Project Update: June 2019

First sampling campaign and other activities.

## 1. Sampling fish

#### 1.1. In the field.

So far, we have recruited seven artisanal fishermen, from the fishing areas called "Bajada Grande", "Puerto Sanchez", "Rincón", "Colastiné Sur", nearby the cities of Paraná, Santa Fe and Rincón (Middle Paraná River, Entre Ríos and Santa Fe Provinces, 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 in the field and by ourselves (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. Taken body measurements of a *Potamotrygon motoro* (Raya), specimen collected by a fisherman collaborator in the fishing spot called "Rincón" (left). Processing of collected digestive tract samples in the laboratory by project's members (right).

Most abundant species obtained: Prochilodus lineatus, Potamotrygon motoro, Rhaphiodon vulpinus, and Salminus brasiliensis.

## 1.2. In the laboratory.

Similar to reported in the first report, here 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 40 samples have been successfully processed.

## 2. Sampling birds

#### 2.1. In the field.

Sampling faeces has been difficult. As a result, we have incorporated the use of mist-nets to capture birds (as reported in the first report). However, even this method is too suitable since the time-effort invested is too much regarding the obtained results. In this sense, we have changed our method to sample bird faeces (again). But, this time it was completely successful! We explored environment and found (and selected) big nests of Phacellodomus ruber (a freshwater bird). We settled plastic sheets under each nets in order to collect faeces. In addition, we recorded plastic material used to build P. ruber nests from abandoned nests (no- ethical concern). We found extremely concentration of plastic debris inside the analysed nests (Figures 2, 3 and 4).

Figure 2. Plastic sheet used to collect faeces under an active nest of *P. ruber*.





Figure 3. Plastic debris (mainly foam and soft plastics) found inside a nest of P. ruber.



Figure 4. Freshwater environment extremely polluted of plastic waste (one of our study areas for the bird study, near the Setúbal Lake, Santa Fe province, Argentina).

## 2.2. In the laboratory.

Feces samples have been weighed (Wf, g) and digested following the same protocol to fish samples (Figure 5).



Figure 5. Processing of feces of birds (Laboratory of Hydro-ecology, INALI, Argentina).

#### 3. Microplastic separation and identification.

## 3.1. Drying and sieving.

Samples were dried and weighed in a drying oven at 60°C per 24hs. Then, samples were sieved through a stainless steel sieve with 350 µm mesh size (45) using a Retsch<sup>TM</sup> 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  $\mu$ m 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<sup>-3</sup>) was added and strongly stirred for about one 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, colour 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 6).

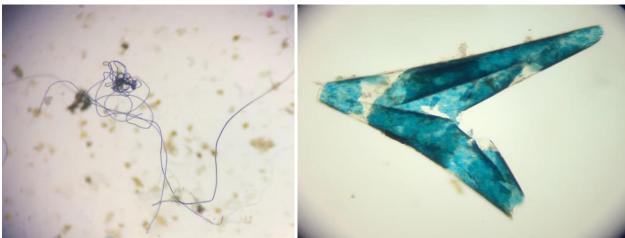


Figure 6. Examples of microplastics found in the digestive tract of fish and bird feces. Blue fibers (left), laminar plastic piece (right).

#### Publication of the first results

At the moment we have obtained (after very hard work!) information enough to write two scientific studies. Both have been sent for their evaluation (submission) to two international peer-review journals. In both manuscripts we explicitly mentioned: "This research was financially supported by The Rufford Foundation (Grant number: 26610-2)".

1) Title: Threats underestimated in freshwater plastic pollution: mini review. Type: paper review.

Journal: Water, Air, & Soil Pollution. Impact factor: 1.7.

Submission code: WATE-D-19-00556R1. Current state: accepted with changes.

2) Title: Massive plastic pollution in a mega-river of the Global South: sediment deposition and fish ingestion.

Type: paper research.

Journal: Environmental Pollution. Impact factor: 4.3.

Submission code: ENVPOL 2019 2780 R1.

Current state: submitted.

#### Presence in the Media.

We have strongly disseminated our first results in the local and national media. This is a very important) since education and awareness are key aspect of this project. Our results (at the moment) are very strong and significant, and as a result the public interest is very high.

Interview in the newspaper: El Diario (Paraná, Entre Ríos Province). 28.04.2019 (Figure 7).

https://www.eldiario.com.ar/31174-encontramos-microplasticos-en-el-100-de-los-ejemplares-de-sabalos-analizados/



Figure 7. Our first results were reported by the newspaper EL DIARIO (front page). Personal interview in the newspaper: INFOBAE (national newspaper of wide circulation, Buenos Aires).

20.04.2019.

https://www.infobae.com/sociedad/2019/04/20/alerta-por-los-niveles-alarmantes-decontaminacion-por-plasticos-en-el-rio-parana/

Interview in Radio LT 10. "Sobre el agua" programme. <a href="https://www.ivoox.com/progr-sobre-agua-domingos-13hs-lt-10-udios-mp3">https://www.ivoox.com/progr-sobre-agua-domingos-13hs-lt-10-udios-mp3</a> rf 33893139 1.html

Interview in Radio UNNE Medios 99.7 Mhz, Radio Universidad Nacional del Nordeste. "La Tarde Nos UNNE" programme. 29.03.2019.

http://medios.unne.edu.ar/index.php?option=com\_k2&view=item&layout=item&id=99 &Itemid=103&lang=es

Interview in Radio Red 101 (101.5), FM Light (97.3), Radio Voz (106.9), Radio Brisas (98.5). 01.04.2019.

https://soundcloud.com/fundacionmedife

Interview in Radio FM Cultura. "Cuestión ambiental" programme. 12.04.2019.

Interview in Radio AM 1220. "Integrantes" programme. 29.05.2019.

Interview in Radio LT10. "Alma de Barrio" programme. 17.06.2019.

Interview in Radio Universidad. "Conciencia ambiental" programme. 18.06.2019.

Note: I have mentioned the RF as financial support in each interview.

#### Open conferences.

- I have delivered some open conferences, mentioning the RF as financial support, in different places.
- 1) "Ríos de plástico" (Plastic rivers), in the frame of Café Científico, organized by El Ministerio de Ciencia, Tecnología e Innovación Productiva (Santa Fe Province). Place: Santa Fe city. 02.05.2019 (Figure 8).
- 2) "Ríos de plástico" (Plastic rivers), in the frame of Café Científico, organized by El Ministerio de Ciencia, Tecnología e Innovación Productiva (Santa Fe Province). Place: San Cristóbal. 26.05.2019.
- 3) "Ríos de plástico" (Plastic rivers), organized by Universidad Nacional del Litoral (UNL, Santa Fe Province). Place: FHUC, Ciudad Univesitaria. 13.06.2019.
- 4) "La contaminación por plásticos en el río Paraná" (Plastic pollution in the Paraná River), organized by Acuario del Río Paraná Centro científico tecnológico y educativo (Rosario). 03.09.2018. (Figure 9).



## A cargo de **DR. MARTÍN BLETTLER**

Lic. en Biodiversidad (FHUC-UNL), MSc. en Ecología de Ambientes Acuáticos Continentales (UEM, Brasil) y Dr. en Ciencias Biológicas (FBCB -UNL) JUE 02/05 · 18 HS.

**DEMOS** 9 de Julio 2239 Santa Fe





Ministerio de Ciencia, Tecnología e Innovación Productiva

Figure 8. Flyer of the public conference in Santa Fe city.



# Charla: "La contaminación por plásticos en el río Paraná"

Desde el Centro Científico, Tecnológico y Educativo "Acuario del Río Paraná" los invitamos a participar de esta disertación del Dr. Martín Blettler, del Departamento de Hidro-Ecología del INALI (Instituto Nacional de Limnológía). La charla estará dedicada a la problemática del uso de los plásticos y la contaminación en el río Paraná.

La disertación está dirigida a público en general y se realiza en el marco del "Programa de reducción del uso de plásticos y el estudio de sus efectos en los ecosistemas de la cuenca del Río Paraná" que lleva adelante el Centro Científico, Tecnológico y Educativo "Acuario del Río Paraná".

El plástico es versatil, liviano, flexible, resistente a la humedad y económico. Esas son cualidades atractivas que nos llevan, a su consumo masivo, sobre todo aquellos productos descartables. Sin embargo, luego de su uso estos materiales se convierten en residuos de muy lenta degradación. La atracción mundial por el plástico y su llegada a los ambientes acuáticos hace que podamos encontrar plásticos a niveles alarmantes tanto en el mar como en los ríos de todo el mundo. Esta problemática se ha detectado también en el río Paraná.

Ante esta realidad de escala planetaria, un grupo de investigadores y becarios del CONICET se encuentran estudiando cuán extendido y grave es el problema sobre el río Paraná, así como el impacto los plásticos sobre peces, aves e invertebrados acuáticos. Sobre este tema y las posibles soluciones a la problemática, estaremos conversando con Martín.

¡Los esperamos!

IMPORTANTE: Capacidad limitada. Se requiere inscripción previa Link de inscripción: https://goo.gl/forms/ofqwuxzeFkqortau1

+ GOOGLE CALENDAR

Figure 9. Flyer of the public conference in Rosario city.