## Project update: June 2024

With the objective to update information on Southern River Otter's (SRO) distribution in north Patagonia, in March and April 2024, a new survey of the species was conducted in various river basins in the province of Chubut. The survey took place in several rivers and lakes of the Futaleufú or Grande River basin, the Carrenleufú or Corcovado and Pico river basin, and the Senguer River basin. In multiple sites across the coast, transects of 600 linear meters were carried, searching for signs of the presence of SRO, such as feces or tracks. Ideally, each site had a separation of 5k. Also, habitat and environmental characteristics were recorded in each site such as: slope, slope after the coast, coast width, riparian zone elevation (only rivers), vegetation cover (percentage), environment after the coast, amount of shelter, presence or absence of food, types of shelters present, submerged and coastal substrate, types of coastal and submerged rocks, human activity, and presence of domestic or wild species.

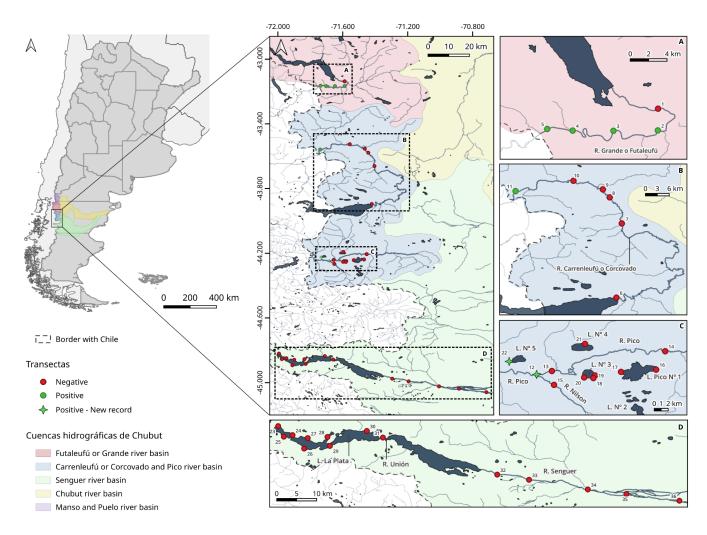
In total, 36 transects were conducted (Figure 1). In 29 of the sites, no traces of SRO were found, therefore they are considered negative and represented as red points on the map of Figure 1. On the other hand, SRO traces were found in 7 of the transects, represented as green points.

In the Senguer River basin, 14 transects were carried out where no signs of presence were found: eight points in La Plata Lake, one in the Unión River, and five in the Senguer River. On the other hand, the species was recorded in the Futaleufú or Grande River basin. Five transects were conducted in the Futaleufú River basin, on the Futaleufú or Grande River, where four were positive. The species was also recorded in the Carrenleufú and Pico River basins. In total, 17 transects were conducted in the basin: six on the Carrenleufú or Corcovado River, three on the Pico River, one on the Nilson River, and seven transects in the different Pico lakes. In this basin 3 sites resulted positive: In one site in the Carrenleufú River a cave and feces where found (Fig 2), one transect in the Pico River (Fig 3) where a mother and its cub where seen, and in Pico Lake N5 a cave and feces where found (Fig 4).

Seven sites in La Plata Lake and four sites in Futaleufú river were accessible only by boat (Fig. 5). Therefore eleven sites were accessed by bote. The other 25 sites were accessible by vehicle or walking. The total campaign was divided in three due to logistic and organization reasons. The first one the Senguer basin and Pico and Corcovado basin were visited in a total of 16 days where 20 sites were surveyed. The second campagne the Pico and Corcovado basin was finished in a total of 8 days where 11 sites were surveyed. Lastly the Futaleufú basin was visited in a total of 4 days where 5 sites were surveyed.

Lastly in every positive site, feces samples were collected. From each sample DNA will be extracted and sequenced with the objective to study the genetic structure of Southern River Otters in north Patagonia. Also, the Southern River Otter's diet will be analyzed through these samples.

In the following months, the feces samples will be analyzed. In October/November of 2024 a campaign to produce photographic and video graphic material will take place. In 2025 the survey of Patagonia Norte will be completed. In the months of March and April the Chubut basin and Manso and Puelo basin will be surveyed in Chubut province, and the Neuquén basin in Neuquén province. The habitat characteristics will be recorded and fecal samples collected to finish the genetic structure and diet analysis of Patagonia norte. Finally with the complete characterization of the coast and distribution of the species, the habitat suitability map will be constructed.



**Figure 1.** Map of the survey area and location of the sites done. Where signs of SRO were found (green dots), where the species was recorded for the first time (green asterisk), and where no signs of SRO were found (red dots).

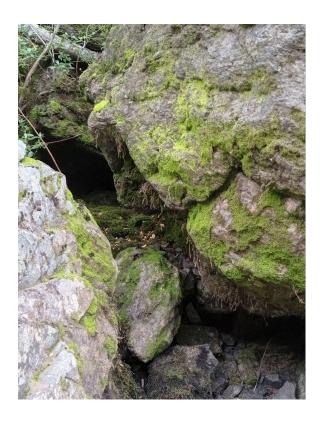


Figure 2: Cave in rocks where feces were found in Carrenleufú river (site 11)



**Figure 3.** (A) and (B) Southern river otter (*Lontra Provocax*) swimming Pico River where the individuals were seen. (C) Pico River (site 12)



**Figure 4:** (A) Fresh feces of Southern River Otter (*Lontra Provocax*). (B) Feces of Southern River Otter (*Lontra Provocax*) with remains of fish. (C) cave between rocks where the feces were found. (D) Cost line of Pico N5 Lake where the cave was found (site 22)



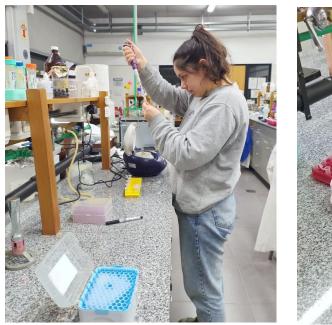
**Figure 5**: Site in La Plata Lake only accessible by boat.

## Project update: December 2024

With the objective to study the genetic structure of Southern River Otters in north Patagonia, feces samples of individuals were collected. We made sure fresh samples were collected (figure 1) and they were preserved in 96% alcohol, then stored in the freezer until DNA extraction. During the months of August, September and October the DNA extraction was performed in a laboratory in the university of Luján in Buenos Aires (figure 2). We tried multiple methods and protocols for the extraction. The phenol-chloroform method showed to be the most efficient. The extraction was successful with the seven samples (100%). From the DNA extraction, the control region (CR) of mitochondrial DNA was successfully amplified at six sites (85.7%). So far, successful amplification of nuclear DNA has not been achieved.

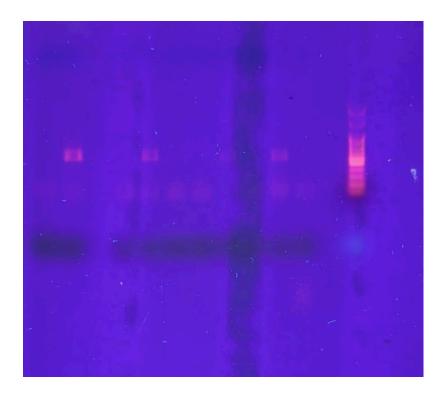


**Figure 1:** Fresh feces and jelly excretions of Southern River Otter (*Lontra Provocax*)





**Figure 2:** DNA extraction from feces in the laboratory



**Figure 3:** Agarose gel electrophoresis with the amplification results.