GULL EGGS AS BIOINDICATORS OF POPs: CASE STUDY IN ATLÁNTIC ISLANDS OF GALICIA NATIONAL PARK AND CHAFARINAS ISLANDS REFUGE

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Introduction

Today many bird species live in habitats strongly affected by environmental pollution [1-3], mainly by chemicals used in the past in agriculture (organochlorine pesticides), industry (water repellents, flame retardants, lubricants) or in everyday life (Teflon, Gorotex, etc.). Many of these substances accumulate in birds through ingestion and may affect survival, body condition, behaviour and reproductive state. Among different chemicals, Persistent Organic Pollutants (POPs) occupy an important place because of their persistence in the environment, high potential for accumulation and biomagnification through the food chain, and their high toxicity.

The use of bird eggs has been proven suitable for monitoring the levels and impact of POPs in a given ecosystem. Specifically, gull eggs have been reported as excellent bioindicators of POPs[4] as they reflect the contamination pattern of an area. Gulls have the following characteristics that make them excellent bioindicators (Figure 1):

- Gulls have a long lifespan (up to 20 years) and accumulate the contaminants through food and are transferred annually to the eggs.
- Gulls are secondary consumers and are apex organisms of the seashore food chain since they do not have any predators and therefore biomagnify the POPs present in the marine foodweb.
- Gulls exhibit high levels of site fidelity, breeding in the same location within each colony and therefore reflect the contamination of a given area.
- Gulls are monogamous and colonial breeders that display mate fidelity life-long and therefore the transfer of POPs from parents to chicks can be assessed.
- Gulls are abundant around the world and therefore geographical distribution of POPs can be determined.
- Many gull species, especially *Larus michahellis,* are non-protected species whose eggs represent a non invasive sampling protocol and of high simplicity, due to the large colonies settled in many coastal areas.

Figure 1. Scheme illustrating the use of gull eggs (Larus michahellis) as bioindicators of pollution, indicating the main sources of POPs, the accumulation in gulls over the years and the annual transfer to the eggs.

The objective of this study is to investigate the presence and impact of chemical contamination caused by POPs using gull eggs (*Larus michahellis*) as bioindicators of environmental pollution. Study sites are National Parks and a Hunting Area, thus protected areas where the presence of POPs can affect the well being of those marine ecosystems. In addition, this study contributes to the Spanish national inventory of POPs in a systematic way.

Materials and methods

During the period 2010-2012, an annual sampling of gull (*Larus michahellis*) fresh eggs during the spawning period has been carried out in the National Park of the Atlantic Islands of Galicia and in the National Hunting Refuge of Chafarinas Islands (Figure 2). These areas represent main gull colonies in Spain. In each colony, 36 eggs were randomly collected in 3 different sites (12 eggs in total for each site). The first egg was sampled since it represents the maximum pollutant transfer levels from female to eggs[5] and for comparison among different colonies. Twelve eggs were pooled so that a total of 3 pooled samples were analyzed from each colony per year.

Figure 2. Locations of the National and Natural Parks sampled, which correspond to the areas with main gull colonies in Spain.

Compounds surveyed are indicated in Table 1 and according to the Stockholm Convention[6], include the initial POPs (except toxaphene), New POPs (except pentabromobenzene) and some proposed POPs, according to the Stockholm Convention (COP 6, Geneva, Switzerland, from 28 April to 10 May 2013). In addition, BDE 209 and long chain perfluorinated alkylated substances (PFASs) were also investigated.

Table 1. POPs monitored in gull eggs (Larus michahellis).

12 initial POPs	New POPs	Chemicals proposed for listing under the Convention
Aldrin	α-hexachlorocyclohexane	Hexachlorobutadiene
Chlordane	β-hexachlorocyclohexane	Short Chain Chlorinated Paraffins
DDT (6 isomers)	γ-hexachlorocyclohexane (Lindane)	
Dieldrin	Chlordecone	
Endrin	Hexabromobenzene	
Heptachlor	Commercial octa brominated diphenyl ether (BDE 183). BDE 209 also analyzed.	
Hexachlorobencene	Perfluorooctanesulfonic acid (PFOS) and other 16 PFAS	
Mirex	Technical endosulfan and isomers	
Marker PCB (PCB 28, 52, 101, 118, 138, 153, 180)	Commercial tetra and penta brominated diphenyl ether (BDEs 28, 47, 99, 100, 153, 154)	
Dioxins (PCDD)		
Furans (PCDF)		

We have developed and validated an integrated analytical protocol that allows the determination of 17 dioxins and furans (PCDD/Fs), 12 planar polychlorinated biphenyls (DL-PCBs), 6 marker PCBs, 17 PFASs, 8 PBDEs, short-chain chlorinated paraffins (SCCP), and 15 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[7]. In the case of PCDD/Fs and DL-PCBs, gas chromatography-high resolution mass spectrometry was used. The concentration of POPs is given as ng/g fresh weight (wet weight, ww).

Results and discussion

All pollutants were detected in eggs of yellow-legged gull in the 2 areas studied. PCBs were the compounds detected at the highest concentrations, with a contribution of 78% of total contaminant load in Atlantic Islands (average concentration 16.8±7 ng/g ww) and 91% in Chafarinas (average concentration 858± 704 ng/g ww). DL-PCBs were present in 6.4% and 3.4% of the total contaminants in Atlantic Islands and Chafarinas, respectively. Emerging POPs such as PBDEs, PFASs and SCCP were detected in all the colonies, with lower contribution (Figure 3). Among PFASs, perflourosulfonic acid was the compound detected at higher concentrations, although other long chain PFAS were also identified. With regards to PBDEs, although the Stockholm Convention only includes penta and octa formulations, we found that BDE 209 was the congener detected at the highest concentrations in all samples, and therefore, it is highly accumulated and transferred to the eggs. OCPs pesticides were sporadically detected, being the degradation products of DDTs and endosulfan the most ubiquitous compounds. Table 2 indicates the mean levels and standard deviation of each chemical family detected over the period 2010-2012 for Atlantic Islands and 2010-2011 for Chafarinas.

Figure 3. Concentrations (ng/g ww) of each chemical family in the National Park of Atlantic Islands (period 2010-12) and Chafarinas Islands (2010-11).

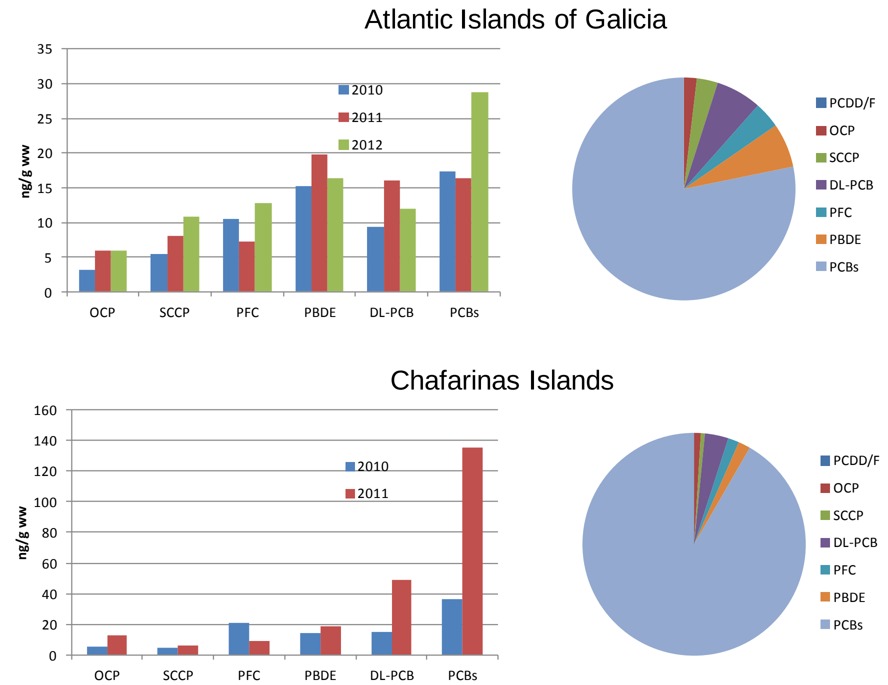

Table 2. Mean concentrations (ng/g ww) of each family of POPs in Atlantic Islands and in Chafarinas Islands over the period 2010-12 (2010-11 for Chafarinas).

	Atlantic Islands	Chafarinas
OCP	4.95±1.5	9.12±5.5
SCCP	8.10±2.6	5.39±1.2
PFC	10.1±2.8	14.9±8.1
PBDE	17.1±2.4	16.4±3.1
DL-PCB	12.4±3.4	31.9±24
PCBs	20.8±6.9	85.8±70
total	75.5±12	165±96

Considering the geographical distribution, the colony of the Chafarinas Island had a higher contamination level (more than 2 times) than the Atlantic Islands, especially for OCPs and PCBs, and this reflects the geographical emplacement of the colony in the south-west Mediterranean which receives the impact of POPs released from north Africa and the Spanish east coast. On the other

hand, the pollution impact of the Atlantic Islands is rapidly diluted due to the open character of the Atlantic Ocean and the strong currents of the area that dilute the contaminant loads.
Finally, PCDD/Fs were detected in both colonies studied at very high levels, exceeding 5-7 times the legislated maximum of PCDD/Fs for chicken eggs. PCDD/Fs and DL-PCBs in Atlantic Islands were of 4.82 pg/g ww and 49.2 ng/g ww, respectively whereas in Chafarinas Islands the levels were of 2.45 pg/g ww and 16.0 ng/g ww. In this case, the higher levels detected in Atlantic Islands reflect the influence of the incineration plants in the area.
When considering the temporal variations over the period 2010-2012, the concentrations of most compounds did not vary significantly within years, indicating that gulls have the capacity to accumulate POPs through their diet and annually transfer them to the eggs. The levels detected in eggs reflect their diet, which in turn reflect the marine ecosystem where they dwell. However, the concentrations of marker PCBs and DL-PCBs in gull eggs of Chafarinas varied by a factor of 3 during the years 2010 and 2011. This may be due to an uncontrolled spill/release affecting that ecosystem during this time period which was evidenced in higher levels in gull eggs.
From the results obtained from the analysis of POPs included in the Stockholm Convention, we show that gull eggs are excellent bioindicators of environmental pollution. This information enables to determine the impact of POPs in each colony and lay the basis for their control. The use as gull eggs in biomonitoring programs are useful because on one hand, permit to determine the impact of POPs in a specific ecosystem. Although results cannot be extrapolated to other species, the presence of POPs in gull eggs indicates that other organisms can also be affected. In the case of National Parks or Refuges, these areas are protected because of the high biodiversity and the need to preserve the area as they are sites with conservation interests. Therefore, knowledge on the presence of POPs can be used to minimize the pollution impact produced by these compounds. Finally, many POPs are neurotoxic, with endocrine disrupting properties and potentially carcinogenic compounds. Therefore, their presence in eggs at parts per million (ppm) levels can have effects on the development and survival of the species. Although *Larus michahellis* is a very resistant species, other birds or animals co-inhabiting in the same ecosystem might be affected by the presence of POPs, and therefore, endangering their development and survival, both at individual or population levels.

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