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IMPACT OF STOCKHOLM CONVENTION POPS IN GULL EGGS FROM THE EBRO DELTA NATURAL PARK

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Impact of Stockholm Convention POPs in gull eggs from the Ebro Delta Natural Park

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Introduction

Persistent Organic Pollutants (POPs) of different chemical families are spread in biotic and abiotic environmental matrices as a result of their historic and current use. Specifically, manufacturing of pesticides and industrial compounds and their use and application in agriculture and in domestic and consumer's products, has caused direct or non-point contamination, even to remote areas due to long range transport. Given their environmental persistence, POP residues are often detected in the environment at relatively high levels, and cause adverse effects in living organisms and wildlife, threatening, in particular, the survival of endangered and protected species.

Residues of POPs in a given ecosystem is amplified in areas with very high human pressures, which together with geographic/environmental conditions favor their accumulation. The Ebro Delta, is the largest wetland in Catalonia and one of the main aquatic habitats in the western Mediterranean (Figure 1). Due to the Delta's ecological importance, it was declared as Natural Park in 1983. The Ebro Delta is a wetland of international interest as it holds high biodiversity and birdlife, and it serves as a rest and wintering place, as well as being a breeding ground for many migratory and resident bird species.

Although the Ebro Delta Natural Park and the breeding colonies have a high degree of protection and conservation from human exploitation and occupation, this area is very sensitive to anthropogenic activities and suffers from habitat contamination, eutrophication, destruction and disturbances due to urbanization, tourism, fishing, intense agriculture and historic release of industrial wastes (e.g., the chloroalkali plant located in Flix). Due to these anthropogenic pressures, the area is endangered by POP pollution.

As other pelagic birds, gulls are useful to determine the spatial distribution of POPs because of the vast breeding distribution and because their feeding ecology reflect the foraging area 1. In addition, POP accumulation in birds' eggs is attributed to the feeding of the female in the breeding area and thus, gull eggs become good indicators of site-specific contamination. The Ebro Delta is an important breeding area of Larids, specifically the scavenger yellow legged gull (Larus michahellis) and the protected species, the Audouin gull (Larus audouinii). Larus michahellis is an abundant seabird due to its opportunistic feeding habits and the few specific requirements for successful breeding. There are approximately 10,000 couples in the Ebro Delta where they scavenge on rubbish tips as well as seek suitable prey in fields, exploit trawler discards or prey smaller vertebrates and bird eggs. On the other hand, the Audouin's gull was considered one of the most endangered gulls in the world in the 1970's and since then their populations have increased. It has a world population of only 22,325 breeding pairs, which are mostly concentrated in the Iberian Mediterranean coast and the North African coast. Spain holds 20,315 breeding pairs, mainly in Chafarinas Islands and the Ebro Delta 2 , with the latter holding nearly 70% of the global breeding population until 2012, being thus a crucial area for the conservation of this threatened species. Unlike most gulls, the Audouin's gull is a specialist epipelagic fish eater or collects fishing discards from the trawler fishery operating in local waters.

The aim of this study was to evaluate and compare the concentration of Stockholm Convention POPs in gull eggs of 2 species cohabiting in the same ecosystem, Larus michahellis and Larus audouinii, over a

period spanning from 2009-2015. Compounds studied included polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin-like and marker polychlorinated biphenyls (DL-PCBs and marker-PCBs), organochlorine pesticides (OCPs), polybrominated diphenyls ethers (PBDEs), perfluorinated alkyl substances (PFASs), and short-chain chlorinated paraffins (SCCPs). Overall, the study is intended to determine the impact of POPs in 2 bird species with different feeding habits and evaluate the effects at the individual and population levels. In addition, the biomonitoring of POPs in gull eggs permit to determine the potential effect to other aquatic bird species sharing habitat, which are expected to be similarly exposed to POP pollution and thus, might be at risk.

Materials and methods

A set of 36 fresh eggs from Larus michahellis and Larus audouinii were collected annually during the period 2009-2015, in the Ebro Delta Natural Park (Figure 2). The first egg was sampled since it represents the maximum pollutant transfer levels from female to eggs and for comparison among years. Twelve eggs were pooled so that a total of 3 pooled samples were analyzed from each colony per year 3.
Compounds analyzed included 17 PCDD/Fs, 12 DL-PCBs, 6 marker-PCBs, 17 PFASs, 8 PBDEs, SCCP, and 20 organochlorine pesticides (OCPs). The developed methods are based in selective extraction depending of the chemical family and gas and liquid chromatography coupled with mass spectrometry or high resolution mass spectrometry in the case of PCDD/Fs and DL-PCBs. All POP concentrations are given in ng/g wet weight (ww) basis.

[Figure 1 here]

Figure 1. Map of Ebro Delta colony, where Larus michahellis (Lm) and Larus audouinii (La) cohabit in the same area.

Analytical method

Analysis of PCDD/Fs and dl-PCBs was performed following the USEPA 1613 and 1688 methods, respectively. Ten grams of freeze-dried sample were Soxhlet extracted using a toluene:cyclohexane solvent mixture (1:1) and the purification of the extracts was accomplished using the automated clean-up Power-PrepTM System (FMS, Waltham, M.A., USA) based on the use of multilayer silica, basic alumina and PX-21 carbon sorbents. PCDD/F and dl-PCB analyses were performed using GC-HRMS (GC-DFS, Thermo Fisher Scientific, Bremen, Germany) operating in selected ion monitoring (SIM) mode at resolving power of 10,000 (10% valley definition). Separation was accomplished using a DB-5ms (Agilent, Palo Alto, CA, USA) fused-silica capillary column (60 m x 0.25 mm I.D., 0.25 μm film thickness)
For SCCP and marker PCB analyses, a simultaneous extraction and clean-up method based on selective pressurized liquid extraction (sPLE), using acidic silica (44%, w/w) as fat retainer was applied. Then, the extracts were fractionated by means of an open glass column using Florisil. PBDEs were Soxhlet extracted for 24 h with hexane:dichloromethane (1:1, v/v). The organic extracts were then purified using a silica gel column modified with sulphuric acid (44%, w/w). Then, the extracts were fractionated by means of an open glass column using activated basic alumina. Analysis of SCCPs, PBDEs and marker-PCBs was accomplished by GC-MS using a DSQII quadrupole mass spectrometer (Thermo Fisher Scientific, Milan, IT) operating in negative ion chemical ionization (methane as moderating gas) for SCCPs and electron ionization for PCBs and PBDEs.
OC pesticides were extracted in an ultrasonic bath using hexane:dichloromethane (1:1) and Florisil solid phase extraction columns of 10 g were used for purification of extracts. Analysis was performed by GC-EI-MS/MS in a GC Agilent 7890A equipped with a 7000A GC-MS Triple Quadrupole, and electron ionization (EI) was set at 70 eV. Acquisition was performed using Multiple Reaction Monitoring and internal standard quantification permitted to detect all compound with a high sensitivity and selectivity.
Finally, PFASs were liquid-solid extracted in an ultrasonic bath and purification was performed using glacial acetic acid and activated carbon. PFASs were determined by Liquid Chromatography coupled to tandem mass spectrometry. Internal standard quantification of all the analytes was performed using labelled compounds.

Results and discussion

The most significant of this study was that all POPs were detected in all samples and during the 7 monitoring years. POPs concentration decreased in the order OC pesticides > marker- and DL-PCBs > PFAS > PBDEs > SCCPs > PCDD/Fs (Figure 2A). For most of the compounds, the concentration was higher in Audouin gull than in yellow-legged gull eggs, which can be attributed to the fish based diet. There was a different POP pattern according to the species studied. The specific POP profile in both species is indicated in Figure 2B, where the predominance of OC pesticides and PCBs is well evidenced. ∑POPs was up 2,000 ng/g ww, with some variations among years.
The significant levels detected, which might pose a risk for the gull colonies, deserve attention regarding trends and identification of sources, so that actions can be undertaken. Firstly, because the colony is located downstream of a chloroalkali industry that operated since1897 in the manufacturing of OC pesticides, PCBs and lately other compounds. Secondly, these discharges produced an accumulation of 700,000 tons of polluted sediment, which has been removed during the last 3 years. Thus, the high levels of OC pesticides is attributed to the pollution of the river due to past discharges from this plant and mobilization and resuspension. The removal of sediments caused an increase of OC pesticides in the years 2014-15, in relation to pre-removal period (2009-10). The OCPs most prevalent detected in gull egg samples were endosulfan, mirex, hexachlorobenzene, hexachlorobutadiene, chlordane, and DDT isomers, at concentrations close to 1000 ng/g ww.

[Figure 2 here]

Figure 2. (A) Concentration of the different chemical families of POPs in gull eggs from the Ebro Delta and (B) POPs profile in each species.

PCBs were found at levels between 1 to 400 ng/g ww, and although the concentration of marker-PCBs were similar in both species, the levels of DL-PCBs were higher in eggs of Audouin gull. The PCB pattern was dominated by PCB 153 and 180, which are the most bioaccumulative compounds. The source of these compounds is also likely to be the chloroalkali plant in Flix.
Regarding the presence of PFAS, higher levels were detected in eggs of Audouin gull than in yellow-legged gull, indicating that a fish based food contributes to the accumulation of these compounds in comparison to an opportunistic diet, which is more variable. The PFOS was the compound detected at the highest concentration, at 39-67 ng/g ww in yellow-legged and up to 85 ng/g ww in Audouin. Long chain PFAS were also found in all samples, especially PFUnA, PFDoA and PFTeDA, whereas short chain PFAS were not detected or at very low concentration. The ubiquitous presence of PFAS in gull eggs indicates that the colony has been exposed to these compounds with slight differences in the concentration pattern according to the sampled year.
PBDEs have also been detected in both species at similar levels, with a clear predominance of BDE-209 in comparison to the other PBDE congeners. This prevalence of BDE-209 can be attributed to a specific use of this compound in Spain. Levels up to 40 ng/g ww were detected in the analyzed gull egg samples, although this compound has not been manufactured in the area nor it is being directly used. Thus, its origin is enduringly diffuse and has led to its accumulation in gull eggs.
SCCPs have been very seldom analyzed in gull eggs. SCCPs have been proposed as candidate substance for listing in the Stockholm Convention and are included in the list of very high concern substance by the European Chemicals Agency because of their ubiquitous occurrence, bioaccumulation and biomagnification potential, toxicity to organisms, and high long-distance atmospheric transport potential. Compared to other chlorinated organic compounds, information on environmental levels and fate of SCCPs is still limited, in particular in seabirds and aquatic organisms. In the present study, high concentrations levels of SCCPs ranging from 5 to 7.5 ng/g ww for Audouin gulls and between 2.8 and 6.5 ng/g ww for yellow-legged gulls were found in gull eggs from Delta Ebro, suggesting a common and diffuse source of contamination. To our knowledge, it is the first time these compounds are identified in gull eggs from the Ebro Delta. Due to the toxicity of SCCPs, the specific effects on gulls, specifically the protected species, is largely unknown.
Finally, PCDD/F were also detected in all species and again, a fish source was plausible given the slightly higher concentration in Audouin gull in comparison to yellow-legged gull. The levels detected where higher than the WHO-TEQ for chicken eggs and indicated that these concentrations might have an effect on the reproduction and survival. In this period, mean total WHO-TEQ concentrations for both species, Larus audouinii and Larus michahellis, were from 16.2 to 14.0 pg WHO-TEQ/g fat for PCDD/Fs.

To evaluate the potential effects of the POPs cocktail on both gull species, the eggshell parameters, reproduction success and chick viability were studied in order to determine the impact on the gull colonies.
To conclude, the biomonitoring of POPs in protected areas like the Ebro Delta is important to protect the biodiversity and to know the levels of pollution and trends in the long term. In addition, POP contamination can affect the population dynamics of many species and the ecologic equilibrium. Thus, it is important to instigate conservation actions to minimize POP contamination in an area historically affected by POPs.

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References:

1. Ramos, R., Ramírez, F., Sanpera, C., Jover, L., Ruiz X. (2009) Mar Ecol Prog Ser 377, 289-297.
2. Oro, D. (1998) BWP Update 2 (1), 47-61.
3. Morales L, Martrat MG, Olmos J, Parera J, Vicente J, Bertolero A, Ábalos M, Lacorte S, Santos J, Abad E. (2012) Chemosphere 88(11), 1306-1316.

Figure 1

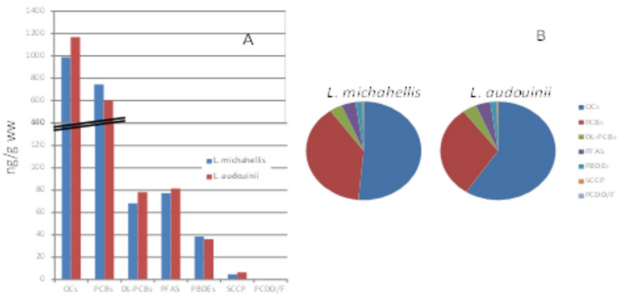

Figure 2