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LIFE SEABIL PROJECT
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LIFE SEABIL TECHNICAL WORKSHOP - FRANCE

SEABIRD'S NECROPSIES: HOW TO USE SEABIRD AS BIOINDICATORS IN THE MARINE ENVIRONMENT

Organized By:
LPO FRANCE

In the context of:
LIFE SeaBiL Project

Associated beneficiaries

Fundings

INDEX

GENERAL INFOS

TITLE OF THE WORKSHOP

Seabird's necropsies: How to use seabirds as bioindicators in the marine environment

DATE

November 26, 2024

LOCATION

LIENSS Laboratory – La Rochelle University – 2 rue Olympe de Gouges, 17000 La Rochelle, France

ORGANIZATION

The workshop was organized by the **Ligue pour la Protection des Oiseaux** (LPO) in collaboration with all other partners of the LIFE SeaBiL project.

ATTENDEES

The event was attended by 26 participants (12 in person, 14 online via Zoom), including representatives from NGOs, universities, research centers, governmental authorities, and other stakeholders from France, Portugal, and Spain

ATTENDEES

ORGANIZATIONS

- Ligue pour la Protection des Oiseaux (LPO France)
- Sociedad Española de Ornitología (SEO Birdlife)
- Portuguese Society for the Study of Birds (SPEA BirdLife)
- LIENSS Laboratory – La Rochelle University
- University of Cadiz (UCA)
- Environment and Climate Change Canada (ECCC)
- National Institute for Scientific Research (INRS)
- Wildlife Rehabilitation and Research Center (RIAS)
- Marine and Food Research Institute (AZTI)
- French Biodiversity Office (OFB)
- National Center for Scientific Research (CNRS)
- Ecole Normale Supérieure de Rennes (ENS Rennes)
- Ornithological Groupe of Normandy (GONm)
- Manche-Mer du Nord network (MMN)
- National Museum of Natural History (MNHN)

LIST OF ATTENDEES

Name	Organization	Regime
Karen Bourgeois	OFB	Presential
Antoine Chabrolle	MNHN	Online
Christophe Aulert	OFB	Online
Diana Matos	NA	Online
Fabrice Gallien	GONm	Online
Florence Nono Almeida	CNRS	Online
Françoise Amelineau	ENS Rennes	Online
Guillaume Le Hetet	LPO	Presential
Ilhas Barreira	SPEA	Online
Inês Lacerda	SPEA	Presential
Javier Franco	AZTI	Presential
Jérôme Fort	LIENSS	Presential
Joao Franco	SPEA	Presential
Karen McCoy	CNRS	Online
Ludovico De Vega	SEO	Presential
Marga L. Rivas	UCA	Online
Maria Victoria Mena Casero	SPEA	Presential
Monica Costa	SPEA	Online
Monica Exposito Granados	UCA	Online
Nuno Oliveira	SPEA	Presential
Paulo Lago Barreiras	SEO	Presential
Philippe Giraud	MMN	Online
Raphaël Lavoie	INRS	Presential
Rute Costa	SPEA	Presential
Victor H. Paiva	Univ. Coimbra	Online
Yada Trapletti	SPEA	Online

OBJECTIVES

OBJECTIVES OF THE WORKSHOP

Role of Necropsies in Seabird Research

Demonstrate the importance of necropsies to provide information on marine litter impacts on seabirds

Harmonized Protocols

Present the standardized protocols to be followed throughout the Bay of Biscay sub-region and the advantages of these protocols

Unified Solution for Wildlife Research

Discuss the main difficulties encountered by wildlife care centres and researchers, with a view to identifying common solutions. And official presentation of the central tissue bank

AGENDA & SESSIONS

OPENING AND WELCOME SESSION

- 9:30 am – Guillaume Le Hétet (LPO)
  - LIFE SeaBil: Project overview and Key results

PRESENTATIONS AND DISCUSSIONS

- 9:50 am – Raphaël Lavoie (INRS)
  - Ecotoxicology and other environmental stressors impact seabirds

- 10:10 am – María Victoria Mena Casero (RIAS)
  - Birds as sentinels of environmental health: exploring pollution through necropsies

- 10:30 am - Discussions with the public

- *11:00 am – Coffee break*

- 11:20 am – Florence Nono Almeida & Karen Mc Coy (CNRS)
  - EcoDIS project: impact of environmental stress on seabird movements and infectious transmission in the Mediterranean Sea

- 11:40 am – Javier Franco (AZTI)
  - Assessment of the incidence of plastic ingestion by seabirds from the Bay of Biscay by bird necropsies and bird pellets

- 12:00 am - Discussions with the public

- *12:30 am – Lunch break*

- 2:20 pm – Françoise Amelineau (ENS Rennes)
    - Northern fulmar and plastic distribution at sea in North Atlantic

- 2:40 pm - Discussions with the public

- *3:00 pm – Coffee break*

- 3:20 pm – Jérôme Fort (LIENSS) & Monica Exposito Granados (UCA)
    - LIFE SeaBiL Necropsies methodologies and results

- 3:45 pm - Discussions with the public

END OF THE DAY

DISCUSSIONS

This workshop was the moment for every partner to discuss the results of the LIFE SeaBiL project and to talk about necropsies as a tool for seabird research and about how to harmonize existing protocols for that. Below is a summary of the key points discussed during the various presentations:

1- LIFE SeaBil: Project overview and Key results - Guillaume Le Hétet (LPO)

After a quick reminder of the context of the LIFE SeaBiL project, its four objectives and the main results were shown. Here is the list with the achievement for every objective.

Objective 1 – Improve the coordination of beach cleaning actions and the monitoring of stranded birds

- Adaptation of an application, accessible to all, for monitoring stranded seabirds: the adaptation of the ICAO application in conjunction with the MARNOBA application for monitoring marine litter (https://lifeseabil.fr/ressources/application-suivi/).
- Creation of guidelines and risk maps in order to raise awareness and obtain better coordination amongst every stakeholder of the beaches monitoring (https://lifeseabil.fr/ressources/guide-carte/). In addition, training sessions and waste collections were carried out in the partner towns to demonstrate the right actions to take to protect biodiversity during this type of event. The project will have carried out 56 waste collections on the beaches, with 1,250 volunteers. For a total of 1.5 tons of waste collected.

Objective 2 – Consolidate the acquisition of knowledge on the impact of plastic waste on seabirds and define an indicator species for Good Environmental Status

- Replication of the work carried out by the northern sea fulmar network and then the LPO Poitou-Charentes

- Identification & monitoring of an indicator species for Good Ecological Status; Transfer of biological material & analyses to the University of La Rochelle
- Creation of a biological tissue bank
- Training & experience sharing (transnational); On tools for monitoring strandings/on the monitoring of marine waste in colonies/on necropsies

Objective 3 – Improve the identification of marine litter sources to optimize the management and prevention of waste

- Implementation of protocol-based monitoring of marine litter; for macro-waste monitoring in Spain (training operators in protocol monitoring); Nano plastic analyses in France by CEDRE; Integrating stranded birds
- Carrying out a territorial diagnosis (FR); Co-construction of an action plan (https://lifeseabil.fr/wp-content/uploads/2024/11/V4-SeaBiL-Plan-daction_compressed.pdf) (28 objective sheets) to reduce waste at source (test areas La Tremblade, CARA[1], St Georges de Didonne, St Palais sur Mer / Collaborators PNMEGMP[2], CDC[3] Oléron, TEO[4], Agglomeration La Rochelle and many others). And sharing expertise on tidal bins with Spain (6 tidal tanks installed in Spain)

Objective 4 – Raise awareness amongst the general public on the issue of marine litter and seabirds in the N2000 areas

- In the field; On the beaches (stands, challenges, panels); Involvement of schools (more than 1,000 pupils involved)
- Media; Through an online communication strategy; Paper publications (Oiseaux mag junior - LPO)

[1] Royan Atlantique Agglomeration Community
[2] Gironde Estuary Marine Nature Park
[3] Community of municipalities
[4] Territories environment Ocean

2- Ecotoxicology and other environmental stressors impact seabirds - Raphaël Lavoie (INRS)

To look abroad and not just at Europe, this Environment Climate Change Canada (ECCC) presentation shows how plastic pollution is dealt with in Canada.

For the context, there is some politics about plastic pollution in the country;

- Zero plastic waste program by 2030
- Regulation banning single-use plastics by 2025
- Promoting science (Canadian Plastics Science Program (CPSP))

This program is designed to study plastics in the environment in a very broad way: from their design, through their use, waste recovery, etc., to their impact on flora and fauna and human health. All this is within the Ecotoxicology and Wildlife Health department at the ECCC.

They use waterbirds to study contaminants in general, as birds are sensitive to any change in their habitat, and they are under the responsibility of the ECCC via the Migratory Birds Convention Act.

One main result shown during the presentation is about a 20-years study on seabirds in the Saint-Laurent, Canada. They showed a decrease in 30% of birds' colony. To find that, they have been monitoring birds' eggs since 1969 and analyzing contaminant concentration. To determine the plastic contamination, they used Herring gull as a bio-indicator, because of its generalist characteristics. The study is still in progress for spatial monitoring to follow contamination through Canada. There are also other studies about plastic in Herring gull colony occurring.

3- Birds as sentinels of environmental health: exploring pollution through necropsies - María Victoria Mena Casero (RIAS)

In Portugal, « *Recuperação e Investigação de Animais Selvagens* » is a rescue centre for biodiversity and mostly seabirds. In addition to caring for and rehabilitating the animals, the hospital raises awareness and educates the public about the environment, as well as carrying out research and scientific monitoring.

One of their main types of research is about necropsies. They use it to monitor ecosystem health, understand mortality causes, detection of threats and crimes and to supply a tissue bank, which can be widely used by everyone.

Necropsies is a tool for now and the future. RIAS does it to train and educate student and volunteers, as well as contribute to conservation beyond borders.

RIAS has also an experience in pollution research. 4,4% of their admissions are about pollution impact. Between 2010 and 2019, they recorded 14 species affected mainly from fishing gears. If sometime, the dead cause is not obvious, they carry out necropsies to find it.

In two of their study about plastic ingestion by aquatic birds, they found more than 22% of the stomachs had anthropogenic litter and predominantly plastics debris, clear of white in colour. They also studied the plastic contaminant' assimilation in gulls and found that the effects are not only physical but also neurofunctional.

4- EcoDIS project: impact of environmental stress on seabird movements and infectious transmission in the Mediterranean Sea - Florence Nono Almeida & Karen Mc Coy (CNRS)

In the context of the very high impact of plastic pollution on biodiversity, this thesis focuses on micro-plastic in the Mediterranean Sea, a hotspot of plastic pollution in the world.

The Yellow-legged Gull is used as a bio-indicator to determine the quantity and type of plastic ingested by seabirds. Necropsies are used to analyze the plastic inside the digestive system, and then to analyze the potential presence of microplastics. Research on plastics has been done everywhere in the bird's body. The results are clear with 96,67% of Gulls with plastic inside, mostly found in ventricle and then digest system and intestine. Moreover, 15% of pellets contain plastic, and the most common type of plastic is micro-plastic (97%)

5- Assessment of the incidence of plastic ingestion by seabirds from the Bay of Biscay by bird necropsies and bird pellets - Javier Franco (AZTI)

In the context of the OSPAR Commission, Northern fulmar (*Fulmarus glacialis*) is used as a sentinel species to evaluate the 'Ecological Quality Objective with' (EcoQO). But the Bay of Biscay is a big area for wintering and migratory seabirds, in addition to resident species for breeding season.

So, in order to assess plastic ingestion by different species in the area and to assess various methodological approaches considering seabirds

community, the AZTI group have been studying two main target groups (wintering and migratory species, and resident species). They used necropsies of carcasses and pellet analysis from 359 birds of 18 species over 10 years. They characterised plastic by colour, type and size before analysed the Frequency of Occurrence, factors influence, etc.

Considering bird pellet analysis, although the number of samples from each colony was low, the Frequency of Occurrence was 10%, like other study from 2010 and 2019. The two main type of plastic found were fragment (50%) and thread (40%), mostly white-clear of black colours. IN pellet, microplastic were the most abundant by size (70%).

The results of the necropsies shown that 14% of the individuals of 13 species have plastics inside their bodies, and that for some of the species 100% of the birds had. The incidence of plastic ingestion by seabirds in the Bay of Biscay has been shown to be like the level found in other marine regions for the same species. Same as in the pellet, fragment (70%) and thread (20%) were the most type of plastic, and the colours was black and white-clear.

The results of this assessment in the Bay of Biscay are relevant about the good option to assess the spatial and temporal variation of marine plastic pollution via pellets analysis, although in some areas sampling is not easy. It concludes that more studies are needed to evaluate the presence of plastic in stomachs. The combined analysis of seabird pellets and stomach contents with necropsies seem to provide a more comprehensive record of marine plastic pollution than a single approach alone.

6- Northern fulmar and plastic distribution at sea in North Atlantic - **Françoise Amelineau (ENS Rennes)**

Northern fulmar are indicator species of plastic pollution in the North Atlantic Ocean (OSPAR Convention). The aim of this OSPAR work is to have less than 10% plastics in stomach birds, a rate which is currently around 50%.

In this context, SEATRACK project wants to calculate the risk of plastic ingestion depending on the location of the birds and plastic pollution. Their hypotheses are (1) there are differences between colonies depending on exposure during non-breeding season and (2) there is a correlation with corresponding EcoQO values for those regions.

By recording models mounted on coded leg rings of 382 individuals from 11

colonies (between 2006 and 2021), *...to be completed by the presentation (on request).*

7- LIFE SeaBiL Necropsies methodologies and results – **Jérôme Fort (LIENSS) & Monica Exposito Granados (UCA)**

For the LIFE SeaBiL, necropsies have been carried out to evaluate the plastic contamination in seabirds wintering along the coast of France, Spain and Portugal and to run preliminary analyses to propose one or several bioindicator species for the area.

The methodology was to use bird carcasses from French, Spanish and Portuguese wildlife rescue centres. The bird must have been dead within 24h and not been fed. They also used stranded birds found on beaches. At the end, 273 carcasses were necropsies and analyzed for plastic content. In addition to this analysis, all the tissues were collected to supply the tissues bank.

In French, 82 seabirds were analyzed, from 6 different species. All birds necropsied were dead of starvation. In Spain, 195 birds, from 15 species, were collected between 2022 and 2024.

The necropsies have been carried out through two phases, following the standard protocol, e.g. OSPAR ecoQO:

1) Extraction of all plastic particles from digestive track using a binocular loupe, plus infrared spectroscopy to validate these particles were plastics and if so, characterize them (number, type, size, colour, etc.)
2) Digestion of the entire digestive tract using 30% hydrogen peroxide for 48-72 hours, followed by filtration and analysis using a Spotlight Fourier-transform infrared spectroscopy (FTIR)

The results in France were quite small and unexpected. Only one of the 82 analyzed birds had confirmed plastic particles in its digestive track (with phase 1). For phase 2, over the 5 individuals tested, 4 of them contain plastics particles.

In Spain, the results were only relevant for three of the 15 species collected, because their sample of individuals tested was larger, the other species having only 1 or 3 individuals collected. The frequency of plastic occurrence for these three species was between 30 and 65%. Microplastics (1mm - 5mm) were the size of plastic most present, at 66%, followed by meso-plastics (5mm - 25mm) at 20%. The most common type of plastic was

fiber (80%), followed by plastic film (15%) and fragments (2%). Finally, the most dominant colors found in plastics were transparent (49%), blue (21%) and black (18%).

8- Main point discussions with the public

During the workshop, several key points were raised concerning details of research methods and the future of the LIFE SeaBiL project.

First, the origin of plastic is often poorly identified, or impossible to determine, which makes the fight against this pollution complex.

The analysis of regurgitation of pellets seems to be a promising method, but it still presents technical challenges (such as access to the pellets, the species, etc.).

As with all scientific research, the financial costs associated with the work are always an issue, so if we are to continue this work, particularly research into microplastics, greater technical and financial resources will be needed in the future.

Secondly, access to tissue banks was identified as a key issue for researchers. They all see it as an effective way of sharing data and information. Everyone would like to establish a simple protocol allowing anyone to access the tissue bank on request. A map of all the samples has been proposed, to show where samples have been sent in Europe, and to create links between the partners. What's more, once the samples have been shared, everyone would like to share the knowledge acquired and the data obtained. A common database, accessible to all, was proposed to facilitate the exchange of information and encourage collaborative work.

Indeed, as in all LIFE projects, the importance of continuing to work together in a coordinated fashion was emphasized, in order to strengthen the impact of research and find sustainable solutions, particularly for the AFTER-LIFE period.

Finally, the question of adapting the work carried out in the Atlantic to the Mediterranean was also discussed. The environmental conditions and species present differ between these two areas, requiring specific adaptation to ensure the relevance of the research, particularly in the choice of indicator species. Similarly, major differences between France and Spain were highlighted in the results, both in terms of conditions and the species studied. It is therefore necessary to adapt the work to the local conditions of the sites/species studied, and therefore to continue

discussions on the harmonization of research protocols on plastic pollution and the impact on seabirds.

CONCLUSION

Studies on the impact of plastic on seabirds are numerous and varied. There are many different techniques, depending on the research objectives.

Necropsy is one method that appears to be effective in determining plastic contamination in seabirds. It appears to be useful for following the causes of death of birds, and the spatial and temporal evolution of plastic pollution in the marine environment. However, **combining necropsies with other analysis methods**, such as the analysis of pellets, would probably be even more effective.

The results of the various necropsies presented by the partners at this workshop seem to be similar; **plastic is very present in the bodies of the seabirds studied**, whether they are migratory or resident. Microplastics (1mm to 5mm) are generally the most present. They are most often in the form of plastic fragments or fibers, and are white, clear or even transparent, or black or grey in color.

This highlights the **need to work together** on these issues, to minimize financial costs on the one hand, but above all **to propose harmonized and effective research methods and protocols** on the other.

To continue to develop research methods and increase our knowledge of the impact of plastics on biodiversity, the project partners emphasized at the workshop the **need to work together and to share and exchange their data (via databases and tissue banks in particular)**. Especially as there is still a lack of knowledge about the precise origin of plastics. With the aim of reducing plastic waste at source, it is therefore important to continue research and development in this area.

APPENDICES

Presentation available on request