Second autopsy report

LIFE SeaBiL (Action B6)

Saving SeaBirds from marine Litter

LIFE20 GIE/FR/000114

Coordinator

Beneficiaries

Financial partners

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1. Summary

This report presents the result of dead seabirds' necropsies made during the project (2022-2024).

405 of those carcasses were analysed for plastic content by the different beneficiaries of the project (82 in France, 195 in Spain and 128 in Portugal). More specifically, necropsies were performed on a total of 33 Atlantic puffins (13 in France and 20 in Spain), 27 Common guillemots (24 in France and 3 in Spain), 23 Black-legged kittiwakes (22 in France and 1 in Spain), 42 northern gannets (5 in France and 37 in Spain), 121 razorbills (4 in France and 117 in Spain), 4 great cormorants in Spain, 15 European storm petrels (14 in France and 1 in Spain), 104 European shags (1 in Spain and 103 in Portugal), 3 Great shearwaters in Spain, 2 Scopoli's shearwaters in Spain, 1 great skua in Spain, 5 gulls in Spain, and 25 Cory's shearwaters in Portugal.

During necropsies, each bird was measured (mass, culmen, tarsus and flattened wing cord lenghs), tissues were collected: feathers, brain, muscle, kidneys, liver, blood and the digestive track. These later were used for plastic content analyses while the other tissues (>2000 tissue samples collected over the course of the project) are stored frozen in the tissue bank set-up at LIENSs as part of the project (Action B5), to be shared with the scientific community. The database linked to the tissue bank is available at https://bird-biobank.oasu.u-bordeaux.fr/admin/login.

2. Report

FINAL SEABIRDS' NECROPSIES' RESULTS - SUMMARY REPORT

LIFE SEABIL "SAVING SEABIRDS FROM MARINE LITTER"
LIFE20 GIE/FR/000114 – B6 action

Coordinating beneficiary

Associated beneficiaries

SUMMARY REPORT:
PROJECT LIFE SEABIL - ACTION B6

LIFE SEABIL "SAVING SEABIRDS FROM MARINE LITTER"
LIFE20 GIE/FR/000114

CONTEXT :

Action B6 — sub-action 2 of the SeaBiL project aimed to analyse microplastics in the seabird carcasses collected as part of sub-action 1, to ultimately propose an MSDF indicator species. In that context, a transnational network has been set up in collaboration with volunteers and care centres (Action B4) and allowed the collection of >500 stranded carcasses during winters 2022/2023 and 2023/20024. Additionally, carcasses of European shags and Corry shearwaters were also collected during the breeding season in Portugal (128 individuals)
405 of those carcasses were analysed for plastic content by the different beneficiaries of the project (82 in France, 195 in Spain and 128 in Portugal). More specifically, necropsies were performed on a total of 33 Atlantic puffins (13 in France and 20 in Spain), 27 Common guillemots (24 in France and 3 in Spain), 23 Black-legged kittiwakes (22 in France and 1 in Spain), 42 northern gannets (5 in France and 37 in Spain), 121 razorbills (4 in France and 117 in Spain), 4 great cormorants in Spain, 15 European storm petrels (14 in France and 1 in Spain), 104 European shags (1 in Spain and 103 in Portugal), 3 Great shearwaters in Spain, 2 Scopoli's shearwaters in Spain, 1 great skua in Spain, 5 gulls in Spain, and 25 Cory's shearwaters in Portugal. Necropsies followed the protocol "*Protocol for transportation & necropsies of stranded seabirds' carcasses and tissues*" established as part of SEABIL – Action B6.

During necropsies, each bird was measured (mass, culmen, tarsus and flattened wing cord lenghs), tissues were collected: feathers, brain, muscle, kidneys, liver, blood and the digestive track. These later were used for plastic content analyses while the other tissues (>2000 tissue samples collected over the course of the project) are stored frozen in the tissue bank set-up at LIENSs as part of the project (Action B5), to be shared with the scientific community. The database linked to the tissue bank is available at https://bird-biobank.oasu.u-bordeaux.fr/admin/login.

Figure 1 : Necropsies of common guillemots at La Rochelle University

Digestive tracks were extracted following the protocol "*Protocol plastic extraction"* validate between ABs. Suspected plastic particles were first isolated using a binocular magnifier. Each of these particles was then analysed with infrared spectroscopy (µFTIR) to confirm these were indeed plastic and to characterize the polymers. Analyses were performed by QUALYSE laboratory (La Rochelle, France), at UCA or together with members of TRAGSATEC (participants of the SeaBiL network). Once confirmed as plastic, each particle was measured using ImageJ software and weighted using a microscale, and the colour determined. Below are results of plastic prevalence found on French stranded birds (analysed by La Rochelle University) and on Spanish and Portuguese stranded birds (analysed by University of Almería).

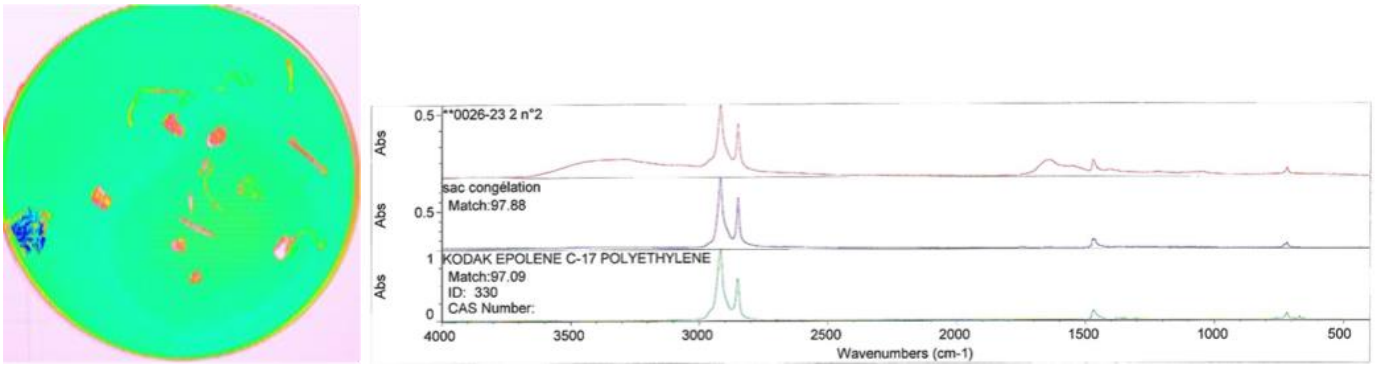

Figure 2 : Example of suspected plastic particles collected on one black-legged kittiwake and analysed by infrared spectroscopy. The left panel show the image observed with the extracted particles. The right panel shows the spectrum measured for one of those particles and compered to a bank of known polymers

Prevalence of plastics in birds collected along the French coasts

Almost all birds had potential plastic particles. As a result, the preliminary mid-term necropsy report for the project suggested a high prevalence of plastics in all species. However, have being analysed by infrared spectrometry, it appeared that almost all these particles were organic (e.g. fish bones).

As a result, over the 82 seabirds analysed in France, only 1 had confirmed plastic particles in its digestive track. This was a black-legged kittiwake with 3 particles of polyethylene.

Prevalence of plastics in birds collected along the Spanish coasts
The analysis of microplastic ingestion among seabird species revealed significant variation in the frequency of occurrence (%FO) across different species.

Alca torda (Razorbill) exhibited a %FO of 28.21%, with 33 out of 117 individuals showing evidence of microplastic ingestion. In contrast, Fratercula arctica (Atlantic Puffin) and Morus bassanus (Northern Gannet) showed higher %FO values of 65.00% and 59.46%, respectively, indicating a greater prevalence of microplastic ingestion in these species.

Some species showed an alarming 100% frequency of occurrence, meaning that all sampled individuals had ingested microplastics. This was the case for Calonectris diomedea (Scopoli's Shearwater), Rissa tridactyla (Black-legged Kittiwake), Gulosus aristotelis (European Shag), and Uria aalge (Common Murre), suggesting that these species may be particularly susceptible to plastic pollution.

However, it is important to note that the sample size for these species was very small (one or only a few individuals), making the frequency of occurrence an unreliable indicator of the true prevalence of microplastic ingestion within their populations.
Infrared spectroscopy results revealed that cellulose was the most frequently identified polymer, accounting for 37.3% of the samples. This was followed by polyethylene (17.3%) and polyethylene terephthalate (3.6%). Other polymers were found in lower frequencies, with occurrence rates ranging between 1.8% and 0.9%. These included synthetic fibers, polymethyl methacrylate, polypropylene, and polycarbonate, among others.

Prevalence of plastics in birds collected in Portugal
In Portugal, protocols were tested in European Shag population during 2022 and 2023 breeding seasons (January to June). Cory's Shearwater sampling and lab analysis were tested in May-June 2023. More than 100 and 25 samples were collected for Shag and Cory's Shearwater, respectively. Microplastic were found in ~92% of the Cory's Shearwater samples. Seven in 53 (11%) and three in 50 (2%) of European Shag nests were found with plastic contents in 2022 and 2023, respectively.

Conclusion
We observed a strong variation among sites and partners in the plastic contamination of stranded birds, suggesting high spatial variability in seabird exposure to plastics.
However, the small sample sizes for some species preclude to draw final conclusions and to propose one or two indicator species as initially planned.
In addition, obtained results as part of the SeaBiL project also contrast with a previous study where plastic prevalence was found to be high on seabird carcasses collected on French beaches (Franco *et al.* 2019), including in some species monitored here.

This suggests inter-annual variations in bird plastic contamination. These spatial and temporal variations might also result from different ages or sexes of stranded birds between sites and years. Indeed, previous studies demonstrated contrasting feeding ecologies between males and females in some seabird species or between life stages (e.g. Phillips *et al*. 2011). While both sex and age (juvenile *vs* adult) were monitored during the necropsies, this information has not been considered in the analysis of plastic prevalence towards the definition of an indicator species.

In addition, stranded birds considered in the SeaBiL project and its different sites were wintering birds which might originate from very distant breeding grounds where their exposure to plastics (at the breeding site or along their migration route) could have been very different, resulting in contrasting spatial winter contaminations.
To account for this, genetic analyses should be performed to determine birds' origin.

Results obtained along the SeaBiL project therefore call for further analyses by focusing on a restricted amount of species which could become indicator species and fill the following criteria:

- 1/ enough birds carcasses from beaches and care centres are available every year to perform a robust analysis and monitor plastic ingestion on the long term,
- 2/ plastic particles have been found in the digestive track.

Also, we recommend that different species could be considered between regions to make sure the previous criteria are met, and to consider the winter distribution of the different species.
Therefore, we recommend extending the analyses on Atlantic puffins, black-legged kittiwakes and northern gannets in France, puffins, kittiwakes, gannets and razorbills in Spain, and European shags and Corry shearwaters in Portugal.
Finally, we recommend considering bird age, sex and origin when these additional analyses will be performed.

Additional microplastic analyses

During the SeaBiL project, and as previously described, we focused on visible particles only, found in the digestive track by bare eyes or using a binocular magnifier. However, it is very likely that seabirds are also contaminated by invisible particles resulting from the degradation of plastics in the bird's digestive track or ingested as such. In this case, the contamination measured though visible particles only could be biased and underestimated. We therefore took advantage of the collected samples during SeaBiL to investigate this "invisible contamination" on a few birds and species of seabird from the French coast. To this end, the different parts of the digestive track (oesophagus, gizzard, intestine), were digested using hydrogen peroxide (H_2O_2) 30% for 48 to 72 hours, filtered using an inox mesh of 25 µm and analysed using an infrared spectroscopy (Spotlight FTIR). These pilot analyses were performed on a total of 5 birds: 2 Atlantic puffins, 1 common murre and 2 black-legged kittiwakes.

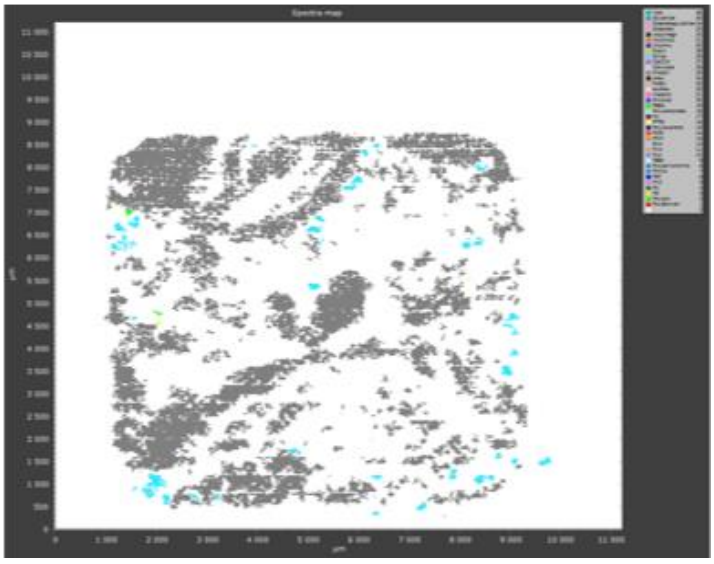

Figure 3: Examples of a filter analysis by Spotlight FTIR after digestion of an Atlantic puffin gizzard

Results showed that common murre had no microplastic particle. However, both puffins and kittiwakes showed some microplastics in their digestive track (see table below).

These first analyses show the importance to go further than investigating for visible particle in order to have an unbiased information about seabird contamination by plastics. We therefore recommend to extend these analyses on more birds and on the different potential indicator species (see conclusion above).
These analyses also confirm that Atlantic puffins and black-legged kittiwakes are indicator candidates and should be studied further.

Table 1: Result of the microplastic analyses.

	Puffin 1		Puffin 2	
Part of the digestive track	Estimated plastic mass (µg)	Polymers	Estimated plastic mass (µg)	Polymer
Oesophagus	2500	Polypropylen	6,4	Polypropylen & Polyethylen
Gizzard	8,8	Polypropylen & PTFE	4	Polypropylen & Polyethylen
Intestine	1,1	PTFE	1,1	Pelyethylen & PET

	Kittiwake 1		Kittiwake 2	
Part of the digestive track	Estimated plastic mass (µg)	Polymer	Estimated plastic mass (µg)	Polymer
Oesophagus	1,85	Polypropylen, Polyeth	0,2	Polypropylen
Gizzard	0,3	Polyethylen	5	Polypropylen
Intestine	0		1	Polypropylen & PTFE