Internship report

4th year

The use of plastic debris as nesting material and microplastic ingestion by a bird species of South America (*Phacellodomus ruber*)

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Contents

Instituto Nacional de Limnologia

The national institute of limnology is located in the Santa Fe city in Argentina. This institute was founded in 1962 by Dr. Argentino Bonetto. It is part of the National Council of Scientific and technical Research (CONICET), and of the *Universidad del Litoral* (UNL). This research institute focus particularly on the chemical, biological, ecological, morphological processes that occur in continental hydro system, focusing on the great Parana river system. Thus, the INALI is composed by different laboratory that study every kind of life in the Parana system (flora, mammals, birds, reptiles, invertebrates). In addition to the research thematic, there are some people that begin to work on more applicated subject like the sustainable fish production.

The lab where I have worked is in charge of two people: Luis Espinola, Doctor of environmental Sciences and Martin Blettler, Doctor of biological sciences. I have been supervised by Martin Blettler during my internship. The team of researcher is composed by doctor and Phd student that work in different fields of investigation. They are Elie Abrial, Anna Pia Rabuffetti, Daiana Pascuale, Eliana Eberle, Nicolas Garello and Florencia Eurich.

Introduction

The plastic, or plastic material, is a synthetic or a semi-synthetic material derived from different kind of primary resources like coal, natural gas, crude oil, cellulose and salt (plasticseurope.org). The molecular aspect of the plastic is a mixture of numerous polycarbonated chains. Plastics can be divided in 2 great families: thermoplastics which are soften when heated and become harder when cooling; and thermoset which remain hard after moulded. And in these two families it is possible to find numerous types of plastic (ABS, PC, PE, EP, etc…). The uses of plastic material are numerous and various. We can find it in clothes, cooking tools, cars, almost everywhere. But this worldwide use creates today a very important and actual problematic about the use of plastic.

Since the creation of the industry of plastic, its production and its consumption increase all over the world. Indeed, the production increased dramatically from 1.7 million of tons in 1950 to 348 million of tons in 2017 (plasticseurope.org). The most important producer of plastic in the world is Asia with 50.1 %, then Europe with 18.5 %, then North America with 17.7 %, the rest of the production is divided between Middle and East Africa, Latin America and Russia (plasticseurope.org). In another hand, the plastic recycling is not so much developed in each country. In addition, "developed" countries send their wastes to "developing" countries even if the waste treatment is not very effective (France Inter, The guardians). So, the quantity of plastic throwed in the environment increases in countries with not a powerful waste management. This phenomenon lead to the emergence of numerous unauthorized dumps. This is the case in the Santa Fe city and around it. Indeed, the waste management exist in the city but it is not very effective and more, the cities near to Santa Fe are actually in a period of strong development with the installation of a lot of people. But, in these little towns the waste management is very ineffective, and this problem leads to the apparition of illegal dumps near to the cities. But, all of them are in wetlands in the floodplains of the Parana and during the high-water level the debris are easily remobilized. Moreover, the copious rains and the very flat topography of the environment enhance the phenomenon of remobilization.

This intense production of plastic material leads also to new problematics never been studied before. As a consequence, numerous studies have emerged in order to better understand this totally new topic. Actually, several studies have been done about the quantity of plastic in seas, but a little part is focus on the freshwater environment (Wagner et al., 2014; Eerkes-Medrano et al., 2015), moreover these studies focus about each kind of organisms (birds, mammals, etc…). These studies are generally divided regarding to the characteristic of the plastic particles. Indeed, the first approach proposes to consider the size of the particle. Thus, it is possible to classified particles into 5 categories (Lipiatt et al., 2013): megaplastic that are the very big pieces of plastic whose size exceeds 1 meter, macroplastics whose size vary between 1 m and 2.5 cm and mesoplastics whose size is comprised between 2.5 cm and 5 mm. These particles are observable by eyes. Then, it is possible to find smaller particles: microplastics whose size is comprised between 5 mm and 1 µm and finally there is nanoplastic that doesn't exceed 1 µm (figure 1)(Lipiatt et al., 2013).
But there is a clear asymmetry in the studies between marine environment and continental environment (Blettler et al., 2018). Moreover, most of the studies that occurs on freshwater system are concentrated in the north hemisphere in countries assimilated as "developed". This poses a significative problem concerning the way of study of microplastic pollution (Blettler et al. 2018).

Finally, there is very few studies about continental organisms and the impact of anthropogenic particles on these organisms (Jagiello et al. 2019). The few that exist are focused on thematics such as entanglement, ingestion and the use of anthropogenic debris by the birds for nests building. But, as mentioned above, the great majority of the paper done about this subject are focusing on the birds in marine environment and about the problematics of entanglement and ingestion but actually there is no study concerning the uses of anthropogenic debris in nests in south American birds (Battisti et al. 2019). So, this is a totally new topic of research.

In that case, we proposed in this report to study the presence of anthropogenic particles in nests of a native bird species of South America and the presence of microplastics in this bird via the analyses of faeces. For this work, the first part is given to the context of this study, next the methodology applied are presented, then the first results obtained are described quickly and finally the protocols, results and limits are exposed in the final part: the discussion.

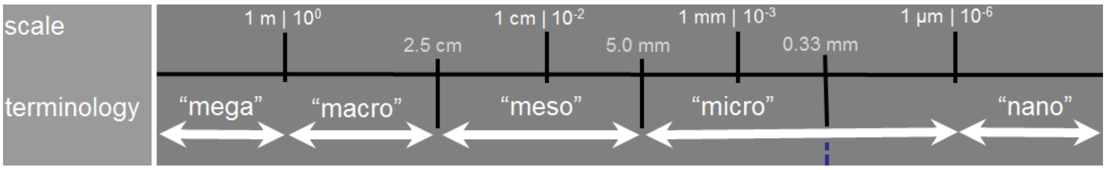

Figure 1: Size classes of plastic particles (taken in Lipiatt et al., 2013)

Study context

1. Parana River

1.1. Hydrology

Classified as the second most important river of South America and the ninth in the world (Latrubesse, 2008). The Parana river has an annual discharge of 18000 $m^3.s^{-1}$ at the ocean (Latrubesse, 2008). The mean discharge at Corrientes (upstream from Santa Fe) is 16595 $m^3.s^{-1}$, the minimum discharge is 4092 $m^3.s^{-1}$ and the maximum register for this station is 54500 $m^3.s^{-1}$ (grdc.com). Discharges are driven principally by the rain which means that the Parana has a pluvial regime with low water level during the winter, and high-water during spring/summer (Agostinho, 2004) (figure 2).

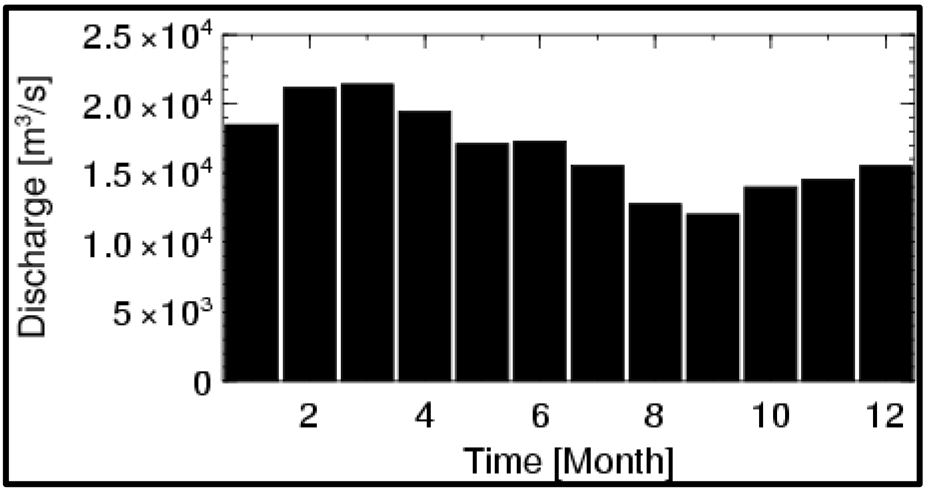

Figure 2: Hydrograph of the Parana river at Corrientes (grdc.com)

1.2. Morphology

The Parana's basin represents 2.8 million of km² and its length is approximatively 4000 km (Latrubesse, 2008). A very general classification propose to divide the river in 4 parts. The first one in the upstream is called Upper Parana and it extends from the confluence between Paranaiba and Grande river to the Guayrà falls (Bonetto *et* Drago, 1968). Then, the High Parana continue up to the Paraguay (2550 km) confluence. Thirdly, the middle Parana extends to Coronda confluence (T). Finally, the Lower Parana

finish to the beginning of the La Plata river in an estuary of 300 km long and 60 km wide representing approximatively a surface of 15000 km² (Bonetto, 1986). La Plata basin results from the joining of the Parana's basin and the Uruguay's basin. Other classification proposed more accurate classification (Thorne, 2002) that are not meaningful for this work.
This river is called an alluvial river, which means that the river forms its channel in the sediments deposited (Church, 2006). Moreover, the geormorphic aspect of the river vary from the upstream to the downstream. The morphological aspect of the upper part of the river tend to be a braided river that have not reach its equilibrium yet (Bonetto, 1986, Thorne, 2002). Then, after the confluence with the Paraguay the river changes into a typical plain river with a floodplain wideness increasing toward downstream. Iriondo (2011) defined the middle reach of the Parana as an anastomosed/braided river with a lot of great island. The river divides into several different channels taking a style with multiple channels. The high morphodynamic of the river and the very flat topography create a peculiar environment with a lot of ponds and lake which can be highly connected during the high-water level period (Iriondo et al. 2007).

1.3. Biodiversity

Due to the length of the river and the countries that it cross, the diversity of habitat is very high. Indeed, tropical humid forest, deciduous trees forest, plains can be found along the river (Bonetto, 1986). Moreover, this biological corridor allows the development of a variety of subtropical fauna in temperate climate (Bonetto, 1986). In another hand, vegetation of the flood plain is represented by numerous ligneous species that are sort according to an altitudinal gradient with the biggest trees on the highest places. Near to the places with water presence, pioneer's species lives. In the lentic waterbodies the majority of the aquatic vegetation's community is composed by floating plants which grows during the spring/summer, and the productivity is increasing in the same season and reach a high maturity at the end of the summer (Iriondo et al., 2007). With the hydrological annual variations, the systems estate is refresh (Iriondo et al. 2007, Nieff, 1975, 1986). This hydrological dynamism of the river is very important in the interaction between lentic and lotic waterbodies, indeed without this connection, these lentic systems are closing and disappearing. Moreover, the connection of lentic waterbodies and the main channel bring an important amount of nutrients in the main stream.
The growth of the aquatic plant and floating plant create numerous microhabitats (Marchese et al, 2013) essential for numerous species of the river.

2. Birds of interest

2.1. Taxonomy and repartition

In the case of this study, the bird named *Phacellodomus ruber* (Vieillot, 1817) (hereafter: Espinero) has been chosen. This specie belongs to the order of Passeriform and to the family of Furnariidae (also called ovenbird) which count 34 genera for 214 species. The Espinero is part of the Furnariinae subfamily (5 genera, 35 species) and of the Synallaxini tribe. This specie is one of the numerous native species of south America. Its distribution ranges from Brazil, north Bolivia, Paraguay and in north Argentina in the south of the province of Tucuman, but also in the province of Entre Rios, Santa Fe and Buenos Aires (Vaurie and Vuilleumier, 1980)(figure 3).

Figure 3: Distribution of the species Phacellodomus ruber (oiseaux.net)

2.2. Morphological aspect

This bird presents a brown rufous uniformous colour on his top except on the crown and the wings which are more reddish, and its down has a creamier color. It can be some color's variations (olive brown, more reddish, etc.) but the majority of the population is brown rufous (Vaurie and Vuilleumier, 1980). Finally, it presents yellow eyes (figure 4).

Figure 4: Phacellodomus ruber, Vieillot (1817)

Espinero lives in diverse environment like humid forest of plains, humid maquis, near to rivers and streams, in bog, but also in artificial zone (oiseaux.net) (figure 5).

Figure 5: Example of polluted living site of the species Phacellodomus ruber

2.3. Ecology

The Espinero is a little bird of 21 cm length and weighing 35 to 51 g (oiseaux.net). They usually live in pairs (male and female) and the breeding period begin during the austral spring, then eggs are laid into the nest from October to January. The nest has a conical shape and it can be massive. Sometimes a new nest is built among the oldest one leading to the breaking of the branches which support the nest. The interior of the chamber is wadded with soft materials, initially with vegetation (moss, roots) but it is possible to find anthropogenic materials. The exterior of the nest is composed of branches and twigs arranged for protected chamber. These twigs are usually from thorny trees. Then, 3-4 eggs are laid in the nest-chamber and incubated for 20-22 days by both parents. After hatching, the young are fed by both parents for 25-29 days (oiseau-birds.com).

Its diet is composed in a majority of insects, but it can feed on some mollusks and tadpoles (aves-argentina.blogpost.com). They usually capture their prey in the down stratum of the vegetation and on the ground (oiseaux-birds.com).

This bird has been chosen for this study because it is a native species, it is present in the province of research (Santa Fe), and their nest are easy to collect. They use also a lot of material in order to build their nest so the probability to find macroplastics is higher. Moreover, the feeding habits of the Espinero increase the chance to find microplastics in faeces.

Concerning the status of protection, the IUCN classify the Espinero Grande as Least concerned (LC) (IUCN).

3. Study Site

The nest of the Espineros has been collected in one site. This is an illegal dump next to the city of San Jose del Rincon. This site is characterized by a high concentration of wastes and by extended marshes. This place is annually flooded because of the increasing of the water level of the Setubal lake. The

Figure 6: Study sites localization and overviews

faeces are collected in a second place which is the ecological reserve (RECU) of the *Universidad Nacional del Litoral* (UNL) which is a semi-urban place surrounded in one side by the Setubal lake and in the other side by a hotel and the university (figure 6). Moreover, there are some dumps around the pond of the RECU. For this study these two sites are considered the same.

Materials and methods

1. Macro and mesoplastic processing.

1.1. Nests collecting method

The sampling campaign of Espinero's nest was done during the autumn in two places: the Rincon dump and in the RECU (figure 6). Nests are checked before collection in order to avoid collecting nest used by birds. The presence of fresh faeces under the nest is a clue of its activity. It is known that this specie uses the same nest during several years, so active nests are not collected in order to avoid any supplementary energetic costs for the bird the next year (Canal et al., 2016). Then, the nest is collecting by cutting down the branch on which it relies, and it is also vaporize with an insecticide in order to mitigate the presence of any dangerous invertebrates (wasps, stink bugs, scorpions, spiders).

1.2. Macroplastic identification

In the laboratory, the nest is weighed using an OHAUS^{TM} SCOUT PRO 6000 g balance, and the length, width and height is measured. Then, the nest was opened, and the intern chambers were exhaustively sorted according to the nature of the particle extracted. Thus, we can find vegetation, cotton under different forms (bandages, clothes, etc.…), different type of plastic mosses used as wadding for mattress (Annex 2) and pillows for example, and pieces of plastic from plastic bag mostly (Annex 2) (figure 7). Considering these information it is possible to classify the particles following the methodology applied by Mishra et al. (2017) which divides the debris as plant matter, animal matter, anthropogenic matter and unidentified materials. This classification has been found has the most appropriate for the component types found in nests.
Once every particle has been removed from the nest, each categories of particles are weighed using an OHAUS^{TM} SCOUT PRO 600 g balance.

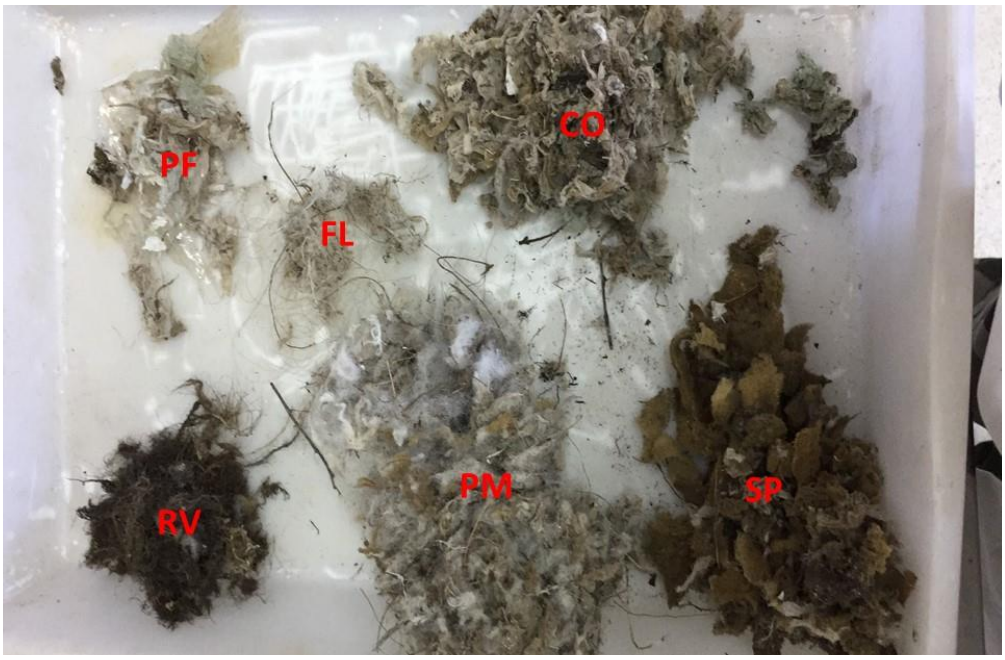

Figure 7: Inner nest composition of Phacellodomus ruber. CO = cotton, FL = Fishing Line, PF = Plastic films, PM = Plastic moss, RV = residues of vegetation, SP = Sponge.

2. Microplastic processing

2.1. Faeces collecting methods

2.1.1. Tarpaulin methodology

To collect faeces from Espinero, nest with fresh faeces under are selected because this clue indicate that the nest is still active which means that a pair of birds still living in the nest. Then, a place of 1 m² approximatively is arranged in order to put a 1*1 m plastic tarpaulin under the nest. This tarpaulin is placed during 48 hours on the field. Later, faeces on the tarpaulin are collected thanks to a little metallic spatula and placed in a little plastic bag and placed in a freezer for further analysis.

2.1.2. Nets methodology

The other methodology to collect faeces from birds implied to catch birds alive. For that, nets are placed in the field in places with vegetation which is useful for hiding the net (Annex 1). Then, each bird captured are extracted from the net and placed in a 100 % paper bag to let them defecates. Then, biometry is done. This biometry includes the measurement of the weight, length of the head + beak, beak's length, beak's width, beak's height, tarsus' length and wing length. Finally, paper bags containing faeces are cut in order to minimize the quantity of paper to digest during the next processing. However, this protocol had been inefficiency concerning two points (Annex 1). The first was that the number of birds captured per sampling campaign was very low. The second point was that the digestion of the paper bag is very ineffective, it remains too much residues for a simple and quick sort of the MP. So, only the first protocol has been retained for Microplastic analysis.

2.2. Method of MPs extraction

The faeces collected with the plastic tarpaulin are counted and then all of them are carefully remove from the sheet and stocked in a plastic bag. Then they were dried at 60 °C for 2 hours minimum second to the protocol proposed by Hardou-Benbouabdellah (2014). Finally, dried samples are weighed and put in a beak with hydrogen peroxide in order to begin the process of wet oxidation (Blettler et al., 2017). For that, faeces are liquified with the minimum of water possible (Gil-Delgado et al., 2017) and then 4 volumes of hydrogen peroxide are added to this mixture. Finally, the sample is put on a hot plate at 60°C and the reaction is allowed to continue until all the organic matter is digested (Blettler et al., 2017).

When the digestion is finished, the content of the beaker is sieved through à 63 microns sieve and washed in a Petri dish with distilled water. Then, every plastic particle is identified using a stereoscopic microscope (Boeco™). Then MPs are placed on a glass lamella beforehand weighed with an AB204-S/FACT balance from the brand METTLER TOLEDO (0.0001 g) during 20 min. Once the extraction of MP of the sample is completed the water present on the lamella is dried and the lamella is weighed again in order to define the weight of microplastic present in each sample. Next, the glass blade is mounted under lamella with water in order to avoid coloration changes induce by other type of resins (Dang, 2018). Observation under a Nikon™ Eclipse E100 microscope are done for identification (type, colors) of the MPs. Finally, measurements (length, width, areas) of the particles are done using the software Adobe Photoshop.

2.3. Laboratory air contamination

Microparticles of plastic are present in all the environment, even in the air (Dris et al., 2016) especially the fibers particles. These fibers can be produced by the friction on the clothes (Lahens et al., 2018). As the sample's processing is not done in completely isolate environment, some particles from the laboratory air can be found in the sample. So, in order to characterize the amount of contamination, Petri dishes of 10 cm diameter with and without protection are placed in the working environment during 24h, 48h. Then the number of particles is counted under a Boeco^{tm} BOE6060.000 binocular and characterize (length and color) under a Nikon^{TM} Eclipse E100 microscope. All the manipulations are done using a 100 % cotton lab coat in order to avoid contamination from the operator's clothes.

Results

1. Nest composition:

The nests showed a mean mass of 1154.6 g (± 400 g) (Table 1), including both natural and anthropogenic material. Regarding the component of the inner nest, the wadding material represent an average of 3.6 % (± 1.5 %) of the total mass of the nest. However, the nest n°3 shows a significative difference with the other nests. Indeed, its mass is very low comparing to the other.

Table 1: General characteristic of sampled nests

	Nest weight (g)	Volume (cm^3)	Particle weight (g)	Pourcentage (%)
Nest 1	1442	na	55.85	3.9
Nest 2	1491	50000	86.36	5.8
Nest 3	498.3	6375	7.28	1.5
Nest 4	1187.2	30000	40.02	3.4

Following the classification proposed by Mishra et Kumar (2017), the proportion of mass of anthropogenic particle in the interior of the nests is clearly dominant in front of the vegetation proportion. The other categories are not represented by any pieces (figure 8).

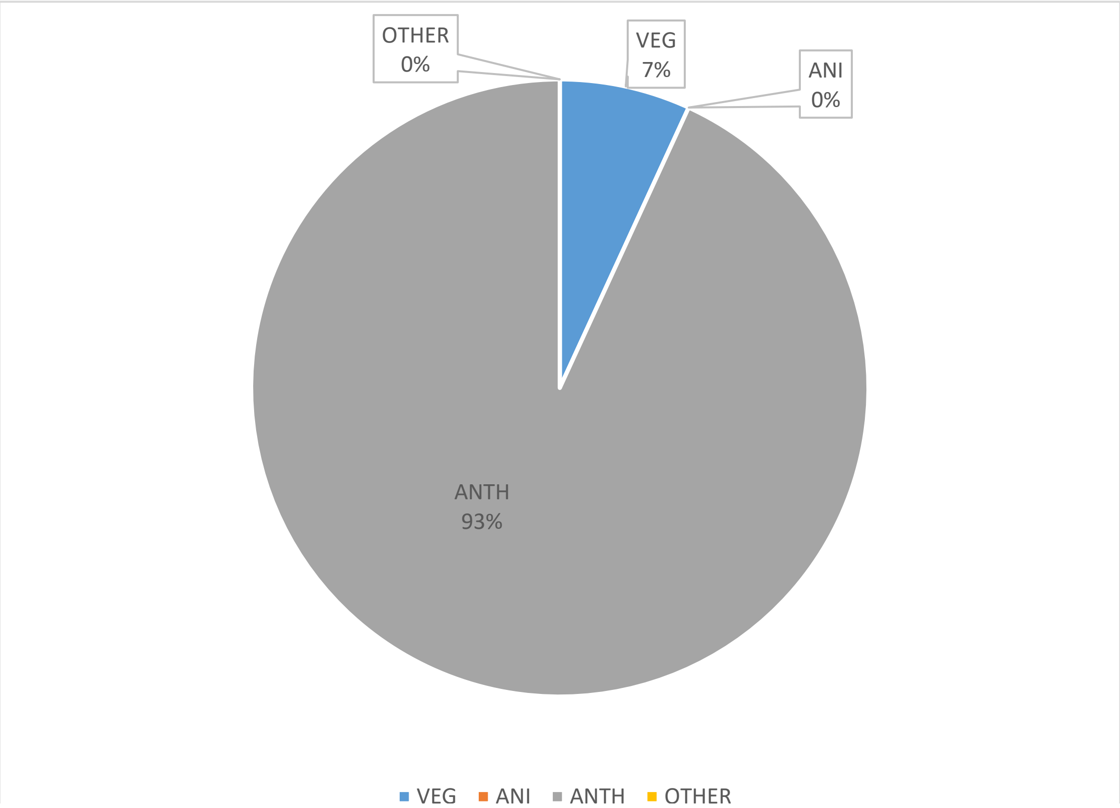

Figure 8: Proportions of mass of type of particles found in Espinero's nests

The wadding material used for the interior of the nest 1, 2, 4 was relatively the same (figure 9). The third nest shows a strong difference regarding to the other, the composition lacks synthetic wadding, cotton and fishing lines. The others show the same composition of nest with a majority of synthetic wadding, then sponge wadding, cotton, vegetation, plastic films and fishing lines.

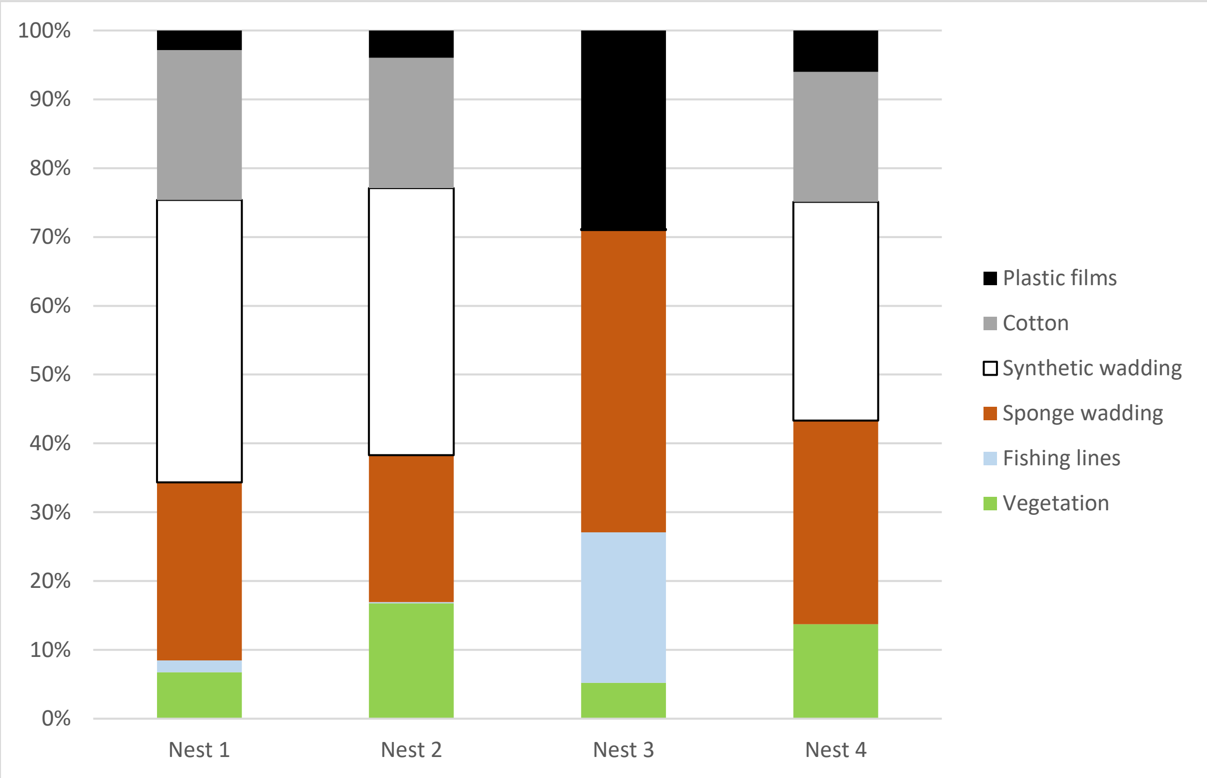

Figure 9: Proportion of masses of wadding materials in Espinero nests

2. Macro and mesoplastics in the nests

2.1. Occurrences

After nest's dissection the particles that can be measured in terms of length and width are considered. In that way only fishing lines, cotton ropes and pieces of plastic are retained for further analysis. The results show that the composition of anthropogenic particles is mainly represented by plastic films from undetermined origin, it represents more or less 50 % of the particles found. The other 50 % are represented by plastic grocery bags, bandages, and fishing lines. In some cases, some cotton ropes are found but in very low proportion. It is also possible to find some rare occurrences of other type of plastic, like bubble wrap or plastic foam (figure 10).

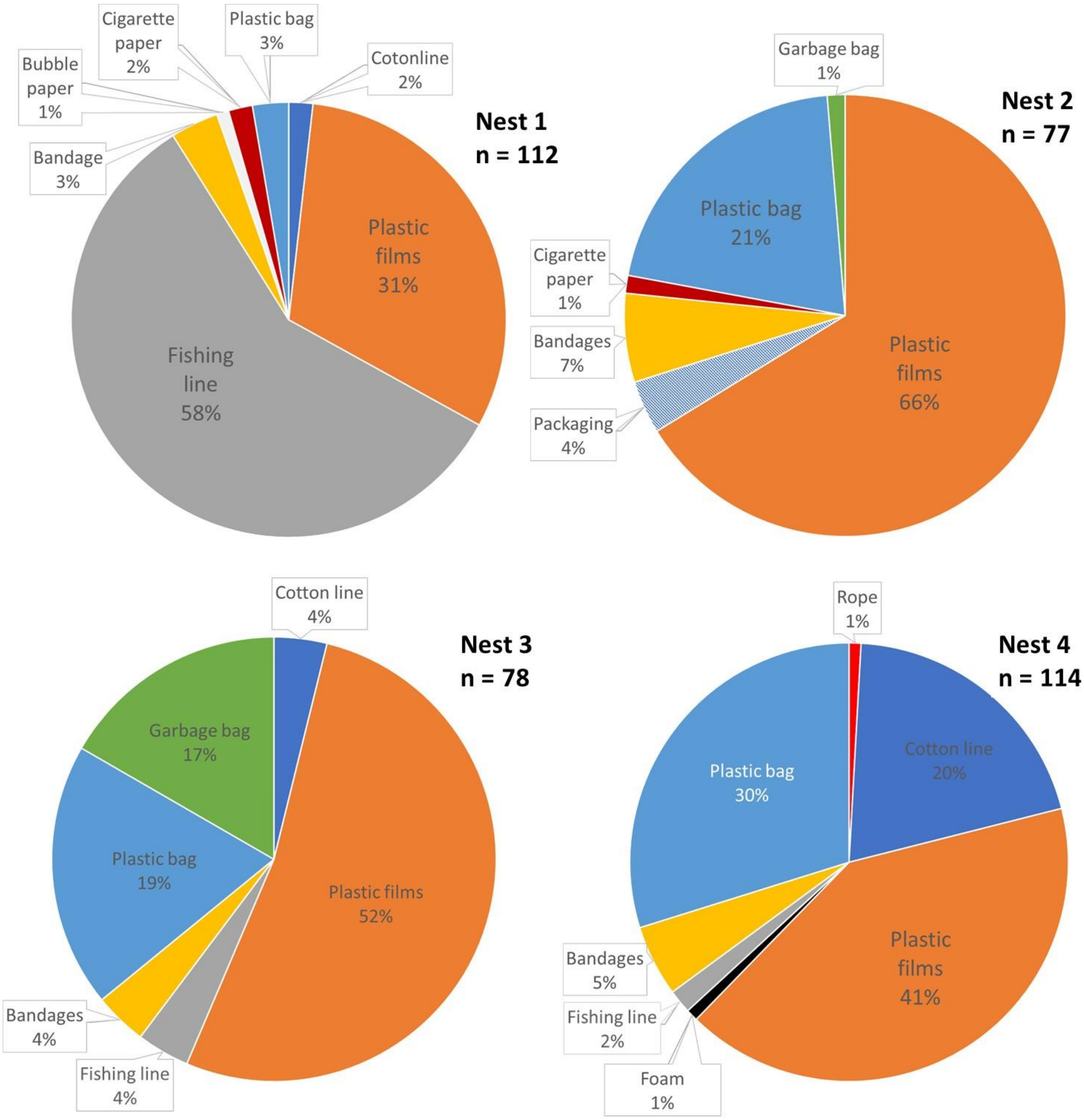

Figure 10: Proportion of occurrences of anthropogenic particles for the four nests sampled

2.2. Particle size

Every fragment has been measured in width and length in order to approximate a surface. It is possible to see tendency in the composition of the particle areas for two nests (n° 2 and 4) which show a similar distribution with the class [5;10[the most represented. Then nest 1 shows a bimodal distribution with a majority of particles comprise between 0 and 1 cm² and 4 and 5 cm² the other classes are less represented. Finally, the nest 3 shows a unimodal distribution with a maximum comprises in the class [1;2[and then the curve decreases when the particle size increase (figure 11).

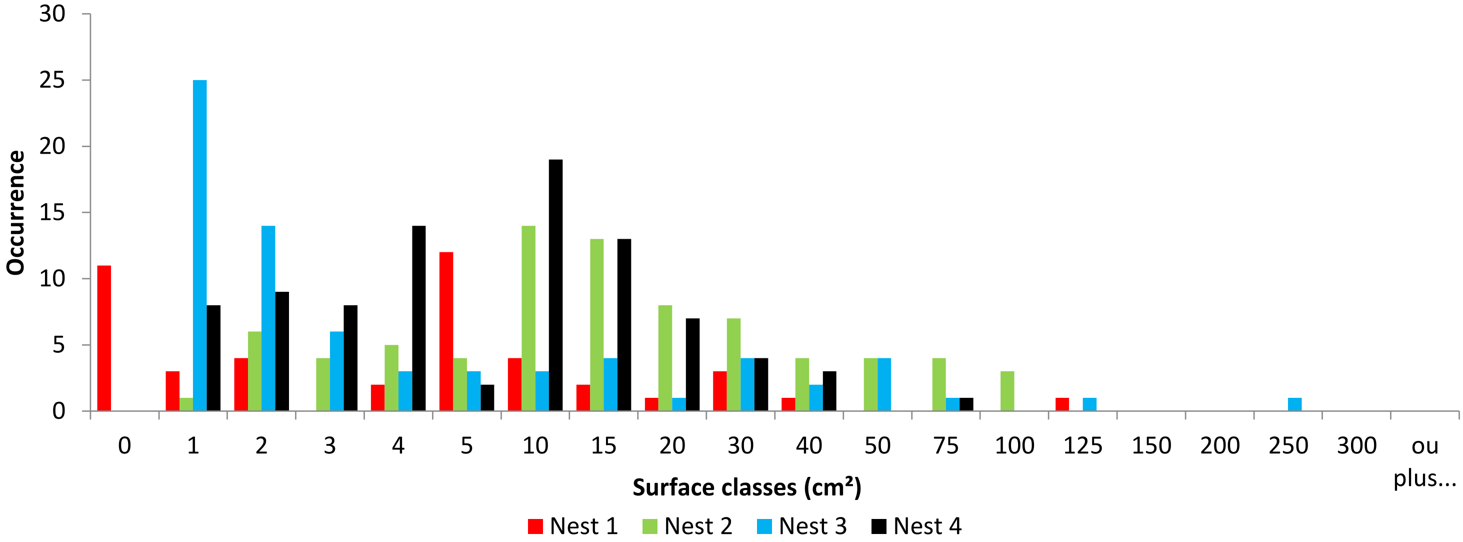

Figure 11: Distribution of plastic particle areas found in Espinero nest

2.3. Colors of particles

Each nest shows a great proportion of white fragment. Then it is possible to find blue and transparent particles. Finally, there are some occurrences of particle of others color (green, red, mix). Globally, each nest shows the same colors (figure 12). It is also possible to link the proportion of colors with the proportion of type of plastic particle.

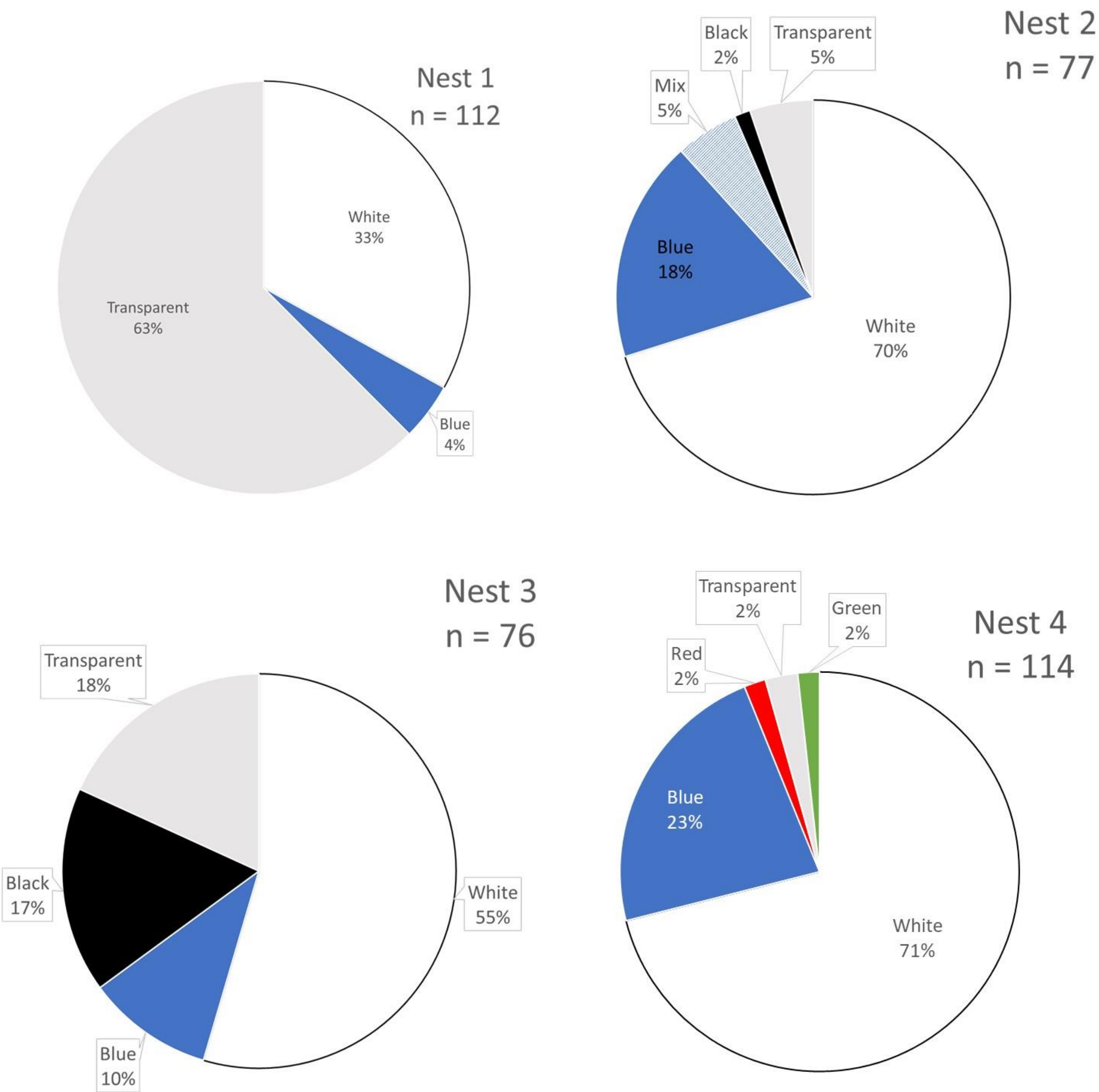

Figure 12: Proportions of occurrences of plastic's colors of the four nests sampled

3. Faeces collecting

The sampling campaign of faeces show that the production of faeces of each nest differ significantly (figure 13). 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.

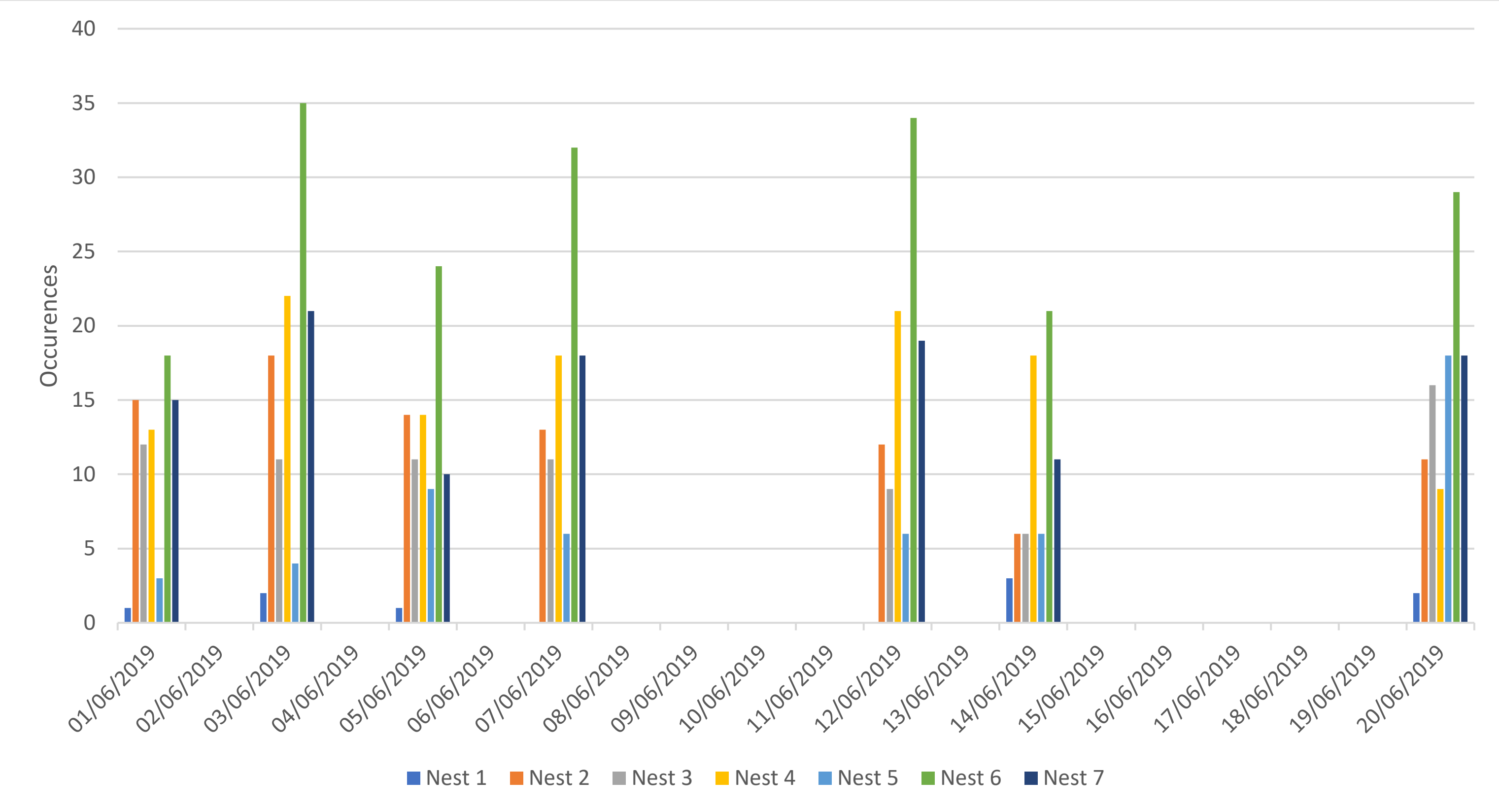

Figure 13:Number of faeces per tarpaulin per sampling campaign

4. Microplastic analyses

This part is still in processing due to technical problem with the new hot plate, and the digestion step is quite long without sources of heat.

5. Aerial contamination

The experiments concerning the laboratory aerial pollution reveal that even when the Petri dish is covered with aluminum sheets, there is contamination of the sample by the particles present in the air. Indeed 8 fibers has been found after 24 hours and 6 fibers after 48 hours. Regarding to the Petri dishes not covered with aluminum more fibers has been found. Finally, as expected, the number of fibers found after 48 hours is more important than after 24 hours (table 2).

Table 2: Samples contamination by aerial fibers

Couleur	24 h covered	24 h uncovered	48 h covered	48 h uncovered
Noire		5	3	5
Bleue	5	7	3	10
Rouge	3	1		2
Jaune				1

Discussion

1. Macroplastics used as building materials in Espinero's nests

1.1. Nest characteristics

The nests sampled are relatively the same except for the third one which is very lightweight. This difference can be explained by the fact that this nest was an old one no longer used by the bird. Indeed, Espinero are known to build new nest above the older one (Sigrist, 2013), and the nest n°3 was the lowest one on the branch sampled. That being, these types of nest remain interesting in order to assess the use of plastic as nesting material.

1.2. Plastic type:

The composition of each nest is also relatively similar as mentioned previously. But, only one nest on the four show a strong difference in the wadding composition (n°3). Indeed 3 components habitually found in the other nests lack in this one. Moreover, the mass of the anthropogenic particle in that nest is also very low regarding to the other one. One explication of this variation is the same as evoked above. Indeed, the older is the nest, the more degraded is the material and it is possible to see that the material lacking is the most easy to degraded (cotton, synthetic wading) and the only materials that remain is the most long to decompose (plastic films, sponge material, fishing line). Moreover, the analyses of the shape of the plastic particle show that the most present particle size is smaller than for the other nest. This can be due to the age of the nest too. Because of the weather and UV radiation the plastic is degraded in smaller pieces. This degradation also leads to the production of the meso- and microplastic (secondary source of microplastic), which seems to be the most important source of microplastic in South American countries (Costa and Barletta, 2016).

Moreover, during the dissection of the other nests it appears that these one was used for several breeding periods by the birds maybe even during the last one. From that, it results a constant supply of new wadding material. So, the results presented here show not only one year of supplying but several of them. It might be interesting to evaluate the input of anthropogenic material for one year by using another methodology than the one proposed here. Moreover, the protocol applied in this internship implied to collect the nest for analyzing the content but if the nest is still active the energetic cost for a pair of birds is significative if they have to build a new entire nest the next year (Canal et al, 2016). A protocol based on the observation with binocular of the activity of the birds during their active period for counting and identifying the particles bring to the nest would be a less impactful methodology for the species (Wang et al., 2009 ; Shivangi et al., 2018) but the main disadvantage of this method is that it is extremely time consuming and some periods of the day are not surveyed (early morning and late afternoon).

Supplementary to the analyses of anthropogenic particles in nest it would be interesting to study the availability of nesting materials in the environment second to the protocol followed by Wang et al. (2009) which established an index which resume the quantity of debris in the environment and it might be interesting for further study to do it but in different environmental condition in order to differentiate study sites second to a gradient of pollution of the places. Moreover, this index might provide a new perspective concerning the choice of the material for the nest's building because it appears that some species adapt their way of building the nest second to the environment (Jagiello et al., 2019). Moreover, it appears that for some species, the use of anthropogenic particle might enhance the fitness and the successful of reproduction (Diamond, 1986 ; Suarez-Rodriguez et al., 2013).

Finally, as mentioned in several studies the problematic of entanglement is quite present and impactful on the nestlings (Townsend and Barker, 2014; Ryan, 2018). But, the debris found seems to be not that much impactful on the nestling of Espinero because there is no very strong ropes that can entangle birds, the only threads that can be dangerous are some pieces of fishing line but the one found in the composition of the nest are not so long and during the dissection of the nests it appears that the nylon threads composed a sublayer of the chamber not in contact with the nestling (personal observation). In another hand, the other anthropogenic materials found in the nests (Sponge, plastic films) can be more probably ingested by nestling and leads to other dangerous problematics like the ingestion of pollutant or digestive tract occlusion for example, these cases can leads to bioaccumulation, decreasing of the appetite and so underfed, and obviously in some cases to the death of the bird.

1.3. Plastic colors:

Concerning the colors of the anthropogenic particles, they are always the same for each nest, there are only few occurrences of particles that don't have an "ordinary" color regarding the other one found in the nests. But, some of the nests shows some prevalence for some color, for example the nest n°1 present a very high number of transparent particles, but this one had a lot of pieces of fishing lines so it influence strongly the results of the colors of anthropogenic particles in the nest. In another hand, the "white color" considers diverse type of anthropogenic particles. Indeed, in that color's class it is possible to find pieces of plastic bag, bandage, but a great majority of these particles are not possible to identify due to an important state of degradation.
In order to enhance the identification the debris, the operator expertise is the easier way to solve this problem. Indeed, for sorting anthropogenic particles the most accurately possible, the operator needs a strong personal database of pictures of different plastic object in order to recognize the origin of the anthropogenic debris. A solution for facilitating the identification might be to clean the particle as proposed by Blettler et al. (2017), but the fragility of the particles forbids this methodology for nests, more, it is too much time consuming to clean each piece, but it can be very tiny.

During the classification of the threads, it appears that fishing line can be inverse with old horsehair. Indeed, with time, the horsehair loses its color, and inversely the fishing line that are habitually transparent become tinted. The same problem appears for the white particles that became tinted with time due to dust, UV radiation or faeces. This bias can influence the results presented in this report.

To conclude this paragraph, it is necessary to underline the fact that there are very few studies about the problematic of anthropogenic litter used in the nest building (Battisti et al., 2019) and the few that are available are focusing in the marine environment.

2. Microplastic protocol limits

2.1. Collection method

It is possible to see that the first nest shows a very low number of faeces for each sampling campaign. This is surprising considering the fact that this nest was defined as active during the recognition campaign. A reason of this, might be induced by the fact that the environment around the nest has been hardly modified during the installation of the tarpaulin and it is possible that it had afraid the pairs and they left the nest.
In another hand, it might be interesting to collect faeces and then collect the nest for dissection. This might allow to see if there is a correlation between the quantity of MaP in the nest and quantity of microplastic found in faeces. After that, if there is a positive correlation between these two factors it would be very useful to manage some experiment regarding to the physiological state of the birds affected by MP because there are papers that evoked the fact that plastic ingestion can affect the health of the organisms (Suarez-Rodriguez et al., 2017; Battisti et al., 2019).

2.2. Sources of contamination

Concerning the extraction of the MP, it appears that it is clearly complicated to avoid any contamination of the sample by fibers and fragment present in the air, the clothes or the stuff used. For example, during the processing of the faeces samples collected following the first, inefficient, protocol applied, a lot of fragments had been found. But it wasn't expected to find this quantity because the species (*Polioptila dumicola,* Vieillot, 1817) does not usually feed on the ground where the most of plastic pieces are. Considering this incoherence and after investigation, it appears that the paper towel used for drying the petri dish, was responsible of the highly contamination. Indeed, the composition of recyclable paper include a lot of plastic particle whose origin is undetermined, but which can strongly affect the results obtained after processing. This observation leads to an important conclusion concerning the difficulty to process samples with no risk of contamination. It is almost impossible to forbid completely the input of foreign particles in samples. This observation is also supported by the results acquired in the little experiment about the laboratory air contamination. The only way to avoid contamination or to mitigate it, consist in covering the containers with aluminum sheets, to operate in very stable condition with not a lot of draught and to use plastic gloves and lab coat. But even with these precautions, there are still fibers that can contaminate samples.

2.3. Digestion protocol

The hydrogen peroxide's methodology choose for this work has been discussed for long for its efficiency. Indeed, owing to our experience acquired during the setup of the experiment it appears that H_2O_2 was not as effective as expected considering the fact that at the beginning of the internship, and for a long time, the hot source wasn't available due to technical problem. Moreover, it appears that the H_2O_2 solution was quite expensive and the volume used for digestion was important, so the cost-effectiveness of this digestion component was not that interesting. So, in order to acquire a good level of digestion the samples are in digestion for more than a week at ambient temperature which was really too long. Moreover, it still remains some particles undigested generally vegetation's structures, fishbones, fat, chitinous carapace which can hide or be mingled with MP. In order to improve the digestion step, an important work of research concerning other methodologies has been done. It results from this research that all methodology shows limits that can be constraining for the identification and extraction step (Avio et al., 2015 ; Enders et al., 2016 ; Lusher et al., 2017). But some of them seems to be more interesting, for example the digestion using a mix of 2 chemical components, KOH and NaClO in combination has been found as very effective with the fewest issue for the plastic particles (Enders et al., 2016 ; Lusher et al., 2017). Indeed, other research found that the experimental conditions are influencing the number of plastics after digestion. An important factor is the temperature (Munno et al., 2018). Indeed, plastic are reacting to the variation of temperature. Most of the time an increase of the temperature is often accompanied by a melting of the plastic particle. This influence of the temperature has been considered in very few studies about microplastic analysis (Munno et al., 2018), this lack is very impacting because second to Munno et al. (2018) a temperature superior to 60 °C can significantly affect the quantity of MP found in sample. Moreover, after the processing of different sample on a defective hotplate which had a temperature upper than 100 °C, a little experiment was managed in order to assess the impact that the temperature can have on plastic particles (Annex 3). The results of this experiment (not shown in this report) showed that the size of each particles was reduced, so if the surface of mesoplastic has been relatively strongly affected by high temperature it is possible that microplastic can be even more affected by the temperature and some of them can disappear bringing an important bias in the analyses of samples. But this parameter is very complicated to control because the chemical compound used for digestion generate an exothermic digestion that vary second the quantity of organic matter available, even sometimes, the reaction is so strong that the temperature overpassed 60°C without hotplate. So, it is necessary to consider this exothermic temperature and the temperature added by the hotplate in order to avoid any losses in the microplastics. Moreover, a too high temperature often leads to strong reaction and to the production of a lots of foam that can overflow the beak and leads to a loss of particles. Even if this step seems to be very complicated to control, the digestion is necessary for the next analyses. Indeed, Dang (2018) shows that the identification and the sorting of the MP is extremely complicated in sediments with no digestion step, other study show the same trend (Avio et al. 2015). In Avio et al. (2015) the rate of particles' extraction is quite low (50 %) with no digestion in comparison with samples digested. This is why the digestion step should be considered as primordial in the analysis of microplastic for the most accurate extraction.

However, even with all the limits that the hydrogen peroxide can have, it has been conserved for the experiment especially for its ease of implementation. Moreover, it appears to us that the effectiveness of this compound was relatively good, after some days on a hotplate, no big pieces of organic matter remains, and with the sieving all of the tinted water and very fine particles were eliminated. And finally, in order to extract the maximum of plastic particles, the content of the sieve is diluted in a beak with distilled water and the solution is poured in a Petri dish in several times in order to maximize the extraction rate of particles.

Conclusion

The objective of this internship was in a first time, to assess of the use of anthropogenic particles in the building of the nest of the *Espinero Grande*. In a second time, a work has been done on the presence of microplastic in the organism of the same species. For these works, the first step was to collect enough data by a strong work of bibliography in order to better understand the world plastic's problematics. Then after readings, it appears that the methodologies used for digesting and extracting the microplastic are very diverse. So, a great part of this internship has been given to find the most appropriate methodology.

Then, after this work of bibliography, a first protocol for collecting faeces has been tried but it appears very quickly that the efficiency of it was not enough for acquire a sufficiently amount of data in the fewest times. So, a new protocol for collecting faeces has been imagine in order to answer this problematic. It appears that this protocol was very effective and cost-effective. Actually, the presence of microplastic in birds' faeces has not been assessed yet. This will be the work of the last month of the internship. But it is expected to find particles because of the feeding habits and some clues found on the field during the sampling campaign support the hypothesis of the presence of microplastic in bird organisms.

Concerning the macroplastic study the results show a very important prevalence of the anthropogenic materials as material for nest building. But at this moment, the number of nests collected is too low for confirming that this is a general phenomenon. Moreover, it could be interesting to manage this same study for diverse species of the Parana system. In another hand, further investigation concerning the preferential uses of anthropogenic particles for nest building might be very interesting. This type of study could enhance the interest of the subject. Moreover, some studies linking quantity of anthropogenic particles and entanglement or quantity of microplastic and general estate of the birds might also improve the knowledge about the potential effect on organisms which is not so much studied.

The Plastic problematic appears as the problem of the 21th century, but also as a very complicated to solve due to the multiplicity of the factor implied in this problem. Indeed, it is an ecological, societal, economical, ethical problematic. Ecological, because the anthropogenical products induce a lot of changes in the ecosystem, for example the plastic is involved in the death of numerous individuals of a great variety of species, the chemical compounds affect strongly the population of diverse species even the human's species but also the simple presence of plastic debris is a threat for the ecosystems. Societal problem, because every individuals of the society is concerned by this subject but under different aspect, for example there are poor people that lived of the wastes, rich people that consumed too much plastic and countries that send their garbage in other countries. Economical, because if the plastic comes to disappear it is an important loss for the countries that produce most of the world consumption. And ethical, because it is necessary to think about the consequences of the acts of each one, but also to consider each actor in this problematic.

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Tables and figures

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Appendices

Appendix 1: Nets' placement

Appendix 2: Parent materials of the anthropogenic particles (mattress, grocery bag, bandage)

Appendix 3: Effect of the temperature on plastic particles

In order to characterize the effect of the temperature on plastic material, two types of plastic has been cut in several pieces. The plastic material of a bottle has been cut in square of 5*5 mm and fishing line (Nylon) has been cut in pieces of 5 mm long. Then morphological characteristic (length, width, area) are measured and the plastic pieces are put in water and boiled at 100 °C for 1 hour. Finally the morphological characteristic are taken again.
The results of this experiments (not shown in this report) showed that a temperature of 100 °C reduce the size of the plastic particles.

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2018-2019

The use of plastic debris as nesting material and microplastic ingestion by a bird species of South America (*Phacellodomus ruber*)

In a world where the use of plastic is constantly growing, the number of studies is following the same trend. But a clear asymmetry is visible concerning the place where these studies are managed. Indeed, most of them was performed in the north hemisphere of the globe. Moreover, very few papers talk about plastics and anthropogenic particle in freshwater ecosystem. In this context, a study about the presence of anthropogenic particle in nest and faeces of a South American bird living near to the greatest river of Argentina has been performed. For that, the analyses of several nests revealed that the use of anthropogenic particles in nest's building is quite important. Moreover, an analysis of the presence of microplastics in bird organisms using a non-invasive protocol of faeces collection is actually performed. But it is expected to find microplastics due to the prevalence of macroplastics in the nest. The main purpose of this work is to assess the importance of the use of anthropogenic particles and to complete the database of plastics in South American freshwater ecosystem.

Keywords: anthropogenic debris, birds, freshwater, faeces, nest

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