Final Project Evaluation Report

Your Details	
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Project Title	Microplastics ingestions by fauna as a serious emerging threat in freshwater systems
Application ID	26610-2
Grant Amount	£5000
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1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

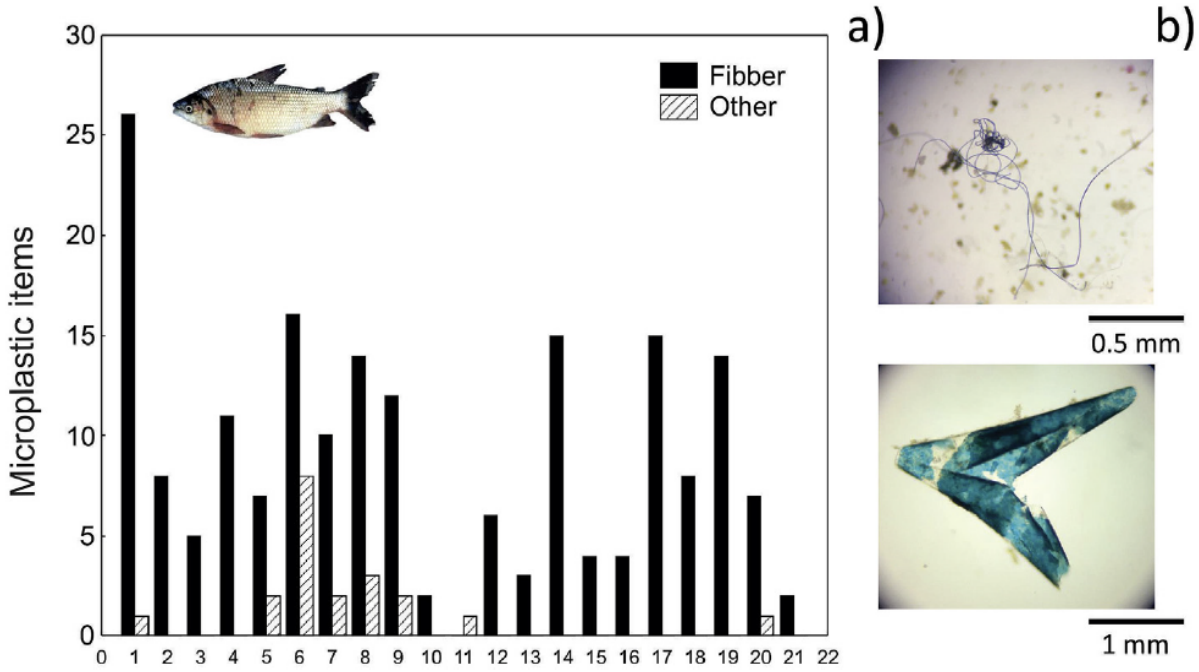
Objective	Not achieved	Partially achieved	Fully achieved	Comments
1) To determine the potential presence of microplastic particles in stomach and gut of five fish species and faeces (and bird pellets: regurgitated parts of food that could contains plastics) of three bird species of the Paraná River (middle section; Entre Ríos province, Argentina).				We decided to select only three fish species (*Prochilodus lineatus*, *Potamotrygon motoro*, *Pterodoras granulosus*) but increasing the number of studied individuals of each specie. This strategy was used in order to increase our chances to publish papers in high level peer-reviewed journals. This was successful since we have published in Environmental Pollution (Blettler et al. Massive plastic pollution in a mega-river of a developing country: sediment deposition and ingestion by fish (*Prochilodus lineatus*), Environmental Pollution. 255: 113348). The originally proposed methodology for birds (analysing MPs presence in regurgitated food) was too complicated and caused some bird deaths. In this sense we changed our methodology (for methodological and ethical reasons). Alternatively, we collected faeces to detect MPs in them. Fortunately, this modification was successful (it was also used for other authors worldwide). In addition, we analysed macroplastics used as nest building material in many bird nests as a measurement of the conspicuous presence of this pollutant in the natural environments.
2) To count and measure each detected microplastic particle (items number, area, length and volume).				As planned, we counted and characterised every MP (determining number, area, length, volume and colour of everyone).
3) To classifying the microplastics into categories (hard plastic				We also classified the MP in categories according to features such as hard plastic fragments, fibres, foams or

fragments, fibres, foams and films).				plastic films.
4) To discuss and propose mitigation measurements with stakeholders.				We recently have discussed with government authorities of the Santa Fe city municipality, even with the elected mayor (Emilio Jatón). This was direct consequence of our great media presence and repercussions of this study. Based in our results, we proposed to the mayor the use of floating barriers (for the first time) in the most polluted urban streams and storm drains in order to catch and remove most of plastic waste flowing from these streams to the main river channel (Paraná River).

Illustrative figures showing laboratory activities and some specific results:

Treatment of fish gut and extraction of microplastics and in the laboratory.

Example of a meso-plastic found in the digestive tracts of the fish *Pterodoras granulosus* detected into the stomach of one individual (1.7 x 3.1 cm).



Microplastic particles (fibers and others) found in the digestive tracts of Prochilodus lineatus. Number of items (a), fibers and a piece of microplastic film (b).

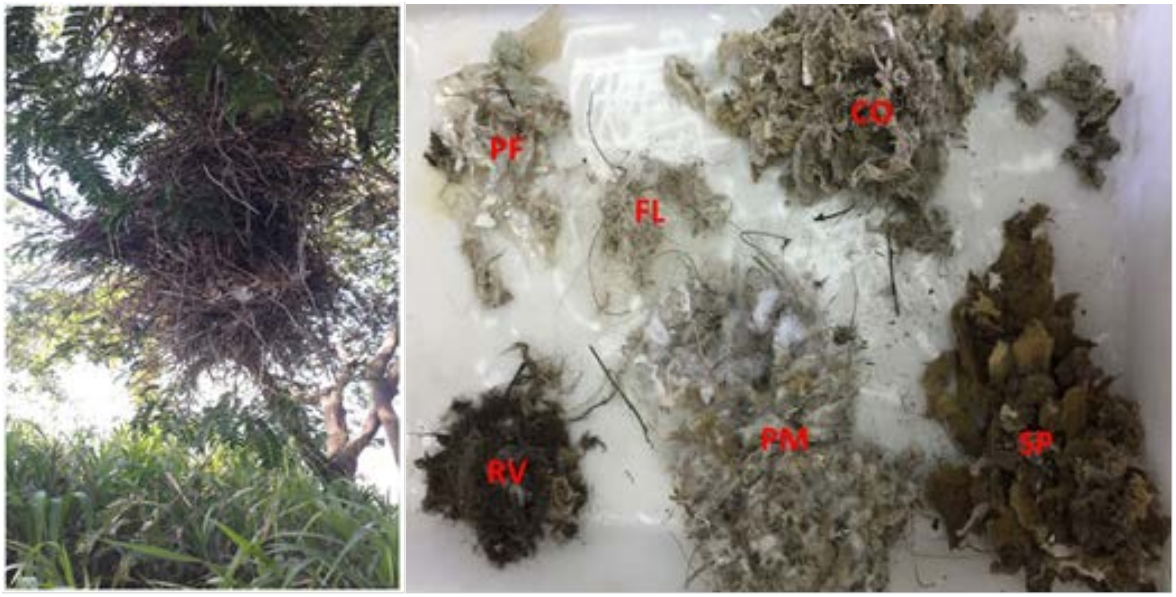
2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

The current financial crisis in Argentina (rising gas prices, strong inflationary process, etc) was a growing threat to successfully complete this study (https://www.forbes.com/sites/afontevecchia/2019/05/06/explaining-argentinas-financial-crisis-macri-cristina-and-the-specter-of-populism/#60d9434c5ea1). To deal with, we made some changes in the budget (see Point 8 below). Prices are changed day by day in Argentina, particularly imported products. Sampling campaigns (performed by us and by local artisanal fishermen) were the most expensive activities. Because of the crisis, we had to take more money than initially expected for sampling campaigns and fishermen payment.

On the other hand, initially we tried to detect ingested MPs in birds analysing their presence in regurgitated food. This methodology was clearly inefficient. Catching birds is not an easy task; mist-net operation requires one experienced operator on site, it is a non-selective method, putting in risk of death the trapped individuals. Because of that, we decided to collect faeces using sheets located under active nest (no risk for birds). This method was successful. However, it was not always possible to use. For example, in case of the freshwater bird *Phalacrocorax brasilianus*, we never found a correct place for the sheets, since the presence of water (flooding) prevented its use. This is the main reason why we could not study this specie, unfortunately.

Additionally, and based on direct and unexpected observations, we also collected some empty nests (empty for ethical reasons) of *Phacellodomus ruber* in order to

analyse the amount of plastic debris used as building material. This was an extra effort of this project, but it really was worth it.



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.

3. Briefly describe the three most important outcomes of your project.

1. We confirmed our initial hypothesis: native freshwater birds and fish ingest MPs in the Paraná River. This is a crucial result since there is no antecedent about.
2. This study becomes a significant educational element for the local population since local community was involved/interested in the project. Politicians have reacted with surprising promptness, probably due to the high media pressure.
3. Two high-impact publications (we expect another one after finishing the processing of all remaining samples):

Blettler MCM, and Wantzen K. M. 2019. Threats Underestimated in Freshwater Plastic Pollution: Mini-Review. Water Air Soil Pollut, 230:174.
https://link.springer.com/article/10.1007/s11270-019-4220-z

Blettler et al. "Massive plastic pollution in a mega-river of a developing country: sediment deposition and ingestion by fish (Prochilodus lineatus).", Environmental Pollution. 255: 113348.
https://www.sciencedirect.com/science/article/pii/S0269749119328520

Note that in both cases RF was mentioned as founding source.

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

Local community was closely involved in the project through the participation of volunteers during field activities, public conferences (in schools, municipality and other public institutions of education and research), media exposition (TV, radio, newspapers, web), the interest of politicians and the potential modification of the current legislation about solid waste treatment and final disposal methods in Santa Fe city. The dissemination of results quickly captured the population attention and concern about the plastic pollution problem in the Paraná River.

For ex., TV: 1) https://www.ellitoral.com/index.php/id_um/201763-la-contaminacion-por-plastico-cada-vez-mas-presente-en-el-ecosistema-martin-bletter-investigador-del-conicet-area-metropolitana.html

2) https://www.youtube.com/watch?v=yvAREvel5Lw

3) https://www.ellitoral.com/index.php/id_um/211210-como-dejar-de-contaminar-la-setubal-el-litoral-podcasts-area-metropolitana.html

4) https://www.elonce.com/secciones/parana/603868-hallazgo-en-peces-quotno-es-que-estemos-ingiriendo-plnastico-al-consumir-pescadoquot.htm

5) even in international media! https://www.ehn.org/freshwater-is-getting-neglected-when-it-comes-to-plastic-pollution-research-2639610931.html

Newspapers:
1) https://ahora.com.ar/hallaron-microplasticos-sabalos-rayas-y-armados-del-rio-parana-n4189153

2) https://www.elonce.com/secciones/sociedad/603625-rno-paranna-encontraron-microplnasticos-en-snabalos-rayas-y-armados.htm

3) https://www.elonce.com/secciones/parana/603868-hallazgo-en-peces-quotno-es-que-estemos-ingiriendo-plnastico-al-consumir-pescadoquot.htm

Radio:
1) http://fmuniversidad.com/web/bletter-se-refirio-a-los-estudios-recientes-en-materia-de-contaminacion/

After the great public diffusion of this project and its results, peoples spontaneously have organised beach clean-up campaigns. Actually, we have successfully organised and participated in one of them (involving more than 100 peoples from Santa Fe city): https://www.unosantafe.com.ar/santa-fe/en-solo-tres-horas-se-removieron-mas-500-kg-basura-la-laguna-setubal-n2517722.html
Local NGOs also have been involved in this clean-up campaigns.

5. Are there any plans to continue this work?

Of course. It is necessary to increase the frequency of sampling campaigns, to expand the sampling area as well as to involve more and more freshwater species potentially impacted by microplastics. As a result of this study, we are currently sure that plastic pollution is a great problem in the Paraná River (we statistically quantified this pollution in the fauna for the first time). We have shown that microplastics can be ingested by freshwater fish and birds.

We need more field data in order to determine how expanded is this pollution in order to answer new questions, such as Which are the most impacted species? What areas are more impacted? Is it possible to reduce/control plastic pollution? How? Tentative methods or techniques to reduce plastic pollution must be urgently used in the area.

We also must evaluate the potential impact of macroplastics in freshwaters since many species of marine vertebrates have been recorded as entangled in macroplastic debris and there is no reason to assume that riverine fauna is not affected by macro-debris. Information about the impact of plastic pollution on fauna in freshwater systems is still very poor worldwide.

6. How do you plan to share the results of your work with others?

To the scientific community: through the publication in international peer review journals and attendance of symposia and congresses. Two scientific publications so far is a big achievement, product of hard work.

To the general public: through the media exposition, making simplified versions of the published papers (available online, in Spanish and English, accessible style of language, shorten, etc), public conferences, and active collaborations with NGO's. Ex. https://www.acercaciencia.com/2019/03/30/la-paradoja-de-la-contaminacion-por-plasticos/

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

Most of the grant money was used to pay sampling campaigns (fuel, nautical oil, artisanal fishermen, technical staff, etc). Other expenditures were performed throughout the whole project (expendable material, glassware, reactive such as H_2O_2, etc).

The length of the project was relatively well predicted in the proposal and every step developed in time. Of course, as usually happen, we have several samples not yet processes in the laboratory as well as information no published yet. But we are still working on it until finish everything. We had to make some adjustments regarding what was said in the original proposal. However, these changes were more related to the budget than to timescale (see below).

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Trinocular Microscope Acromatic BM-700 Boeco	2210	2129	-88	
Canoe (plus paddles and life vest)	950		-950	Since other expenses were higher than expected, we did not buy the canoe
Fuel and oil for car and boat, plus engine maintenance	590	650	+60	Fuel notoriously increased the price
Fishermen payment	600	1178	+578	Fishermen demanded more money than expected.
Chemical solutions (distilled water; H2O2; KOH)	410	180	-230	
Portable cooler	110		-110	It was not necessary since we transported the samples immediately to the Lab.
Freezer Whirlpool Hztal. Bco. WHA22KBDIM		316	+316	No considered in the original budget but absolutely necessary to preserve the fish samples, avoided the use of chemical products.
Hot Plate DB 270 (laboratory instrument)		363	+363	We already had one but unfortunately it broke. Because of this, we have to buy a new one. This is a key element to digest OM from gut and faeces.
Binoculars (10x 50mm)	95		-95	As we finally collected faeces from nest, binoculars were not necessaries.
Safety glasses, protective gowns, gloves and caps	35	38	+3	
TOTAL	5000	4847	-153	We plan to use this remaining money to buy more reactive (H2O2), that we will need to process the remaining samples as soon as possible.

Local exchange rate: 1£= ARG 75.9 (source: Banco Nación Argentina; http://www.bna.com.ar/)

9. Looking ahead, what do you feel are the important next steps?

The next step is to evaluate the impact of macroplastic pollution on aquatic vertebrate fauna (by entanglement, ingestion, etc). Many native vertebrates have a key importance from an ecological, commercial and cultural point of view. Several species play a significant role in the local cultural and people commonly identify with these species for cultural or economic reasons, particularly indigenous communities.

On the other hand, we need to design and to evaluate (effectiveness, efficiency) alternatives methods and techniques to reduce plastic pollution in freshwater environments.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

The Rufford Foundation logo was showed at the beginning of public conferences and mentioned during TV and radio interviews. Furthermore, the RF was explicitly mentioned in the section of "Funding information" of the published papers.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Martín Blettler (leader of the team). I was involved in each activity: planning and execution of sampling campaigns, samples processing (identification and classification of plastic debris), analysis of data, writing of RF reports and papers publication. I was the main media communicator; however other members also participated in this activity. I leaded the public conferences; but all members of the team were present and involved in feedback discussions in close interaction with the audience. Meetings with politicians involved in Solid Waste Treatment of the Santa Fe city: Dr. Luis Espinola and I. NGO's activities were carried out by all members of the team.

Elie Abrial. He has processed fish samples and classified microplastic particles under microscope. In addition, he has been involved in all field activities.

Rodrigo Lorenzón. He has processed bird samples and classified microplastic particles under microscope. He has been involved in all field activities. He also collected most of the nests.

Luis A. Espínola. He has participated in field activities, in data processing (statistical analyses), and in one paper writing.

Daiana Pascuale. She was involved in field and laboratory activities.

Nicolás Garello. He was involved in field and laboratory activities. He has been actively involved in public conferences, recruiting volunteers, etc.

12. Any other comments?

We would like to express our sincerely gratitude to RF. This study would not have been possible without the RF support. We sincerely believe that the present study was successful at scientific level (two peer review publications, legislative (meetings with the mayor of Santa Fe city to modify the current solid waste management policy, educational (creation of groups of volunteers, open conferences in public institutions), and social level (great deal of media attention, much more than expected, and NGO's collaboration). Definitely, these achievements encourage us to continue working in this direction.