

Final Evaluation Report

Your Details	
Full Name	Roberto Fiorini Torrico
Project Title	Behaviour, hormones and energetic condition: an assessment on how golden-headed lion tamarins (GHLTs) cope with degraded forests
Application ID	29410-1
Date of this Report	16/6/2023

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Establishment of phenology plots				<ul style="list-style-type: none"> - We established 47 plots within the home range of four lion tamarin groups. - Plots were distributed across crops and forest fragments in different regeneration stages. - 803 plants were measured and identified in the floristic survey.
Behavioural observation of wild GHLTs				<ul style="list-style-type: none"> - We conducted 11 months of focal observations of three groups of lion tamarins. - All observations were voice-recorded and later transcribed to a spreadsheet with the help of an assistant. - We documented the behaviour of 21 adult and subadult lion tamarins using a thorough ethogram to assess energy intake and expenditure, and social interaction. - We recorded 1165 10-min focal samples that together added up to 194.2 hours of observation.
Collection and processing of GHLTs faeces				<ul style="list-style-type: none"> - We collected a total of 289 faecal samples: 257 samples concurrent with behavioural observations and 32 samples during two capture events performed by BioBrasil's team. - Ethanol (80%) was evaporated from all samples which were later oven-dried for 4 hours at 50 °C. - We packed samples in sealed Mylar bags which were stored at -20 °C until shipping and analysis.
Food availability assessment				<ul style="list-style-type: none"> - We monitored the phenophase of 130 marked trees from 21 species, commonly consumed by lion tamarins, during 11 months of fieldwork. - We determined the temporal changes in ripe fruit availability and the flower availability of <i>Symphonia globulifera</i> through an index that integrated

				phenology scores and density of preferred plant food items obtained based on the floristic survey.
Validation of lab tests and hormone concentration measurements				<ul style="list-style-type: none"> - We obtain all applicable permits to ship the faecal samples to the Interdisciplinary Laboratory of Clinical Analyses of Murcia University (Interlab-UMU) in Spain. - Hormone metabolite extractions and measurements were carried out successfully. - We performed an analytical and a biological validation of the measurements of faecal glucocorticoids (GCs) and triiodothyronine (T3) metabolites. - To measure the metabolites in the extractions we used commercially available enzyme immunoassay (EIA) kits.
Statistical analysis, reporting and concluding remarks for conservation				<ul style="list-style-type: none"> - Statistical analyses were concluded and used for the preparation of two chapters of my doctoral thesis which will be defended by the beginning of August 2023. - Chapters will be corrected according to the reviewers' feedback and subsequently formatted for their submission to specialised journals.

2. Describe the three most important outcomes of your project.

This project linked the behavioural patterns of wild GHLTs to the levels of faecal GC and T3 levels while considering the temporal changes in the availability of preferred plant food items. By doing so, we uncovered the strategies adopted by GHLTs to face the energetic challenges of their disturbed environment. Importantly, our results indicated that faecal GCs are associated to energy-saving strategies and faecal T3 correlate to energy-intake strategies.

A) More specifically, the time devoted to pausing between locomotion and resting following high activity levels corresponded significantly with changes in GC excretion. This suggests that GC secretion modulate the expression of behaviours that prevent depleting the energy reserves and thus contribute to alleviating the stress response to energetic challenges.

B) On the other hand, time dedicated to feeding on ripe fruits and the abundance of *S. globulifera* flowers which provide nectar, as expected, were more closely related to variation in faecal T3 levels, as thyroid hormones are known to be mediators of the metabolic rate in various vertebrate species. This finding, in

particular, shows the crucial role of fruit intake for energy acquisition by GHLTs and, given that peak bloom of *S. globulifera* coincided with low fruit availability, it is likely that flowers of this species serve as a fallback food during periods of low fruit intake.

C) The biological validation revealed significant differences for both the levels of GC and T3 metabolites between samples collected during the capture events and the full-day focal follows of the GHLTs' groups. Additionally, faecal T3 corresponded with a body condition score and faecal GCs with the time of collection, which denotes, respectively, the influence of energetic condition and circadian rhythms on these metabolites. Taken together, these results proved the biological relevance of the non-invasive techniques applied in this study to investigate the levels of faecal GCs and T3 in GHLTs.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

- Initially we intended to study four groups of GHLTs. However, given restrictions imposed by one of the private landowners, we were not able to monitor the phenology plots inside one of the group's home ranges and had to remove all tree markings. That is how we ended up monitoring three groups. Nonetheless, thanks to that, we gathered more repetitions of focal observations for each individual.
- We were forced to delay the onset of the full-day focal follows of GHLTs due to the COVID-19 pandemic. To return to activities, we updated hygiene protocols at the field station and organised logistics with Project BioBrasil team, who collaborated with this project in its whole development.
- Dye marks on GHLT tails, which enabled the identification of individuals during the behavioural observations and faeces collections, started to wear off sooner than expected. To tackle this difficulty, we anticipated the capture of lion tamarins and tested a new harmless dye on the tamarins' tails. Capture was time-consuming but overall successful. Focal follows had to be rearranged during that period; however, new dye marks remained until the end of data collection.
- As data was being collected, we realised that a single person to transcribe all the voice recordings and field sheets was not sufficient. So, we decided to include a data entry assistant with whom I regularly met to examine the resulting spreadsheets.
- We had difficulties finding a new freeze-dryer to preserve the faecal samples, since the one at my university was broken. The best choice, at that moment, was to adapt our preservation protocol with the available equipment. So, instead of freeze-dryer we used a laboratory oven to dry the samples.
- Changes in the preservation protocol need to be validated. So, we collected an additional set of samples to perform an analytical validation which ensured that the combination of ethanol and drying in an oven did not

significantly affect the level of metabolites of interest in the faeces. This step showed low variation between samples preserved with and without alcohol.

- Authorisations for the transportation of samples from Brazil to Spain had to be updated due to the changes in preservation protocols. Furthermore, we faced several uncertainties to be able to define the best way to ship the samples to Spain. We carried the samples ourselves with all the applicable permits.

4. Describe the involvement of local communities and how they have benefited from the project.

In February 2021, I participated in a photo exhibition in Colonia de Una (BA), organised by Project BioBrasil, which was open to the whole community in the town. In this activity we showcased several photos depicting nature in the Atlantic Forest, including some taken during this field work. In the exhibition, we connected the pictures of the Atlantic Forest fauna and flora to the lion tamarin's behaviour through a story about the GHLT life and surroundings.

Between September and October 2021, I was involved in the organisation of an online course in "Biodiversity and Environmental Management in Family Farming" offered to rural extension workers and environmental technicians that work with family farmers inside the scope area of the Bahian Lion Tamarin Conservation Initiative (BaLTICI). This area coincides with the distribution range of GHLTs living in similar landscapes to our project's GHLTs. In this course, I was responsible for teaching a series of classes and preparing instructional material concerning ecosystem services in agriculture. In our classes, we reviewed some concepts of ecology using as examples the Atlantic Forest, the GHLTs' habitat, and the shaded cocoa agroforests – Cabruca. Our goal was to provide people linked directly to family farmers with straightforward information about the importance of healthy ecosystems for the provision of services in the agriculture and to talk about the potential monetary and tax benefits that come from ensuring the provision of ecosystem services and protecting Cabruças and forest remnants on farms.

5. Are there any plans to continue this work?

After the fulfilment of all PhD demands, I will devote myself to publishing these results. I will continue collaborating with BaLTICI to ensure this study will be used to update conservation plans and actions for the species across a much larger area. I will also offer all necessary assistance or expertise to extend the non-invasive monitoring of physiological markers to other type of habitats, like Cabruças, which are currently threatened by land use intensification. During my field observations, we witnessed a frequent rate of inter-group encounters and several migrations of known and unknown individuals, such group instability limited to some degree the access to food sources for the study groups. However, it is still unclear how social context between groups with overlapping home ranges affects the behavioural and physiological responses of GHLTs and whether intergroup encounters and resource competition are associated to habitat saturation which may result from the isolation and the relentless fragmentation of forest remnants in our study area. So, my

intention is to continue working with Project BioBrasil with the aim of further investigating the interplay between food availability, energetic condition and social context, as well as the consequences of habitat fragmentation and loss of forest connectivity. Relevant information from this and future projects will continue to be shared locally in the program of educational outreach conducted by Project BioBrasil.

6. How do you plan to share the results of your work with others?

I intend to publish the results in scientific journals because I consider that they will contribute to a better understanding of how energetic stressors in degraded habitats may threaten the persistence of the species. Furthermore, I plan to use these results to improve conservation plans and propose management measures for GHLTs residing in fragmented forests. This will be possible thanks to my collaboration with BaLTCI, an institutional network that has developed a 10-year strategic plan for the long-term conservation of GHLTs. This network unites both academic institutions nationally and internationally, and local stakeholders, local farmers' representatives, and governmental and non-governmental instances involved with GHLTs conservation.

7. Looking ahead, what do you feel are the important next steps?

I believe that the persistence of GHLT populations in degraded forests depends partly on their capacity to migrate and reach different environments. Unfortunately, in our study area it was evident that groups suffered from low vagility caused by low levels of landscape connectivity around the study area. As a result, frequent encounters with adjacent groups limited the amount of food and partners groups had access to. Furthermore, there is an increased chance that migration of sexually mature individuals among a reduced number of groups could lead to inbreeding depression. So, I consider important to investigate the degree of kinship between members of the same group. On the other hand, management plans should be aimed at increasing the connectivity between forest fragments and consequently between isolated GHLT subpopulations. Such plans should be carefully devised so that negative impacts for targeted subpopulations can be prevented, and human conflict can be handled with interdisciplinary and well informed measures. In summary, I visualise two important steps: to determine the degree of kinship within and between adjacent GHLT groups and to propose measurements that increase landscape connectivity.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes, I used the Rufford Foundation logo and name on several occasions:

- June 2020 at the 25-year anniversary of the environmental engineering career organised by Universidad Católica Boliviana in Bolivia: online meeting to talk about prospects in the research field for environmental engineering students.

- November 2021 at the 14th annual Zoo Science Symposium organised by Antwerp Zoo Centre for Research and Conservation in Belgium, title of the presentation: "Feeding strategies and energetic condition of golden-headed lion tamarins: prospects and highlights of fieldwork."
- December 2021 on the podcast "Educa Rap Dicas Socioambientais" organised by Universidade Federal do Recôncavo da Bahia in Brazil: interview about ecosystem services.
- December 2022 at the 1st Meeting of the postgraduate program in Ecology and Biodiversity Conservation that took place at Universidade Estadual de Santa Cruz in Brazil: I talked about my exchange experience in Spain which had the goal of analysing the metabolites in the faecal samples collected for this project.

9. Provide a full list of all the members of your team and their role in the project.

Leonardo de Carvalho Oliveira – advisor and Scientific Director of the Research Institute Bicho do Mato – Leonardo's role was to guide the project's scientific and academic development.

Kristel M. De Vleeschouwer – co-advisor and Director of Project BioBrasil – Kristel's role was to provide scientific expertise, and to coordinate the sampling effort and logistics throughout the completion of this project.

Zaqueu da Silva Santos – field assistant – Zaqueu's role was to assist with the establishment of plots, the floristic composition, the behavioural observation, the collection of faecal samples, and the monthly phenology monitoring.

Edinilson dos Santos – field assistant – Edinilson's role was to assist with the establishment of plots, the floristic composition, the behavioural observation, the collection of faecal samples, and the monthly phenology monitoring.

Rodrigo Bruno de Oliveira – botanist – Rodrigo's role was to collaborate with the establishment of phenology plots and determine the floristic composition within the study area.

Valentina Fortunato – data entry assistant – Valentina's role was to assist with the transcription of voice recordings of observed behavior, groups composition, and monthly phenology.

José Joaquín Cerón Madrigal – Lab collaborator and Head of the Interdisciplinary Laboratory of Clinical Analysis – José's role was to monitor the execution of lab techniques to measure the hormones in the faecal samples and assist with the interpretation of results.

Damián Escribano Tortosa – Lab collaborator – Damián's role was to assist with the preservation of samples, the measurement of hormones, the validation of hormonal analysis, and the interpretation of results.

Ana Claudia Fandi – education outreach partner and Executive Coordinator of Project BioBrasil – Ana's role was to guide and propose educational activities that are relevant to the local environmental awareness and the conservation of GHLTs.

10. Any other comments?

I would like to thank The Rufford Foundation for their financial support. This project would not have been possible without your help.







