Project Update: September 2019

Results.
Currently, we have finished all sampling campaigns (field activities) and we are focusing our efforts on the sample processing and results analysis.

Results under peer review publication:

The second paper:

Blettler et al. "Massive plastic pollution in a mega-river of a developing country: sediment deposition and ingestion by fish (Prochilodus lineatus)." Environmental Pollution (ENVPOL_2019_2780_R2) is under the second round of revision in the prestigious journal "Environmental Pollution" (Impact factor: 4.3).

Results recently obtained.

Fish:
We are still processing information obtained from the sampling campaigns.

In order to evaluate the presence and abundance of microplastic ingested by fish, we have selected 2 species so far, from high to low polluted areas:

Species	Common name	Number of fish	Average length of caught fishes (cm)	Average weight of caught fishes (g)	Pictures
Potamotrygon motoro	South American freshwater stingray, raya	10 in the less polluted area 5 in the highly polluted area	31,1	1860,6	
Pterodoras granulosus	Armado	12 in the highly polluted area	38,7	1008,5	

Table 1. Studied fish so far.

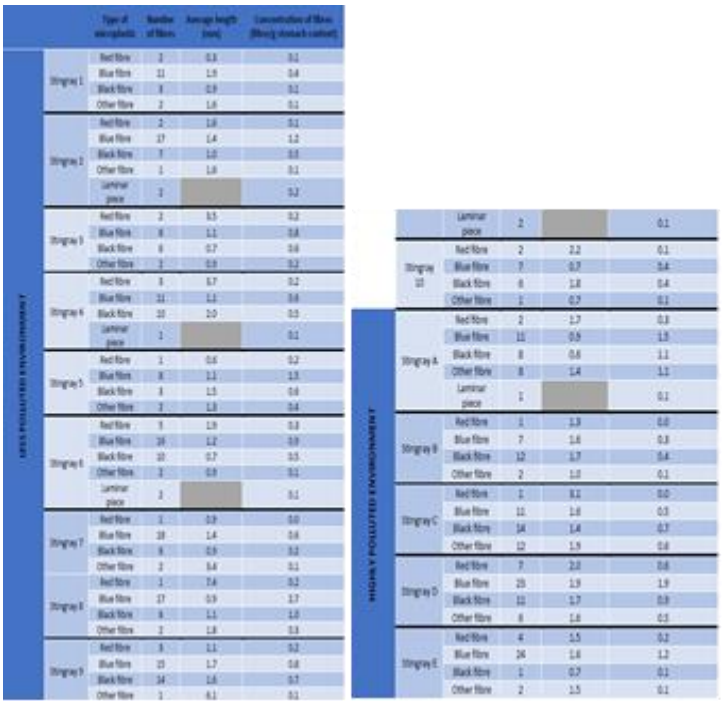
Freshwater stingrays are considered as Data Deficient by the IUCN. Populations of freshwater stingrays are decreasing in the Paraná River, which is linked to human activities, because of the excessive fisheries. (Lucifora et al. 2017). Armado is a fish with a great economic importance in the region, because it is appreciated for its consumption (Almirón et al. 2001).

In order to be able to compare different species with each other, the content from the entire digestive tract is extracted. In addition, it has been shown that for species with distinct parts in the digestive tract, the concentration of microplastics differs between each part. It is therefore necessary to take all the parts in order to quantify all the microplastics ingested (Jabeen et al. 2017).

Fish were dissected after a ventral median incision extending from the cloacal region to the gills (de Melo Germano et al. 2014). All operations were carried out under a vertical laminar flow hood. After washing all the equipment, the stomach and intestines were placed in a petri dish.

The methodology used in this study was based on other well-known studies such as Avio, Gorbi, et Regoli (2015). A BoecoTM binocular magnifying glass was used to identify the microplastics. All the microplastics found were then isolated on a slide, as well as all the items which were not really identified. The slide was observed under a NikonTM binocular microscope with a magnification range of X400 to ensure that it was really microplastic. Once this verification was completed, pictures of the observation were taken under the microscope. The pictures were then analysed to determine the morphological characteristics (length of each microplastic, and area). This measurement was made using Adobe Photoshop software.

Each MP was therefore characterised by its type (length, shape and color) (Table 2).



		Number	Concentration of microplastic (MP/g of stomach content)
Armado K	blue fibres	127	1,1
	black fibres	106	0,9
	red fibres	154	1,4
	other fibres	13	0,1
	laminar pieces	27	0,2
Armado N	blue fibres	70	0,5
	black fibres	85	0,6
	red fibres	60	0,4
	other fibres	21	0,1
	laminar pieces	53	0,4
Armado O	blue fibres	70	0,8
	black fibres	50	0,6
	red fibres	8	0,1
	other fibres	4	0,0
	laminar pieces	18	0,2
Armado P	blue fibres	90	1,0
	black fibres	136	1,5
	red fibres	65	0,7
	other fibres	11	0,1
	laminar pieces	57	0,6
Armado S	blue fibres	108	0,7
	black fibres	110	0,7
	red fibres	40	0,2
	other fibres	20	0,1
	laminar pieces	12	0,1
Armado T	blue fibres	92	1,2
	black fibres	65	0,9
	red fibres	21	0,3
	other fibres	7	0,1
	laminar pieces	49	0,7

Table 2. Microplastics ingested by Rayas y Armados.

Figure 1. Example of a meso-plastic detected into the stomach content of an Armado fish (Petri dish). The Figure 2 shows the number and type of ingested microplastics by stingrays in the studies areas.

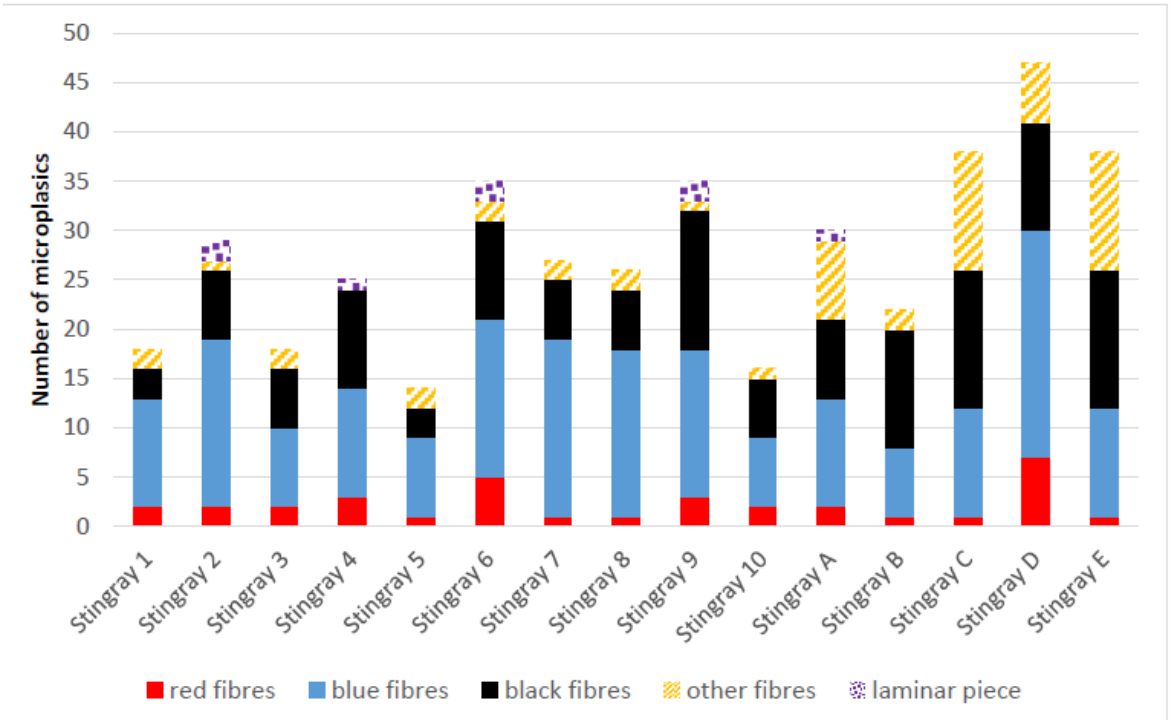


Figure 2. Number and type of ingested microplastics by stingrays.

98.1% of the microplastics were fibres (410), only eight laminar pieces could be found. Among them, 45.7% were blue, 31.1% black and 8.1% red color. The rest were transparent and yellow fibres (Figure 3).

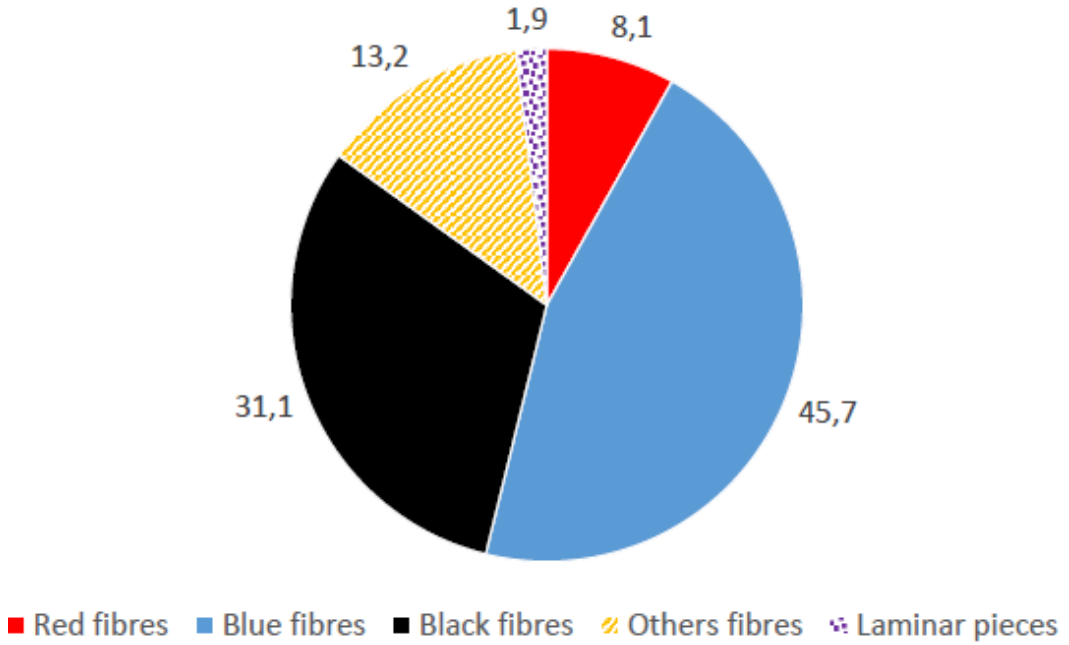


Figure 3. Types of microplastics ingested by freshwater stingrays (%).

Contrary to expected, the Figure 4 shows that there are no differences between the

fibre lengths in both environments.

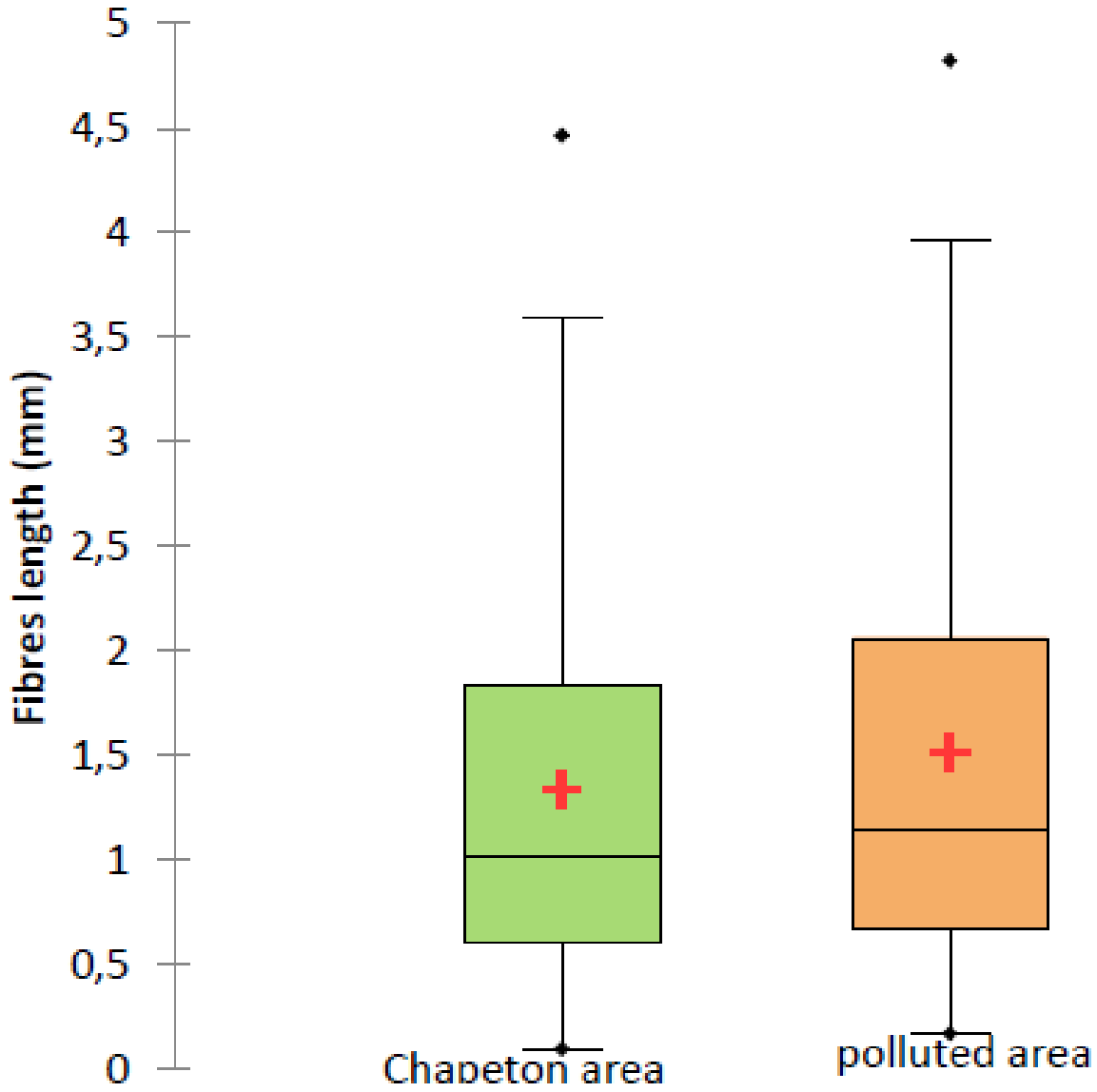


Figure 4. Box plots comparing the length of fibres from a highly and a less polluted environment.

All Armados were caught in the highly polluted environment. Due to the used method is very high time-demanding, only six Armados were analysed. 100% of the analysed Armados contained microplastics. A total of 1749 microplastics were found. On average, each fish contains 291 microplastics. 1533 microplastics correspond to fibres, and 216 correspond to laminar pieces. (Figure 5). 88% of the microplastics were fibres, and 12% were laminar pieces. Among them, 32% were blue, 32% black and 20% red color. The rest were transparent and yellow fibres (Figure 6).

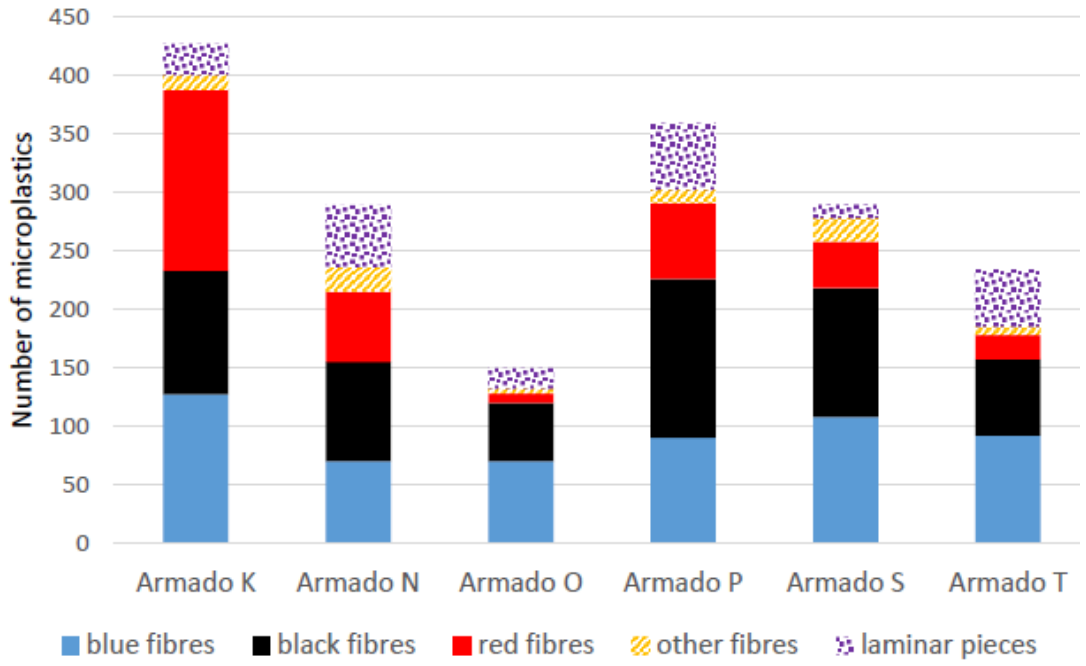


Figure 5. Quantity and type of microplastics ingested by Armados.

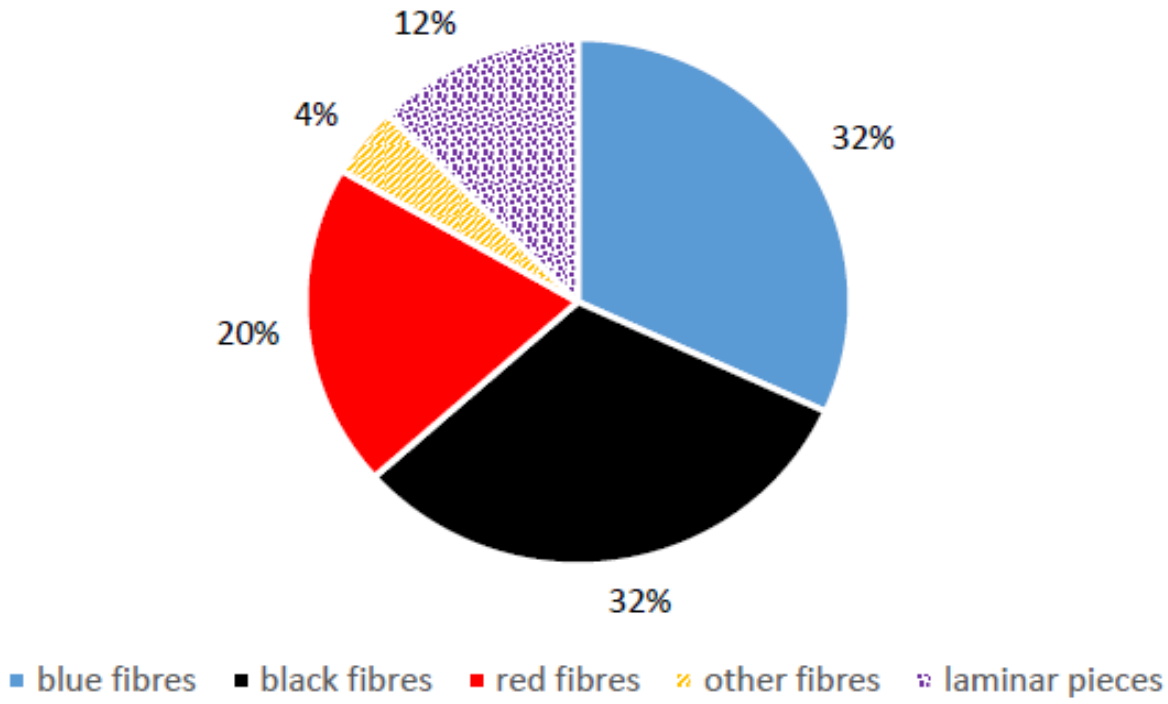


Figure 6. Type of microplastics ingested by Armados.

Birds:

The objectives of this part of the project were i) to study the presence and abundance anthropogenic particles in the nest of the Espinero Grande, and ii) to determine the presence of microplastic ingested by the same bird through the feces analyses. The bird named *Phacellodomus ruber* (Vieillot, 1817) (hereafter: Espinero) has been studied so far. This bird has been chosen for this study because it is a native species so it has an ecological importance, it is present in the province of research (Santa Fe) so there is no necessity of travelling a lot for studying the bird, and their nest are easy to collect because they are usually near to the ground at man height.

Nest sampling.

The nest of the Espineros has been collected in an illegal dump next to the city of San Jose del Rincon. The Rincon's dump shows a part in which the wastes are thrown and a part of extended marshes highly connected to the water level of the Setubal Lake (Figure 7a).

Figure 7. Example of an Espinero's nest with presence of macroplastics (a). Placement of lamina "traps" to collect feces, under active nests of Espinero (b).

Faeces sampling.
The ecological reserve (RECU) of the Universidad Nacional del Litoral (UNL) is a semi-urban place surrounded in one side by the Setubal Lake and in the other side by a hotel and the university. This reserve belonged to floodplain of the Parana, it has conserved a kind of hydrological dynamism and the characteristic vegetation. There is a pond at the center of the reserve and a great part of the RECU become marshes during the rainy season (figure 7b). This place has been chosen for its ease to access and because the Espinero is present.

Macroplastic identification.
In the laboratory, the nests were weighed using an OHAUSTM SCOUT PRO 6000 g (0.1 g) balance, and the length, width and height were measured. Then, the nests were opened, and the intern chambers were sorted according to the basic nature of the particle extracted. Thus, the particles were classified into four categories which were used by Mishra et Kumar (2017), these proposed to sort the material of vultures' nests into: plant matter, animal matter, anthropogenic matter and other matter. After several readings of diverse it appears that this last classification was the most adapted to the study of Espineros' nests (Figure 8).

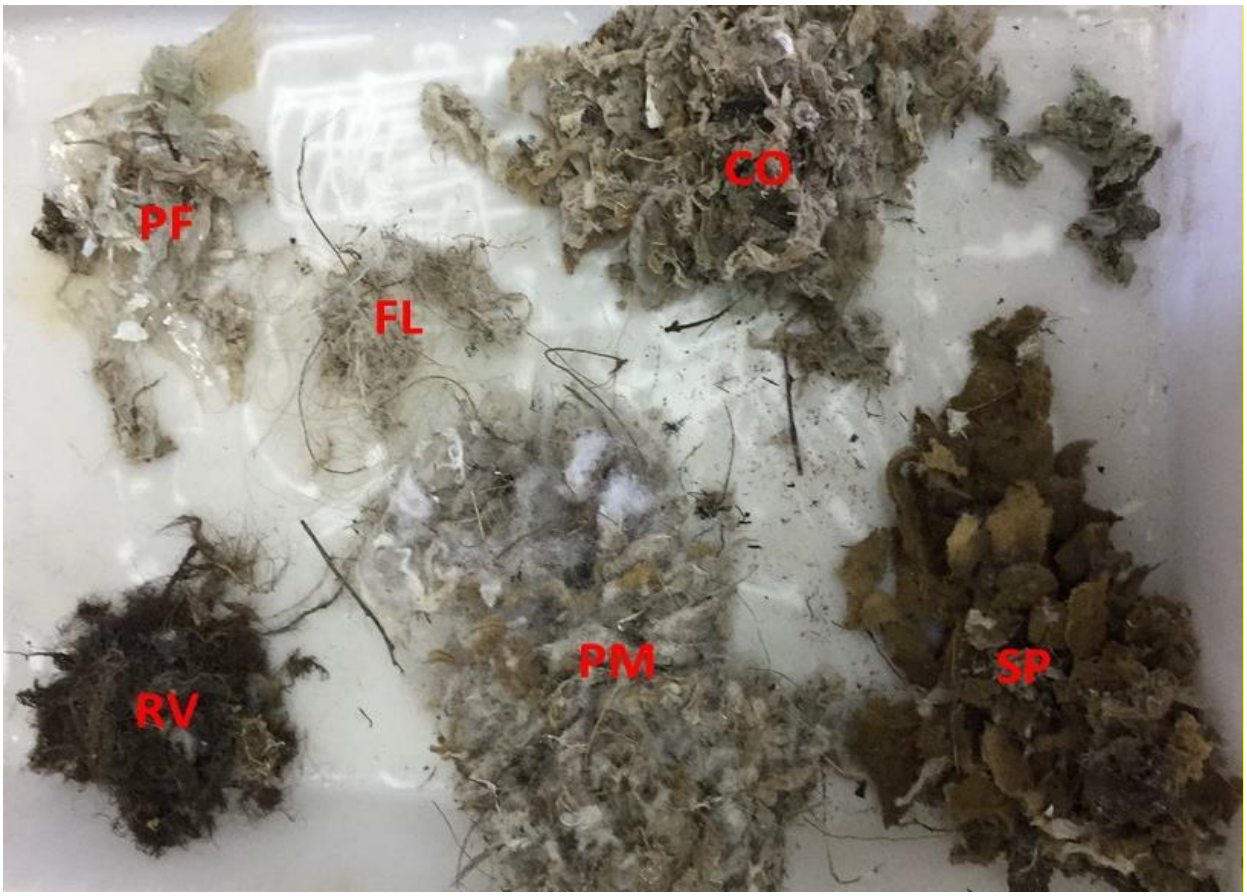


Figure 8. Example of macroplastic recorded in a nest of Phacellodomus ruber. CO = cotton, FL = Fishing Line, PF = Plastic Films, PM = Plastic Moss, RV = Residues of Vegetation, SP = Sponge.

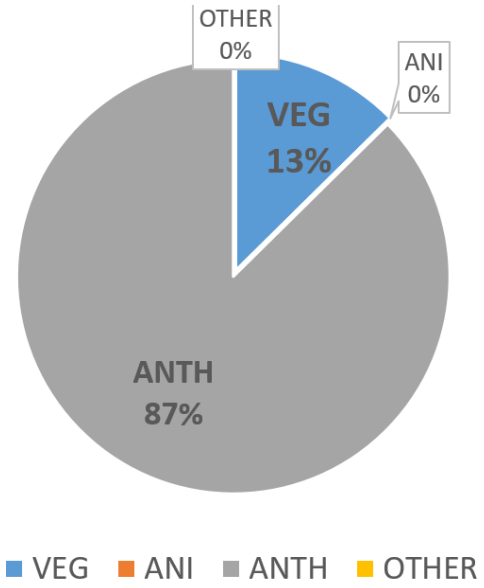


Figure 9. Proportions of mass of type of particles found in Espinero's nests (VEG=vegetation matter; ANI=Animal matter; ANTH=Anthropogenic matter; OTHER=unidentified matter).

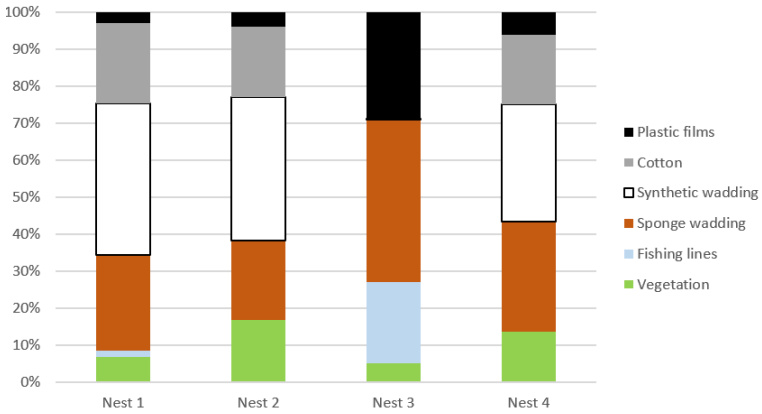


Figure 10. Proportion of masses of wadding materials in Espinero nests.

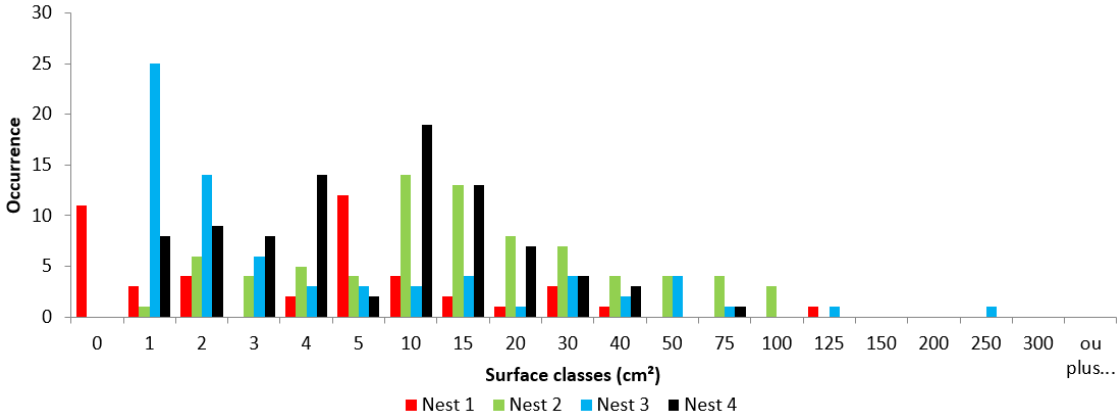


Figure 11. Distribution of plastic particle areas found in Espinero nests.

The sampling campaign of faeces show that the production of faeces of each nest differ significantly (Figure 12). Indeed, the nest 1 is the less productive concerning the number of faeces produced per 48 h for each sampling campaign. On the contrary the nest 6 is the most productive, maybe due to the big size of the nest. The other one produces the same amount of faeces. The mean number of faeces per nest is 13.5 faeces. The minimum is 0 faeces and the maximum is 35 faeces.

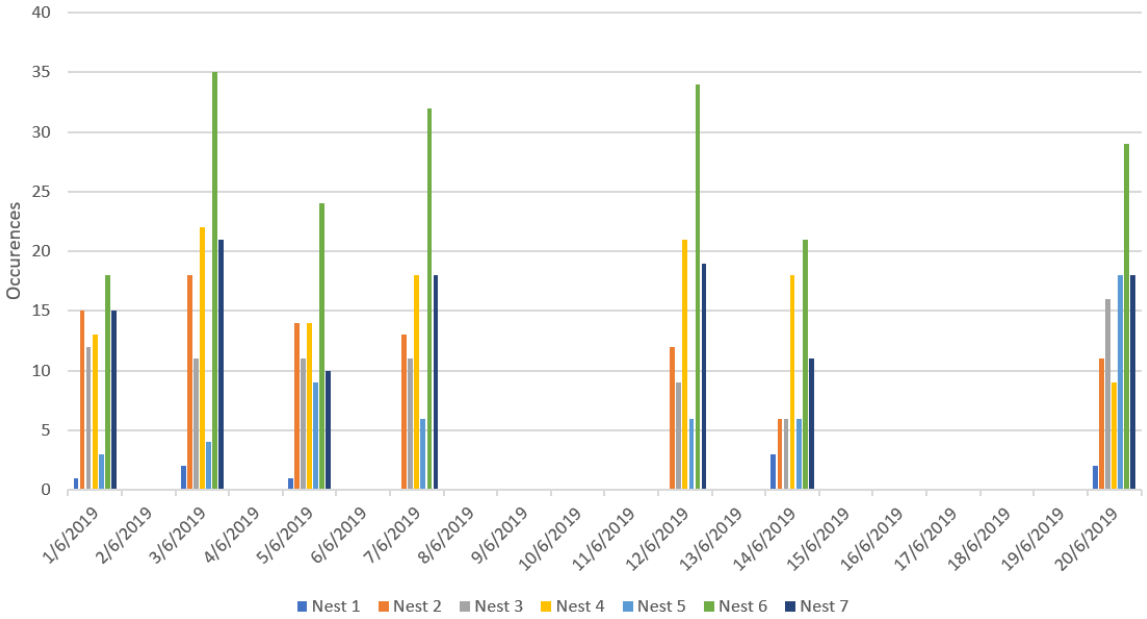


Figure 12. Number of faeces per tramp and per sampling campaign.

Presence in the Media.

We still have strong presence in the media since our results are significant and surprising. As a result, the public and media are very interest in them. For ex.:

i) Programa "Integrantes", AM 1220, journalist Pedro Moreno, 13/09/19 interview with Martín Blettler, topic: plastic pollution and plastic debris used as building material by native birds. https://www.mixcloud.com/ecomedios1220/integrantes-con-pedro-moreno-13-09-2019/;

ii) 05.08.2019 Radio LT10, show: Todo Pasa por LT10, Journalist Daniel Jovellano, interview with Martín Blettler, issue: plastic particles ingested by native fish of the Paraná River;

iii) 05.08.2019 Radio LT9, show: Dame Radio, interview with Martín Blettler, issue: river plastic pollution;

iv) 08.08.2019 FM 87.9 Cadena Oh y web Sinmordaza.com, show: "El Camino en Radio", journalist: Claudia Del Barco, interview with Martín Blettler, issue: plastic pollution and birds.

I want to note that in all cases I particularly mentioned The RF as the financial source.

We are still working hard in this project. So far, the results are very exciting, surprising and significant from a social and scientific point of view. We are working in a new scientific publication right now.

Appendix

Pictures of a much polluted area in Rincón (illegal dumping very near to the Setúbal Lake and the Paraná River), Paraná River Island and urban streams. This area was part of our sampling area, particularly to collect nests of the Espinero bird.