

## EARTHWATCH INSTITUTE FIELD REPORT

**Project Title:** Health Status, Disease Diagnosis, Population Ecology and Use of Space of Wild Mammal Species in the Cerrado-Pantanal Corridor

**Principal Investigators:**

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**Position/Affiliations:**

- (1) M.Sc.
- (2) M.Sc.
- (3) Ph.D.
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**Research Site:**  
Pantanal, Rio Negro farm (19°34'54"S and 56 °14'62"W).

**Local Management Status of the Research Site:**  
The Rio Negro farm is a preserved area with low livestock density. It also presents a private area of preservation (RPPN- Particular Reserve of Natural Patrimony).

**Scientific names of primary species being studied:**  
Small mammals - rodents and marsupials.

**Key Research Objectives:**

### Long term objectives

1. To select host-parasite relationships that can be used as indicator of the health status;
2. To evaluate if changes in landscape can be associated with the emergence of diseases using Geographic Information System (GIS);
3. Training students for Environmental Medicine: wildlife handling, disease diagnosis and GIS analysis.

## Short term objectives

1. To identify and to quantify the endoparasites of small mammals in the Pantanal;
2. To investigate the interaction host-parasite and how parasites influence their host populations;
3. To determine the population dynamics and the reproductive patterns of small mammals and evaluate the risk of disease outbreaks in relation to the population fluctuations;
4. To determine the small mammal's diet and their importance concerning fruit dispersion;
5. To compare the health status between areas which present different degrees of disturbance;

## **Date this report was completed:**

April 28 2005

## **Data Collection and Results**

- a) Give a concise account of the data you have collected during the past field season.
- b) What progress have you made towards achieving your original objectives?
- c) Please provide a summary of your results (even if they are preliminary).

During the last field season (February – November 2004), we continued to monitor the populations of small mammal species through a capture-mark-recapture study that began in early 2003. We had a total of 391 captures from six species of small mammals, three of them were rodents (*Oecomys mamorae*, *Thrichomys pachyurus* and *Clyomys laticeps*) and three were marsupials, (*Gracilinanus agilis*, *Thylamys macrura* and *Monodelphis domestica*). A total of 198 individuals were marked. Total capture success (number of animals captures divides by the total number of traps) was 4,23%. This value is in accordance with those usually obtained for small mammals in Brazil.

The species *O. mamorae* seems to have an annual population cycle that follows the seasonal dynamic of the Pantanal region. The females presented signs of active reproduction during the whole year, suggesting a continuous reproduction along the year. However, we found a bigger rate of females presenting reproductive signs in the beginning of the wet season (December), when the resource availability increases. *T. pachyurus* has been presenting a slight decline since the beginning of the study (early 2003), but there was an increase in the rate of reproductive females starting in December of 2003.

As a result of its reproductive cycle, *G. agilis* presented an annual population cycle, as the majority of the Brazilian opossums. For this species, the breeding season occurs from November to February, together with the period of highest fruit availability.

We collected 244 fecal samples of rodents to evaluate the presence of helminths and to analyse the species' diets. Additionally, we collected and identified eggs found in helminth adult females, in order to do an identification table of the helminths' eggs, which is now ready. This table will be used to identify the eggs found in the small mammals' faeces collected during 2004. Next year, an additional egg identification table will be made for parasites of *O. mamorae*. The diet analysis is being performed by Dr. Nilton Cáceres and are still being analysed.

We have been studying the Pantanal region since August 2000. In the beginning of the study, we did small mammal species inventories together with an assessment of parasite prevalence in two distinct areas: the Rio Negro farm and Alegria farm. The former is a Conservation

International Research Center and the latter has intense economic activities based on cattle ranching. Since 2000, we have been collecting blood samples of several species of wild animals in order to evaluate if they were infected by the hemoparasites *Trypanosoma cruzi* and *T. evansi*. The parasite *T. cruzi* is responsible for Chagas' disease, which infects a large number of animals, besides humans (FNS/MS, 1999; Nunes et al., 1994) and *T. evansi* is the agent of a severe disease in horses and dogs ("Mal das Cadeiras" disease), which also parasitises many other mammals (Stevens et al., 1989; Nunes et al., 1993; Franke et al., 1994; Silva et al., 1995).

We found both *T. cruzi* and *T. evansi* in several species of small mammals in Rio Negro farm and Alegria farm. In the former, the most infected species was *O. mamorae*, followed by the marsupial *G. agilis*. Data from both areas were compared and preliminary results have shown that human action resulted in significant alteration on the relative frequency of small mammals and on the prevalence of both trypanosomatid species, mainly regarding *T. cruzi*. Cattle ranching seems to be associated to a higher prevalence of *T. evansi* in Alegria farm.

From individuals of *T. pachyurus* captured in Rio Negro farm and Alegria farm, we identified 9 species of nematodes: seven of them were found in Rio Negro farm and six in the Alegria farm.

The results obtained in 2004 were important for characterising the population ecology and reproductive patterns of small mammals in the Rio Negro farm. Additionally, the comparison between Rio Negro farm and Alegria farm has shown that alterations in the environment tend to cause changes in the epidemiological profiles of the mammal species. This may have important consequences both to the cattle ranching and wildlife.

### **Significance/Benefits of Research**

a) What is/are the significance/benefits of your research at the following levels?

- local (in the area of the research site)
- national
- international

(For example, do your findings, or do you expect your findings will contribute to management strategies or biodiversity conservation action plans at any of these levels?)

b) How do your findings contribute to issues of sustainability?

Host/trypanosomes interactions and the relative frequency of small mammals seem to be a reliable indicator of environmental degradation. Therefore, interactions between parasites and their mammal hosts may be used as a bioindicator of environmental changes and also be used to define conservation, animal and public health policies - mainly at a local scale.

The assessment of the prevalence of parasites with public health and economic value is really important locally. These results are the preliminary data needed for public health policies. Also these first findings will guide our work to a higher scale, we are now starting to access the processes and patterns of disease emergence. The results of this next phase will shed light on the knowledge about disease emergence, and as the processes share some common variables, these results can be applied to other systems as well.

Our research on population ecology and health status of small mammals also provides basic information, which is essential for developing management strategies or any biodiversity conservation action plan. Small mammals are prey of several other vertebrates and they are important pieces for the well functioning of the Pantanal ecosystem.

## Dissemination of Results

### *Presentations:*

- 1) Title: "Population ecology of small mammals in the Rio Negro farm". Undergraduate monograph, by Cecília Siliansky. Local: Institute of Biology of the Federal University of Rio de Janeiro. Audience: professors and students from the Biology undergraduate course. About 50 people attended to each of those presentation. Dates of presentation: June and December/2004
- 2) Title: "Study of nematodes parasites on the rodent *Thricomys pachiurus* (Rodentia:Echimyidae) in areas in the Pantanal". High school monograph, by Filipe Cunha. Local: Oswaldo Cruz Foundation (FIOCRUZ). Audience: professors and students from FIOCRUZ. Date of presentation: April 2005.
- 3) Title: "Comparative study of nematodes of *Thricomys* spp. (Rodentia: echimyidae) in the biomes of Pantanal, Cerrado and Caatinga". By Filipe Cunha. Local: Oswaldo Cruz Foundation (FIOCRUZ). Audience: professors and students from FIOCRUZ. Date of presentation: May 2004. About 20 people attended.

### *Conferences, Workshops, Congresses and Courses:*

- 1) Nov. 2003: Annual EW conference, Boston, MA. CRI poster presented by Vitor Rademaker (Small mammal project).
- 2) June 2003: First Workshop of Pantanal Researchers, sponsored by EW and CI-Brazil
- 3) Nov. 2004: Annual EW conference, Boston, MA. CRI poster presented by Vitor Rademaker and Natalie Olifiers (Small mammal project).
- 4) Dec 2004: XII Annual Meeting of Scientific Initiation. Poster presented by Cecília Siliansky  
Falta o de Mastozoo e o poster da Luana

## **SECTION II: RESEARCH GOALS, VOLUNTEERS AND COMMUNITIES**

### **1. Introduction**

Free-living animals are frequently infected by parasites that can be transmitted to domestic animals (Losos and Gwamaca, 1973; Sherlock et al., 1984; Arias et al., 1997; Reid, 1999). Some examples are the foot-and-mouth disease (Howard, 1985; Paes, 2001) and trypanosomiasis (Silva et al., 1995).

Disease occurrence in wild animal populations has become an important indicator of the health status of an ecosystem (Keymer et al., 1988; Nielsen, 1995). Nevertheless, the objective of health vigilance is not to treat parasitized and/or sick animals, but to monitor and assess these species role in the ecosystem context (Daszak et al., 2000). Even when a disease does not threaten population viability, it can cause pain and distress to a very large numbers of individuals, as in the case of trypanosomiasis in coatis (*Nasua nasua*; Silva et al., 1999) and myxomatosis in rabbits (*Oryctolagus cuniculus*; Sainsbury et al., 1995).

The study of the impact of a disease on a given host population has to include the observation of infection (Simpson, 1996; Hazel et al., 2000; Comer et al., 2000) and of the disease effects (Simpson, 1997; Silva et al., 1999; Valenzuela et al., 2000), and also the quantification of which infectious agents affect host demographic parameters, especially survival and fecundity (Pence and Windberg, 1994; Stoner, 1995; Schubert et al., 1998; Shibata and Kawamichi, 1999).

From a conservation perspective, small mammals are an important group of organisms. They have short life cycles and respond rapidly to environmental alterations. They are important prey items for a majority of carnivores. In addition, they have medical and veterinary importance through their role as pathogen reservoirs, maintaining the sylvatic cycles (those affecting wild animal populations) of some important diseases, e.g. leishmaniasis and hantavirus infections. Because of these characteristics, small mammals are considered good biological indicators and monitoring their populations and health status has become an important tool for developing management and conservation strategies.

Like many species of small mammals, the coati is a reservoir of some trypanosomiasis (Ferrioli & Barreto, 1968; Nunes & Oshiro, 1990; Nunes et al., 1993; Lainson et al., 1989). In some regions of Pantanal, it has been revealed a high prevalence of infection by the tripanosomatid *T. evansi* among coatis and domestic animals. Given its ecological and parasitic importance (see Gompper & Decker, 1998), associated with its high abundance and widespread distributions in the Pantanal region, the coati is an adequate species to be studied in order to investigate the parasitic cycles in carnivores.

Our main objective is to monitor the health status of wild mammal populations in the Pantanal. We will identify important host-parasite systems that can be used to indicate the health status of mammal populations, and we will offer training to students in wildlife handling, population ecology, and disease diagnosis. Acquired information will be fundamental for future management plans for conservation as well as to develop control strategies of wildlife diseases.

The project began in 2002 and became part of the CRI in January 2004. In 2005, we will start to monitor the population of coatis (*Nasua nasua*) as an additional target species on the study of parasitic cycles in Pantanal.

### Long term objectives

1. To select host-parasite relationships that can be used as indicator of the health status;
2. To evaluate if changes in landscape can be associated with the emergence of diseases using Geographic Information System (GIS);
3. Training students for Environmental Medicine: wildlife handling, disease diagnosis and GIS analysis.

### Short term objectives

1. To identify and to quantify the endoparasites of small mammals;
2. To select a target species for parasitism study;
3. To investigate the interaction host-parasite and how parasites influence their host populations;
4. To determine the population dynamics and the reproductive patterns of small mammals and to evaluate the risk of disease outbreaks in relation to the population fluctuations;
5. To determine the small mammal's diet and their importance concerning fruit dispersion;
6. To compare the health status between areas which present different degrees of disturbance;

## **2. Methods**

In order to evaluate the parasitism and its effects on small mammal populations, we monitored the small mammal populations through a capture-mark-recapture study on one area and removed individuals for parasitological study from another area in the Rio Negro farm.

### *Capture–Mark–Recapture*

The small mammals captures were performed every three months during seven trap-nights on three forest fragments in Rio Negro farm. We designed a sample plot unit consisting of three linear transects randomly located inside a forest fragment and one transect placed on the edge of the same forest fragment. Each transect had 10 trapping stations spaced 20 meters apart. On each station two traps were placed on the floor and also one trap on a tree. We used fixed four plot units in the Rio Negro farm, distributed randomly. The traps were baited with a mixture of peanut butter, banana, oat and bacon.

Animals captured were marked by ear tagging. Captured animals had their weight and body length measured, the sexual condition observed and marsupials had their degree of dental development estimated, before being released. We took a small blood sample of the target species to perform the health analyses

### *Removal-Release Captures*

The captures were performed every six months during seven trap-nights on two plot units per site, described in the previous section. The animals captured were removed to the laboratory and tranquilized with Zoletil<sup>®</sup>50 (Virbac) to take an aseptic blood sample through vein puncture. The blood samples were used to perform detailed parasitological studies (serologic surveys for hemoparasites and patterns of hematology). The animals were ear tagged and kept in captivity for one or two days for recovering, and then they were released on the same trapping station.

### *Population analysis*

We studied the population dynamics of the most frequently captured species. The target species will be used as a model for parasitological studies. Population abundance, survivorship and recruitment rates will be calculated using Pollock's 'robust' design (Pollock, 1982; Pollock, 1991; Pollock 2000). The breeding season will be analyzed as the period in which females present signs of reproductive activity. Mean fecundity will be estimated as the number of female offspring per adult female.

In order to evaluate parasite influence in host population, we investigated relationships between health parameters (parasite burden, prevalence and intensity, hematology parameters and blood biochemical analysis) and host population parameters (abundance, fecundity and survivorship).

### *Health Analysis*

The faeces samples collected during the fieldwork were weighted and stored individually in alcohol-formalin acetic solution (AFA). A sample was taken and examined under the stereoscopic microscope for worm eggs. The results will be expressed by the estimated number of eggs per grams of feces.

Blood samples collected and sera were sent to Laboratory of Trypanosomatids Biology, Fiocruz/RJ for the search of anti-*T. evansi*, anti-*T. cruzi* and anti-*L. chagasi* antibodies by indirect fluorescent antibody test.

The packed cell volume, total red blood cell and total white blood cell will be counted in Neubauer chamber. The differential leukocyte counting will be performed on blood smears stained with a mixture of May-Gruenwald, Giemsa and methanol.

### *Geographic Information Systems*

At the Laboratory of Trypanosomatids Biology, Fiocruz/RJ, the health data of a small area will be plotted in a detailed view of the landscape by computerized mapping system. Appraisal will be carried out by a geographical analysis of health data that attempts to detect clustering of disease cases in these small discrete areas.

### *Formation of human resources*

*A practical professional training on biological sample collection, hematology, parasitological tests, necropsy practice, use of GIS and technical for population estimate will be offered to undergraduate and graduate students by the technical staff. Local people will receive training for wildlife handling as well as laboratory techniques.*

## **3. Results**

### **Highlights**

*2000-2002 species inventories:*

- During the species inventories, 11 species (7 rodents and 4 marsupials [opossums]) were captured,

- Most species were found in “cordilheira” forests (strains of closed vegetation), but one marsupial was restricted to gallery forest (forest in the vicinity of rivers), one semi-aquatic rodent to flooded habitats, and two other rodents were more common in grasslands;
- Two rodents that were abundant and widely distributed were chosen as target species for parasite studies, the arboreal rice rat, *Oecomys mamorae*, and the spiny rat, *Thrychomys pachyurus*,
- A cattle-impacted area had a higher percentage of domestic animals and small mammals infected by horse trypanosomiasis, an epizootic disease caused by *Trypanosoma evansi* (a parasitic flagellate),
- Both target species were infected by tripanosomatids, but *Oecomys mamorae* had a lower level of helminthes infection than *Thrychomys pachyurus*., perhaps because of the former’s arboreal habits.
- The number of helminthes species infecting *Thrychomys pachyurus* was lower in the cattle-impacted region, suggesting a shift in parasite species dominance in the environmentally impacted area.

#### 2003-2004 expeditions:

- The rodent *Oecomys mamorae* seems to have an annual population cycle that follows the climate seasonal dynamic of the Pantanal region.
- *Thrychomys pachyurus* has presenting a slight decline in abundance since the beginning of the study;
- As a result of its reproductive cycle, the marsupial *Gracilinanus agilis* presented an annual population cycle, as the majority of the Brazilian opossums;
- We found both *T. cruzi* and *T. evansi* in several species of small mammals in Rio Negro farm and Alegria farm. In the former, the most infected species was *O. mamorae*, followed by the marsupial *G. agilis*.
- During all field procedures, the volunteers, PIs, and assistants used individual biossecurity equipment that included masks with high performance filters. These masks are used to protect against potential hantavirus contamination.

During the last field season (February – November 2004), we had a total of 391 captures from six species of small mammals, three of them were rodents, *Oecomys mamorae*, *Thrychomys pachyurus* and *Clyomys laticeps* and three were marsupials, *Gracilinanus agilis*, *Thylamys macrura* and *Monodelphis domestica*. A total of 198 individuals were marked. Total capture success (number of animals captures divides by the total number of traps) was 4,23%. This value is in accordance with those usually obtained for small mammals in Brazil.

We monitored the populations of small mammal species through a capture-mark-recapture study that began in early 2003. In the last field season, we continued to monitor the populations of small mammal species through a capture-mark-recapture study. All species presented a 1:1 sex ratio but *G. agilis*, which showed different sex ratios during the study period, reaching 2,5 males for 1 female in May of 2003. As a result of its reproductive cycle, *G. agilis* presented an annual population cycle, as the majority of the Brazilian opossums. For this species, the breeding season occurs from November to February.

The species *O. mamorae* seems to have an annual population cycle that follows the climate seasonal dynamic of the Pantanal region. The females presented signs of active reproduction during the whole year, suggesting a continuous reproduction along the year. However, we found a bigger rate of females presenting reproductive signs in the beginning of the wet season (December). *T. pachyurus* has presenting a slight decline since the beginning of the study (early

2003), but there was an increase in the rate of reproductive females starting in December of 2003.

We collected 244 faecal samples of rodents to evaluate the presence of helminths and to analyse the species' diet. Additionally, we collected and identified eggs found in helminth adult females, in order to do an identification table of the helminths' eggs, which is now ready. This table will be used to identify the eggs found in the small mammals' faeces collected during 2004. Next year, an additional egg identification table will be made for parasites of *O. mamorae*. The diet analysis has been performed by Dr. Nilton Cáceres and results are still preliminary.

We have been studying the Pantanal region since August 2000. In the beginning of the study, we did small mammal species inventories and parasite studies in two distinct areas: the Rio Negro farm and Alegria farm. The former is a Conservation International Research Center and the later has intense economic activities based on cattle ranching. Since 2000, we have been collecting blood samples of several species of wild animals in order to evaluate if they were infected by the hemoparasites *Trypanosoma cruzi* and *T. evansi*. The parasite *T. cruzi* is responsible for Chagas' disease, which infects a large number of animals, besides humans (FNS/MS, 1999; Nunes et al., 1994) and *T. evansi* is the agent of a severe disease in horses and dogs ("Mal das Cadeiras" disease), which also parasites many other mammals (Nunes et al., 1993; Franke et al., 1994; Silva et al., 1995).

We found both *T. cruzi* and *T. evansi* in several species of small mammals in Rio Negro farm and Alegria farm. In the former, the most infected species was *O. mamorae*, followed by the marsupial *G. agilis*. Data from both areas were compared and preliminary results has shown that human action resulted in significant alteration on the relative frequency of small mammals and on the epidemiological profiles of both trypanosomatid species, mainly regarding *T. cruzi*. Cattle ranching seems to be associated to an increase in the transmission of *T. evansi* as expressed by higher prevalence rates in Alegria farm.

From individuals of *T. pachyurus* captured in Rio Negro farm and Alegria farm, we identified 9 species of nematodes: seven of them were found in Rio Negro farm and six in the Alegria farm.

## **4. Discussion**

### **(a) Engagement**

For several times volunteers formulate simple questions that come up with good ideas for the development of the research. It happens every time we work with different people that have different skills and expertise. But volunteers were particularly important in this first phase of the project, in which we had few people to help us in the field. We really needed the help of volunteers to perform almost all kinds of tasks in the field. Volunteers helped us to set up the traps, check and bait them every day, to collect and store the faecal samples, to take note of all scientific data and to log it in the computer, to prepare the bait and equipment needed in the field, etc.

Our project involves local people and students from high school to graduate courses. There is a high school student who is trainee of our laboratory, and did his monograph concerning helminth fauna of *T. pachyurus*. Also, we are currently training one young local student that will help us in the fieldwork. Additionally, there is an undergraduate student that developed her undergraduate monograph on population ecology of small mammals in the Rio Negro farm. One of the PI's is currently doing his Ph.D. in The Pennsylvania State University, using data

collected on small mammal's health in Pantanal. For the next field season, another PI will be starting her Ph.D. in the University of Missouri-Columbia. She will study the ecology and health of the coatis. For the next season, we also expect to train one veterinarian student to help us in this project.

The study helps local community to understand that all species are important in the ecosystem. In our project people learn that Pantanal presents several species of wild rodents, which are seed dispersers, prey items for other species and are a key component in the maintenance of parasites in the wild.

## **(b) Science**

Monitoring the small mammal health and its parasites allow us to identify the risk of disease transmission and spread. The results of the study of the relationship between small mammals and its parasites in areas with different degrees of disturbance will be useful in accessing the impact of human activities in natural populations and the possible outcomes of these activities. This project will also provide cues to CI's Project Cerrado-Pantanal Corridor. The results could help in conducting necessary management activities in the areas to be connected by the corridor.

Acquired information will be fundamental for future management plans for conservation as well as to develop control strategies of wildlife and emerging diseases. Host/trypanosomes interactions and the relative frequency of small mammals seem to be a reliable indicator of environmental degradation, although more studies are needed to confirm it. Therefore, interactions between parasites and their mammal hosts may be used as indicator of environmental changes and also be used to define conservation, animal and public health policies - mainly at a local scale.

Additionally, our research on population ecology and health status of small mammals also provides basic information, which is essential for developing management strategies or any biodiversity conservation action plan. Small mammals are prey of several other vertebrates and they are important pieces for the well functioning of the Pantanal ecosystem.

During the past season we had some logistic challenges. The main logistic challenge concerned the costs associated with the study area. Rio Negro farm is a quite isolated area, which requires us to rent an airplane during the wet season. We had to adjust our budget and fieldwork design in order to overcome problems regarding the costs associated transportation and PIs accommodation. For the next field season, we will be working in a new area: the Nhumirim farm. Costs associated with this new area of study are relatively lower and we hope to be able to invest more in the scientific research *per se*.

A scientific challenge we were expecting was that for the next years, we will not be able to study deeply all species we usually capture in Pantanal. During this first year working with Earthwatch, we chose some target species for developing the population and health studies. Additionally, for the next season, we will begin to work with coatis (*Nasua nasua*), which were chosen as a target species for investigating parasitic cycles in carnivores.

In the next phase of the project, population ecology studies will be developed in association with experimentation *in situ*, in order to investigate the influence of helminths on the health status of the small mammal target species.

## **(c) Dissemination**

This is the first long-term study of population ecology in the Rio Negro farm. Our project provides 1) a species inventory of small mammals occurring in the area and 2) information on the health status of several mammals species. Additionally, the population ecology of the small mammals species were never studied in the area before. Therefore, our project is able to provide basic and fundamental knowledge for management plans in the area.

## 5. Presentations, Meetings, Conferences/Workshops and Seminars

### *Presentations:*

- 1) Title: "Population ecology of small mammals in the Rio Negro farm". Undergraduate monograph, by Cecília Siliansky. Local: Institute of Biology of the Federal University of Rio de Janeiro. Audience: professors and students from the Biology undergraduate course. About 50 people attended to each of those presentations. Dates of presentation: June and December/2004
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### *Conferences, Workshops, Congresses and Courses:*

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- 4) Dec 2004: XII Annual Meeting of Scientific Initiation. Poster presented by Cecília Siliansky

## Partnerships

- 1) Genetics Division, Cancer National Institute/RJ
- 2) Laboratory of Helminthology, Federal University of Rio de Janeiro
- 3) CNPq
- 4) Conservation International

- (1) Genetic Division (INCA) helped us to identify some species of rodents that can only be identified by genetic analysis;
- (2) Laboratory of Helminthology helped us to identify the helminths species of small mammals captured. When publishing scientific papers, results of our research will be used in partnership with researchers from institutions (1) and (2).
- (3) CNPq helped us by providing funding for acquiring equipment that are essential for fieldwork and laboratory analyses.
- (4) Conservation International has helped us with logistics in the field.