

**Assessing genetic diversity and gene flow for Neotropical otter (*Lontra longicaudis*)  
in the San Juan River Basin, Costa Rica**  
PARTIAL REPORT

**1. Project Activities**

1.1 Research Collection Permits: Before starting the field activities, we requested a non-invasive sampling collection permit through the National Commission for the Management of Biodiversity (CONAGEBIO) of Costa Rica. CONAGEBIO authorized the non-invasive sampling collection under resolution numbers: R-031-2021-OT-CONAGEBIO, R-032-2021-OT-CONAGEBIO, and R-025-2022-OT-CONAGEBIO.

1.2 Activities and timescale: This section indicates the original project's activities, and timescales to achieve the objectives and includes relevant comments on factors that affect their achievement. Additionally, it shows the current achievements of the activities according to the project timeline.

Activities and timeline	Comments
Meetings and workshops (January-February 2021).	Although we conducted meetings and workshops, these activities were re-scheduled to May 2021, mainly because of the COVID travel restrictions and suspended activities in Costa Rica. In addition, meetings with professors and universities were not possible due to the suspension of academic activities in the country. All meetings and workshops were performed in collaboration with park rangers, volunteers, and locals at field stations managed by SINAC (El Ceibo-Braulio Carrillo National Park) and Biological Stations management by NGOs such as Caño Palma Biological Station, La Selva Research Station, Tirimbina Biological Reserve, and the Bijagual Ecological Reserve.
Fieldwork; first sampling period (February-July 2021), second sampling period (January-May 2022).	Fieldwork activities for the first sampling period were re-scheduled to May 2021. However, due to COVID restrictions and the high prevalence of positive cases in the country, we decided to focus our sampling efforts on two areas within the San Juan River Basin (SJR). These areas are considered of particular interest for conservation for their high biodiversity of flora and fauna and increased economic and tourist activities. In addition, due to all the sanitary regulations in Costa Rica and noticing the active presence of park rangers and locals within different Protected Areas and rivers, we decided to implement community-based monitoring with them through specific areas. We trained 22 people between park rangers, locals, and volunteers through 5 workshops distributed between the government biological stations and NGOs. After completing all meetings and training, we returned to the US for health security. For the second sampling period, which was re-scheduled from March to June 2022, the COVID regulations and sanitary

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	<p>standards in Costa Rica were lowered, and we conducted the sample collection in person.</p> <p>Additionally, to optimize fecal DNA collection and storage techniques and maximize the success of PCR amplification, we decided to collect fresh feces and anal jellies through two methods; a) collection of fecal material from otter samples stored in dimethyl sulfoxide saline solution (DETs buffer) and b) fecal external swabs stored in ATL buffer.</p> <p>After two sampling periods, 512 non-invasive genetic otter samples were collected, which 162 unique samples stored in DETs buffer (with no duplicates), and 350 samples (DETs buffer, n=175; swab protocol, n=175) using both DETs and swab protocols. Due to COVID restrictions for people in Costa Rica, the number of samples collected during 2021 was lower (n=138) than in the second sampling period (n=374).</p>
<p>Perform genetic analysis (August-November 2021) from the first sampling period and genetic analysis (June-September 2022) from the second sampling period in the Laboratory for Ecological, Evolutionary, and Conservation Genetics (LEECCG) at the University of Idaho (UI).</p>	<p>All samples are stored in the Laboratory for Ecological, Evolutionary, and Conservation Genetics (LEECCG) at the University of Idaho (UI). Furthermore, the first set of 74 non-invasive genetic samples from the first sampling period have been processed for DNA extraction and genotyping. This set of 74 samples is composed of samples collected in silica (n=25) and donated by Caño Palma Biological Station, as well as 49 samples collected (DETs buffer, n=25; swabbing stored in ATL buffer, n=24) as part of this project from May to August 2021. Currently, DNA extraction for the rest of the samples collected during the first sampling period and all collected during the second sampling period is ongoing.</p>
<p>Conduct data analysis from the first sampling period (October-December 2021).</p>	<p>A set of nine nuclear DNA microsatellite loci and a sex identification marker were amplified in duplicate a single PCR multiplex for the first group of 74 non-invasive genetic samples. Amplification success was estimated by each sample's total number of positive amplifications, divided by the total number of loci attempted across two PCR replicates. We classified successful samples as positive amplification with an amplification success <math>\geq 40\%</math> of the loci attempted. Preliminary results for this first set of 74 samples showed the highest amplification success rate for swabbing protocol (54%), followed by DETs buffer (33%), fecal samples stored in silica samples were not successfully amplified.</p>
<p>Dissemination of preliminary results (December 2021).</p>	<p>We will participate in the 15th IUCN/SSC OSG International Otter Congress from the 19th to the 23rd of September 2022. The congress will be online, and we will present preliminary results on optimizing fecal DNA sampling and storage techniques for Non-invasive genetic sampling of Neotropical otters in Costa Rica.</p>
<p>Dissemination of results from the first sampling period (January-February 2022).</p>	<p>We will participate in the 15th IUCN/SSC OSG International Otter Congress from the 19th to the 23rd of September 2022. The congress will be online, and we will present preliminary results on optimizing fecal DNA sampling and storage techniques for Non-invasive genetic sampling of Neotropical otters in Costa Rica.</p>

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Develop Landscape Genetic modeling and final data analyses (October 2022-April 2023).	A set of environmental data layers has been identified to model the dispersal routes of neotropical otters within the SJRB; these layers were obtained from the Digital Atlas of Costa Rica published in 2008 by the Technological Institute of Costa Rica.
Dissemination of the final results (May-August 2023).	We are still working on DNA extraction and genotyping for all 2022 otter samples.
Defend PhD thesis (August 2023).	

## 2. Involvement of local communities

**2.1 Community-based monitoring.** We trained and developed workshops for those local volunteers and park rangers interested in the project. This community-based monitoring was partially successful regarding the number of samples collected due to the high prevalence of COVID positives cases in Costa Rica and sanitary regulations in all 2021 years. However, training and workshops were entirely successful in terms of new knowledge acquired by park rangers and locals because they were trained in non-invasive sampling methods, GPS unit management, identification of fresh otter feces, and the natural history of the species and its ecological function in rivers. It is important to highlight that park rangers and locals accomplished the first sampling collection period.

**2.2 World Otter Day Workshop.** World Otter Day is an annual international celebration to raise awareness of the conservation of the 13 species of otters. One of the activities of this project was to hold the first World Otter Day in Costa Rica and Central America. To accomplish this, we carried out an Otter workshop and educational activities with 14 young students between 7 to 12 years old from the Tirimbina School in collaboration with the academic and research department of the Tirimbina Biological Reserve. The Tirimbina School plays a vital role within the community for local youth's education and environmental awareness because it is the only elementary school in the zone, and the students are in frequent contact with nature.

## 3. Conservation networks

Since the beginning of the project, we have maintained active communication with researchers, students, government areas, and NGOs in Costa Rica and throughout species distribution. In Costa Rica, this conservation network includes government sectors such as the National System of Conservation Areas or SINAC, Caño Palma Biological Station, La Selva Research Station, Tirimbina Biological Reserve, and the Bijagual Ecological Reserve, which are NGOs.

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#### **4. Challenges Faced**

4.1 During all of 2021, COVID restrictions and sanitary regulations in Costa Rica were substantial mainly because of the high prevalence of COVID positives cases throughout the country. Due to the pandemic, we faced four significant challenges.

- a) It delayed all government procedures, including processing the collection of non-invasive genetic samples for the project. Additionally, high bureaucracy within government offices slowed progress on paperwork and permits.
- b) The suspension of academic activities in the country changed all the plans to conduct workshops with students and researchers from the Universities.
- c) Despite park rangers and volunteers carrying out the community-based monitoring, communication with them was limited in some collection sites due to the lack of telephone signal or internet access in the area.
- d) Restrictions in the field due to sanitary regulations affected our sampling process, and we were forced to reduce the spatial extent of our study area.

4.2 The high precipitation was another challenge during the first sampling season. Although the zone is known for its high precipitation, the precipitation in 2021 was extraordinary compared to the 2022 rain season. High precipitation in sites such as the Sarapiquí sub-basin, Tortuguero National Park, and Braulio Carrillo National Park triggered river floods, covering all logs and rocks that otters use to mark with feces and anal jellies, which might have reduced the number of available samples.

#### **5 Future Directions**

- a) We are building a database with all articles focused on molecular analysis for *Lontra longicaudis* to identify and test loci previously used in this species. This database will allow us to select the best loci to complete the optimization of the neotropical otter multiplex.
- b) Complete DNA extraction for all samples collected and develop a PCR multiplex with 7-12 loci for Neotropical otters for individual identification, and test additional sex identification markers for analyzing otter's population genetic parameters and test landscape genetic hypotheses.
- c) We are addressing a new research direction by adding molecular diet analysis to my thesis project. A reference database of potential otter prey is currently being developed. In addition, we are contacting researchers who have worked with potential otter prey for future collaborations.
- d) We will apply for additional funds to support analyses of the extra samples collected and add on molecular diet analysis.

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**Photo 1.** Instruction on using GPS units to volunteers within the Bijagual Ecological Reserve. Sampling season 2021.



**Photo 2.** PowerPoint presentation on non-invasive genetic sampling collection to the park ranger chief at the Ceibo Station. Sampling season 2021.



**Photo 3.** Meetings with the research staff from La Selva Research Station to introduce them to the project. Sampling season 2021.



**Photo 4.** Workshop with locals and workers from Tirimbina Biological Reserve related to the natural history of otters. Sampling season 2021.



**Photo 5.** Meeting with Caño Palma Biological Station volunteers to discuss doubts about the non-invasive genetic sampling protocol. Sampling season 2021.



**Photo 6.** Example of non-invasive genetic sample collected using both protocols. Puerto Viejo river. Sampling season 2022.



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**Photo 7.** Collection of fresh feces within the riparian zone in the Sarapiquí river. Sampling season 2022.



**Photo 8.** Collection of non-invasive genetic otter samples in the Puerto Viejo River. Sampling season 2022.



**Photo 9.** Recording coordinates of non-invasive genetic samples collected within the Tirimbina river. Sampling season 2022.



**Photo 10.** Identification of otter feces deposited on a rock near the Tirimbina Biological Reserve. Sampling season 2022.



**Photo 11.** Activities during the World Otter Day at the Tirimbina School. Sampling season 2022.



**Photo 12.** World Otter Day picture with young students from La Tirimbina school. Sampling season 2022.