

## Project Update: October 2022

### Objective No. 1. To estimate the distribution of the Neotropical otter in the study area.

#### - Environmental DNA (eDNA)

In June 2022, the vacuum pump, and the filtration equipment (kitazato flask, funnel, handle and hoses) were acquired to filter DNA in water samples. In June and July, the first tests of the equipment were carried out with different water samples to estimate the filtering time. On August 12, 2022 the Akumal Monkey Sanctuary in Quintana Roo was visited, where three Neotropical otter specimens are in captivity. The purpose of the visit was to collect six water samples (1 l each) from the pools where the otters are kept, as well as faeces samples, as a positive control for environmental DNA analysis. The samples were filtered with the filtration equipment using 0.40  $\mu\text{m}$  cellulose membrane filters. Filters were stored in sterile Eppendorf tubes in a freezer until extraction.



Fig. 1. Activities related to the eDNA technique. Above: Collection of feces and water samples at Akumal Monkey Sanctuary. Below: water samples, filtration process and filtrates obtained in the eppendorf tubes. Photo credits: Jaime Cifuentes and Fabiola Corona.

In September 2022, the first DNA extraction tests were carried out on the water samples collected at the Akumal Monkey Sanctuary. DNA extraction was also performed from the anal gel found in the otter faeces samples. For DNA extraction we use the NORGEN cell and tissue extraction kit, following the steps indicated in the kit. Subsequently, the first PCR amplification was performed, both DNA found in the pools and in the feces sample, using LutcytF (5'-CCACAATCCTCAACAACCTCGC-3') and LutcytR (5'-CTCCGTTGGGTGTATGTATCG-3') otter-specific primers designed to amplify the partial sequence of otter cytochrome b. The PCR was performed at 35 cycles and at three different temperatures (49.0 °C, 50.1 °C and 52.7 °C). Finally, we ran the agarose gel to visualise the nucleic acid content of the samples. On this occasion, a good amplification was obtained for the positive control (otter faeces), but not for the samples obtained

from the water. Therefore, modifications to the PCR conditions will be made to obtain the expected results.



Fig. 2. DNA extraction activities in water and feces samples and sample preparation for PCR amplification. © José Manuel García.

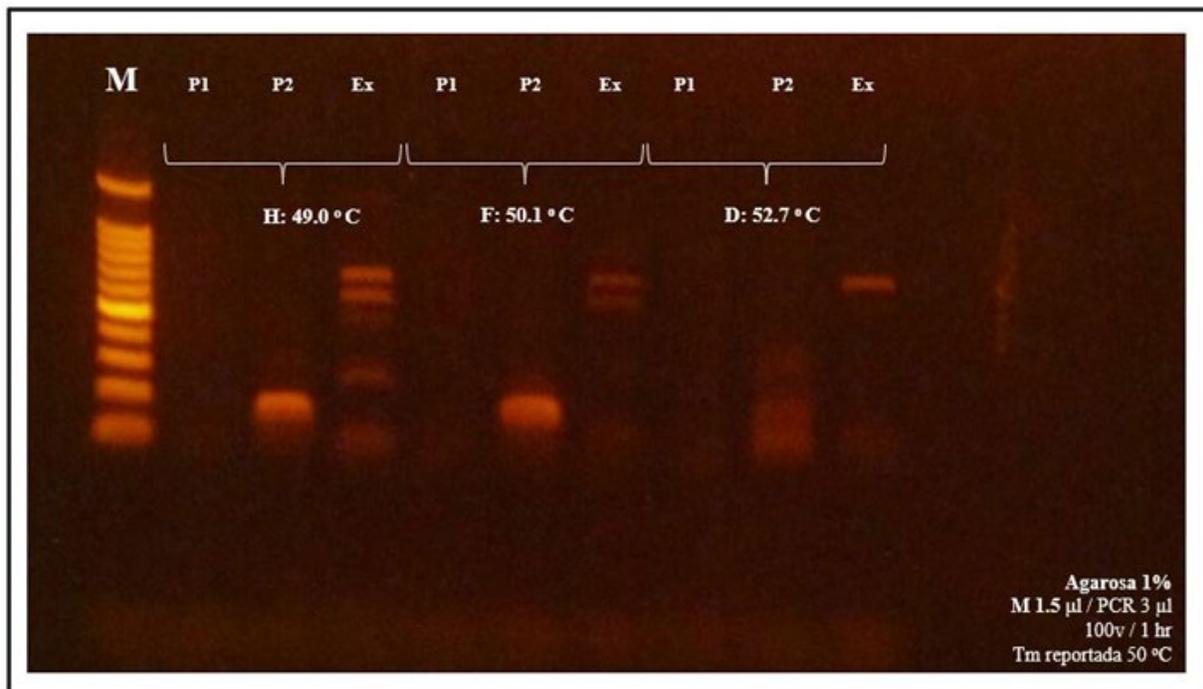


Fig. 3. Result of the amplification visualized in the agarose gel. © José Manuel García.

- Camera trap stations

At the end of August 2022, 12 photo-trapping stations were installed in Río Hondo (n = 6) and in Laguna de Bacalar (n = 6). The cameras were installed in the places where indirect evidence of the presence of otters (footprints, faeces) was found, adjusting themselves in the trunks of pucté, red mangrove or buttonwood trees, at a height of approximately 1 m, to avoid immersion in case the water level rises. In addition, the cameras were installed with a metal casing to prevent uninstallation by people outside the project. Each camera targeted the area of the water surface and the shoreline of the body of water. The configuration assigned for each camera was two photos and one video of 10 seconds. It is important to mention that the camera traps were monitored by local people who have supported the field trips for this research.



Fig. 4. Camera trap stations installed in the study area. © Fabiola Corona and José Negrete.

At the end of September 2022, the trap cameras were reviewed at each of the sites, changing the microSD memories and batteries, according to the level of charge. All cameras were found in place; however, two of the cameras installed in Río Hondo were damaged due to the entry of ants and other insects. The images and videos obtained were reviewed in an image and multimedia viewer on the computer. So far, the presence of Neotropical otters has not been recorded at photo-trapping stations; however, the presence of other organisms such as birds and lizards were recorded. The reviews of the images and videos will be carried out after each review of the stations. In addition, eight more camera trap stations are expected to be installed to cover the pending study area, that is, the middle basin of Río Hondo and in the Guerrero Lagoon. The camera trap stations will be left active until December 2022, checking them at least once a month.



Fig. 5. Photographic captures of two camera trap stations installed in the Bacalar lagoon during August-September 2022.

### Other activities

- Presentation of preliminary results at the XIII SOLAMAC Congress and 19 RT, from September 11 to 15 2022, in Praia do Forte, Bahia, Brazil.

During the congress, two oral presentations were made on the preliminary results of the study. One of the presentations was about the results obtained during interviews with local people about the otter and its habitat. And the other presentation consisted of the results on the presence of otters in the study area. During the presentations there was feedback from other experts, so we will take their comments into account in our analyses.





Fig. 6. Presentations made during the SOLAMAC Congress, in Praia do Forte, Bahia, Brazil.  
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