conservation in Misiones were: lack of awareness of the species and Yellow Fever outbreaks. Deforestation, hunting, habitat loss, invasive species and other such threats which are commonly reported for threatened species were not considered as important as these two major challenges. For this reason, and to take advantage of the areas of participants' expertise, this workshop focused mainly on all aspects of Yellow Fever outbreaks. On the other hand, the workshop would immediately start raising awareness and communicating about the species in a one-day presentation to the main stakeholders that took place in Iguazú on March 28, 2013. Communication and raising awareness would continue to be tackled after the workshop.

VII. Understanding Yellow Fever Outbreaks

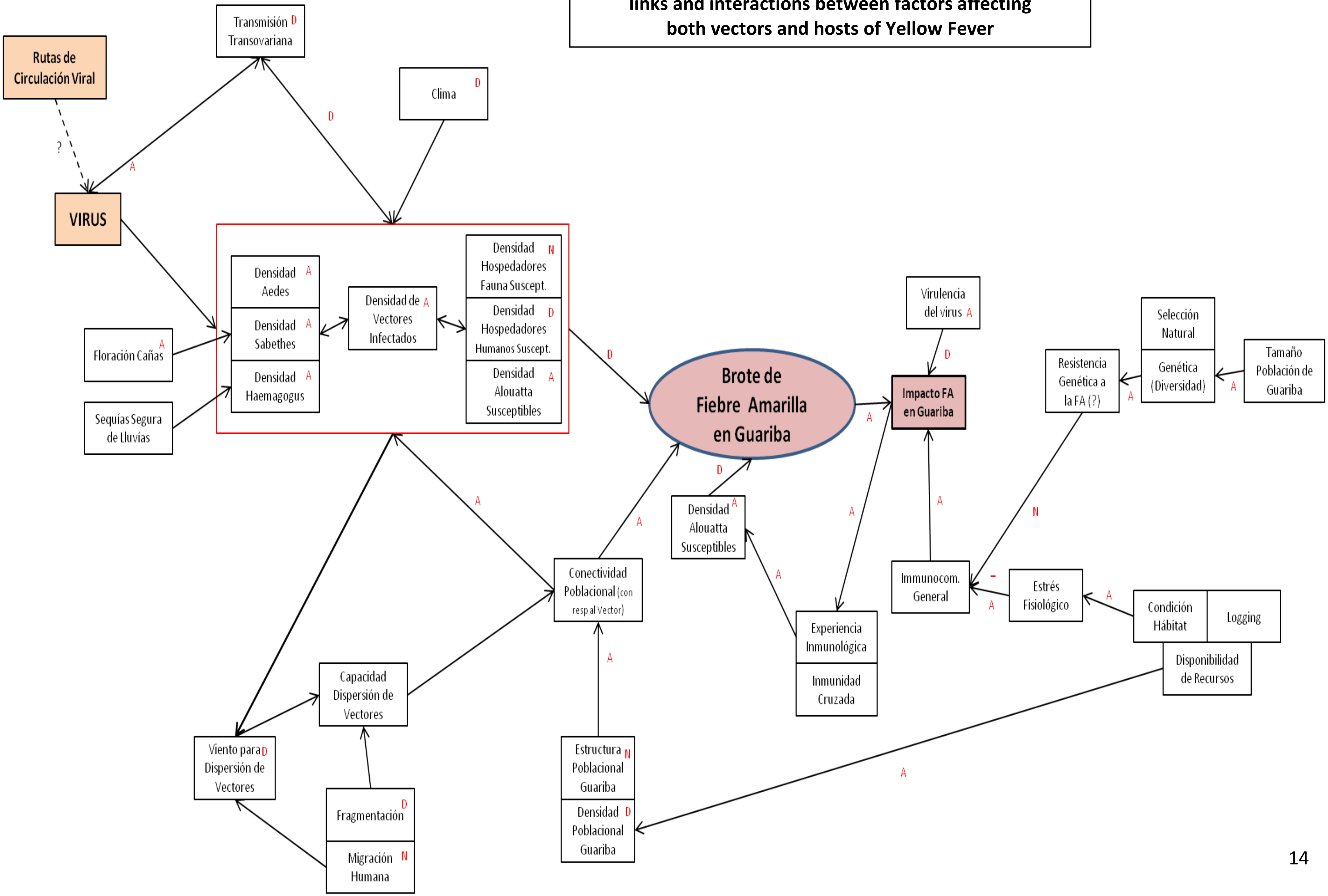
To better understand Yellow Fever outbreaks, the group was asked to create a flow chart including the disease vectors and the hosts. This exercise was done in plenary, though sometimes participants broke into small groups to work on a particular issue. Links and interactions were changed and re-grouped until the participants produced a flow chart they were satisfied with.

Participants were then asked to examine each link and issue, and state whether the relationship or issue was based upon solid data, if it was an assumption made on the basis of data available for different but related situations, or if the information was completely lacking. This step was crucial to understanding the state of knowledge of Yellow Fever dynamics and to reach the Objective (3).



Workshop participants creating the flow chart

Flow chart created by participants illustrating all links and interactions between factors affecting both vectors and hosts of Yellow Fever



VIII. Workshop Recommendations

Once the flow chart was completed and carefully dissected to better understand the state of our knowledge, participants were divided in two groups: (1) host and (2) vector. Each group was asked to look at the flow chart and see where actions could be taken to improve our knowledge and help stop the Yellow Fever outbreaks or minimize their effects on brown howlers.

Actions were then listed on flip charts and prioritized. Recommendations and actions are presented in order of priority according to the participants.

Participants prioritizing workshop recommendations

<u>**Objective:**</u> **To have a surveillance system for Yellow Fever in Misiones.**

Action 1. Implement a regular surveillance system for alerting suspected Yellow Fever outbreaks in monkeys and people.

<u>Focal person</u>: Ilaria Agostini [Instituto de Biología Subtropical (IBS) – Universidad Nacional de Misiones – CONICET; Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA)], Brown Howler Monkey Conservation Group.

<u>Time frame</u>: 6 months once the funding has been secured; annual surveys (funding limit). Participatory program (Jaguar example).

<u>Collaborators</u>: Vanessa Barbisan Fortes (Universidade Federal de Santa Maria, RS – Brazil). Collaboration with – Ministerio de Salud de la Nación Argentina (MSAL), Ministerio de Ecología y Recursos Naturales Renovables de la Provincia de Misiones (MERNR), Administración de Parque Nacionales de Argentina (APN), Fundación Vida Silvestre Argentina (FVSA), Dirección de Fauna

Silvestre – Secretaria de Ambiente y Desarrollo Sustentable de Argentina (DFS – SayDS), IBS-CeIBA, NGOs, Municipal authorities. Need Public Health authorities financial support, and training of local health professionals and local communities awareness.

Financial support : MSAL, MERNR, APN, Municipal authorities.

Difficulty : The main difficulty consists in maintaining the collaboration among participants and organizations in the long term.

Objective: Acquire better knowledge of brown howler monkeys in Misiones.

Action 2. Estimate the population abundance of brown howler monkeys in Misiones.

Focal person: Ingrid Holzmann (IBS – CeIBA).

Time frame: 1-year once the funding has been secured.

Collaborators: Martín Kowalewski (Estación Biólogica de Corrientes - EBCo), Ilaria Agostini (IBS – CeIBA), Mario Di Bitetti (IBS – CeIBA), Luciana Oklander (IBS – CeIBA), Carlos De Angelo (IBS – CeIBA), Júlio César Bicca-Marques (Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS, Brasil).

Financial support : To be defined.

Difficulty: The most difficult part is securing the funding.

Objective: Health assessment of brown howler monkeys in Misiones.

Action 3. Health studies of all brown howler monkey populations in Misiones to evaluate parameters such as physiological stress, innate and acquired immunity, hematology, etc., to be able to evaluate and compare different populations especially before and after Yellow Fever outbreaks.

Brief description: Two studies are proposed: (1) A longitudinal study, with a strong focus on individuals, in which different groups from sites with Yellow Fever outbreaks and where the outbreaks were not evident will be followed in time to collect data on genetic parameters, health status, immunological experience, reproductive output and behavior and (2) A cross-sectional one that would compare populations that suffered different degrees of impact after the last Yellow Fever epidemic. The focal information to be retrieved from those populations will be immunological experience (screening of antibodies against Yellow Fever and other flaviviruses), and genetic variability (in general, MHC, and other relevant markers). Concomitant mosquito surveys would be conducted at the sites of both studies (related directly with actions 4 and 6).

Focal person: Pablo Beldomenico [Laboratorio de Ecología de Enfermedades (LEcEn)- Instituto de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral - CONICET (ICiVet Litoral, CONICET-UNL) and Global Health Program, Wildlife Conservation Society (GHP-WCS)].

Time frame: From the acquisition of funding, at least 2 years.

Collaborators: LEcEn (ICiVet Litoral, CONICET-UNL), GHP-WCS), Martín Kowalewski (EBCo), Ilaria Agostini (IBS-CeIBA), Instituto Nacional de Medicina Tropical de Argentina (INMeT), Instituto Nacional de Enfermedades Virales Humanas (INEVH), Mariela Martínez (INMeT – CONICET), Silvina Goenaga (INEVH - INMeT), Juan Pablo Arrabal (IBS - CeIBA), Sebastián Costa (IBS – CeIBA), Ezequiel Vanderhoeven (CeIBA – Güira Oga), Marcela Uhart (UC Davis).

Financial support: Will apply for a grant of the Morris Animal Foundation (the next call for Wildlife/Exotics health and welfare proposals will be in mid-August 2013 with proposals due in mid-November 2013) and others to be defined.

Difficulty: The most difficult part is securing the funding.

Objective: To understand Yellow Fever dynamics.

Action 4. **Isolate Yellow Fever virus from adult and larvae of mosquitoes**.

Focal person: Eduardo Lestani (INMeT).

Time frame: Start now.

Collaborators: INMeT and INEVH.

Financial support : MSAL, CONICET, WCS, Fundación Mundo Sano, and others.

Difficulty: Easy execution.

Objective: To understand Yellow Fever dynamics.

Action 5. Conduct a thorough literature and archive review to enhance our understanding of the interactions (environmental and anthropogenic) involved in the maintenance and dynamics of Yellow Fever outbreaks in South America.

Brief description: The basic idea of this review would be to challenge the dogmatized traditional understanding about the responsible mechanisms for Yellow Fever maintenance and occurrence in South America. The working hypothesis would be that human primates and African mosquitoes (*Aedes* spp.) are more important than currently appreciated in the maintenance and dynamics of Yellow Fever in South America and that a “One Health” perspective is more appropriate than the traditional compartmentalization into urban and wild cycles.

Focal person: Pablo Beldomenico (LEcEn - ICiVet Litoral, CONICET-UNL), Eduardo Moreno (Ministério da Saúde do Brasil).

Time frame: Start now, within 1 year.

Collaborators: Collaboration between Brazilian and Argentinean researchers.

Difficulty: Easy execution.

Objective: To understand Yellow Fever dynamics.

Action 6. Capture adult mosquitoes where monkeys sleep or capture adult mosquitoes through monkey baited capture stations.

Focal person: Eduardo Lestani (INMeT).

Time frame: Start now, within 1 year.

Collaborators: INMeT and INEVH.

Financial support: MSAL, CONICET, WCS, Fundación Mundo Sano, and others.

Difficulty: Easy execution.

Objective: Acquire better knowledge of brown howler monkeys in Misiones

Action 7. Define the current and potential distribution of brown howler monkeys in Argentina.

Focal person: Ilaria Agostini (IBS – CeIBA), Ingrid Holzmann (IBS – CeIBA), Katia Ferraz (IUCN SSC – Conservation Breeding Specialist Group – Brazil).

Time frame: Depends on Action 2 as well as historical data review.

Collaborators: Asociación Primatológica Argentina (APRIMA), Júlio César Bicca-Marques (PUCRS, Brasil).

Financial support: Depends on funding obtained for Action 2.

Difficulty: Feasible execution.

Objective: To understand Yellow Fever dynamics.

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

Focal person: Silvina Goenaga (INEVH – INMeT).

Time frame: 6 months-1 year.

Collaborators: INMeT, INEVH; Júlio César Bicca-Marques (PUCRS, Brasil), David Santos de Freitas (PUCRS, Brazil).

Financial support : MSAL, CONICET, WCS, and others.

Difficulty: Feasible execution.

Objective: To understand Yellow Fever dynamics.

Action 9. Systematic review about the virulence of the Yellow Fever virus from different strains in different vertebrate hosts in Misiones and Brazil.

Focal person: Silvina Goenaga (INEVH – INMeT).

Time frame: Start now.

Collaborators: INEVH.

Difficulty: Easy execution.

Objective: Acquire better knowledge of brown howler monkeys in Misiones.

Action 10. Understand what defines the carrying capacity of brown howler monkeys and their habitat requirements (limiting factors, food, threats).

Brief description: This study is constituted by two parts: a general vegetation survey in the sites where brown howlers are localized (see actions 2 and 7) and several long-term studies (e.g. could be subjects of PhD theses) focusing on across-sites comparisons of relationships between brown howlers and the environment in which they live.

Focal person: Martín Kowalewski (EBCo).

Time frame: No time frame, long term project.

Collaborators: Vanina Fernández (EBCo), Gabriel Zunino (UNGS), Júlio César Bicca-Marques (PUCRS, Brasil), Vanessa Barbisan Fortes (UFSM, Brazil), Erin Vogel (Rutgers University, USA), Jessica Rothman (City University of New York, USA).

Financial support : To be defined.

Difficulty: Will require long term ecological data.

Objective: To understand Yellow Fever dynamics.

Action 11. Identify suspected vertebrate hosts, and places of Yellow Fever virus circulation in Misiones through screening of antibodies against Yellow Fever or other Flaviviridae.

Focal person: Silvina Goenaga (INEVH – INMeT).

Time frame: 6 months-1 year.

Collaborators: INMeT, INEVH, Júlio César Bicca-Marques (PUCRS, Brasil).

Financial support : MSAL, CONICET, WCS, and others.

Difficulty: Feasible execution.

Objective: Acquire better knowledge of brown howler monkeys in Misiones.

Action 12. Study of the metapopulation genetic diversity (i.e., population structure, connectivity, bottle necks, etc.).

Focal person: Luciana Oklander (IBS – CeIBA).

Time frame: Depends on monitoring programs being implemented (see Actions 1 and 3).

Collaborators: Marta Mudry and Mariela Nieves (Grupo de Investigación Biología Evolutiva – Universidad de Buenos Aires), Carina Argüelles (IBS-Posadas), Inés Badano (IBS – Posadas; LABIMOL – Universidad Nacional de Misiones), David Santos de Freitas (PUCRS, Brazil).

Financial support : To be defined.

Difficulty: Will depend on the funding available.

IX. Workshop implementation and Next Steps

Communication Strategy

One of the main challenges mentioned by workshop participants was the lack of involvement of the local community and authorities in brown howler monkey conservation. On the 28th of March, workshop participants gathered together at the Hotel Saint George in Puerto Iguazú to give a presentation about workshop results to local stakeholders and authorities. For two hours, the workshop participants gave a detailed presentation about the reason for undertaking the workshop, the process participants went through, as well as final recommendations and future steps.

Representatives from national authorities (Administración de Parques Nacionales) and provincial authorities (Ministerio de Ecología y Recursos Naturales Renovables) that are involved in decision-making processes concerning biodiversity conservation in the region attended this final meeting. Also present were representatives of the **Instituto de Medicina Tropical (INMeT)**, a national research institute dedicated to public health issues; **Grupo de Investigación de Biología Evolutiva de la Universidad de Buenos Aires**; **Fundación Vida Silvestre Argentina,** a WWF-associated NGO active in the field of biodiversity and ecosystem conservation in Argentina; **Güira Oga,** a rescue and rehabilitation center for wildlife; **Conservación Argentina,** an NGO committed to forest conservation; and **Temaiken** a foundation dedicated to environmental education and conservation. Finally, some press/radio media covered the event and advertised its main results on the following days.

Several questions and points of discussion have been raised by the stakeholders that participated in this final plenary meeting. Some of them are summarized below, with the relative answers given by workshop participants.

Presentation about workshop results to local stakeholders and authorities at the Hotel Saint George in Puerto Iguazú.

A representative from the Administración de Parques Nacionales, Guillermo Gil, asked:

1) Has a vaccination against Yellow Fever ever been developed for howler monkeys?
   REPLY: Vaccination in wildlife species is highly debated. Most of the time, the costs are greater than the benefits, since it implies a great logistic effort. On the other side, it could have negative side effects for the individual and the population that have to be taken into account.

2) Given that the future projected situation of brown howlers in the region is rather critical, should people intervene to avoid extinction before the indicated 100 years period?

   REPLY: Before thinking of intervening on the population with extreme and questionable management options, it is worth it to try to avoid extinction by controlling the major threats to the population, such as the impact of recurrent Yellow Fever epidemics in the future.

3) Is there any gene flow and interchange among brown howler populations between Argentina and Brazil?

REPLY: There are no reliable data on this issue. However, it seems that if an interchange exists it would be probably very low due to the geographical barriers to dispersal (i.e. large rivers marking the border between Argentina and Brazil).

4) Although it is not common to find individuals of brown howlers in captivity, is there any idea about what to do with such individuals? Would some kind of *ex situ* management action be feasible? Would it be worthy to think of a reintroduction with these individuals?

   REPLY: Brown howler individuals are very rare in captivity. In addition, any *ex situ* management action would not be appropriate because although the brown howler population of Misiones has been drastically reduced, it still inhabits the region at small numbers. So, this is not a suitable condition for a re-introduction, since the population still exists and before thinking about an *ex situ* management option, the factors that caused population extinction should be known and removed.

5) Do brown howlers live in Paraguay too?

   REPLY: There are no brown howlers in Paraguay.

Claudio Maders, the coordinator of provincial park-rangers in charge of protected areas of the central portion of Misiones (the one including most of the brown howler distribution in Argentina) stated that in his opinion, the situation is really alarming because since 2008, park rangers have not recorded any direct observation of this species in the protected areas and surroundings.

6) Maders asked about what is currently known about the species situation in Brazil.

   REPLY: Brown howlers are known to persist in many protected areas in the Atlantic Forest of Brazil, although it has been highly affected by Yellow Fever, especially in Rio Grande do Sul. Also, even though the species is frequently present at high densities (compared to Misiones), it should be reminded that the Brazilian Atlantic Forest is heavily reduced in surface and fragmented, the fate of these populations is unsure in the long-term.

Manuel Jaramillo, coordinator of the Selva Paranaense Program of Fundación Vida Silvestre Argentina, argued that this species does not have any visibility in the public opinion, and gained some public attention only after the Yellow Fever epidemics occurred in 2008-2009. Further, it is not a species that people recognize as typical of Argentina. Jaramillo then asked some questions:

7) In case yellow fever outbreaks do not occur, would the brown howler population reach the carrying capacity?

   REPLY: One of the objectives that came out of the workshop process is to investigate what defines the carrying capacity of brown howlers and what are their habitat requirements in Misiones. We are aware that brown howlers in this region lived at relatively low densities, even before the occurrence of recent Yellow Fever epidemics. The factors that determine these low densities are completely unknown and would be worth investigating. Is it that the carrying capacities for this particular habitat is low, or are there any factors that prevent brown howlers to reach the carrying capacity (e.g. difficulty of recovery after recurrent Yellow Fever outbreaks).

8) Compared with other species, at least one would know what is necessary to do to conserve this species, i.e. to work hard to prevent Yellow Fever outbreaks, and not so much involving issues such as habitat loss or forest fragmentation, is it right?

REPLY: Yes. From the threats analysis we carried out, it is clear that to increase the chances of persisting in the long-term for this species, the main actions should be focused on preventing Yellow Fever outbreaks and minimizing their impact on the population. Other potential threats, such as habitat loss, are currently considered to be far less important for the region.

9) What if some brown howler individuals have survived the Yellow Fever epidemics and they get resistant to this disease. One could think of translocating some of these individuals to other affected areas?

REPLY: As discussed for vaccination, such an important intervention is difficult to justify, since costs could overcome the benefits. Possible drawbacks may include high risks for the translocated individuals, the disruption of social structures, and loss of recovery potential for populations where resistant individuals are naturally present. Besides, Yellow Fever is not the only parasitic disease circulating in the area, translocation of animals may carry new parasites into "healthy" areas.

10) I just realized that the Yellow Fever virus uses the same vector as the dengue virus. A couple of years ago in Iguazú it was said on the media that there were 500 cases of dengue in people, while actually the number of people that got infected was 10,000. This suggests that the dengue is a disease that acts against very powerful interests, such as tourism income in Iguazú. This is why the information about the severity of this disease is frequently manipulated. If the Yellow Fever is somewhat associated to the dengue, then how could we manage not to get the information about Yellow Fever manipulated in a similar way? To highlight the link between a threat to human health, such as dengue, to Yellow Fever and the conservation of brown howlers could be useful, but the information should be carefully managed not to openly arouse conflicts of interest.

REPLY: This is an important issue to take into account. It is true that conflicts of interest can arise between politics of tourism development in the region and dissemination of information about the actual impact of diseases. Probably, the development of relationships with national and provincial governmental authorities and an increase in their commitment to the brown howlers' conservation cause could help with managing this potential problem.

11) Is there any reason why the distribution of brown howlers in Misiones is so restricted? Any known limiting factor? It is quite peculiar that the species is so limited to a relatively small area given that in Brazil is highly represented.

REPLY: As said before (reply to question 6), we also found this distribution pattern hard to explain on the basis of mere forest habitat extension currently available in Misiones. This is why we decided to investigate what defines a suitable habitat for brown howlers, and then evaluate the possible reasons for differences found between the potential and the actual distribution ranges of brown howlers in the region.

The president of the NGO Conservación Argentina, Diego Varela, argued that:

12) A carrying capacity of around 400 individuals seems too small for brown howlers in Misiones.

    REPLY: The numbers presented in the *Vortex* models are just based on an educated guess given the small information on population size and distribution for brown howlers in Misiones. It is not the number the most important here, but the understanding of possible dynamics of the brown howlers population under different scenarios. Numbers can be changed in the future as soon as we obtain better estimates for each parameter.

Finally, Paula González, from the foundation Temaiken, asked:

13) What is the role of the Ministerio de Ecología y RNR of Misiones in all this process? She said she is concerned they are not taking a lead role.

    REPLY: Workshop organizers have already established contact with representatives of this Ministry, which have been invited to this final meeting. Although the presence of the Ministry has not been significant in this event, we are aware that they are the key institution for implementing most of the actions we recommend. After we complete the workshop report (by June 2013), we will set a meeting with the Minister in Posadas (Misiones capital) to present all the outcomes of this event and our plans for the future, illustrating all the possible chances for collaboration and soliciting commitment from this authority.

14) This species would perfectly apply for funding from the "*Ley de Bosque*" funds of Argentina.

    REPLY: This is an excellent suggestion and we will certainly inquire about this funding possibility for any of our actions.

Overall, these issues raised by participants led to a discussion and exchange of ideas between stakeholders and the group of researchers that had developed the population viability analysis for brown howlers during the previous days. This discussion was fruitful and offered an idea of what is currently the perception of other institutions and NGOs involved in biodiversity conservation in the region. Also, it has raised awareness for the first time about the critical situation of this relatively neglected endangered population.

On the last day of the workshop the following actions in the short and medium term to promote and communicate results of the workshop were agreed upon.

Scientific communications

- Eduardo Moreno will prepare a publication on results from the workshop for a public health journal.
- Ilaria Agostini will prepare a publication for Neotropical Primates.
- Pablo Beldomenico will lead the writing of a review on the ecology of Yellow Fever in South America.

Presentations in conferences and meetings

- Martín Kowalewski is going to present the main workshop outcomes at the II Latin American Congress of Primatology and XV Brazilian Congress of Primatology in Recife in August 2013.
- Ilaria Agostini, Ingrid Holzmann, Martín Kowalewski, Luciana Oklander and Mario Di Bitetti will present results of the workshop and Species Conservation Strategies methodology at the first meeting for the development of Primate National Conservation plan that will take place in Misiones, approximately in September 2013 (dates have to be defined).

Dissemination of report to stakeholders

- Arnaud Desbiez and Ilaria Agostini will prepare a summary for the IUCN SSC Primate Specialist Group.
- Summary and report need to be widely distributed to the Administración de Parques Nacionales, Ministerio de Ecología y RNR de Misiones, Ministerio de Salud de la Nación, Instituto de Medicina Tropical, forestry companies such as Alto Paraná S. A., NGOs such as Fundación Vida Silvestre Argentina, Conservación Argentina, Temaikén, Güira Oga, Fundación Mundo Sano, and local municipal authorities.

Fundrasing

- Ilaria Agostini will contact all the institutions that have donated or expressed interest to donate to the workshop and send the report and hopefully engage them to continue supporting the work.
- In less than six months some of the workshop participants (Ilaria Agostini, Ingrid Holzmann, Mario Di Bitetti, Martín Kowalewski, Pablo Beldoménico, Eduardo Moreno, and possibly any other interested who will join the group) will get together to write proposals to seek funding for key aspects of the workshop.

Implementation Strategy

In order to ensure that recommendations of the first Brown Howler Monkey Conservation Workshop are effectively implemented, the group decided to create the Brown Howler Monkey Conservation group (BHMC group). Ilaria Agostini will be the point of contact for the group. All workshop participants are automatically members of this group, and more members can be added to the group as they get involved in the actions.

Several communications tools have been established to ensure efficient communication between group members. The Dropbox that was well used before the workshop to disseminate papers and reports related to howler monkey conservation and yellow fever will be maintained as a tool to exchange data. A Google group was also established.

The BHMC group also agreed to hold an annual meeting to revise and evaluate the progress of the implementation of the recommendations. New participants will be welcome to join the group at that time.

X. Vortex Report

POPULATION VIABILITY ANALYSIS

Modelers:

Arnaud Desbiez (Royal Zoological Society of Scotland; IUCN/SSC CBSG Brasil)

Phil Miller (IUCN/SSC CBSG)

Model input group:

Ilaria Agostini, Ingrid Holzmann, Martín Kowalewski and Mario Di Bitetti

Introduction

During the Brown Howler Monkey workshop participants divided into two groups to examine and estimate parameters and scenarios for two of CBSG modeling tools: *Vortex* and *Outbreak*. *Vortex* was used to examine the impact of Yellow Fever (YF) as a catastrophe affecting brown howler monkey populations. A base line model was created (and will be improved upon in the following years), a sensitivity analysis of some parameters performed, and then the impact of YF with different values of severity and frequency was examined. Finally, participants tried to model what they felt was a realistic scenario of brown howler monkey distribution and population abundance in Misiones at the moment. We examined what would happen if YF outbreaks were not evenly distributed.

Vortex Simulation Model

Vortex is a Monte Carlo simulation of the effects of deterministic forces as well as demographic, environmental, and genetic stochastic events on wild populations. *Vortex* models population dynamics as discrete sequential events that occur according to defined probabilities. The program begins by creating individuals to form the starting population and stepping through life cycle events (e.g., births, deaths, dispersal, catastrophic events), typically on an annual basis. The fate of these individuals is followed throughout their “lives”. Events such as breeding success, litter size, sex at birth, and survival are determined based upon designated probabilities. Consequently, each run (iteration) of the model gives a different result. By running the model hundreds of times, it is possible to examine the probable outcome and range of possibilities.

Vortex is not intended to give absolute answers, since it is projecting stochastically the interactions of the many parameters used as input to the model and because of the random processes involved in nature. However, it provides a clear view of general population trends under different scenarios and insights on the relative importance of different variables that could affect the population trajectories. Interpretation of the output depends upon our knowledge of the biology of howlers, the environmental conditions affecting the species, and possible future changes in these conditions. For a more detailed explanation of *Vortex* and its use in population viability analysis, see (Lacy 1993, Lacy 2000, Miller & Lacy 2003a).

Baseline Model: Biological Growth Potential

Input Parameters for Simulation Modeling

A general baseline population model for Brown Howler monkeys was built and it was later tailored to represent the populations of Misiones. The baseline population model was designed to investigate the viability of a non-existent but biologically accurate howler population without any anthropogenic threats. The baseline model reflects the biological potential of Brown Howlers. Alternative values for demographic parameters were then explored through sensitivity testing.

Scenario settings

Duration of simulation: Life expectancy of howlers is approximately 10-18 years in the wild. The population was modeled for 100 years (approximately 15 generations) so that long-term population trends could be observed. One hundred years is far enough into the future so as to decrease the chances of omitting a yet unknown event, but also not too short to fail to observe a slowly developing event.

Number of iterations: 1000 independent iterations were run for each scenario.

Reproductive system and rates

Breeding system:

Long term polygyny (well established fact in all howler monkey publications).

Age of first reproduction: ♀: 5 years; ♂: 6 years

Vortex defines reproduction as the time at which offspring are born, not simply the age of sexual maturity. The program uses the mean age of first reproduction rather than the earliest recorded age of reproduction.

Females reach sexual maturity around 36 months, then gestation lasts 6 months, so on average the age of first reproduction could be around 4 years (Crockett & Eisenberg 1987). In *A. caraya* on average females between 4-5 years of age have their first offspring (Martín Kowalewski, pers. comm.). To be conservative the age of first reproduction for females was set at 5 years. It was estimated that it would take longer for males to mate and reproduce since they must be able to secure a troop of females first. There was a lot of discussion regarding this parameter which will be tested in the sensitivity analysis (Minimum = 5; Maximum = 8 yrs). From observations in *A. caraya*, the average age for first reproduction of males was set at 6 (Martín Kowalewski, pers. comm.)

Maximum age: 16 years

Vortex assumes that animals can reproduce (at the normal rate) throughout their adult life. Longevity was set as the maximum age of reproduction. In captivity a maximum of 20 years has been reported (http://www.demogr.mpg.de/LONGEVITYRECORDS/INDEX2.HTM), but in the wild it is probably lower. In *A. caraya* a female of 16 years old was observed producing an infant (Martín Kowalewski, pers. comm.).

Maximum number of offspring per year: 1

Female breeding success: 50%

According to Strier et al. (2001), the average Inter-birth Interval was 21.2 months in a population of brown howler monkeys that was followed during a four years period. Therefore it was considered that adult females produced 1 infant every two years. Different studies report different annual birth rates, the highest is recorded by Miranda (2004) with 0.72 infants per adult female per year. For this reason Environmental Variation (EV), i.e. the annual fluctuations in mean demographic rates that result from random variability in environmental conditions, was set at 11. This means that the percentage of female breeding is on average 50% but can be as low as 28% and as high as 72%.

Mortality rates

Mortality rates: According to Strier et al. (2001), 74% of brown howler monkeys in the study survived their first year of life, other researchers in the group seem to confirm this data and a mortality rate of 25% was set for both males and females from age 0-1. From the ages 1-3 in a long term study of *A. caraya* there was a 40% mortality rate (Martín Kowalewski, pers. comm.), however other researchers in the group found this slightly high. All agreed that between ages 3-4 they become sub-adults and mortality rates drop. They feed independently (no more leaf poisoning and their digestive system is developed), there is less risk of predation, their participation in group social life increases and they can act as helpers. So as sub-adults (both males and females) get older mortality rates decrease. However, males aged 4 years old must disperse which increases their chance of mortality compared to females.

Mortality rates of adult howler monkeys are very low. For example, Miranda (2004) followed 6 groups with on average 6.3 individuals for 3 years and only 1 individual disappeared. However it was estimated that for both males and females the mortality rate would increase after the age of 10. For females after 10 years of age the mortality rates will increase by 5% each year and for males by 20% each year. This is because after 10 years of age the likelihood of the male having been expelled from his group increases and if a male is expelled from a group his mortality rates increases highly.

Table 1. Mortality rates of males and females used in the baseline model.

Mortality rates Ages	MALES Baseline model Value (EV)	FEMALES Baseline model Value (EV)
from age 0 to 1	25 (5)	25 (5)
from age 1 to 2	15 (5) (max 25)	15 (5) (max 25)
from age 2 to 3	10 (2.5) (max 20)	10 (2.5) (max 20)
from age 3 to 4	5 (2)	5 (2)
from age 4 to 5	10 (2.5)	2 (1)
from age 5 to 6	5 (2)	1 (0.5)
after age 6	1 but after 10 ((A<=10)*1) + ((A>10)*20*(A-10))	1 (0.5) but after 10 1 + ((A>10)*5*(A-10))

Species description

Definition of extinction: Extinction is defined in the model as no animals of one or both sexes remain.

Concordance of environmental variation (EV) between reproductive rates and survival rates: YES

Environmental variation (EV) is the annual variation in reproduction and survival due to variation in environmental conditions. Making EV concordant between reproduction and survival means that good years for reproduction are also good years for survival and vice versa. EV for survival and reproduction were linked in the model, since environmental variation not only affects howlers directly but also their food (leaves, flowers, fruit), which is considered to affect their survival and reproduction.

Inbreeding depression: *Vortex* includes the ability to model the detrimental effects of inbreeding through reduced first-year survival of inbred individuals. Inbreeding is thought to have major effects on reproduction and survival, especially in small populations. As population size of howlers continues to decline and populations become fragmented genetic considerations become very important.

The median value estimated from analysis of studbook data for 40 captive mammal populations was 3.14 lethal equivalents (LE) (Ralls et al. 1988). Wild populations that live in potentially more challenging environments are more vulnerable to inbreeding than captive populations. Crnokrak and Roff (1999) examined 157 datasets for wild populations of 34 taxa and found that 90% showed evidence of inbreeding depression, with average effects being significantly higher (7x) in the wild than observed in captivity. O'Grady et al. (2006) found an average overall effect of 12.3 LE over the life history of wild mammal and bird populations, with 6.3 LE of this impacting the production and survival of offspring to age one year. Based on these studies, the impact of inbreeding was modeled as 6 LE on juvenile mortality, with 50% of the effect of inbreeding due to recessive lethal alleles.

Population description

Number of populations: In the baseline model only one population is considered (no metapopulation structure).

Dispersal among populations: In the baseline model only one population is considered, with no immigration or emigration.

Initial population size (N): According to a best guess the population of Misiones could be around 200 individuals. For the purpose of the baseline model it is considered as one population, but in reality it is composed of several smaller fragmented populations with unknown connectivity among them.

Carrying capacity (K): 420

The carrying capacity was considered as a little more than double the initial population (N=420) according the participants. This number is not based on research but rather a best guess. No environmental variation was added to the carrying capacity, as variations in population size are accounted for by environmental variation in reproduction and survival.

Number of catastrophes: Catastrophes are singular environmental events that are outside of the bounds of normal environmental variation affecting reproduction and/or survival. Natural catastrophes can be tornadoes, floods, droughts, disease, or similar events. These events are modeled in *Vortex* by assigning an annual probability of occurrence and a pair of severity factors describing their impact on survival (across all age-sex classes) and the proportion of females successfully breeding in a given year. These factors range from 0 (maximum or absolute effect) to 1 (no effect), and are imposed during the single year of the catastrophe, after which time the demographic rates can rebound to their baseline values.

Examples of potential catastrophes: Yellow Fever outbreak and tornado. For howlers the most important known catastrophe is a YF outbreak. YF outbreaks in Misiones occur on average every 15 years, on average. It is therefore estimated that there is a 6% probability each year of an outbreak occurring.

YF does not affect breeding however it has a severe effect on survival killing between 60 to 80% of the population during an epidemic. To model the variability of the severity between 60-80% the following function was used (Figure 1): =0.3+(0.05*(SNRAND(Y+(R*100)))).

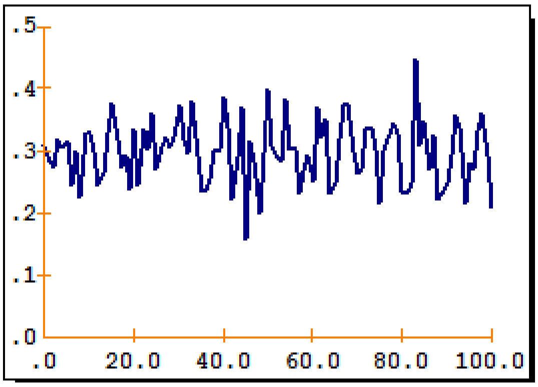


Figure 1. Severity of YF outbreak on % survival (Y axis) throughout the years (X axis).

<u>Harvest</u>: No harvest was included in the baseline model.

<u>Supplementation</u>: No supplementation of individuals from other unrelated populations, wild or captive, was incorporated into the baseline model.

Parameters used in the baseline model (Table 2) were tested in the sensitivity analysis.

Table 2. Summary of parameter input values used in the baseline model.

Parameter	**Baseline value**
Number of populations	1
Initial population size	200
Carrying capacity	420
Inbreeding depression	6 LE
% of the effect of inbreeding due to recessive lethal alleles	50
Breeding System	Long-term Polygyny
Age of first reproduction (♀ / ♂)	5 / 6
Maximum age of reproduction	16
Annual % adult females reproducing (SD)	50% (11)
Average litter size	1
Density dependent reproduction?	No
Maximum litter size	1
Overall offspring sex ratio	50:50
% adult males in breeding pool	90
% mortality from age 0-1 (EV) (♀ / ♂)	25(5)/ 25(5)

% mortality from age 1-2 (EV) (♀ / ♂)	15(5)/ 15(5)
% mortality from age 2-3 (EV) (♀ / ♂)	10(2.5)/ 10 (2.5)
% mortality from age 3-4 (EV) (♀ / ♂)	5(2)/ 5 (2)
% mortality from age 4-5 (EV) (♀ / ♂)	2(1)/10 (2.5)
% mortality from age 5-6 (EV) (♀ / ♂)	1(0.5)/10(1)
% mortality from age 6-10 (EV) (♀ / ♂)	1(0.5)/1 (0.5)
% mortality from age 11-16 (EV) (♀ / ♂)	+5% each year/+20% each year

Baseline Model Results

During the workshop a series of key questions and concepts were explored using *Vortex*. For these exercises the following parameters were used to interpret results:

r_{stoch} (SD) – The mean rate of stochastic population growth or decline and standard deviation, demonstrated by the simulated populations, averaged across years and iterations, for all simulated populations. This population growth rate is calculated each year of the simulation, prior to any truncation of the population size due to the population exceeding the carrying capacity.

$P(E)_{100}$ – Probability that the population will go extinct. Extinction is defined in the model as no animals of one or both sexes remaining. $P(E)_{100}$ is determined by the proportion of 1000 iterations that go extinct within 100 years.

MTE – Is the mean time to population extinction, in years, over a 100-year period.

N-all – Is the mean population size reported for all simulated populations with standard deviation (SD) across iterations.

N-Ext – Is the mean population size reported only for simulated populations that have not gone extinct.

GD- Genetic diversity

Results with and without YF outbreaks are reported (Table 3; Figure 2&3). It is important that caution be used when interpreting the results from the baseline model. The baseline model represents the biological potential of howlers based on the parameters previously described (Table 2). No harvest rates from hunting, no increase in mortality due to disease or fire, and no habitat loss have been included. This does not represent a realistic situation, but provides the basis upon which future models including other threats can be made. Without YF outbreaks the stochastic population growth rate is positive (r_{stoch}=0.026) and the population could potentially increase almost 3% a year. However the stochastic growth rate with YF is negative, this means that according to this model overall the population of Misiones has a low chance of survival in the long term due to recurrent YF outbreaks. However since the standard deviation of the stochastic growth rate is very high, the population has a 20% chance of not going extinct in 100 years.

Table 3. Model results for 1 population of howler monkeys (N=200; K=420) with and without Yellow Fever (YF) outbreaks.

	r_{stoch}	SD	N-all	PE	GD
Without YF	0.026	0.052	408.51	0%	0.972
With YF	-0.051	0.296	71.56	80%	0.8466

Considering results without YF: sex ratio (all age classes) is female biased and the sex ratio of males to females is 44:56. However if only adult individuals are considered than the sex ratio of adult males to adult females is 37:63. Adult individuals (sexually mature individuals) represent 56% of the population.

These results were perceived as realistic by the workshop participants.

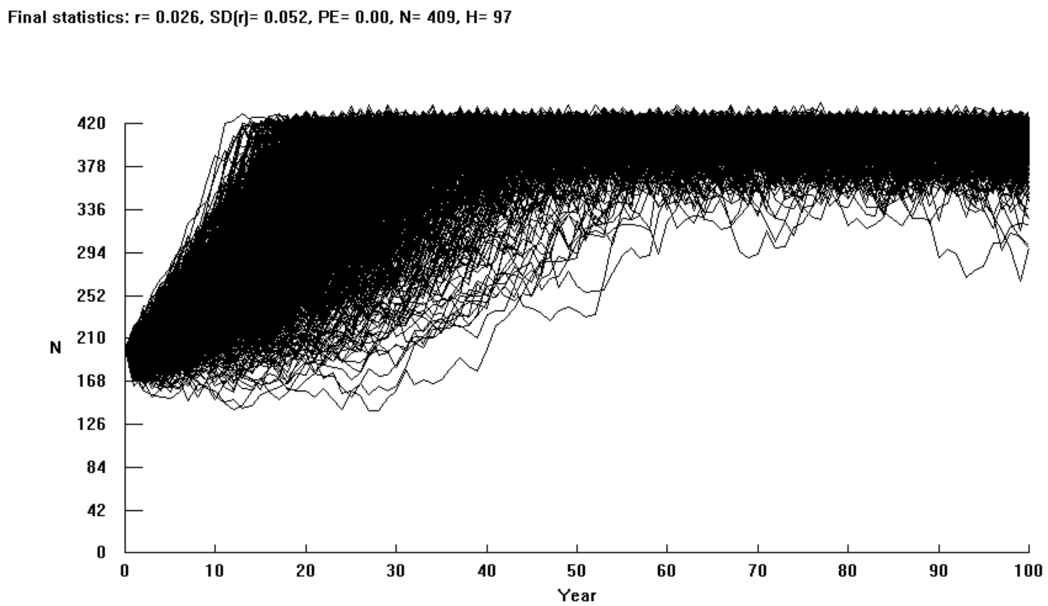


Figure 2. Trend of population of brown howler monkey during 100 years in the absence of YF outbreaks.

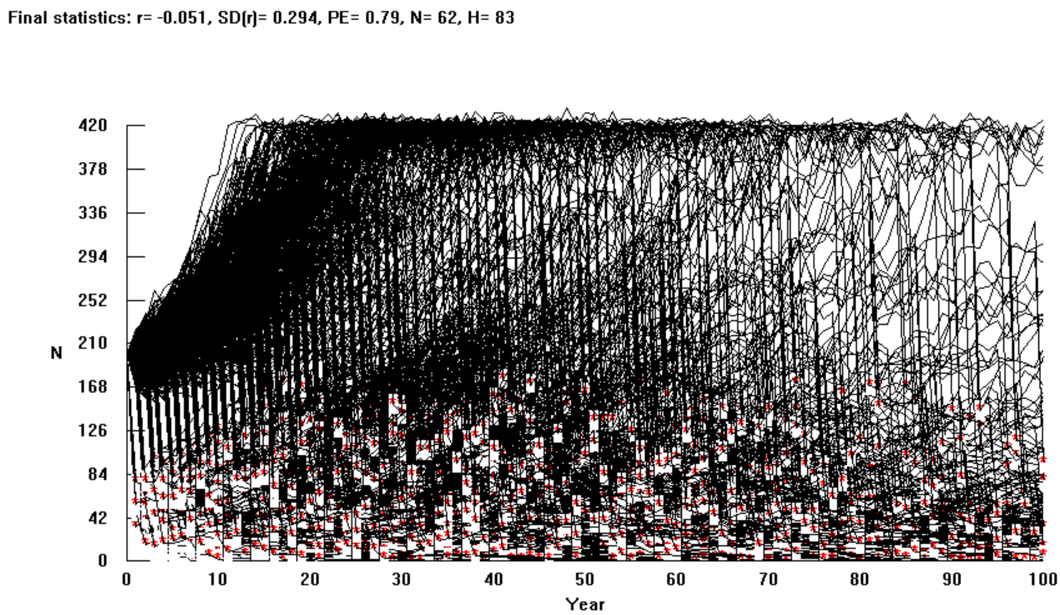


Figure 3. Trend of population of brown howler monkey during 100 years including YF outbreaks.

Sensitivity Analysis

Demographic Rates

Sensitivity analysis is a tool used to evaluate the robustness of a model to variations in parameter values. The more robust the model is to variations in a particular parameter, the less sensitive the model's results are to the input values of that parameter. This tool is used, in the current context, to uncover particularly sensitive parameters that could significantly alter the results and conclusions derived from the model. The most sensitive parameters require greater certainty in the input values to produce more confident results.

Sensitivity analyses using highest and lowest values for each parameter were performed on demographic rates to evaluate the effect of model parameters on the stochastic growth rate (r_{stoc}) of howler populations. Mortality rates were increased and decreased, 1 year was subtracted to age of first reproduction and a few years were added/subtracted to maximum age of reproduction (Table 4).

Table 4. Values of parameters used in the sensitivity analysis without YF outbreak.

	Minimum values	**Baseline**	**Maximum Values**
% mortality age 0-1 (EV) (♀ / ♂)	15	**25(5)/ 25(5)**	35
% mortality age 1-2 (EV) (♀ / ♂)		**15(5)/ 15(5)**	
% mortality age 2-3 (EV) (♀ / ♂)	Decrease of 5 for subadults	**10(2.5)/ 10(2.5)**	Increase of 5 for subadults
% mortality age 3-4 (EV) (♀ / ♂)		**5(2)/ 5 (2)**	
% mortality age 4-5 (EV) (♀ / ♂)		**2(1.5) /10(2)**	
% mortality age 5-6 (EV) (♀ / ♂)		**1 (0.5)/10 (1)**	
% mortality adult (EV) (♀ / ♂)	Decrease of 5 for adults	**1 + function**	Increase of 5 for adults

Age of first reproduction (♀ / ♂)	4/5	**5/6**	
Annual % of adult ♀ reproducing	40 (10)	**50 (11)**	60(10)
Maximum Age of Reproduction	13	**16**	20

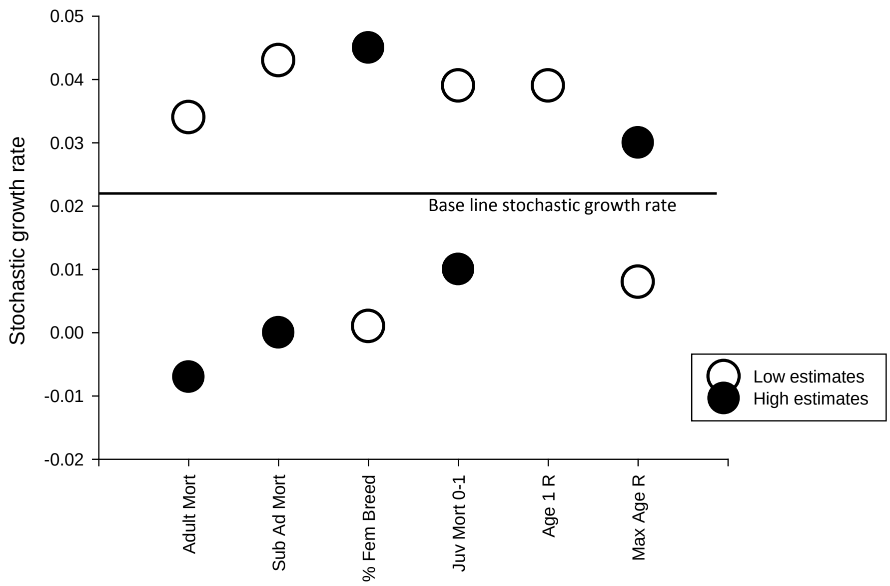


Figure 5. Impact of high and low estimates on stochastic growth rate (parameters to the left have the highest impact).

Table 5. Results from sensitivity analysis.

	r_{stoc}	SD(r)	PE	N-all	SD Nall	GenDiv	SD(GD)
Base line model (no YF)	0.026	0.052	0	409	16.05	0.9719	0.0041
Low mortality 0-1	0.039	0.053	0	413	11.86	0.9726	0.0033
High Mortalility 0-1	0.01	0.051	0	362	66.43	0.9671	0.0087
Low mortality sub adult	0.043	0.047	0	417	8.58	0.9741	0.0034
High Mortalility subadult	0	0.059	0	220	103.5	0.9494	0.0206
Low Mortality Adults	0.034	0.048	0	414	11.47	0.9744	0.0034
High Mortality Adults	-0.007	0.06	0.008	133	90.03	0.9284	0.0444
First age of reproduction	0.039	0.053	0	413	10.96	0.971	0.0039
% of ♀ breeding Low	0.001	0.047	0	232	99.37	0.9555	0.015
% of ♀ breeding high	0.045	0.042	0	418	6.97	0.9727	0.0032
Max Age of Repr Low	0.008	0.053	0	337	84.7	0.9645	0.0109
Max Age of Repr High	0.03	0.053	0	412	12.66	0.9721	0.0037

The sensitivity analysis demonstrates that adult mortality has one of the highest impacts on the population. Current long term studies (Strier et al. 2001, Miranda 2004, Martín Kowalewski, pers. comm.) show that adult mortality is extremely low in healthy howler monkey populations. Therefore the impact of a YF outbreak on a population is devastating as this mortality rate highly increases.

Sensitivity Analysis of Yellow Fever Outbreaks

According to the workshop participants YF outbreaks are the biggest current threat to brown howler monkeys. According to them they occur on average every 15 years, which was translated in *Vortex* as a 6% probability each year of an outbreak occurring. As for its impact, YF does not affect breeding, however it has a severe effect on survival: killing between 60 to 80% of the population during an epidemic. A sensitivity analysis of the severity and frequency of YF outbreaks was performed.

Sensitivity to severity of outbreak was modeled (Table 6; Figure 6), and mortality due to YF ranged from 90% ± 10% to 20% ± 10%. A frequency of 10 to 1% probability each year of an outbreak occurring was tested (maintaining the severity used in the base line model, killing between 60 to 80% of the population during an epidemic) (Table 7; Figure 7).

Conclusion

Both severity and frequency of YF have an impact on the rate of extinction of brown howler monkeys. The change in survival rates after each outbreak had more impact than frequency of outbreaks. However this is also because the severity of the outbreak used in the baseline model was high (70% mortality). Measuring the impact of YF outbreaks on survival of individuals from populations of howler monkeys is important for population viability modeling purposes.

Table 6. Impact of variation of severity of YF outbreaks on a population of brown howler monkeys (N=200/ K=420).

Impact on survival	stoc-r	SD(r)	PE	N-all	SDN_{all}	GenDi	SDGD	MeanTE
90% mortality	-0.08	0.485	0.964	2.38	23.94	0.808	0.147	35.1
80% mortality	-0.067	0.374	0.934	3.03	21.8	0.832	0.153	49.1
70% mortality	-0.052	0.295	0.802	12.66	51.31	0.821	0.141	56.2
60% mortality	-0.037	0.234	0.604	30.01	75.05	0.851	0.115	68.3
50% mortality	-0.022	0.182	0.332	71.04	103.4	0.876	0.111	75
40% mortality	-0.013	0.144	0.118	111.4	118.3	0.890	0.107	79.1

30% mortality	0.002	0.103	0.014	229.6	131.9	0.940	0.058	75.1
20% mortality	0.012	0.077	0.00	334.9	91.97	0.963	0.015	0.00

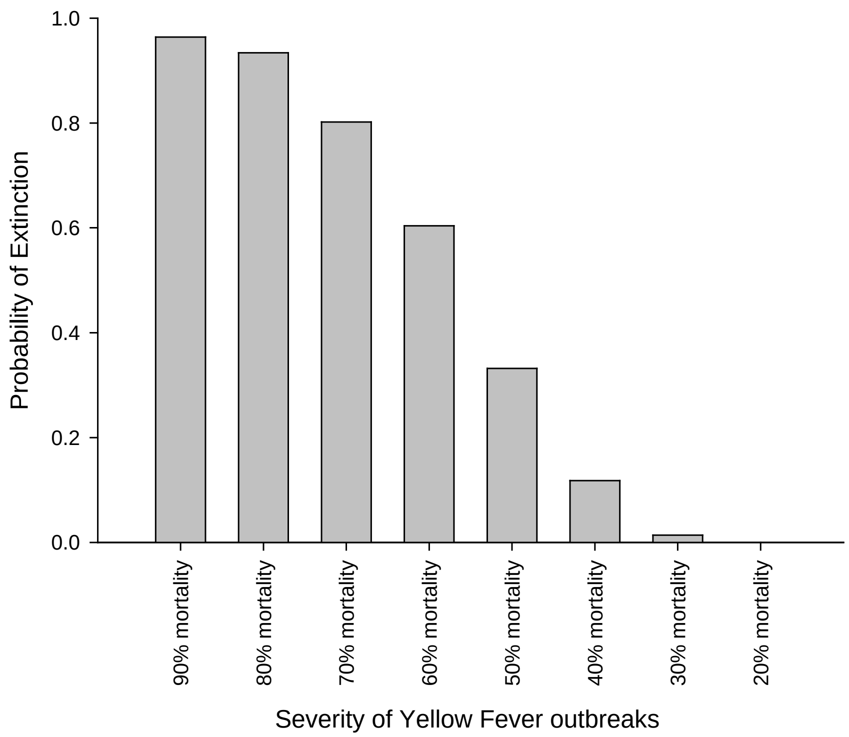


Figure 6. Sensitivity analysis of severity of YF outbreaks.

Table 7. Impact of variation of frequency of YF outbreaks on a population of brown howler monkeys (N=200/ K=420).

Frequency of outbreaks	stoc-r	SD(r)	PE	N-all	SDN_{all}	GenDi	SDGD	Mean TE
Frequency 1%	0.012	0.133	0.024	286.3	148.2	0.95	0.048	73.8
Frequency 2%	-0.004	0.187	0.128	163.9	155.7	0.915	0.081	70.6
Frequency 3%	-0.015	0.214	0.296	110.4	142.9	0.901	0.089	69.6
Frequency 4%	-0.03	0.25	0.492	47.79	96.17	0.850	0.129	65.8
Frequency 5%	-0.038	0.267	0.616	29.43	71.59	0.837	0.140	61.2
Frequency 6%	-0.05	0.292	0.766	11.87	40.33	0.826	0.133	57.7
Frequency 7%	-0.064	0.318	0.898	6.01	36.99	0.826	0.109	52.6
Frequency 8%	-0.071	0.329	0.934	1.97	12.47	0.803	0.118	49.5
Frequency 9%	-0.081	0.346	0.958	1.05	6.39	0.795	0.125	44.1
Frequency 10%	-0.091	0.36	0.982	0.98	9.6	0.815	0.154	41

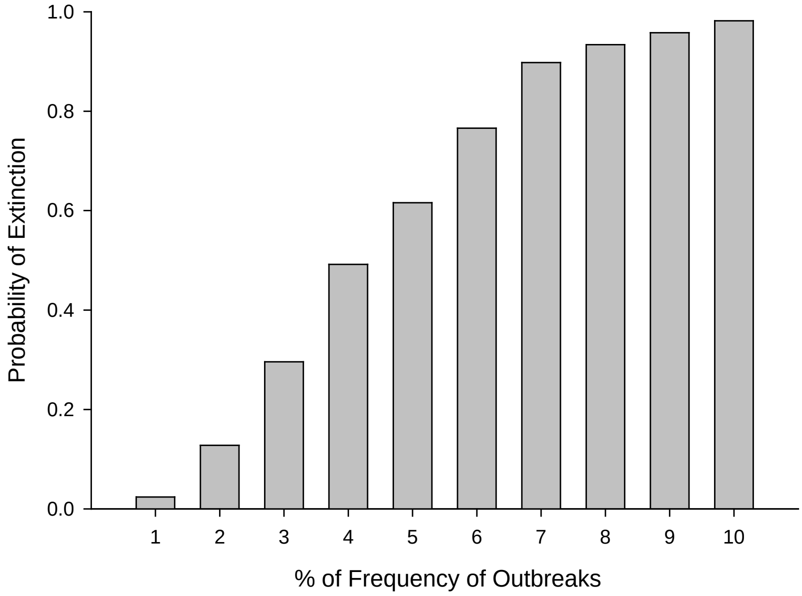


Figure 7. Sensitivity analysis of frequency of YF outbreaks.

Case study Misiones

Due to forest conversion to other land uses and to the presence of roads and towns, the Atlantic Forest of Misiones is becoming highly fragmented. The remnant brown howler monkey population is thus becoming increasingly structured into subpopulations with an also increasing (and probably high) degree of isolation among them. Participants tried to imagine how the population of brown howler monkey might potentially be distributed in Misiones (Figure 8a), what population sizes would be (N) and could potentially be (K) (Figure 8b). In reality the population is fragmented and might not be impacted by YF outbreaks in the same way.

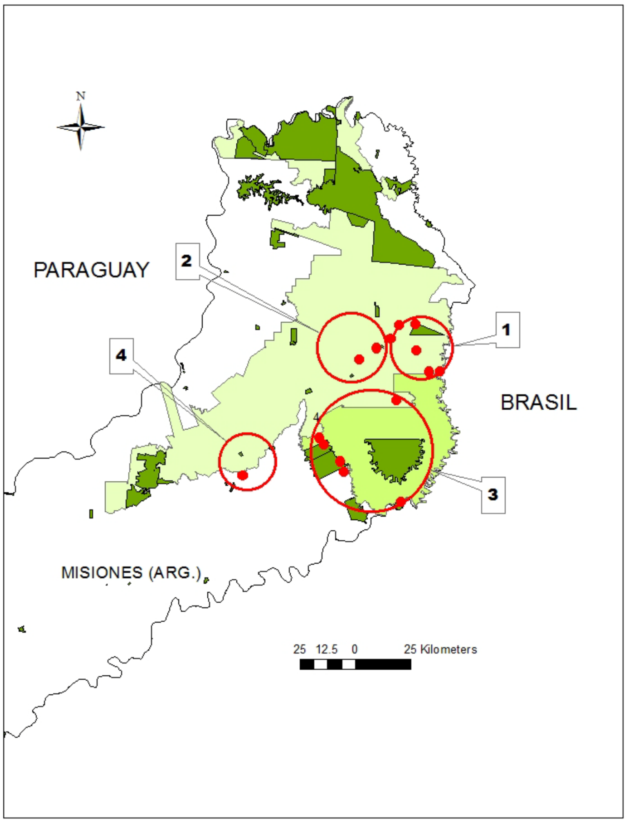


Figure 8a. Distribution of remnant populations of brown howlers in Misiones estimated by the workshop participants. Red circles represent the potential subpopulations (numbered from 1 to 4) currently present in Misiones. Red points represent locations where the species presence has been confirmed (at least before the YF outbreaks).

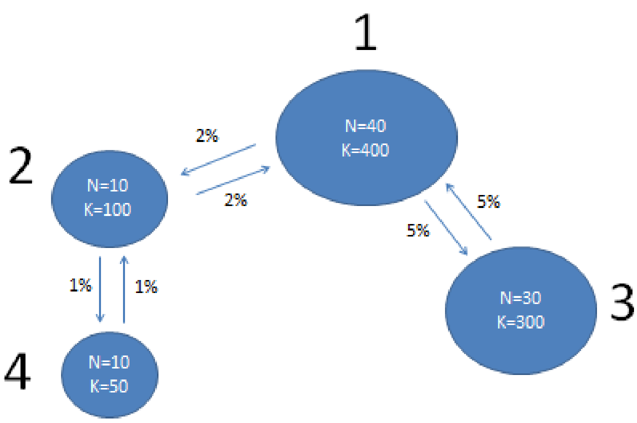


Figure 8b. Distribution, size (N), potential carrying capacity (K) and connectivity of brown howler monkeys estimated in Misiones by the workshop participants.

Dispersal between populations was estimated and modeled. Participants estimated that males dispersed more than females and dispersal probably occurs between 4 and 6 years of age. Dispersal rates varied between fragments based on the perceived degree of connectivity and no mortality was considered. Of the dispersing individuals 70% were males and 30% females. The following function was used to represent this: D*(0.30+(0.40*(S=1))). Dispersal rates between populations are represented in Figure 8.

Conclusion:

According to the model if YF does not impact all fragments equally then fragmentation of the brown howler populations could decrease their probability of extinction (Table 8; Figure 9).

When all the populations are impacted by the same YF outbreak the probability of extinction is 96%. However, if the outbreak only hits one or two of the populations then the probability of extinction decreases. Probability of extinction is highest when the YF outbreak impacts the largest populations (populations 1 and 3) but particularly when it hits populations 1 & 3 simultaneously (Table 8; Figure 9). Although dispersal rates are low a dynamic of source/sink populations can prevent the extinction of brown howler monkeys if YF does not impact all fragments equally.

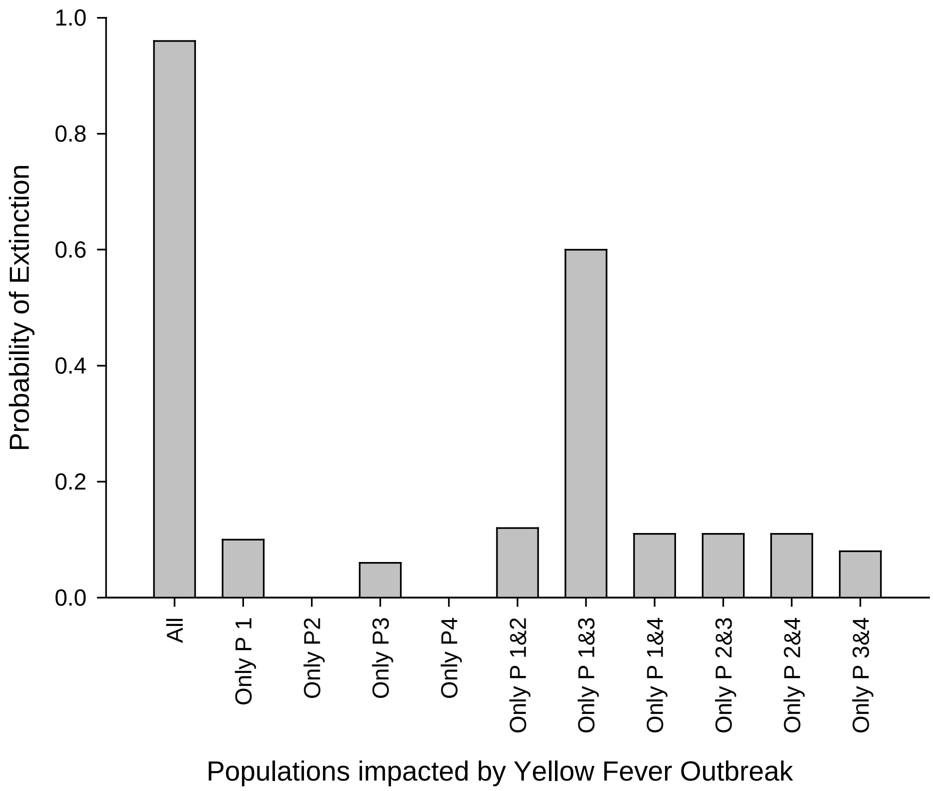


Figure 9. Probability of extinction when YF outbreaks hit all or only some populations.

Table 8. Results for the four populations and the metapopulation when YF outbreaks impact all or only some of the populations.

	Population	**stoc-r**	**SD(r)**	**PE (%)**	**N-ext**	**SD(Next)**	**GD**	**SD(GD)**	**MTE**
All populations impacted by YF	Pop 1	-0.05	0.29	98%	26	36.75	0.67	0.24	41
	Pop 2	-0.03	0.27	99%	10	7.33	0.70	0.05	25
	Pop 3	-0.04	0.29	97%	19	34.61	0.67	0.21	40
	Pop 4	-0.04	0.26	100%	3	0.00	0.72	0.00	22
	Metapop	-0.06	0.20	**96%**	30	56.92	0.72	0.14	55
Only population 1 impacted by YF	Pop 1	-0.01	0.30	28%	29	35.51	0.84	0.09	43
	Pop 2	0.00	0.14	49%	31	27.67	0.78	0.12	53
	Pop 3	0.00	0.09	15%	82	73.69	0.83	0.11	80
	Pop 4	-0.01	0.15	73%	17	13.03	0.72	0.12	47
	Metapop	-0.01	0.08	**10%**	125	113.65	0.86	0.10	85
Only population 2 impacted by YF	Pop 1	0.01	0.08	0%	190	110.78	0.92	0.04	0
	Pop 2	0.02	0.29	13%	22	19.36	0.87	0.07	22
	Pop 3	0.02	0.08	0%	189	90.71	0.92	0.03	0
	Pop 4	-0.02	0.15	81%	13	12.08	0.69	0.14	47
	Metapop	0.01	0.05	0%	400	205.38	0.93	0.03	0

Only	Pop 1	0.00	0.09	9%	94	79.25	0.87	0.08	82
population	Pop 2	0.01	0.13	13%	50	30.65	0.85	0.10	51
3	Pop 3	0.00	0.29	20%	32	38.16	0.84	0.09	42
impacted by	Pop 4	-0.01	0.16	58%	17	13.70	0.76	0.11	44
YF	Metapop	0.00	0.07	6%	173	132.79	0.89	0.07	87
Only	Pop 1	0.02	0.08	0%	205	105.06	0.93	0.03	0
population	Pop 2	0.02	0.12	1%	64	30.19	0.90	0.05	32
4	Pop 3	0.02	0.08	0%	200	87.66	0.93	0.03	0
impacted by	Pop 4	-0.02	0.27	74%	5	3.45	0.75	0.10	21
YF	Metapop	0.02	0.05	0%	471	204.46	0.94	0.03	0
Both pops	Pop 1	-0.01	0.30	32%	29	33.03	0.82	0.10	43
1&2	Pop 2	-0.01	0.27	86%	9	10.34	0.76	0.13	27
impacted by	Pop 3	0.00	0.09	14%	89	79.70	0.83	0.11	79
YF	Pop4	-0.02	0.15	87%	15	11.75	0.67	0.12	48
(same YF)	Metapop	-0.01	0.10	12%	114	107.72	0.84	0.11	81
Bothe pops	Pop 1	-0.05	0.30	88%	14	20.15	0.73	0.18	38
1&3	Pop2	-0.01	0.14	69%	32	26.79	0.72	0.17	53

impacted by	Pop 3	-0.05	0.30	94%	22	23.63	0.77	0.18	36
YF	Pop 4	-0.01	0.15	79%	18	13.90	0.70	0.15	49
(same YF)	Metapop	-0.03	0.15	**60%**	43	45.41	0.73	0.18	66
Both pops	Pop 1	-0.01	0.30	29%	28	34.56	0.83	0.10	44
1&4	Pop 2	0.00	0.14	57%	31	27.20	0.77	0.13	52
impacted by	Pop 3	0.00	0.09	14%	82	74.54	0.83	0.10	76
YF	Pop 4	-0.03	0.26	97%	5	3.04	0.66	0.13	21
(same YF)	Metapop	-0.01	0.09	11%	118	116.04	0.85	0.09	81
Both pops	Pop 1	0.00	0.09	13%	90	80.85	0.85	0.10	77
2&3	Pop 2	0.01	0.28	48%	15	17.56	0.81	0.10	27
impacted by	Pop 3	-0.01	0.29	28%	29	32.87	0.83	0.11	45
YF	Pop 4	-0.02	0.15	86%	16	11.57	0.68	0.15	48
(same YF	Metapop	-0.01	0.09	11%	124	118.41	0.86	0.09	80
Both pops	Pop 1	0.00	0.09	14%	83	77.15	0.84	0.10	76
2&4	Pop 2	0.01	0.28	48%	14	15.36	0.80	0.10	28
impacted by	Pop 3	-0.01	0.29	29%	30	36.07	0.82	0.10	45
YF	Pop 4	-0.02	0.15	82%	16	11.32	0.67	0.13	46
(same YF)	Metapop	-0.01	0.10	11%	117	117.19	0.85	0.10	79

Both pops	Pop 1	0.00	0.09	11%	86	72.19	0.85	0.11	82
3&4	Pop 2	0.01	0.13	19%	48	31.74	0.84	0.09	51
impacted by	Pop 3	-0.01	0.29	26%	28	31.98	0.84	0.09	41
YF	Pop 4	-0.02	0.27	83%	6	5.93	0.71	0.11	20
(same YF)	Metapop	0.00	0.08	8%	150	121.40	0.87	0.10	85

XI. Outbreak Report

SIMULATION MODEL OF YELLOW FEVER POPULATION EPIDEMIOLOGY

Modelers:

Phil Miller (IUCN/SSC CBSG)

Model input group:

Eduardo Moreno, Eduardo Lestani, Pablo Beldomenico, Silvina Goenaga, Mariela Martínez.

Introduction

In addition to the basic study of Yellow Fever (YF) outbreaks as catastrophic events in the PVA software *Vortex*, a major goal of this workshop was to apply new disease epidemiology simulation modeling tools to the issue of YF transmission dynamics among brown howler monkey populations in northeastern Argentina. Towards that end, we have used the software package *Outbreak* (Lacy et al. 2012) to build a simple model of yellow fever dynamics in brown howler monkeys, and to investigate model sensitivity to a set of key epidemiology model input parameters.

This Microsoft Windows software package simulates SEIR-style disease dynamics, using the basic conceptual algorithms of Anderson (1982) and May (1986) as a foundation. Using this approach, individuals are classified as Susceptible, Exposed, Infectious, or Recovered/Resistant. To model infectious processes, the state of each individual in the population is tracked, and the probabilities of transition among states are specified as functions of the number of individuals currently in each state and of other relevant parameters such as contact rate and the latent period of infection. All disease parameters are input by the user allowing for customization of disease epidemiology. In addition, demographic information such as breeding rates and non-disease mortality for general sex-specific stages (juveniles, sub-adults, and adults) are user-specified and used to project total population size. Along with basic disease analysis, the user may include vaccination and culling as a means of managing disease dynamics in the population.

With the objective of defining the information related to the dynamics of YF in *Alouatta* that has to be entered into *Outbreak*, workshop participants collaborated for the creation of a diagram illustrating the main factors that determine the disease. Within this framework the researchers also identified input variables for which data are available in Argentina, or those for which data could be extrapolated by assumption on the basis of studies carried out in other regions with known circulation of the yellow fever virus in South America and other parts of the world. Starting from this diagram, the best ways of adjusting the use of the software to a disease characterized by vectors-mediated transmission, such as YF, were discussed. At the same time, for each variable, the participants listed the main challenges in obtaining data on these parameters.

General Approach for Model Construction

We decided to use the advantages of both *Vortex* and *Outbreak* to create a more realistic model for investigating the dynamics of YF in brown howler populations in Argentina. To do this, we used a new technology called metamodeling (Pollak and Lacy 2013; Miller & Lacy 2003b; Bradshaw et al. 2012; Lacy et al. in review). A metamodel is composed on two or more independent, discipline-specific models that exchange data in order to reveal emergent complex properties of a system. In this approach, the output of one model can modify inputs to another model. This approach offers considerable potential to combine the methods and strengths of multiple disciplines into a more integrated analysis of complex systems.

Specifically, we linked our baseline *Vortex* model of brown howler population demography to alternative *Outbreak* models of YF epidemiology. This metamodel approach allows us to analyze the population-level impacts of simulated YF outbreaks in a more detailed fashion compared to a method using *Outbreak* alone. For information on the input parameters for the *Vortex* demographic model, please refer to the section titled "Input Parameters for Simulation Modeling" in Chapter X.

Input Parameters for Stochastic Disease Epidemiology Models

For our analysis, we looked at a small population of brown howlers that would typically represent a localized assemblage of social groups. We assume throughout a starting population of 30 brown howlers, age 1 year and older. Furthermore, we assume that this population occupies an area of habitat that can support approximately 50 brown howlers through time. This is known as the carrying capacity of the habitat.

Pre-Susceptible State

We assume that all newly-born individuals become susceptible to YF immediately after birth, except in cases when a mother has recovered from infection with the pathogen (Haddow et al. 1951; Monath 2001). In that case, newborn individuals acquire temporary immunity from infection with Yellow Fever. This temporary immunity lasts somewhere between 1 and 180 days for each newborn animal, with a uniform probability of reverting to the Susceptible state during this period of time.

Susceptible State

Yellow Fever introduces specific difficulties when studied using the *Outbreak* analysis package, since the pathogen is transmitted between individuals by an intermediate vector – the mosquito – instead of by direct contact among infectious and susceptible animals. Therefore, we had to think about transmission dynamics among howlers in a relatively more abstract fashion.

Encounter rate – Considering that YF is a disease transmitted by vectors with multiple potential hosts and vectors, the traditional concept of contact rates among howler monkeys cannot be defined with accuracy. One infected mosquito can rather easily transmit the virus to an entire howler social group, given that mosquitoes need several blood feedings to conclude their reproductive cycle (Dekker et al. 2005; Forattini & Gomes 1988; Gomes et al. 2010; Ostfeld & Keesing 2000).

Therefore, it is impossible not to consider the encounter rate as a function of a combination of factors, namely: (i) the ability of dispersal of mosquitoes (Moreno & Barata 2012), (ii) the densities of host and vector species (Goenaga et al. 2012), and (iii) the proportion of blood-feeding events where howler monkeys are used as food sources (Gomes et al. 2010). Unfortunately, all these factors remain unknown to those studying the howler monkey – mosquito – YF dynamic system.

During the outbreaks of YF that occurred in Argentina in 2008 and 2009 (Goenaga et al. 2012; Holzman et al. 2010), the virus was observed to spread rapidly among some identified *Alouatta* populations in the Misiones region. However, it is unknown whether all *Alouatta* groups actually came into contact with the virus or if they were exposed and survived the disease.

We assumed in our baseline model that an infected individual would "encounter" those individuals within its own social group, composed of approximately 7 – 10 animals (Agostini et al. 2012; Crockett & Eisenberg 1987; Di Fiore & Campbell 2007). This value represents infected mosquito vectors moving among and seeking blood meals from those individuals making up a social group.

This parameter is highly uncertain in our models. Therefore, in order to identify the importance of this variable in the modeling process, workshop participants arbitrarily set minimum and maximum values of encounter rates as 1:1 and 1:20, i.e. one viremic mosquito can transmit the disease from a minimum of one to a maximum of 20 animals.

Transmission rate – For this variable, the participants considered a function of the natural infection rate of mosquitoes with YF (Vasconcelos 2003). Although records of these rates for other sites in Brazil and Africa are available (Cardoso et al. 2010; Souza et al. 2011; Vasconcelos 2001), the participants decided not to use them, considering that they would differ greatly from the likely values for the outbreaks recorded in Argentina. In fact, the natural infection rate of mosquitoes in the latter case should be much higher given the velocity of disease spread identified in the 2008-2009 YF outbreak (Goenaga et al. 2012; Holzman et al. 2010). Workshop participants chose 0.2 as the best estimate for transmission rate given contact; in other words, there is a 20% chance that a viremic mosquito will successfully transmit YF virus when biting a naïve howler monkey.

Again, the accurate value of this parameter is highly uncertain in our models. We therefore chose this parameter as a subject of sensitivity analysis, choosing alternate value of 0.5 and 0.8 in addition to the baseline value of 0.2.

Maternal-offspring (vertical) transmission – This variable was not considered important for YF.

Contact with and transmission of virus through an external environmental source – To estimate this value, the participants noted that the YF virus shows a cycle in this region with a minimum of approximately 14 years (5,110 days) between epidemics (Camara et al. 2011). This may be considered a true minimum value, as this conclusion is based on a relatively short time period of observation. In this model the specific inter-epidemic interval for Argentina was assumed to be more on the order of 30 years (10,950 days) (Goenaga et al. 2012). We assume here that these epidemic events are triggered by exposure to and infection by the virus from a vector outside of the howler monkey population, i.e., a mosquito that has acquired the virus after biting an infected mammal other than local brown howler monkeys. Therefore, the probability of acquiring the pathogen from an external environmental source is simply the reciprocal of the inter-epidemic interval: 1/5110 = 0.000196 to 1/10950 = 0.000091.

There is a considerable amount of uncertainty surrounding this parameter estimate. As part of our epidemiological sensitivity analysis, we reduced the value of this parameter by an order of magnitude to 0.0000091.

<u>Exposed State</u>

Latent period of infection (Incubation period) – To estimate this variable, the participants considered two concepts. First, as a simplified case, they considered only the incubation period for primates of the genus *Alouatta*, using data available from the literature (Laemmert & Kumm 1950; Monath 2001). Under this assumption, the latent period is estimated to be 3 to 6 days. As an alternative assumption for the sensitivity analysis, participants selected the time needed to complete the full cycle, i.e. the incubation period in *Alouatta* individuals, added to the extrinsic incubation period in the mosquito (Johansson et al. 2010). This assumption leads to a latent period of 15 to 20 days. The sensitivity analysis, then, includes these two options as alternative input.

<u>Infectious State</u>

Infectious period – To estimate this variable, workshop participants evaluated two alternative concepts. First, as a simplified case, they considered the period of viremia in individuals of *Alouatta* spp. using data available from the literature (Laemmert & Kumm 1950; Monath 2001; Moreno et al. 2011). Using this assumption, the infectious period is assumed to be 3-6 days. Second, participants selected as the infectious period the time it takes for an infected mosquito to transmit the disease (Johansson et al. 2010; Mondet et al. 2002). Assuming that an infected mosquito can transmit the disease during its entire life span, the researchers therefore estimated the maximum duration of the infectivity period as 30-60 days (Mondet 1997). These two alternatives were used as input for part of the sensitivity analysis.

Disease outcome – First, we assume that no infectious individuals remain in this state indefinitely; all animals either clear the infection after some period of time or die. We assume this infectious period lasts from 10 to 20 days (Laemmert & Kumm 1950).

Second, there is no specific information that defines a universally applicable mortality rate among individuals of the genus *Alouatta* that become infected with the YF virus. Nevertheless, it is known that populations are severely impacted by the pathogen, given the historical records of high mortality showed for this genus in the description of YF outbreaks that have recently occurred in South America (Almeida et al. 2012; Bicca-Marques & de Freitas 2010; Holzman et al. 2010; Moreno et al. 2011).

Specifically, we assume that individuals that survive the infection develop permanent immunity to future infective events (Monath 2001; Vasconcelos 2003). Therefore, the probability of surviving individuals returning to the Susceptible state is 0.0. Workshop participants simulated three different mortality scenarios as part of the epidemiological sensitivity analysis: a mild event characterized by 20% mortality of infectious animals, a medium-level event characterized by 50% mortality, and a severe event with 80% mortality.

Recovered State

Permanent resistance – Once again, we assume that an animal that recovers from infection with the YF virus is permanently immune from further infection (Monath 2001; Vasconcelos 2003). Therefore, the proportion of Recovered individuals that acquire permanent immunity in our models is set to 1.0.

Table 1 presents a summary of the input parameters used for this analysis. Each scenario featured 500 iterations, and was run for 100 years.

Table 1. Input parameters used for Outbreak models of YF epidemiology in brown howler monkeys of Argentina. See accompanying text for detailed explanation of input parameter definitions.

Parameter	Baseline Value	Alternate Values
Pre-Susceptible		
Newborns with permanent immunity	0.0	
Duration of maternally-derived immunity	180d	
Susceptible		
Encounters per day	10	1, 20
Transmission rate given encounter	0.2	0.5, 0.8
Encounter rate with outside source	9.1×10^{-5}	9.1×10^{-6}, 2×10^{-4}
Transmission rate given external encounter	0.2	0.5, 0.8
Exposed		
Incubation period	3 – 6d	15 – 20d
Infectious		
Infectious period	3 – 6d	30 – 60d
Probability of recovering to Susceptible state	0.0	
Probability of recovering to Resistant state	0.5	0.2, 0.8
Probability of dying from the infection	0.5	0.8, 0.2
Recovered / Resistant		
Proportion acquiring permanent immunity	1.0	

Baseline Model Output

The baseline *Vortex* howler monkey demographic model, linked to the baseline *Outbreak* model of YF disease epidemiology, showed a strong rate of population decline over the 100-year duration of the simulation (Figure 1). The annual stochastic population growth rate was -0.045, and the risk of population extinction by the end of the simulation was 0.988. In contrast, a baseline *Vortex* model featuring no disease showed a mean stochastic growth rate of 0.002 and an extinction probability of 0.136. The decline in population abundance seen in the “No Disease” scenario is most likely due to a combination of demographic instability due to the small population size and the gradual increase in juvenile mortality brought about by inbreeding depression.

We can look at the more detailed dynamics of disease by studying *Outbreak* model output for a given year within a single iteration of the baseline disease model. In the first outbreak episode of a simulation (Figure 2), a rapid increase in exposure to the pathogen (point A on the graph) is quickly followed by a similar increase in the infectious component of the population (point B). Because we specify approximately 50% disease-based mortality in our baseline model, exposure and infection are quickly followed by a reduction in overall population abundance by approximately 50% -- with all of those surviving individuals being resistant to further infection (point C). Newborn individuals with maternally-derived immunity to the disease begin appearing about Day 92 (point D), and gradually lose that immunity and begin transitioning to the Susceptible state beginning on Day 155 (point E).

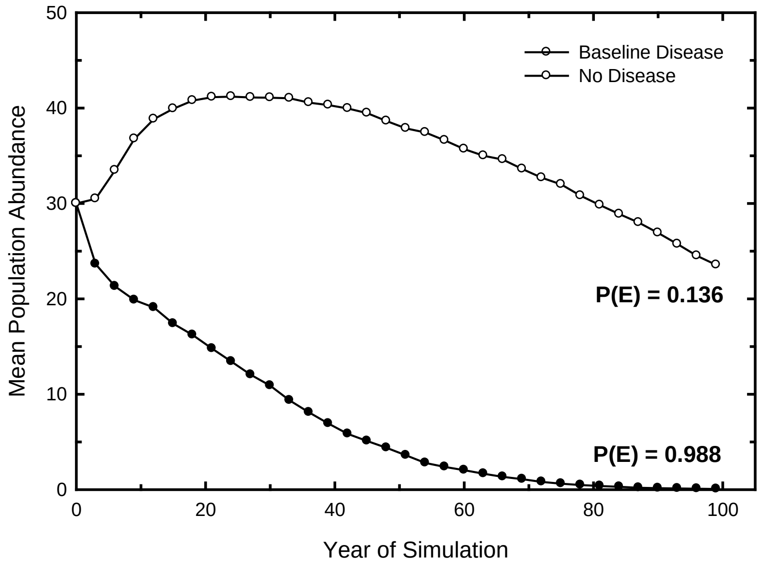


Figure 1. Population abundance trajectories for a simulated brown howler monkey population. Trajectory for baseline disease model, filled circles; baseline demographic model with no disease, open circles. Population extinction risk values are included as labels for each scenario. See accompanying text for additional information on model structure and input parameter values.

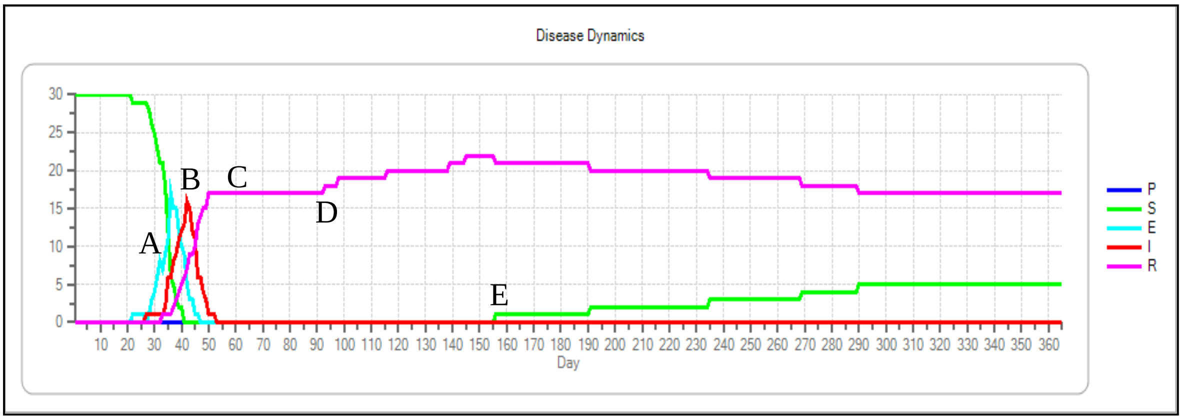


Figure 2. Trajectory of disease dynamics in Year 1 of a single iteration of the baseline brown howler monkey simulation model using *Outbreak* alone. Specific points in the trajectory are designated by letters A – E and are described in more detail in the text. Disease state definitions: P, pre-susceptible; S, susceptible; E, exposed; I, infectious; R, recovered/resistant.

The dynamics of disease outbreak shown in Figure 2 take place during a single year of the simulation. Detailed inspection of the results from the *Outbreak* analysis reveal that such YF outbreaks occurred approximately 10 – 14 times in the 100-year simulation. The specific dynamics shown here may not be a fully accurate representation of yellow fever in the Argentina population of brown howler monkeys, but we believe the model discussed in this report is a reasonable description of the disease and its effects on the howler population. In addition, it serves as a good starting point for exploring the sensitivity of such a model to changes in selected input parameter values.

Epidemiological Sensitivity Analysis: Impact on Metamodel Abundance Trajectories

As discussed in the preceding section on model input parameters and summarized in Table 1, there is a significant amount of measurement uncertainty associated with many of the input parameters used in our *Outbreak* model of yellow fever disease epidemiology. This type of measurement uncertainty, which is distinctly different from the annual variability in demographic rates due to environmental stochasticity and other factors, makes it difficult to generate accurate predictions of population dynamics and future abundance with any degree of confidence. Nevertheless, an analysis of the sensitivity of our models to this measurement uncertainty can be a valuable aid in identifying priorities for detailed research and/or management projects targeting specific elements of disease epidemiology and/or ecology.

A total of 10 additional model scenarios were constructed, each with a specific parameter value changed according to the information presented in Table 1. For each of these parameters we construct new simulations, with a given parameter set at its prescribed alternative value, with all other parameters remaining at their baseline value. The results of this sensitivity analysis are shown in Table 2 and Figure 3.

It is important to recognize that the analysis discussed here is not a formal sensitivity analysis in the sense of that described by Caswell (2001) and others. The ranges of parameter values are not directly comparable, so we do not have a precise measure of the unit changes in population growth rate required to make direct comparisons of sensitivity across the parameters selected for this analysis. Nevertheless, the analysis begins to offer insight into which parameters appear to drive the dynamics of YF outbreaks, therefore helping to prioritize research and/or management to more effectively deal with this acute threat to brown howler populations in Argentina.

Table 2. Input parameter values and model results for epidemiological sensitivity analysis using the linked *Vortex* – *Outbreak* metamodel discussed in the text.

Scenario	r_s	P(E)	T(E)	N_{100}
Baseline	-0.045	0.988	46.5	0.06
Fixed encounter: 1 individual/day	-0.014	0.486	75.4	8.81
Fixed encounter: 20 individuals/day	-0.045	0.992	47.3	0.05
Encounter rate with external source: 9.1×10^{-6}	-0.015	0.542	72.0	7.70
Encounter rate with external source: 2.0×10^{-4}	-0.058	0.998	37.2	0.02
Transmission rate given encounter: 0.5	-0.060	1.000	35.9	
Transmission rate given encounter: 0.8	-0.066	1.000	32.9	
Incubation period: 15 – 20 days	-0.046	0.992	46.9	0.05
Infectious period: 30 – 60 days	-0.047	0.996	46.5	0.07
Disease mortality: 0.2	-0.021	0.630	73.9	4.85
Disease mortality: 0.8	-0.092	1.000	21.4	

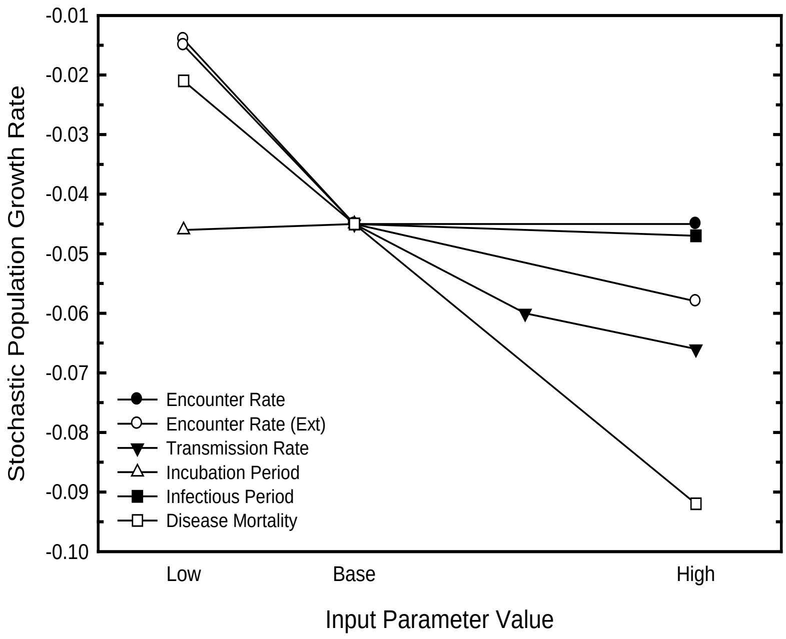


Figure 3. Epidemiological sensitivity analysis of a simulated population of brown howler monkeys subject to yellow fever outbreaks. Those curves with the steepest slope indicate the model parameters with the greatest overall sensitivity. See accompanying text for more information on model structure

The results of this sensitivity analysis can be discussed in two different ways: the impact on the frequency of outbreaks, and the impact of those outbreaks on population viability. As expected, the frequency of outbreaks is strongly affected by the encounter rate, both within the population of howlers and between the howler population and an outside source. When either encounter rate is reduced, the number of outbreaks drops to just one or two events during the 100-year duration of the simulation – a dramatic reduction compared to the baseline frequency of one event every 7 – 10 years on average. However, because of the relatively severe 50% mortality impact of any one outbreak as defined in our baseline model, even a low outbreak frequency leads to a reduced growth rate and increased extinction risk compared to the baseline disease model. In a similar fashion, increasing the transmission rate following contact has a major impact on the simulated population, leading to a significant reduction in population growth rate and an increased extinction risk. With a constant contact rate but a higher transmission rate, the frequency of outbreaks increases significantly. Interestingly, as time progresses in the simulation and the number of surviving and resistant individuals begins to increase, the intensity of the outbreak is reduced (e.g., Figure 4).

Again, as expected, the extent of mortality resulting from the yellow fever outbreak has a large impact on long-term population viability. Since we are not modifying the contact or transmission rates under alternative estimates of mortality, the frequency of outbreak events remains the same as the baseline scenario. However, the population-level impact of changing disease-based mortality is considerable (Figure 5). Reducing the disease-based mortality to 20% increases the stochastic population growth rate by about 50% to -0.021 – although this still leads to a high risk of population extinction (0.63) and a very small final population size (five individuals).

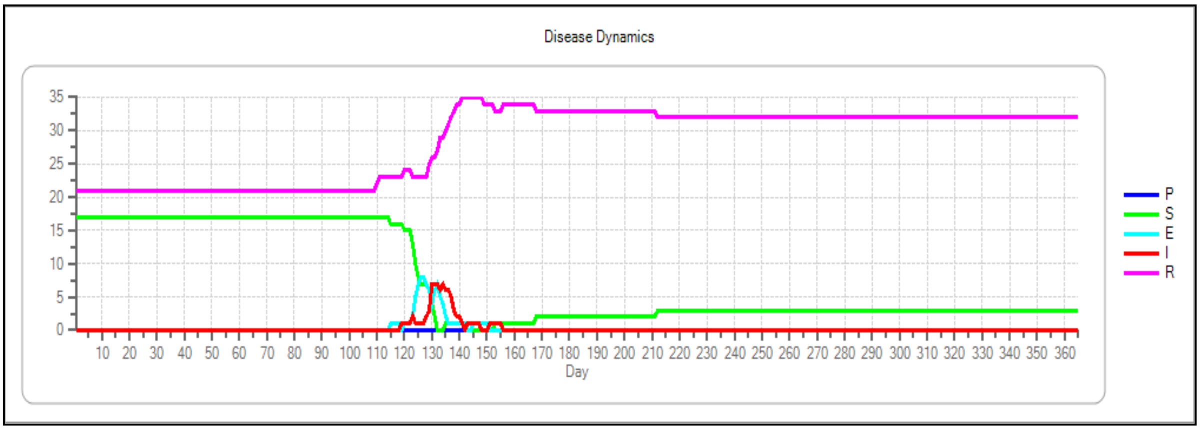


Figure 4. Trajectory of disease dynamics in Year 50 of a single iteration of a brown howler monkey simulation model using *Outbreak* alone. The transmission rate given either within population or external contact is 0.5, higher than the baseline value of 0.2. Note the relatively high proportion of resistant individuals at the start of the outbreak on Day 115, leading to a yellow fever outbreak of lower overall intensity. Disease state definitions as in Figure 2.

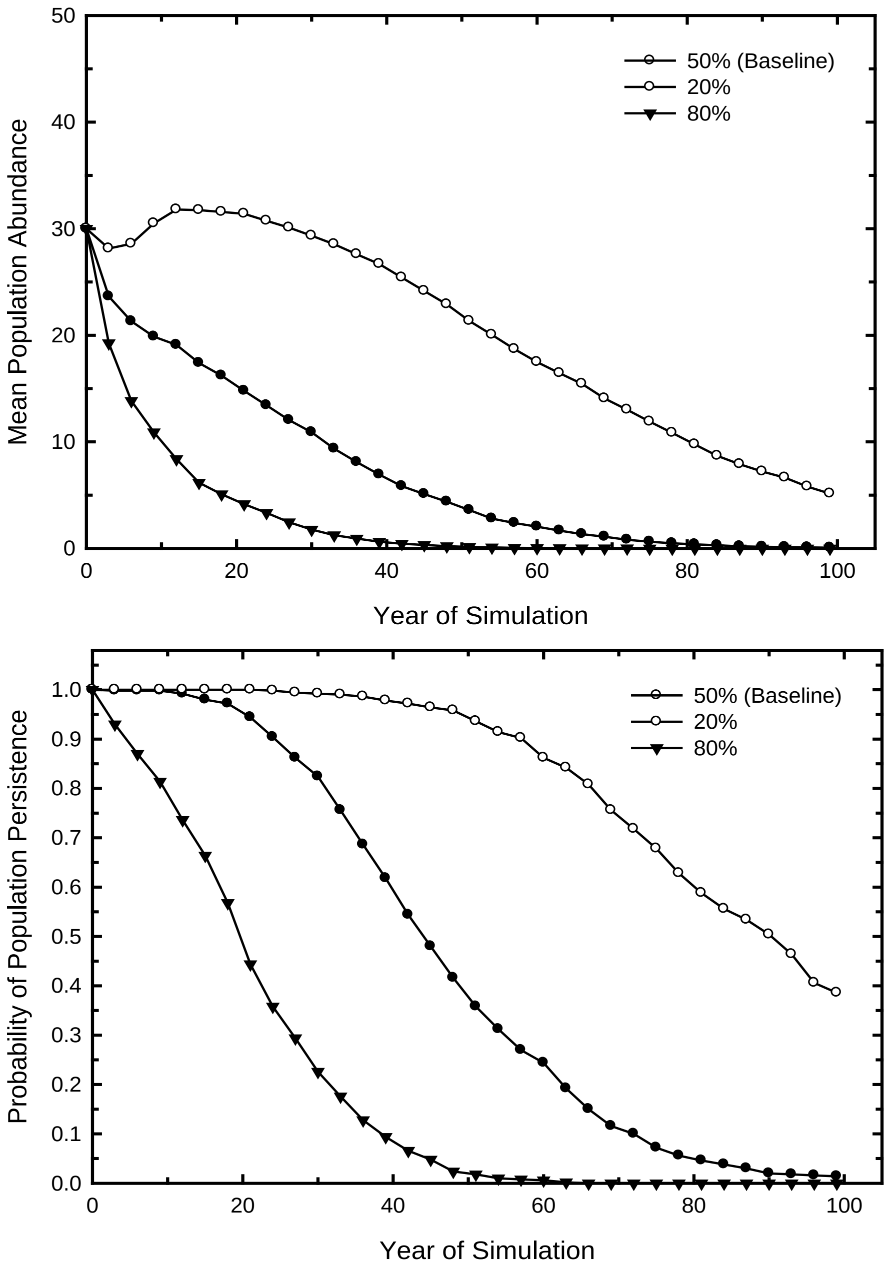


Figure 5. Mean abundance (top panel) and extinction risk (bottom panel) trajectories for a simulated brown howler monkey population in the presence of yellow fever. Curves in each panel feature different levels of disease-based mortality, with 50% mortality rate defining the baseline disease model. See accompanying text for more information on model structure and input.

In contrast to these highly sensitive parameters defining transmission dynamics and disease-based mortality, some of the other characteristics defining the disease do not appear to influence long-term disease dynamics or population impacts. Specifically, changing the baseline values for the incubation (latent) period of the pathogen and the infectious period lead to only very small impacts on population outcome. Because of the rather short time periods involved in these processes of incubation and infectivity relative to the one-year timestep for the linked *Vortex* – *Outbreak* metamodel, transmission dynamics are not affected when latent and infectious period are changed from their baseline values.

Directions for Future Analysis Using the Metamodel Structure

While the metamodeling framework used here represents a step forward in the complexity and sophistication of tools we can use to study infectious disease in endangered wildlife populations, the models discussed in this section remain quite simple. There are three primary directions in which additional modeling using these tools can proceed:

<u>Detailed description of disease epidemiology</u> – We have only begun to think about and explore different aspects of yellow fever disease epidemiology in brown howler monkeys. There may be many mechanisms of disease transmission that remain highly uncertain and highly simplified in our models, such as density dependence of encounter and transmission events, interactions between howlers and other mammalian hosts of the yellow fever virus, etc. We hope that this initial study will stimulate additional work in the field and with the model to help us to better understand system dynamics.

<u>Influence of brown howler metapopulation structure on disease dynamics</u> – The *Vortex* analyses discussed in this report feature an explicit consideration of the metapopulation structure of brown howler monkeys in Misiones. Population structure can be a critical factor in determining the dynamics of disease spread in this species. At the present time, including such a metapopulation structure in a *Vortex* – *Outbreak* metamodel of yellow fever in brown howler monkeys is significantly complex and beyond the scope of the current workshop. However, it is important to explore this possibility in the future so that we can perhaps develop even more sophisticated (but more realistic) models of disease transmission and population-level impacts.

<u>Evaluation of alternative management activities</u> – This initial exploration of disease dynamics helps to generate insight into possible mechanisms to manage yellow fever outbreaks and their severity. Future work could be devoted to creating alternative management scenarios that could be tested for their effectiveness in achieving population viability goals in Argentina.

Conclusion

The analyses presented here represent one of the first detailed applications of a metamodel linking the well-known population viability analysis software package *Vortex* to a sophisticated model of infectious disease epidemiology (see Keet et al. 2009 for a similar application of this technique). For the first time, workshop participants systematically reviewed the state of information on yellow fever dynamics in brown howler populations, and assembled that information into a dataset suitable for use as input to the *Outbreak* model of disease epidemiology. When linked to *Vortex* through the new metamodeling technology, species and disease experts were able to evaluate the dynamics of yellow fever and the consequences of gaps in our understanding of the pathogen and associated disease.

Workshop participants were successful in creating a basic model of yellow fever epidemiology that generated reasonable predictions of disease dynamics in brown howler populations. Sensitivity analysis methodology was used to evaluate the implications of measurement uncertainty in a set of epidemiological input parameters to 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. Based on the insights gained from this simple and preliminary analysis, we hope to challenge these tools further to create more realistic models of yellow fever – particularly in spatially structured landscapes – so that populations of brown howler monkeys may persist in Misiones well into the future.

XII. Institutions

Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA)

The main organization undertaking the organization of the First Brown Howler Monkey Workshop in Argentina is the NGO Centro de Investigaciones del Bosque Atlántico (CeIBA). CeIBA is a non-profit civil association founded on April 30th, 2005 in Puerto Iguazú, Misiones, Argentina. Its general mission is to generate scientific knowledge on ecology, conservation and management of the Upper Paraná Atlantic Forest, so as to contribute to the sustainable development of Misiones. Moreover, other goals of this association are: human resources training in research, conservation, and management issues; enhancement of conservation and sustainable management of regional natural resources; promoting collaboration between different institutions devoted to research and conservation of natural resources at local, national and international level; dissemination and transfer of knowledge to the community, governmental agencies, institutions and scientific areas. CeIBA consists of over 50 members, including researchers, doctoral and postdoctoral students, belonging to several Argentinean and foreign institutions. All members are involved in projects aimed at promoting the conservation and sustainable management of the regional natural resources. In particular, research topics include studies on biodiversity, etnobotany, forest eco-physiology, animal ecology and behavior, and studies on the effects of forest fragmentation and management upon ecosystem functioning.

Instituto de Biología Subtropical (IBS) – Sede Iguazú

The Instituto de Biología Subtropical (IBS) is a research center of the Universidad Nacional de Misiones (UNaM) and the Argentinean National Research Council (CONICET). This institute is working with two different head offices, one in Posadas and the other in Puerto Iguazú. IBS is aimed at generating and transferring knowledge on basic and applied biology, with particular emphasis on diversity (taxonomic and genetic), functioning and management of subtropical ecosystems of Argentina. The research activities are developed in a framework of integrative and multidisciplinary studies. The main goals of the IBS include generating scientific knowledge and providing adequate responses to the demands of different sectors bounded to the use and management of natural and cultural resources, and promoting the sustainable management of the region.

The specific objectives are: (1) promoting the development of scientific research, (2) developing technologies, (3) contributing to train high-level human resources personnel, researchers and technicians, (4) promoting the diffusion and transfer of knowledge and developed technologies to the community, in institutional and scientific areas, (5) offering advice for official and private institutions, as well as NGOs. These objectives aim to contribute to the understanding and conservation of cultural and biological diversity in a framework of sustainable management of the natural resources of subtropical ecosystems of Argentina.

IUCN/SSC Species Conservation Planning Sub-committee (SCPSC)

The Species Survival Commission (SSC), created in 1949, is the largest of IUCN's six volunteer commissions. With some 8,000 scientists, government officials, and conservation leaders worldwide, the SSC membership is an unmatched source of information about species conservation. SSC members provide technical and scientific advice to governments, international conventions, and conservation organizations throughout the world. SSC also provides the best available information critical to the development of tools for species conservation such as the IUCN Red List of Threatened Species and more recently guidelines for Species Conservation Planning which can be found in *Strategic Planning for Species Conservation: A Handbook* (IUCN/SSC 2008).

IUCN/SSC Conservation Breeding Specialist Group (CBSG)

With over 300 volunteer members, the IUCN/SSC Conservation Breeding Specialist Group (CBSG) is one of the largest Specialist Groups comprising the Species Survival Commission (SSC). CBSG has over 20 years of experience developing, testing and applying scientifically based tools and processes for risk assessment and decision-making in the context of species management. These tools, based on small populations and conservation biology, human demography, and the dynamics of social learning are used in intensive, problem-solving workshops to produce realistic and achievable recommendations for both *in-situ* and *ex-situ* population management. CBSG's workshop processes provide an objective environment, expert knowledge, and neutral facilitation to support the exchange of information across diverse stakeholder groups in order to reach some agreement on the important issues facing both humans and wildlife. With this understanding, meaningful and practical management recommendations can be made.

The PVA Workshop is a very efficient and systematic process of collection of quantitative techniques used to help with management decisions for threatened species. Detailed data on species biology, genetics, and ecology are integrated and analyzed by sophisticated computer models to evaluate the risk of wildlife population decline or extinction under alternative future management scenarios. This kind of workshop is a useful way of learning a new technique for threatened species management and conservation. In fact, PVA outcomes can be used in forthcoming management plans and actions in the field, and as such serve as an excellent tool for scientists and wildlife managers in their quest to make better decisions about conservation.

CBSG generally model and simulate wildlife population dynamics using the computer software *Vortex* (Lacy 1993). This is a long-standing widely used PVA computer package that employs a Monte Carlo simulation of the impact of deterministic forces as well as demographic, environmental, genetic stochastic events, and catastrophes on wildlife population dynamics. It is an attempt to model many of the extinction vortices that can threaten persistence of small populations. *Vortex* models population dynamics as discrete, sequential events that occur according to probabilities that are random variables following user-defined distributions. This modeling tool is mostly used to simulate population trends and evaluate current and future risks of population decline or extinction under alternative management scenarios. In essence, it provides a neutral platform upon which the user can examine the current status of a given species and determine which factors, if changed or manipulated, may have the greatest effect on causing or preventing extinction.

In addition to *Vortex*, recently an epidemiological model of infectious disease has been developed using *Outbreak* (Lacy et al. 2012). This software simulates disease dynamics under an individual base model of transitions among susceptible, exposed, infectious and recovered individuals. *Outbreak* provides several options for modes of transmission (random contact within populations, spatially based transmission, contact with environmental sources of disease) and provides options for management through vaccination or culling (Lacy et al. 2012).

XIII. Participants

The list of participants and their expertise was:

- **Dr. Arnaud Desbiez** (IUCN/SSC-CBSG Brasil/ Royal Zoological Society of Scotland; PVA *Vortex* modeling and facilitation); adesbiez@hotmail.com
- **Dr. Phil Miller** (IUCN/SSC – CBSG Headquarters; *Outbreak* modeling); pmiller@cbsg.org
- **Dr. Pablo Beldomenico** (Lab. de Ecología de Enfermedades, Instituto de Ciencias Veterinarias del Litoral, CONICET - Universidad Nacional del Litoral, Argentina; Global Health Program – Wildlife Conservation Society, Argentina; wildlife eco-epidemiology); pbeldomenico@wcs.org
- **Dr. Ilaria Agostini** (CeIBA, IBS sede Iguazú UNaM-CONICET; primate behavioral ecology and conservation); agostini.ilaria@gmail.com
- **Dr. Ingrid Holzmann** (CeIBA, IBS sede Iguazú UNaM -CONICET; primate behavioral ecology and conservation); holzmanningrid@yahoo.com.ar
- **Dr. Mario Di Bitetti** (CeIBA, IBS sede Iguazú UNaM -CONICET; primate behavioral ecology and conservation, mammal community ecology); dibitetti@yahoo.com.ar

- **Dr. Martín Kowalewski** (Estación Biológica de Corrientes – CONICET; primate behavioral ecology and conservation); martinkow@gmail.com

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- **Lic. Silvina Goenaga** (Instituto Nacional de Enfermedades Virales Humanas; INMeT; doctoral student, virology); silvinagoenaga@hotmail.com

- **Lic. Mariela Martínez** (INMeT – CONICET; doctoral student, wildlife eco-epidemiology); marielafmartinez@gmail.com

XIV. Glossary of Abbreviations

Administración de Parque Nacionales de Argentina (APN)

Asociación Civil Centro de Investigaciones del Bosque Atlántico (CeIBA)

Asociación Primatológica Argentina (APRIMA)Conservation Breeding Specialist Group (CBSG)

Consejo Nacional de Investigaciones Científicas y Técnicas CONICET

Dirección de Fauna Silvestre – Secretaria de Ambiente y Desarrollo Sustentable de Argentina (DFS – SayDS)

Estación Biológica de Usos Múltiples de Corrientes (EBCo)

Fundación Vida Silvestre Argentina (FVSA)

Global Health Program, Wildlife Conservation Society (GHP-WCS)

Instituto de Biología Subtropical (IBS)

Instituto Nacional de Enfermedades Virales Humanas (INEVH)

Instituto Nacional de Medicina Tropical de Argentina (INMeT)

International Union for Conservation of Nature (IUCN)

Laboratorio de Ecología de Enfermedades (LEcEn)- Instituto de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral - CONICET (ICiVet Litoral, CONICET-UNL)

Ministerio de Ecología y Recursos Naturales Renovables de la Provincia de Misiones (MERNR)

Ministerio de Salud de la Nación Argentina (MSAL)

Population Viability Analysis (PVA)

Sociedad Argentina para el Estudio de los Mamíferos (SAREM)

Species Conservation Planning Sub-committee (SCPSC)

Species Survival Commission (SSC)

Universidad Nacional de Misiones (UNAM)

XV. Bibliography

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

Agostini I, Aprile G, Baldovino MC, Brividoro M, Di Bitetti M, Fantini L, Fernández VA, Fernández-Duque E, Holzmann I, Juárez CP, Kowalewski M, Mudry MD, Nieves M, Oklander LI, Peker SM, Pfoh R, Raño M, Steinberg ER, Tujague MP, Zunino GE (2012). Orden Primates. In: *Libro rojo de mamíferos amenazados de la Argentina* (RA Ojeda, V Chillo, GB Diaz Isenrath, Eds.), pp. 81- 86. Sociedad Argentina para el Estudio de los Mamíferos (SAREM).

Agostini I, Holzmann I, Di Bitetti MS (2010a). Are howler monkey species ecologically equivalent? Trophic niche overlap in syntopic *Alouatta guariba clamitans* and *Alouatta caraya. American Journal of Primatology* 72:173-186.

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

Agostini I, Holzmann I, Di Bitetti MS (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.

Almeida MAB, Santos E, Cardoso JC, Fonseca DF, Noll CA, Silveira VR, Maeda AY, Souza RP, Kanamura C, Brasil RA (2012). Yellow fever outbreak affecting *Alouatta* populations in Southern Brazil (Rio Grande do Sul State), 2008–2009. *American Journal of Primatology* 74:68–76.

Anderson RM (1982). Transmission dynamics and control of infectious disease agents. In: *Population Biology of Infectious Diseases* (RM Anderson & RM May, Eds.), pp. 149-176. Berlin: Springer.

Bicca-Marques JC & Freitas DS (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.

Bradshaw CJA, McMahon CR, Miller PS, Lacy RC, Watts MJ, Verant ML, Pollak JP, Fordham DA, Prowse TAA, Brook BW. (2012). Novel coupling of individual-based epidemiological and demographic models predicts realistic dynamics of tuberculosis in alien buffalo. *Journal of Applied Ecology* 49:268-277.

Camara FP, Gomes ALBB, Carvalho LMF, Castello LGV (2011). Comportamento dinâmico da febre amarela silvestre no Brasil (1954-2008). *Revista da Sociedade Brasileira da Medicina Tropical* 44(3):297-299.

Cardoso JC, Almeida MAB, Santos E, Fonseca DF, Sallum MAM, Noll CA, Monteiro HAO, Cruz ACR, Carvalho VL, Pinto EV, Castro FC, Neto JPN, Segura MNO, Vasconcelos PFC (2010). Yellow Fever Virus in *Haemagogus leucocelaenus* and *Aedes serratus* mosquitoes, Southern Brazil, 2008. *Emerging Infectious Diseases* 16(12): 1918-1924.

Crnokrak, P. & Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity*, **83,** 260-270.

Crockett CM (1998). Conservation biology of the genus *Alouatta*. *International Journal of Primatology* 19(3):549-578.

Crockett CM & Eisenberg JF (1987). Howlers: variations in group size and demography. In: *Primate Societies* (BB Smuts, DL Cheney, RM Seyfarth, RW Wrangham, T Struhsaker, Eds.), pp. 54–68. Chicago: University of Chicago Press.

Dekker T, Geier M, Cardé RT (2005). Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *The Journal of Experimental Biology* 208:2963-2972.

Di Bitetti MS, Placci G, Brown AD, Rode DI (1994). Conservation and population status of the brown howling monkey (*Alouatta fusca clamitans*) in Argentina. *Neotropical Primates* 2:1-4.

Di Fiore A & Campbell CJ (2007). The Atelines: variation in ecology, behavior, and social organization. In: *Primates in Perspective* (CJ Campbell, A Fuentes, KC MacKinnon, M Panger, SK Bearder, Eds.), pp. 155-185. New York: Oxford University Press.

Forattini OP & Gomes AC (1988). Biting activity of *Aedes scapularis* (Rondani) and *Haemagogus* mosquitoes in southern Brazil (Diptera: Culicidae). *Revista Saúde pública, S. Paulo,* 22(2):84-93.

Goenaga S, Fabbri C, Duenas JCR, Gardenal CN, Rossi GC, Calderon G, Morales MA, Garcia JB, Enria DA, Levis S (2012). Isolation of Yellow Fever Virus from mosquitoes in Misiones Province, Argentina. *Vector-borne and Zoonotic Diseases* 12(11):1-8.

Gomes AC, Torres MAN, Paula MB, Fernandes A, Marassá AM, Consales CA, Fonseca DF (2010). Ecologia de *Haemagogus* e *Sabethes* (Diptera: Culicidae) em áreas epizoóticas do vírus da febre amarela, Rio Grande do Sul, Brasil. *Epidemiologia e Serviços de Saúde, Brasília*, 19(2): 101-113.

Haddow AJ, Dick GWA, Lumsden WHR, Smithburn KC (1951). Monkeys in relation to the epidemiology of yellow fever in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 45(2):189–224.

Holzmann I, Agostini I, Areta JI, Ferreyra H, Beldomenico P, Di Bitetti MS (2010). Impact of Yellow Fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology* 71:1–6.

IUCN/SSC (2010). *IUCN Red List of Threatened Species. Version 2010.4.* <www.iucnredlist.org>

IUCN/SSC (2008). *Strategic Planning for Species Conservation: A Handbook. Version 1.0.* IUCN Species Survival Commission, Gland, Switzerland, 104 pp.

Johansson MA, Arana-Vizcarrondo N, Biggerstaff BJ, Staples JE (2010). Incubation periods of Yellow Fever Virus. *The American Journal of Tropical Medicine and Hygiene* 83(1):183–188.

Keet DF, Davies-Mostert H, Bengis RG, Funston P, Buss P, Hofmeyr M, Ferreira S, Lane E, Miller P, Daly BG (2009). *Disease Risk Assessment Workshop Report: African Lion (*Panthera leo*) Bovine Tuberculosis*. Conservation Breeding Specialist Group (CBSG SSC / IUCN) / CBSG Southern Africa. Endangered Wildlife Trust.

Kinzey WG (1982). Distribution of primates and forest refuges. In: *Biological Diversification in the Tropics* (GT Prance, Ed.), pp. 455-482. New York: Columbia University Press.

Lacy RC (1993). Vortex: a computer simulation model for population viability analysis. *Wildlife Research* 20: 45-65.

Lacy RC (1993-1994). What is Population (and Habitat) Viability Analysis? *Primate Conservation* 14-15:27-33.

Lacy RC (2000). Structure of the Vortex simulation model for population viability analysis. *Ecological Bulletins* 48**:**191-203.

Lacy RC, Miller PS, Nyhus PJ, Pollak JP, Raboy BE, Zeigler SL (2013). Metamodels for transdisciplinary analysis of wildlife population dynamics. *PloS ONE*, in review.

Lacy RC, Pollak JP, Miller PS, Hungerford L, Bright P (2012). *Outbreak version 2.0*. IUCN SSC Conservation Breeding Specialist Group. Apple Valley, MN, USA.

Laemmert HW & Kumm AW (1950). The susceptibility of howler monkeys to Yellow fever virus. *The American Journal of Tropical Medicine and Hygiene* Vol. 1-30(5): 723-731.

May RM (1986). Population biology of microparasitic infections. In*: Mathematical Ecology: An Introduction* (TG Hallam & SW Levin, Eds.), pp. 405-442. New York: Springer-Verlag.

Miller PS & Lacy RC (2003a). *Vortex: a stochastic simulation of the extinction process. Version 9 User's Manual*, pp. 150. Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN).

Miller PS & Lacy RC (2003b). Metamodels as a tool for risk assessment. In: *Experiments in Consilience: Integrating Social and Scientific Responses to Save Endangered Species* (FR Westley & PS Miller, Eds.), pp. 333-351. Washington, DC: Island Press.

Miranda JMD (2004). Ecologia e conservação de *Alouatta guariba clamitans* em floresta ombrófila mista no estado do Paraná, Brasil. MSc thesis, Universidade Federal do Paraná, Curitiba.

Monath TP (2001). Yellow Fever: an update. *Lancet Infectious Diseases* 1 (1): 11-20.

Mondet B (1997). Condições de sobrevivência em laboratório de *Haemagogus janthinomys* Dyar, 1921 (Diptera: Culicidae). *Revista da Sociedade Brasileira da Medicina Tropical* 30(1): 11-14.

Mondet B, Vasconcelos PFC, Travassos da Rosa APA, Travassos da Rosa ES, Rodrigues SG, Travassos da Rosa IFS, Bicout DI (2002). Isolation of Yellow Fever Virus from nulliparous *Haemagogus* (*Haemagogus*) *janthinomys* in Eastern Amazonia. *Vector Borne and Zoonotic Diseases* 2(1):47-50.

Moreno ES, Rocco IM, Bergo ES, Brasil RA, Siciliano MM, Suzuki A, Silveira VR, Bisordi I, Souza RP (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.

Moreno ES & Barata RCB (2012). Methodology for definition of Yellow Fever priority areas, based on environmental variables and Multiple Correspondence Analyses. *PLoS Neglected Tropical Diseases* 6(7): e1658.

O'Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn DW, Frankham R (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* 133: 42-51.

Ostfeld RS & Keesing F (2000). The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Canadian Journal of Zoology* 78:2061–2078.

Pollak JP & Lacy RC (2013) *MetaModel Manager. Version 1.0.* Brookfield, Illinois: Chicago Zoological Society.

Ralls K, Ballou JD, Templeton AR (1988). Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conservation Biology* 10:769-775.

Souza RP, Petrella S, Coimbra TSM, Maeda AY, Rocco IM, Bisordi I, Silveira VR, Pereira LE, Suzuki A, Silva SJS, Silva FG, Salvador FS, Tubaki RM, Menezes RT, Pereira MT, Bergo ES, Hoffmann RC, Spinola RMC, Tengan CH, Siciliano MM (2010). Isolation of yellow fever virus (YFV) from naturally infected *Haemagogus (conopostegus) leucocelaenus* (Diptera, Culicidae) in São Paulo state, Brazil, 2009. *Revista do Instituto de Medicina Tropical de São Paulo* 53(3):133-139.

Strier K, Mendes SL, Santos RR (2001). Timing of births in sympatric brown howler monkeys (*Alouatta fusca clamitans*) and Northern muriquis (*Brachyteles arachnoides hypoxanthus*). *American Journal of Primatology* 55: 87–100.

Vasconcelos PFC, Rosa APAT, Rodrigues SG, Rosa EST, Monteiro HAO, Cruz ACR, Barros VLRS, Souza MR, Rosa JFST (2001). Yellow Fever in Pará State, Amazon region of Brazil, 1998-1999: entomologic and epidemiologic findings. *Emerging Infectious Diseases* 7(3 Suppl):565-9.

Vasconcelos PFC (2003). Febre amarela. *Revista da Sociedade Brasileira de Medicina Tropical* 36(2):275-293.

XVI. Appendix (list of papers on brown howler monkeys in Misiones published in peer-reviewed journals and books; workshop agenda)

LIST OF PAPERS ON BROWN HOWLER MONKEYS IN MISIONES PUBLISHED IN PEER-REVIEWED JOURNALS AND BOOKS:

1. Agostini I., Holzmann I., Di Bitetti M. S. (2012). Influence of seasonality, group size, and presence of a congener on activity patterns of two howler monkey species in Northeastern Argentina. *Journal of Mammalogy* 93(3):645-657.

2. Holzmann I., Agostini I., 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.

3. Agostini I, Holzmann I, Di Bitetti MS (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.

4. Holzmann I, Agostini I, Areta JI, Ferreyra H, Beldomenico P, Di Bitetti MS (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.

5. Agostini I, Holzmann I, Di Bitetti MS (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.

6. Di Bitetti MS (2003). Outlook for Primate Conservation in Misiones (Chapter 17). In: *The State of the Hotspots: The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook.* Galindo Leal C & De Guzmao Camara I (eds.), Island Press, Center for Applied Biodiversity Science at Conservation International, Washington.

7. Di Bitetti MS, Placci G, Brown AD, Rode DI (1994). Conservation and population status of the brown howling monkey (*Alouatta fusca clamitans*) in Argentina. *Neotropical Primates* 2:1-4.

WORKSHOP AGENDA:

Brown Howler Population Viability Analysis

25 – 28 March 2013

Monday, 25 March

MORNING

- Workshop opening
- Participant introductions
- IUCN/SSC/CBSG/SCP subcommittee presentation
- Presentation of modeling tools (*Vortex*/*Outbreak*)
- Start of scientific presentations

AFTERNOON

- Scientific presentations (continued)
- Creation of a Vision working groups/plenary
- Threat analysis (plenary)

Tuesday, 26 March

MORNING

- Presentation of modeling tools (*Vortex*/*Outbreak*) continued
- 2 working groups: (1) Refinement of *Vortex* baseline model
  (2) Begin exploration of Yellow Fever epidemiology
- Plenary group dynamic to understand Yellow Fever outbreaks using results from the models
- Group creation of a flow chart

AFTERNOON

- Continuation exploration of Yellow Fever epidemiology with flow chart; add importance and state of knowledge of the relationships in the flow chart
- 2 working groups: (1) Refinement of *Vortex* model
  (2) Yellow fever epidemiology *Outbreak*

Wednesday, 27 March

MORNING

- Plenary presentation of flow chart
- List of recommendations based on flow chart create recommendations (Working groups)
- Presentation of recommendations (Plenary)
- Prioritization of recommendations (Plenary)

- Discussion: Preliminary model findings and implications for research, management (plenary)

AFTERNOON

- Final refinements of Models
- Discussions on implementations of the final recommendations and communication of the workshop
- Preparation of agenda for public presentations on following day

Travel back to Puerto Iguazú

Thursday, 28 March

MORNING

- Preparation of presentation

AFTERNOON

- Hotel Saint George (Av. Córdoba 148, Puerto Iguazú): Public presentations on workshop process, results