Project Update: March 2019

First sampling campaign and other activities.

1. Sampling fish

1.1. In the field.

So far, we have recruited four artisanal fishermen, two of them in the fishing spot called "Bajada Grande" and the others two in "Puerto Sanchez", nearby the Paraná city (Middle Paraná River, Argentina). Information related to site location, area of fishing, catch per unit of effort, fish species (size, weigh, number, etc.) etc. have been recorded in detail. Digestive tracts (stomachs and guts) has been removed from each fish (using tweezers, scissors, scalpels, and avoiding cross-contamination), stored and transported in coolers with ice and preserved in a freezer in the laboratory (Figure 1).

Figure 1. Fish point of sale in "Puerto Sanchez" managed by one of own fisherman collaborators (left). Fish measurements and digestive tract removal (right).

1.2. In the laboratory.

Content of stomachs and guts have been carefully extracted (washing with distilled water) and digested with a solution of hydrogen peroxide (H_2O_2) 30% to 60 °C until its total digestion. Once the digestive tracts are empty, they will be measured (TLd, cm) and weighed (Wd, g), as well as their content (Wsc, g).

Samples have been observed under zoom microscope (10x) in order to recover microplastic particles, which have been identified, counted, and classified under microscope (40x).

International sample protocols (gathering and processing) have been adopted in order to perform direct comparisons with other studies worldwide.

So far, approximately 20 samples have been successfully processed (Figure 2).
Currently, we are strengthening and scaling up existing fisherman collaboration (a key aspect of this project).

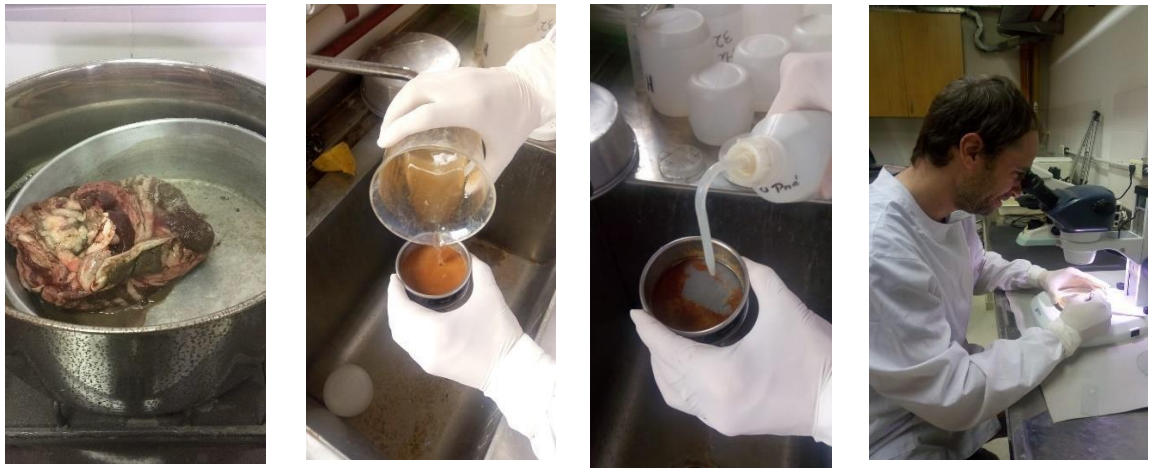

Figure 2. Samples (fish digestive tract) processing. From the left to the right: removal digestive tract, stomach content after 30% hydrogen peroxide digestion, sieving through a stainless steel (350 µm mesh size), microscope examination.

2. Sampling birds

2.1. In the field.

Some bird roosting has been identified in the Paraná River floodplain. However, to sample the faeces has not been an easy task for some reasons (bird roosts are located in inaccessible sites, the setting of the transparent greenhouse (to collect faeces) is not an easy task, etc.). As a results, we are incorporating the use of mist-nets to capture other bird species, expanding our study achieves to more species (smaller ones), but still working with the species (biggest ones) originally selected in the project. Once a bird was removed from the net it was placed in the bottom of a sock so that it would sit upright on the mesh screen. The remaining length of the sock was then tied in to a simple knot to prevent the escape of the bird and to keep it placed on the bottom of the sock closer to the fecal collection bag. Typically, little time was necessary to wait for an individual to defaecate, and faecal samples were collected. Taking extremely care, we experienced no bird injuries from this technique (Figure 3).

Figure 3. Mist-nets settlement (left). Small bird capture and released after faeces collection (right).

2.2. In the laboratory.
Faeces samples have been weighed (Wf, g) and digested following the same protocol to fish samples.

3. Microplastic separation and identification.

3.1. Drying and sieving.
Samples were dried and weighed in a drying oven at 60°C per 24 hr. Then, samples were sieved through a stainless steel sieve with 350 µm mesh size (45) using a Retsch™ sieve shaker. All materials left above the sieve were transferred to a pre-weighed 1L beaker and weighted.

3.2. Wet peroxide oxidation.
30% hydrogen peroxide at 4:1 proportion was added to the sample. The mixture was placed on a hot plate set to 60 °C and the reaction was allowed to continue until all organic material disappeared. Hydrogen peroxide was completely washed from the sampling through a 63 µm mesh size, using distilled water.

3.3. Density separation.
After the full dissolution of the organic matter, a concentrated saline NaCl solution (1.2 g cm^{-3}) was added and strongly stirred for about 1 minute. Subsequently, the supernatant with the plastic particles was extracted and washed with distilled water for further processing. This full step was repeated as many times as it was needed in order to ensure the absence of plastic particles between sand sediments.

3.4. Microscope examination.
Careful visual sorting of residues was necessary to separate the plastics from other materials, such as shell fragments, fish bones and scale fragments, as well as other no- natural items (metal paint coatings, glass, aluminum foil particles, etc.). This procedure was performed under a stereo zoom microscope. After that, plastic particles were picked up and examined to their description with regards to size, color and shape. Classification of microplastics was performed under binocular microscope with a magnification range of 40−100×. Microplastics were classified in hard plastic fragments, fibrils, foam, and films. Microscopic examination was repeated three times, to make sure all plastic particles were fully identified.

All recorded material was photographed using a 5.0 megapixels coupled to the microscope (Figure 5).

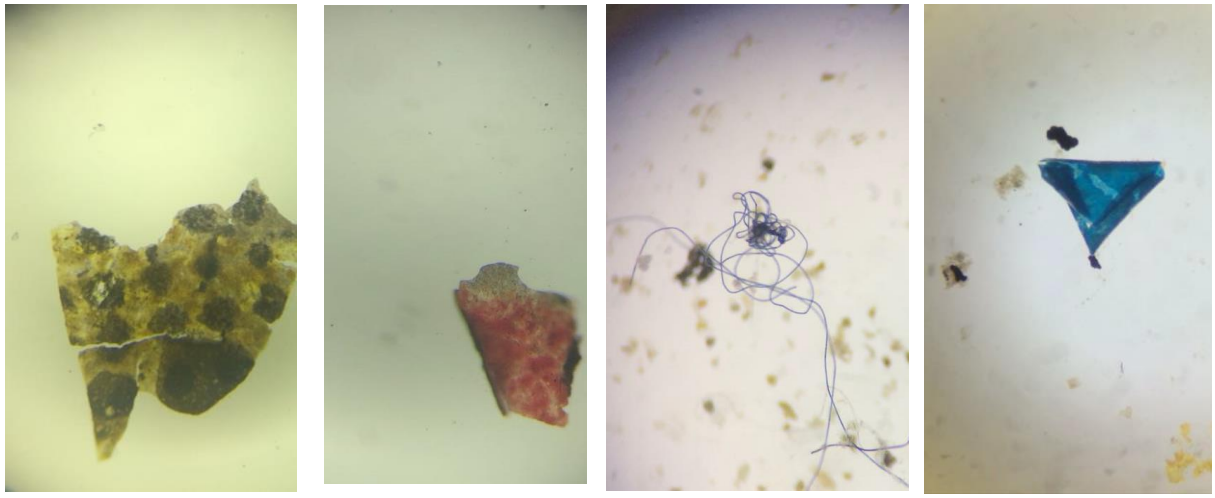

Figure 5. Examples of microplastics found in the digestive tract of fish and bird faeces (films and fibbers; size range: 1.7 to 0.3 mm).