Individual Internship

4th year

Microplastic fragments in digestive tracts of commercial fish *Prochilodus lineatus* in a mega-river (Paraná River, Argentina)

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Acronyms

BS : Bachelor Student
GESAMP : Group of Experts on the Scientific Aspects of Marine Environmental Protection
GIT : Gastro Intestinal Tract
H_2O_2: Hydrogen peroxide
INALI : Instituto Nacional de Limnología
KOH : Potassium hydroxide
MP : Microplastic
PE : Polyethylene
PET : Polyethylene terephthalate
PP : Polypropylene
PS : Polystyrene
PUR : Polyurethane
PVC : Polyvinyl chloride
TL : Total length
TS : Total standard
TW : Total weight
Ws : Stomach weight

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Plastic particles are recently becoming more and more considered as a threat to humanity and biodiversity. Many alarmist messages submerge news and our daily life. Recently, in June 2018, the famous magazine *National Geographic* published an edition with a particularly striking front page: ***Planet or Plastic?*** **(Annex 1).** This campaign is intended to raise awareness about the global plastic trash crisis.

Introduction

This internship took place in South America, in Argentina at approximately 500km from the capital Buenos Aires, in the city of Santa Fe (653,000 inhabitants) which is the capital of the Province of Santa Fe. This city was built on the bank of the Salado River and The Setubal Lake.

The initial subject of the internship was:

Occurrence of microplastic debris in fish fauna (Paraná River, Argentina).

As the original title was too broad to be covered during the internship, the subject was later on refined to the title:

Microplastic fragments in digestive tracts of commercial fish Prochilodus lineatus in a mega-river (Paraná River, Argentina).

The Paraná River is ranked ninth among the largest rivers in the world according to its mean annual discharge to the ocean (18,000 m^3/s), and is therefore classified as a "mega-river" by Latrubesse (2008). This mega-river has an anabranching main channel in the middle course, sandy bottom sediments and exposed sandy bars (Latrubesse, 2008). It is interesting to know that in Guarani (indigenous ethnic group), Paraná means "Parent of the sea", this local name is used because of its impressive floodplain width (10 to 60 km). Paraná River has its sources in Brazil and extends over 4000km to the Atlantic Ocean (nearby Buenos Aires city).

- The objectives of this training course were:

1) ***Quantitative data:*** To determine presence (particle number, shape and color) of plastic debris in *Prochilodus lineatus* (Sábalo) **(Annex 2)** digestive tracts. This objective includes field and laboratory activities, including sampling campaigns.

2) ***Site effects:*** To determine potential relationships between degree of pollution and sampling site (upstream, nearby or downstream of the Paraná city).

The final aim of this internship is to write a scientific article to be published in an international peer reviewed journal. Due to time constraints and in order to remain in the field of the subject previously fixed, the scientific article will focus only on the first objective: ***Quantitative Data***. However, in this report the second objective will be mentioned.

If we take a few minutes to look around us, the verdict is unequivocal: Plastic is everywhere, rare are the objects which don't contain it. We have to face to plastic pollution, the largest environmental challenge (Provencher et al., 2016), its production doesn't stop to increase. Between 2015 and 2016, global production passed from 322 to 335 million tons, representing an increase of 4% in only one year (PlasticEurope, 2017). This is a global and current problem, by 2050 it's estimated that plastic pollution will be multiplied by two (Geyer et al., 2017).

Different types of plastics are produced all over the world, polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PUR) and polyethylene terephthalate (PET) (GESAMP, 2015). Their origins are diverse, among them we find micro beads from cosmetics products, fibers coming from clothes, fragments of agricultural plastics (Horton et al., 2017) but also food wrappers, bags, bottles (Blettler et al., 2017). According to GESAMP (2015) plastic debris size can be separate into 5 categories. The prefix mega corresponds to a debris > 10^2m, macro 10^{-2}-10^2m, meso 10^{-4}-10^{-2}m, micro 10^{-6}-10^{-4}m and nano 10^{-8}-10^{-6}m. In this study we will focus on microplastics (MPs) in freshwater system for two reasons: i) they are ingested by fish fauna (Wright et al., 2013; Collignon et al., 2014) and ii) presence of microplastics in freshwater environment is much less studied than in marine environments (Blair et al., 2017). Presence of plastic in freshwater system and specifically MP is a problem that the scientific community took a long time to take into account. However, since 2010, we have been witnessing a relevant increase of the number of publications regarding this topic (Lusher et al., 2016; Connors et al., 2017). This theme or rather this ecological emergency has been brought to the forefront and today it is attracting increasing attention.

Concerning Latin America, although plastic global production represents only 4 % (PlasticEurope, 2017), waste management remain ineffective and plastic is mainly present along the Argentinean coastline (Gonzales Carman et al., 2015). Only few studies exist about the presence and the occurrence of microplastic in Argentina, that's why this curse is the hallmark of the entire world and it's essential to take the problem in hand. Some of these studies were completed in the Paraná estuary (Pazos et al., 2017), in the Setubal Lagoon located in the Middle Paraná floodplain, nearby Santa Fe city (Blettler et al., 2017). Indeed, the Paraná River is highly valued by its large fisheries. Actually, many families depend on fish resources as an essential source of protein (Anderson and Wiener, 1995). *Prochilodus lineatus* is one of the most important and common species for the economy of the zone used for the consumption (Almirón et al., 2015), his biomass can attain 1000kg/ha in lentic zones of Middle Paraná (Baigún et al., 2013). These individuals are mainly caught in the Delta of Paraná, between 1994 to 2010, 294288 tons were used for exportation (Iwaszkiw et Lacoste, 2011).

In addition to its economic role, *Prochilodus lineatus* has an ecological role as well. Indeed, through its trophic diet this species plays a major role in the trophic system transferring energy from the first levels to large fish-eating. In this way this species is fundamental in the process of transformation of organic matter.

Moreover, trophic transfer of MPs in freshwater species has already been demonstrated (Duis and Coors, 2016; Chae et al., 2018), and therefore they potentially can also affect human health (Eerkes-Medrano et al., 2015). Nowadays much more attention is paid by consumers, politicians and managers requiring information about contaminants in biota in order to assess the health and well-being of organisms and the consumers (Burger et al., 2007).

This study aims i) to determine presence (particles number, shape and color) of plastic debris on *Prochilodus lineatus* (Sábalo) digestive tracts, and ii) to determine potential relationships between degree of pollution and sampling site (nearby or downstream of the Paraná city). It is expected to answer some important questions such as: Is *Prochilodus lineatus* ingesting MPs in the Middle Paraná River? Are fish nearby a large city more polluted that those from relatively pristine zone (e.g. upstream a city)?

The host organization

This internship took place in the National Institute of Limnology (INALI) located in Santa Fe City in the north-east of Argentina. This is the first institute created by the National Council of Scientific and Technical Research (CONICET) in 1962.

All of the projects and studies carried out at INALI mainly concern the Paraná River and its watershed. INALI studies both the most protected areas which remain in a wild state, almost pristine and areas affected by anthropogenic actions giving particular attention to the contamination of ecosystems and present biodiversity.

One of the strengths of the INALI is its ability to mix different trades to lead the research's projects as well as possible. Moreover this institute cooperates with researchers from Uruguay, Brazil, Canada, Spain, England, France, United States of America and Germany. Indeed, this institute operates in a wide range of fields: chemistry of water, hydro-ecology, bacteriology, phytoplankton, zooplankton, aquatic flora and fauna through fishes, amphibians, reptiles and birds. In total, the INALI brings together more than sixty-two people. Moreover, since 2002 the INALI has been in partnership with the University of Littoral (Ciudad Universitaria CP 3000, Santa Fe Province, Argentina), allowing it to train professionals in all of these fields of actions.

In the Hydro-ecology laboratory, where I had the chance to work, there are 3 researchers. Briefly, the fields of their study are:

Mario AMSLER: Director of the Hydroecology's lab. Water Resources Engineer. Specialty: Fluvial Hydraulics – Fluvial Geomorphology – Ecohydrology.
Martín BLETTLER: My professional tutor. Specialty: Hydrodynamics, Morphodynamics, Ecohydrology, Ecohydraulic, Benthic Invertebrates, Plastic pollution.
Luis ESPINOLA: Specialty: Ecology of freshwater fish.

There are also, two post-PhD students **Elie ABRIAL** (from IMACOF) and **Ana Pía RABUFFETTI,** four PhD students: **Eliana EBERLE, Florencia EURICH, Nicolás GARELLO** and **Daiana PASCUALE,** one BSc student **Augustini EBER**, **Elise FERLAY** and I, foreign student intern.

Materials and methods

The tasks carried out can be separated into two categories: theoretical and practical.

- ***Theoretical***

Above all, a full bibliographic research, state of the art was done using Scopus. At the beginning, keywords chosen were “microplastic” AND “freshwater” (123 documents) to have an overview about this complex topic, then other more specific searches were done using “microplastic” AND “freshwater” AND “fish” AND “pollution” (21 documents). These readings allowed me, in order to know what is known and what is less known. But it also permit me to find methods already used to facilitate identification of microplastic from Gastro Intestinal Tract (GIT).

- ***Practical***

The planned activities involved two types of sampling campaigns. The first one, playing the role of “preliminary campaign”, involved the sampling and the collection of several fish species in order to select later the most appropriate species for this study. The fish individuals were measured, weighed and dissected so as to extract their Gastro Intestinal Tract. The second campaign consisted in recovering fishes from sales points managed by artisanal fishermen. After that, all the samples were processed and analyzed in laboratory in order to achieve the targets and contest at the preliminary hypothesis. Before any consideration, it is necessary to know that although there are a few differences between this 2 campaigns there are totally comparable.

Study area

This entire study was carried out on the Argentinean coastline, in Paraná River, in two different places, first one in Lagoon *"Chapeton"* and second one in *"Paraje la Jaula",* downstream the city Paraná (Fig.1).

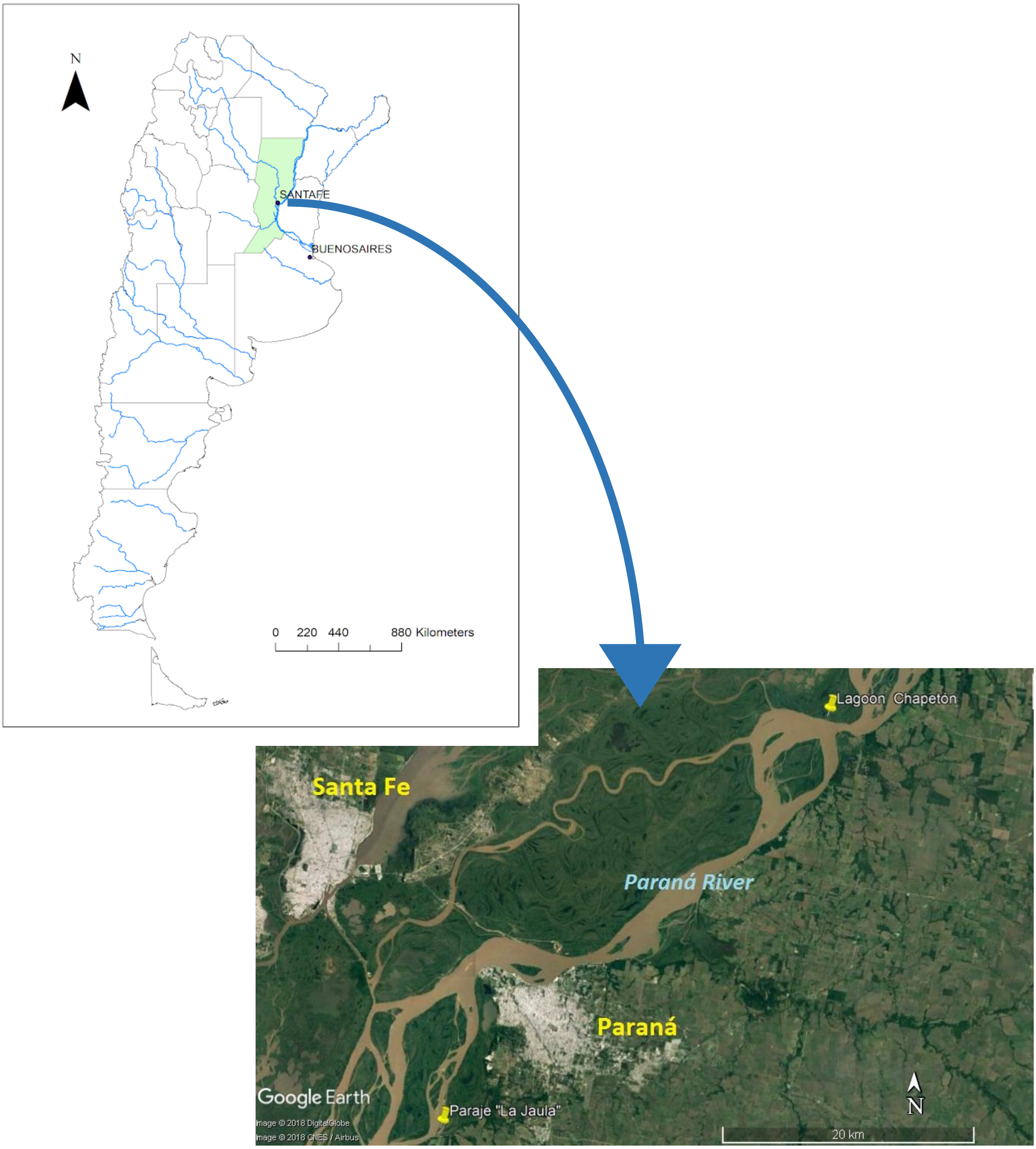


Fig. 1. *Study Area (Arcgis Map and Google Earth Pro).*

❖ *First sampling*

The first study area (Fig.2) is located at 50 km from the city of Santa Fe, and corresponds to a sector of the main channel of the Paraná River in its middle section. This zone is relatively untouched by human activities, and therefore less impacted by plastic pollution. Specimen of *Prochilodus lineatus* were sampled in this "lagoon zone". This experimental fishing was performed in May of 2018 using fillet with different meshes. *P. lineatus* was caught through the mesh 10 (meaning 100 mm distance between opposite knots (mesh opening)). The nets were set at sunset and removed to the first hours of the following morning, staying in the water during 12 hours.

Fig. 2. *Upstream lagoon zone – First sampling area. Author: FERLAY Elise*

❖ *Second sampling*

The second area (Fig.3) is located in a place appreciated by fishermen for its abundance of fish, close to Paraná City and therefore more affected by plastic pollution. Digestive tracts from *P.lineatus* were obtained from artisanal fisherman. This sampling strategy was employed to avoid unnecessary fish killing since the fish were originally caught for human consumption and respecting local policies.

Fig. 3. *Downstream zone – Second sampling area. Author: GINON Lea*

Contamination prevention

In order to avoid contamination from MPs potentially present in the laboratory, the use of cotton lab coats, gloves and mask was mandatory. Furthermore, lab coats were white during all the handling in order to distinguish potential fiber which would come from it. Moreover glassware and working place were cleaned with solution of ethyl alcohol (96%) before starting all experiments in order to conserve a sterile environment. From the beginning of the operations until the observation under the microscope, the samples were covered with aluminum foil to avoid contaminations by fibers in the air. At the same time a control sample was made following all the previous steps.

Chemicals and others materials

In order to detect the plastic debris, it is necessary to digest the organic material with a solvent. Many articles refer to hydrogen peroxide (H_2O_2) (Avio et al., 2015; Pazos et al., 2017; Lei et al., 2018) and other experiments more recent use potassium hydroxide (KOH) (Karami et al., 2017; Khün et al., 2017; Bessa et al., 2018) to dissolve organic matter. In the present study, the idea is to use the methodology used by Pazos et al. (2017) since these authors have studied plastic ingestion in fish of the Paraná River (estuary area), therefore, the selected solvent is solution of hydrogen peroxide (H_2O_2) (30%). This aqueous solution acts as an oxidant according to the following formula. The use of this solvent is to facilitate the visual identification of microplastics debris.

$$H_2O_{2\,(l)} + \text{Organic Matter} \longrightarrow CO_{2\,(g)} + \text{Others}$$

Sample processing

❖ *First sampling*

Fishes were identified *"in situ",* following the keys and existing bibliography for fish from the Paraná River basin (Almirón et al., 2015). In total, 5 *P. lineatus* were captured. They were measured (Total Length (TL, cm) and Standard Length (SL, cm)) and weighed (Total Weigh (TW,g)) (Table 1). After that, for each of them, the GIT was extracted and put into alcohol and brought to the laboratory.

Table 1. *Characteristics of P.lineatus from the first campaign (TL,SL,TW,feeding habitat)*

Species	TL (cm)	SL (cm)	TW (g)	Feeding habit
Prochilodus lineatus	31	25.5	516.3	Illiophagous
Prochilodus lineatus	33.5	29	777.2	Illiophagous
Prochilodus lineatus	32.5	27.2	593.2	Illiophagous
Prochilodus lineatus	30.5	26.7	607.9	Illiophagous
Prochilodus lineatus	30.3	26.2	560.2	Illiophagous

❖ *Second sampling*

For the second campaign, GIT were brought frozen from the fishing site to the laboratory, where their stomach were weighed (Ws, g) (Table 2).

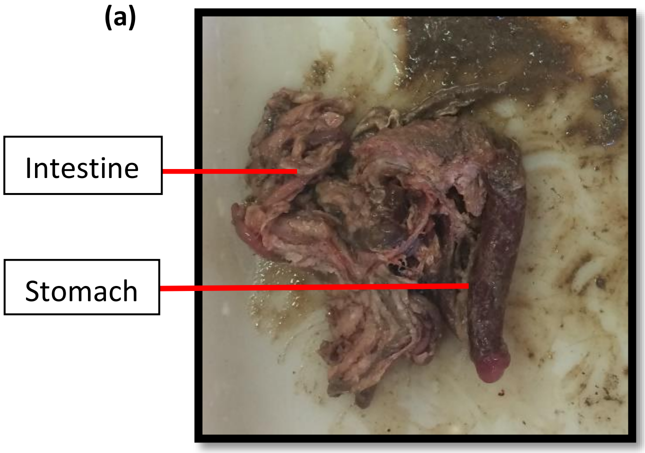
Table 2. *Characteristics of P.lineatus from the second campaign (Ws and feeding habitat)*

Species	Ws (g)	Feeding habit
Prochilodus lineatus	163.6	Illiophagous
Prochilodus lineatus	109.3	Illiophagous
Prochilodus lineatus	197.2	Illiophagous
Prochilodus lineatus	197	Illiophagous
Prochilodus lineatus	172.4	Illiophagous
Prochilodus lineatus	138.3	Illiophagous
Prochilodus lineatus	154.2	Illiophagous
Prochilodus lineatus	139.5	Illiophagous
Prochilodus lineatus	141.2	Illiophagous
Prochilodus lineatus	153.4	Illiophagous
Prochilodus lineatus	168	Illiophagous

❖ *Laboratory procedure*

In order to make direct comparisons, we employed a similar methodology as the one used by Pazos et al. (2017). Previously, after defrosting (Fig. 4a) the digestive system (stomach and intestine) had been cleaned with distilled water and filtered through a 63 µm filter to retain only the internal contents that may contain microplastic. The contents of the digestive tract were placed in a glass container previously filled with 60mL of H_2O_2 (30%).These containers were placed on a hot plate at 60 °C (Fig. 4b), acting as a catalyst, until no organic matter remains. To make sure that the solution does not dry out, hydrogen peroxide has been added regularly. After digestion of all organic matter, the solution was filtered with a 63 µm filter (Fig. 4c,d). Finally, after filtration, the samples were placed in a petri dish (previously checked to follow a pattern for observation) and observed under a Boeco™ zoom stereo microscope (Fig. 4e) and a Nikon™ binocular microscope with a magnification range of ×10–40. Plastic parts were identified following the criteria advanced by Norén (2007): (i) No cellular or organic structures are visible in the pastic/fibre, (ii) Clear and homogeneously coloured particles, (iii) if the particle was a fiber, it should be equally thick, not taper towards the ends and have a three-dimensional bending. Plastic presence was referred as number of particular per fish individual.

(a)



(b)

(c) (d) (e)

*Fig. 4. Laboratory procedure – **(a)** GIT after defrosting **(b)** GIT mix with hydrogen peroxide on hot plate **(c,d)** Filtration after treatment with hydrogen peroxide with a 63 µm filter **(e)** Observation under a Boeco™ zoom stereo microscope. Author: GINON Léa*

Results

For lack of time, the results presented hereafter will be those coming from the first fishing campaign, and only a part of those of the second campaign.

❖ *First campaign*

For the first fishing campaign, 5 fish were captured. The gut analysis contents allowed the identification of 60 elements including 57 fibers (Fig. 5a), which represent 95% of the amount of elements and 3 “other types of MPs” referred to as “others” (pieces of regular and irregular shapes) (Fig. 5b).

(a) (b)

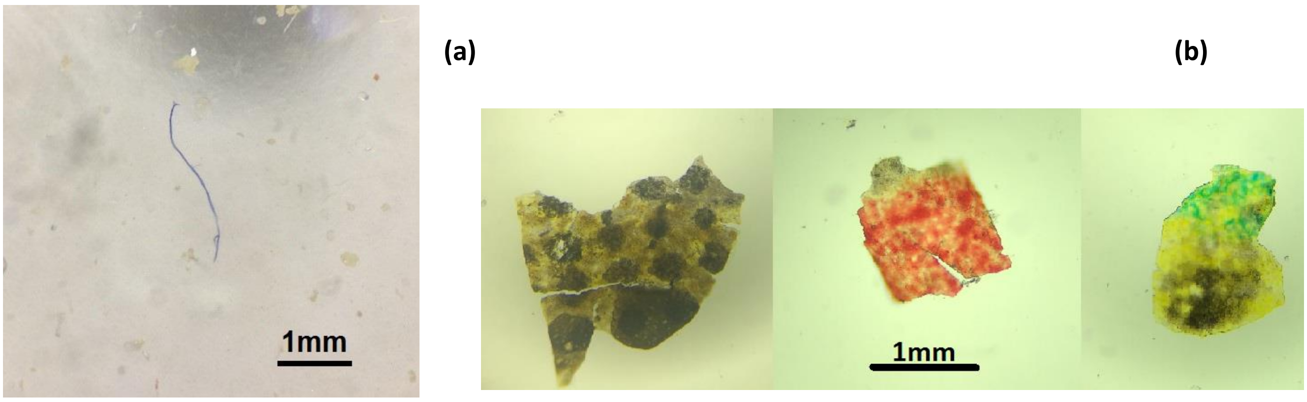


*Fig. 5. **(a)** Blue fiber and **(b)** “others” found in GIT of P.lineatus Author: GINON Léa*

Among fibers there were different colors, which are in the descending order, blue (45 fibers), red (7 fibers) and transparent (4 fibers). On average in each fish we found 12 MPs debris, details of each of them are presented in Fig. 6. It's important to highlight that in all individuals analyzed, some type of the MPs was found. Fibers were present in 100% of the samples, meanwhile "others" were found in 40% of the fish analyzed. In the control samples a maximum of 2 were found. Concerning fibers their sizes ranged between 0.5 and 4.5mm with an average of 1.5mm, "others" micro-plastics were lower than 2mm (average 0.75mm).

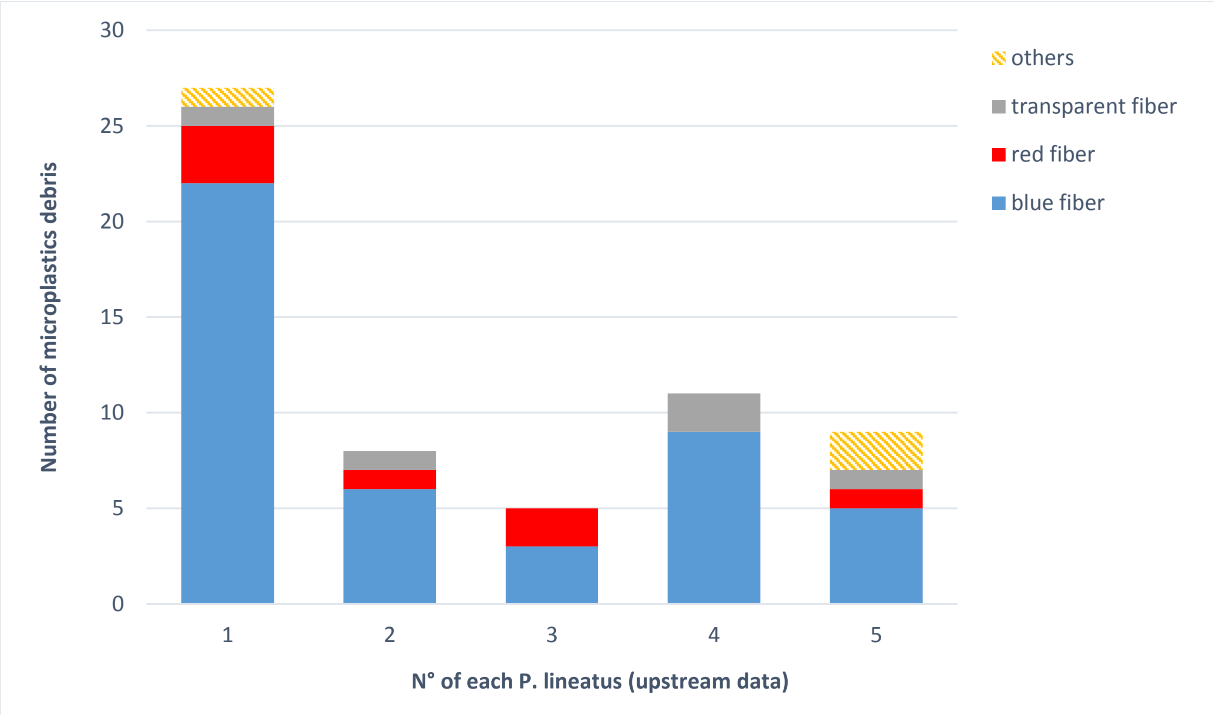


Fig. 6. *Occurrence of microplastics debris (fibers and "others") found in GIT of each P.lineatus captured during the first campaign (upstream zone)*

❖ *Second campaign*

For the second campaign, a total of 16 fishes were analyzed. In this report, I'm going to present the data from 8 of those fishes because three of them are still being treated with hydrogen peroxide and the others are still in the freezer. A total of 79 debris were found (Fig. 7), with an average of 9.9 per fish. Among this debris, 62 were fibers with the same predominance of blue color (38) followed by red and transparent ones (12 for each). Thanks to these results we can also notice that 1 GIT didn't contain fiber plastic. Concerning this zone, much more "others" were present with a percentage of 21.5% and a record of 8 found in one of them. Concerning fibers their sizes ranged between 0.5 and 3.5mm with an average of 0.91mm, "others" micro-plastics were also lower than 2mm (average 0.56mm).

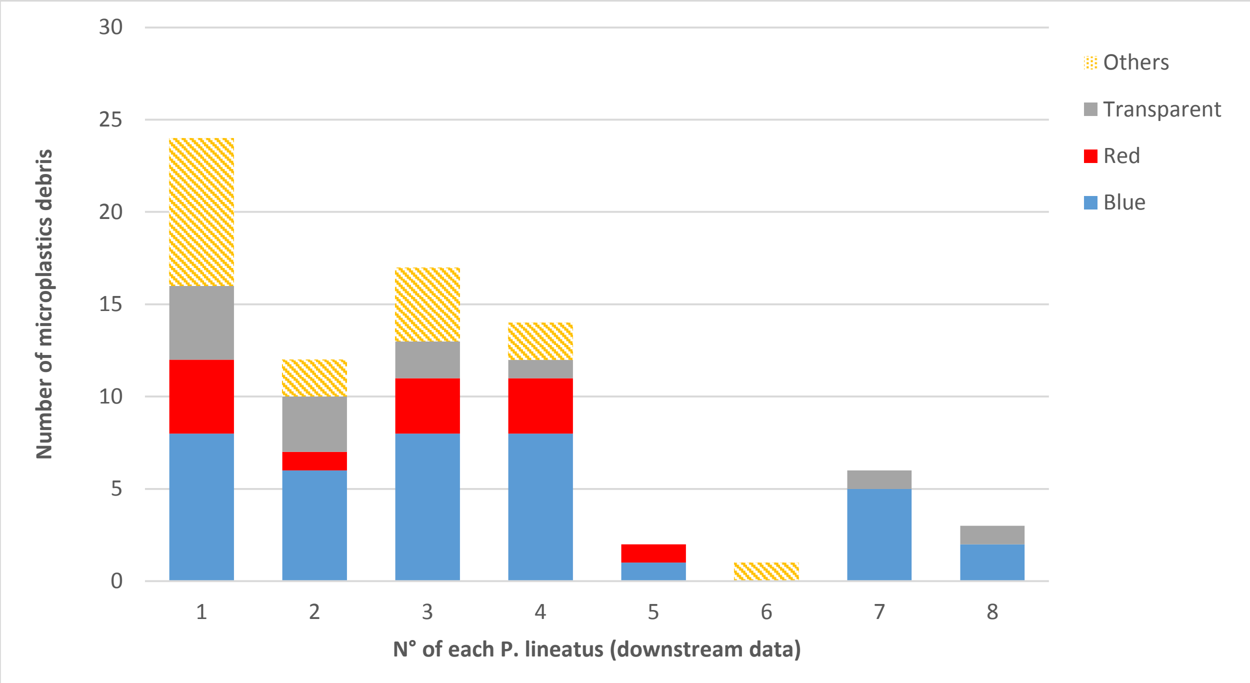


Fig. 7. Occurrence of microplastics debris (fibers and "others") found in GIT of each P.lineatus analyzed for the second campaign (downstream zone)

Discussion

To date, knowledge about the interactions of microplastics with *P. lineatus* is non-existent, so it was important to lead this report.

According to the results of this study, we found MPs in 100% of fish gut contents, indicating that this pollutant interacts largely with fish community in the Middle Paraná River. This result coincides with others studies such as the work leaded by Pazos et al. (2017) in the Paraná estuary where 100 % of fishes' gut contents showed MPs, or in the Adriatic Sea with a benthic flatfish presenting a percentage of 95% of MPs (Pellini et al., 2018). The omnipresence of MPs in each of these individuals is in concordance with the study leaded by Browne et al. (2010) who reveal that MPs debris are more present than larger debris, therefore it results that the risk of ingestion is multiplied.

Moreover, it's important to notice that *Prochilodus lineatus* is a benthic species (Monasterio de Gonzo, 2003). This species ingests large quantities of sediments where microplastics are mainly present. In a study leaded by McGoran et al. (2016) about the presence of microplastic in the digestive tracts of two River Thames fish species (*Platichtys flesus* and *Osmerus eperlanus*) and in another work comparing 4 different fishes habitats by Baalkhuyur et al. (2018), the results showed that it's the benthic/demersal species which ingest more plastics than the others, suggesting that benthic species are exposed to more microplastic.

Among all MPs ingested, another important observation was the abundance of found fibers. Indeed, if we collect data from both campaigns, fibers were in 86% of the fishes. In the study leaded by Pazos et al. (2017), similar results were demonstrated, 96% of MPs were fibers. Other studies also reported such percentage (Boerger et al., 2010; Lusher et al., 2013).

Among fibers, we found a significant quantity of transparent fibers. Presence of this type of color fiber demonstrates that the ingestion of plastic is not an active but a passive or accidental ingestion (Blettler et al., 2017). Obviously due to its illiophagous diet, which means that it feeds of organic and inorganic detritus and living microscopic organisms (diatoms, bacteria and others) located at the bottom of waterways (Sverlij et al., 1993), ingestion of plastic in general can only be passive. Therefore, it results that it's completely impossible for this species to distinguish fiber with another type of food.

The origins of the fibers are diverse, in many studies it was determined that fibers are mainly originated from domestic washing (McGoran et al., 2016; Compa et al., 2018; Halstead et al., 2018) with an average of 1900 fibers released from each piece of clothing during a wash cycle (Browne et al., 2011). As well, fibers can come from fishing industry (Pruter, 1987), female hygiene products and nappies (Lusher et al., 2013). To determinate the origin of fibres, it would be necessary to analyze them with transmission Fourier Transform Infrared (FT-IR) spectroscopy as for the work leaded by (Blettler et al., 2017; Compa et al., 2018).

Concerning "Site effect", at the beginning of this study we expected to notice a correlation between abundance of MPs and the sampling area. However, results showed that on average we found more plastic per fish in the "lagoon zone Chapeton", area less impacted by pollution than in the other one. This result can be explained by the fact that fish doesn't accumulate plastic in his organism and the quantity of MPs is just an indirect instantaneous measure (Pazos et al., 2017). Due to the lack of data, we can't expect and make a conclusion about the trend above and below the city especially when others authors testified the contrary saying that abundance of plastic debris can be explained by the population density (Barnes, 2005).

Conclusion

❖ *Synthesis*

This study provides a contribution to the knowledge and understanding of plastic occurrence in these commercial fishes. While plastic pollution by fish ingestion has been also reported by authors in the Paraná River (In the estuary of this River by Pazos et al. (2017)), this is the first record in the Middle section of this mega-river. This work confirmed the presence of microplastics debris in GIT of *Prochilodus lineatus.* MPs was present in 100% of each of these individuals. Specifically, fibers (86%) were the most abundant item with a predominance of the blue color.

Before the beginning of the experiments, I expected to encounter meso-plastic debris (5mm-2.5cm) visible to the naked eye. However, during the dissection no one was detected. Furthermore, after treatment with hydrogen peroxide, I expected to find an equal distribution between fibers and "others", but the results showed the main presence of fibers, with 86%.

This study noticed the occurrence of microplastic in gut contents. However, to look further into this approach, it could be interesting to know if it’s possible to conclude from stomach content whether fishes are accumulating plastic throughout their lives or are maintaining plastic only during the digestive process.

Furthermore, another step of this study but not the least will be to study if the presence of plastic in digestive tract can attain eatable part of the fish for consumers. However, the impacts of microplastics on humans are not very well documented (Eerkes Medrano et al., 2015), at date there are no data concerning the potential risks of this type of particles in food (Hollman et al., 2013).

Even if, a lot of things remain to be discovered, the fact that there are plastic debris in fish fauna with commercial interest is enough to conclude that it’s now essential and necessary to take this ecological emergency in hand as soon as possible to limit damages and non-reversible consequences.

❖ *Personal assessment*

The research work

This internship at INALI enriched me both professionally and personally.

First of all, on a professional level I was able to learn what research is. I wanted to do this internship not only because the subject interested me but also because I was curious to know this area of activity, and how a research team work. Research is not a work that occurs only during working hours, but anytime. It demands a lot of competences such as autonomy because you have to manage your time and your progress. Also, it is obligatory to be rigorous in one's work and mainly when we follow an experimental method. Then, it requires great capacities of synthesis through the fastidious bibliographic work. Before starting the experiments, I had to immerse myself in the subject by reading many articles and report in order to separate what is already known from what remains to be done. To finish it’s necessary to be patient because until the end you’re not sure to obtain the results that you were looking for.

However, in the field of research, in spite of the strong willpower of discovery, one of the limiting points and not the least remains the financing. This lab receives very little help to finance projects. The experimental outputs are often at the expense of the laboratory staff and it is often difficult to carry out projects for lack of financial means. For example, the experiment I did took a lot of time because the hot plate was too small to hold all the samples. As a result, the experiments took a lot of time to be done. Nonetheless, despite these obstacles, research has the merit of arousing curiosity, starting on an unknown ground and finding correlations, explanations for hitherto unknown points revives the idea in us of why research exists and why it’s essential that it continues to exist.

One of the negative points of this internship is the lack of time, indeed the request of the professional tutor was to realize a scientific article, without having realized this type of rending before, it was very difficult to arrive here. Moreover, this report is the fruit of only two months of internship, the results are thus not completely established. In my opinion, to have time to complete this task completely and perfectly, another couple of months would have been necessary.

The country

This internship also allowed me to develop one of the capacities that an engineer must possess to wit, his adaptation. River management in Argentina differs from that of France. Indeed, Argentina is five times larger in terms of area and is less populated than France. This difference has repercussions on the organization of the territory and therefore on the waterways. During the first fishing campaign, we spent three days on one of the islands of Middle Paraná. So, I could appreciate the wild state that could keep this river. In this area, the river is not channeled or affected by human activities. It's another vision of the river that I could observe. As well, during this first campaign, the fish once caught were opened to determine their sex and to extract their intestinal contents for my study. Some of them were still alive, I had never face to this kind of situation before, and I had to overcome my apprehension in order to succeed in this activity.

Working in another country and moreover in another continent, allowed to realize the differences in operation and the various ways of seeing things. This internship allowed me to open my mind and understand that an engineer must adapt to the environment in which he finds himself to respond in the best possible manner to the needs and not to follow a pre-established method. On a personal level, I have met in this laboratory a wonderful united team, welcoming and human. Even if they are all working in different fields, everyone is there to help and participate in the advancement of others. In this lab, researchers, PhD students and post-PhD students are a good example of the definition of team spirit.

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Annexes

Annex 1. Front page of the edition of the *National Geographic* (June 2018)

Annex 2. *Prochilodus lineatus* (Sabaló)

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2017-2018

Microplastic fragments in digestive tracts of commercial fish *Prochilodus lineatus* in a mega-river (Paraná River, Argentina)

Abstract:

The presence of microplastics (MPs) in gut contents of *Prochilodus lineatus*, freshwater commercial fish of the Paraná River (South America) was studied. 13 individual of this species were captured in two sites. MPs was found in 100 % of the fishes, where fibers represented 86% showing that this pollutant interacts largely with this fish community. There was no relationship found between the sampling area and the abundance of MPs. This study is the first record in the Middle section of this mega-river.

Key words:

Microplastics ingestion, Paraná River, *Prochilodus lineatus*, South America.

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