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Distribution of perfluorinated compounds in Yellow-legged gull eggs (*Larus michahellis*) from the Iberian Peninsula

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ABSTRACT

This study is aimed to evaluate the presence and distribution of Perfluorinated Compounds (PFCs) in Yellow-legged gull eggs (*Larus michahellis*) collected from 8 National or Natural Parks from the Iberian Peninsula. In each colony, 12 eggs were randomly collected and pooled from 3 areas of the colony and analyzed using liquid–solid extraction and liquid chromatography coupled to tandem mass spectrometry. Perfluorooctanate sulfonate (PFOS) was the only compound detected in the eggs and its presence was higher in the colonies situated in NE Iberian Peninsula due to the more industrial and mass urbanization in this area compared to the SW Mediterranean or Atlantic colonies. Accordingly, the Medes site, followed by the Ebro Delta and Columbretes, all situated in the NW Mediterranean coast, contained the highest PFOS levels (40.5–54.0 ng/g-ww). In all other colonies, PFOS was detected at levels of 10.1–18.6 ng/g-ww. Egg shell biometry was studied and it was found that the presence of PFOS did not affect the development of the egg.

1. Introduction

Perfluorinated Compounds (PFCs) have aroused concern because of their increasing distribution in the environment and their still unknown effects on biota. PFCs, such as perfluorinated sulfonates and perfluoro carboxylic acids, are a group of chemicals used as anti-adherent in pans, as waterproof and breathable material (e.g. type Gore - tex ®) and as surfactants in many industrial products (inks, paints, flame retardants, foams, etc.). Although their use is currently regulated (UNEP, 2009), residues have been found in many biological matrices due to their high accumulation potential (Giesy and Kannan, 2001).

Birds are very sensitive to chemical pollution because of their dietary habits and their relatively long life (Furness and Camphuysen, 1997). In a pioneer study performed in 2001, Giesy and Kannan reported the presence of perfluorooctane sulfonate (PFOS), a highly bioaccumulative compound, in the blood, plasma and egg yolk of several bird species across USA at levels up to 2220 ng/mL (plasma of Bald eagles, *Haliaeetus leucocephalus*) (Kannan et al., 2001). In another study PFOS was detected in liver of the Great cormorant (*Phalacrocorax carbo*) at 1873 to 2249 ng/g, and it was associated to industrialization of the sampled area (Kannan et al., 2002). The environmental prevalence of PFCs is demonstrated when Haukås detected PFOS in the liver of the Arctic species Black guillemot (*Cepphus grylle*) at a level of 13.5 ± 2.8 ng/g-ww (wet weight) and in the liver of Glaucous gull (*Larus hyperboreus*) at 65.8 ± 22.4 ng/g-ww (Haukås et al., 2007). Whole bird eggs represent a non-invasive matrix to monitor the presence of contaminants and can be used as indicators of pollution. Spatial studies have been carried out to evaluate the occurrence of PFCs in bird eggs in Common shag (*Phalacrocorax aristotelis*) from Norway (Herzke et al., 2009), in Cormorant (*Phalacrocorax carbo*) from Germany (Rüdel et al., 2011), in Egrets (*Egretta garcetta*) from China (Wang et al., 2008) and Korea (Yoo et al., 2008) and several bird species from North America (Custer et al., 2009, 2010; Rattner et al., 2004; Gebbink et al., 2009, 2011). All these studies show that, despite the species analyzed and the geographical settlement, PFCs are ubiquitous contaminants ranging from a few to thousands ng/g-ww and prove the suitability of bird eggs as indicators of PFCs pollution. In addition to spatial studies, temporal trends have been carried out using bird eggs. In 2 colonies from northern Norway, PFCs concentrations in Herring gull (*Larus argentatus*) eggs showed a nearly 2-fold significant increase from 1983 to 1993, followed by a leveling off in 2003 (Verreault et al., 2007). Temporal trends of PFCs using Swedish Peregrine Falcon (*Falco peregrinus*) eggs revealed a high increase of PFOS over the period of 1974 to 1984, remaining constant thereafter (Holmström et al., 2010). The accumulation of PFCs in birds can have serious implications as these compounds are transferred from the female bird to her eggs during laying period and this may lead to toxicological effects at the developing, individual or population level (Pusch, 2005; Verboven et al., 2008).

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The Iberian Peninsula is an area highly affected by the historical and present use of organohalogenated compounds in both industry and agriculture, and has lead to their accumulation in biota. A study performed in 1991 identified the presence and profiles of PCBs and organochlorinated pesticides in larid (*Larus michahellis*, *Larus audouinii* and *Gelochelidon nilotica*) eggs from Cuenca, Ebro delta and Chafarinas islands (Gonzalez et al., 1991). Other studies proposed gull eggs as biological indicator of PCBs, dioxins and furans (Pastor et al., 1995a) and hexachlorocyclohexane and DDTs (Pastor et al., 1995b) and suggested the need to collect the first egg to avoid variability in the levels due to the sampling procedure. A sampling conducted in 1996 in Doñana showed that Greater flamingo eggs (*Phoenicopterus roseus*) contained traces of PCBs and chlorinated pesticides at generally moderate levels, not affecting the colony wellbeing (Guitart et al., 2005). Also in Doñana, dioxins and furans were detected in Black kite (*Milvus migrans*) at levels exceeding the toxicity thresholds according the No Observed Effect Level (NOEL) (Gómara et al., 2008). Even in pristine areas like the island of Menorca, DDTs, PCBs, dioxins and furans were detected at concentrations that could reduce offspring, and induce embryonic mortality and deformity in two raptor species (*Pandion haliaetus* and *Milvus milvus*) (Jiménez et al., 2007).

Unlike other persistent organic pollutants (POPs), no data is available on the occurrence and distribution of PFCs in birds from the Iberian Peninsula. The objective of the present study was to determine the geographical distribution of 5 environmental relevant PFCs using gull eggs as biomarkers of pollution. Yellow-legged gull (*Larus michahellis*) represents a common and widespread species with a unique habitat. The collection of eggs does not cause any effect on their already vast populations. In addition, gull eggs have been recently proposed as a non-invasive biomonitoring tool for environmental pollutants (Stockholm Convention, United Nations Environmental Programme (UNEP) and the Oslo Paris Convention (OSPAR)). The sampled areas were 8 main gull colonies of the Iberian Peninsula and represented Mediterranean and Atlantic environments. The potential risks of PFCs on eggshell thickness and egg size is discussed, as described earlier for DDTs (Lundholm, 1997; Ratcliffe, 1967; Verboven et al., 2008).

2. Materials and methods

2.1. Chemicals and reagents

Native compounds of perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorobutane sulfonate (PFBS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) were supplied by Wellington Laboratories (Ontario, Canada). Stock standard solutions were prepared in acetonitrile at a concentration of 5 ng/μL and were stored at −18 °C. Perfluoro-n-(1,2,3,4-$^{13}C_4$) octanoic acid (m-PFOA) and sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octane sulfonate (m-PFOS), also from Wellington Laboratories, were used as internal standards. HPLC grade water and acetonitrile were supplied by Merck (Darmstadt, Germany) and glacial acetic acid from Panreac (Barcelona, Spain).

2.2. Sampling areas and preparation for analysis

Fresh eggs of Yellow-legged gull (*Larus michahellis*) were collected from 8 National or Natural Parks of the Iberian Peninsula in March–May 2009 to determine the occurrence of PFCs (Fig. 1). These sites represent the most important gull colonies of the Iberian Peninsula (Bermejo et al., 2009). These included the northern Mediterranean colonies (Medes Islands, Ebro Delta and Columbretes Islands), the southern Mediterranean (Dragonera Island, Grosa Island and Chafarinas Islands) and the Atlantic (Berlengas Islands and Cies Islands). The western Mediterranean was split into northern and southern areas because hydrographical data indicate the existence of two major units influenced respectively by the Algerian current and the Northern current (Millot, 1987, 1999). The limit between currents varies seasonally and annually (Pinot et al., 2002), but the 37.8 isohaline, as established in MEDATLAS II climatology (http://www.ifremer.fr/sismer/program/medar/), seems to be a convenient frontier between the two regions (Fig. 1).

At each colony, 36 eggs were randomly collected in 3 different subcolonies (12 eggs in total per subcolony). This sampling approach is proposed by UNEP and OSPAR guidelines since it covers the geographical variability of the eggs within each colony and does not cause any effect in the colony. Eggs were halved and one half was used for individual analysis and another half was pooled with the other 12 eggs of each subcolony. As a result, a total of 3 pooled samples were analyzed per colony, except in Columbretes islands and Dragonera, where only 12 eggs were found and so one pooled sample was analyzed. In Berlengas islands, 4 pooled samples were analyzed. The PFCs were analyzed in individual and pooled egg samples to validate the sampling procedure (Fig. 2). The first egg was sampled since it represents the maximum pollutant transfer levels from female to eggs, as demonstrated for PCBs, dioxins and furans (Pastor et al., 1995b), and for comparison among different colonies. Embryonated eggs were not used.

2.3. Egg and eggshell parameters

Eggs were transported inside cool boxes to the laboratory. The following egg parameters were measured before being opened: (i) the length and width and (ii) the weight of the whole egg. Afterwards, eggshells were dried at room temperature for one month and then the following was measured: (iii) the weight of the dried shell and (iv) the eggshell thickness. The eggshell thickness was measured at three points around the equator, using a Ratio digital calliper with a precision of ±0.01 mm.

The eggshell thickness index (I) values were calculated for all the eggs according to the method given by Ratcliffe (1967).

The egg volume was calculated using the formula $V = Kv^{*}L^{*}Wd^{2}$ given by Hoyt (1979), where Kv is a species-specific constant whose value is 0.000477 for *Larus michahellis* (Oro, 2008), L = length (mm) and Wd = width (mm) of the egg.

Desiccation index (Di) was calculated for each egg as a measure of functional quality according to the formula given by Helander et al. (2002). Di expresses the mean density of the entire egg content at the time of laying because the egg immediately begins to lose weight by diffusion of water vapour. A low Di value indicates desiccated eggs.

2.4. Extraction

The PFCs were solid–liquid extracted from wet samples using acetonitrile, based on the method of Meyer et al. (2009). About 1 g of sample was weighted in polypropylene tubes and internal standards (m-PFOS and m-PFOA) were added at a concentration of 100 ng/g-ww and incubated for 18 h at 4 °C. Nine ml of acetonitrile was then added and the sample was thoroughly mixed using a vortex mixer. Samples were then extracted in an ultrasonic bath for 10 min at room temperature (3 times). Between each period of 10 min, the samples were thoroughly mixed. Afterwards the samples were centrifuged at 2500 rpm for 5 min. The supernatant was transferred to a new vial and evaporated to dryness. Then 1 mL of acetonitrile was added to the dried sample and incubated for 10 min in the ultrasonic bath. The samples were purified by adding 25 mg of activated carbon and 50 μL of glacial acetic acid and were vigorously mixed for 1 min. Afterwards, the samples were centrifuged for 10 min at 10,000 rpm. One mL of the supernatant was transferred to a clean micro vial and 350 μL were diluted with 150 μL of HPLC water and analyzed.

Fig. 1. Map showing the Yellow-legged gull colonies that were sampled in March–May 2009. In each colony, 36 eggs were collected, divided in 12 eggs in 3 subcoloniesof each colony. The dashed line shows the limit between the north and south basins in the western Mediterranean.

2.5. Instrumental analysis

The PFCs were measured using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). The system consisted of Acquity Ultra performance LC system (Waters, USA) connected to a Triple Quadruple Detector Acquity Ultra performs LC. An Acquity UPLC BEH C_{18} column (1.7 μm particle size, 50 mm × 2.1 mm, Waters, USA) was used as mobile phase residue trap to remove any contamination from the mobile phases. Ten μL of extract were injected. The analysis was performed on a LiChroCART HPLC RP-18e column (125 mm × 2 mm × 5 μm particle size, Merck, Germany). The mobile phase consisted of 2 mM NH_4OAc (A)/acetonitrile (B). Gradient elution was starting from 70% A and 30% B, increased to 90% B in 5 × min and to 100% B in 0.10 min and held for 1 min, at a flow rate of 0.4 mL/min. The chromatographic time was of 6 min. Then, initial conditions were regained in 1 min and the system was stabilized for 3 min at initial conditions. The various PFCs were measured under negative electrospray ionisation. The 2 transitions from parent to product ion used to identify each compound as well as the dwell time, cone voltage, collision energy and retention times are summarised in Fernández-Sanjuan et al. (2010). To identify the target compounds, the retention time and these 2 transitions were used. Internal standard quantification was performed using m-PFOS to quantify PFOS, PFHxS and PFBS and m-PFOA to quantify PFOA and PFNA.

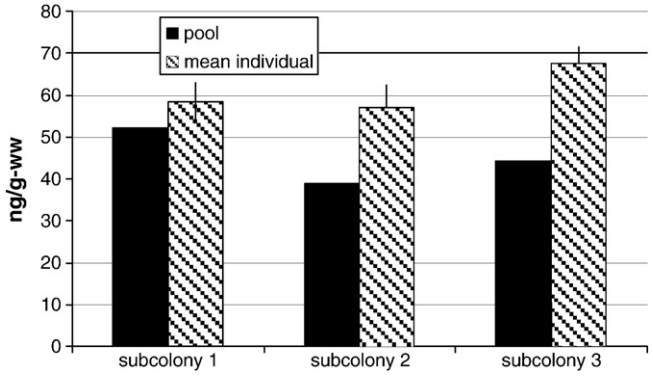

Fig. 2. Graph comparing the PFOS levels in the pooled mixture of 12 eggs and the mean concentration and relative standard deviation in individual Yellow-legged gull eggs ($n = 12$) for the 3 subcolonies in the Ebro delta.

2.6. Quality control parameters

The samples were extracted and analysed in batches together with a procedural blank to control any external contamination during the whole analytical process. A five point calibration curve was built over a concentration range of 1.25 to 50 ng/mL. The samples and quality controls were spiked with the internal standards at a concentration of 100 ng/g-ww. Recovery studies were performed twice by two operators and in duplicate using chicken eggs spiked with native compounds at concentrations of 10, 100 and 250 ng/g-ww. Instrument detection limits (LOD_{inst}) were calculated using the lowest concentration standard solution at 1.25 ng/mL for each compound and were calculated using three times the value of the signal-to-noise ratio (the ratio between the peak intensity and the noise 1 min after the peak signal). Method detection limits (LOD_{method}) were calculated in the same way, using spiked chicken eggs at 10 ng/g-ww.

3. Results and discussion

3.1. Sampling and method performance

The first step was to test the performance of the sampling procedure. Fig. 2 compares the PFOS values from analyzing 12 individual eggs with the PFOS concentration from the pooled sample ($n = 12$) from Yellow-legged gull eggs collected from 3 subcolonies of the Ebro delta site. We compared each pooled sample (i.e. single observation) with the mean of the individual eggs of each subcolony by a modified *t*-test. In all cases the pooled samples did not differ from the individual egg samples (subcolony 1: $t_{10} = 1.691$ $p = 0.122$; subcolony 2: $t_{10} = 2.199$ $p = 0.052$; subcolony 3: $t_{11} = 1.356$ $p = 0.202$; Fig. 2). On the other hand, the PFOS concentration from the individual egg did not differ between the three subcolonies ($F_{2,31} = 0.848$, $p = 0.437$). This indicates the very low variability of PFOS within the individuals of a colony and therefore, the sampling of 36 eggs is indicative of the pollution level within the whole colony.

Regarding the analytical performance, good resolution and selectivity were obtained indicating that lipids were totally removed during the extraction step and no analyte suppression was observed. In addition, the solvent trap installed prior to the analytical column avoided any external contamination from the HPLC system and only traces of PFNA were detected below the LOD. Acquisition was performed in a 6 min chromatogram, which permitted a high sample throughput and solvent saving. The method was particularly

Table 1
Description of the colonies sampled, ordered from northeast to northwest of the Iberian Peninsula.

Colony	Category	Coordinates		Distance from mainland (km)	Altitude in the middle (m)	Feeding habits (chicks)	Reference
Medes	Marine reserve	3º13'E	42º02'N	0.87	63	Refuse tips, pelagic and benthonic preys	Ramos et al., 2009
Ebro Delta	Natural park	0º40'E	40º35'N	0	0	Pelagic preys, crops and terrestrial preys and refuse tips	Ramos et al., 2009
Columbretes	Nature reserve	0º41'E	39º54'N	53.78	Not available	Most pelagic, but also brackish and freshwater preys	Ramos et al., 2009
Dragonera	Natural park	2º18'E	39º35'N	0.94	175	Increasing trophic spectrum and refuse tips feeding	Bermejo et al., 2009
Grosa	Natural park	0º42'W	37º43'N	1.57	34	Refuse tips, brackish, freshwater and pelagic preys	Ramos et al., 2009
Chafarinas, Rey	Hunting ground	2º25'W	35º10'N	3.3	73	Most pelagic preys, but also benthic, terrestrial preys and refuse tips	González-Solís et al., 1997
Chafarinas, Congreso sur		2º26'W	35º10'N	6.67	6		
Berlengas	Nature reserve	9º30'W	39º24'N	10.32	74	Most refuse tips	Personal communication
Cies	National park	8º54'W	42º13'N	2.54	70	Most pelagic preys, but also benthic preys and refuse tips	Moreno et al., 2010

repetitive (<14%, $n=18$ injections during 5 days). Table 2 provides the quality parameters of the method used. As regards to the extraction efficiency, by extracting 1 g of chicken eggs samples, PFCs recoveries were between 86 ± 1 and 127 ± 4% at 10 ng/g-ww spiking level, between 84 ± 3 and 125 ± 10% at 100 ng/g-ww spiking level and from 82 ± 3 to 116 ± 9% at 250 ng/g-ww spiking level. Recoveries were performed by 2 independent chemists and results were equal, indicating the robustness of the method. m-PFOS and m-PFOA were used to evaluate the extraction efficiency in Yellow-legged gull egg samples and recoveries were of 92 ± 7% and 85 ± 10%, respectively. Chicken eggs used for spiking experiments did not have any PFC contribution. Another important issue is the sensitivity of the method. The LOD obtained were between 0.09 and 0.30 ng/g-ww, similar to those calculated for Zebra mussels (*Dreissena polymorpha*) (Fernández-Sanjuan et al., 2010). Overall, the method proved highly robust, repetitive and selective for spiked chicken samples and thus, able to be used for monitoring PFCs in the eggs of Yellow-legged gull.

3.2. Spatial distribution of PFCs in Yellow-legged gull eggs

Among the 5 PFCs studied, PFOS was the only compound detected in Yellow-legged gull eggs from all colonies at mean levels from 10.1 to 54.0 ng/g-ww. This is in accordance to previous studies where PFOS accounted for >90% of perfluorinated sulfonates in Herring gull eggs (*Larus argentatus*) across the Laurential Great Lakes of North America (Gebbink et al., 2011) and was the predominant perfluorinated alkyl substance in Falcon eggs (*Falco peregrinus*) (Holmström et al., 2010). As regards to perfluorocarboxylic acids, PFOA and PFNA were not detected although longer chain length PFCAs (C10–C15) should be explored as they have been reported in eggs from gull species and other wild birds at higher concentrations compared to the PFOA and PFNA (Löfstrand et al., 2008; Gebbink et al., 2009; Holmström et al., 2010).

From a north to south and east to west distribution, Fig. 3 shows the concentration of PFOS in gull eggs from each colony in the Iberian Peninsula, where each value is the average concentration of 12 pooled eggs in 3 subcolonies collected at each colony. The presence of PFOS in Yellow-legged gull eggs was colony dependant and differed among them (Fig. 3; $F_{2,17}=63.42$, $p<0.001$). Differences were found between northern Iberian Peninsula colonies (Medes Islands, Ebro delta and Columbretes), that contained the highest PFOS levels, and the southern Mediterranean colonies (Dragonera, Grosa island and Chafarinas) and the Atlantic colonies (Berlengas and Cies) (Tukey's HSD test, $p<0.001$). Among the southern Mediterranean and Atlantic areas, no differences were found (Tukey's HSD test, $p=0.371$).

Seawater pollution, feeding ecology and distance to land play an important role in the accumulation of PFCs (Schiavone et al., 2009). In addition to that, we found the PFOS exposure scenarios were colony-specific and responded to emission and environmental pressures.

Considering seawater pollution, the Mediterranean is an enclosed basin that has limited exchange of deep water with outer oceans and where the water circulation is dominated by salinity and temperature differences rather than winds. Such geographic and climatological configuration make the basin a sink of pollutants, derived from river discharges, wastewater discharges through treated (emmisaries) or

Table 2
Quality parameters of the method such as response factor (F), regression coefficient (R^2), repetitivity, recovery at 10, 100 and 250 ng/g, limits of detection, LOD ng/g-ww and blank levels.

Compound	F (slope)	R^2	Repetitivity %	%R[a]			LOD (ng/g-ww)	Blank levels[b]
				Low (10 ng/g)	Medium (100 ng/g)	High (250 ng/g)		
PFBS	2.44	0.997	14	107 ± 15 93 ± 1	89 ± 12 96 ± 3	97 ± 12 93 ± 4	0.10	n.d.
PFHxS	5.2	0.996	11	105 ± 13 86 ± 1	85 ± 10 84 ± 3	94 ± 10 82 ± 3	0.20	n.d.
PFOA	3.52	0.984	7	108 ± 7 116 ± 5	110 ± 7 107 ± 9	102 ± 4 108 ± 5	0.09	n.d.
PFNA	1	0.988	10	118 ± 9 127 ± 4	125 ± 10 115 ± 9	116 ± 9 116 ± 4	0.13	<LOD
PFOS	1.83	0.999	7	118 ± 11 105 ± 4	92 ± 11 94 ± 4	99 ± 2 104 ± 3	0.30	n.d.

n.d. = not detected.
[a] The percentage recoveries in spiked chicken samples were performed by two operators and in duplicate.
[b] Blank levels were measured with chicken eggs spiked with the internal standards.

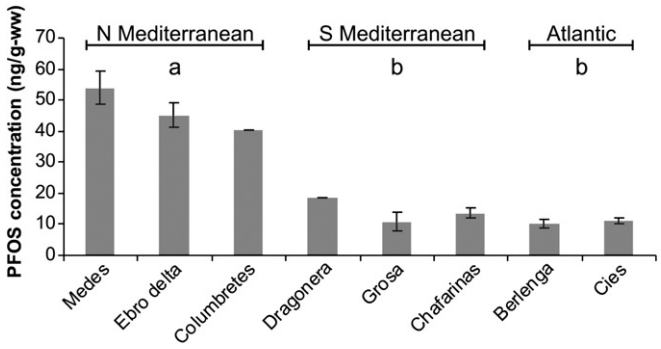

Fig. 3. Mean concentration of PFOS in each colony for the three marine areas of the Iberian Peninsula. Each measurement corresponds to 3 pooled samples ($n = 12$ eggs each) of the 3 sites of each colony, except for Dragonera and Columbretes where only 12 eggs were obtained, and Berlengas which measurement corresponds to 4 pooled samples. Different letters indicate significant differences ($p < 0.001$) among marine areas.

untreated wastewaters, and run-off (Sánchez-Avila et al., 2009). As a result, pollution in this region has been extremely high in recent years. The United Nations Environment Programme has estimated that 650 million tons of sewage, 129,000 tons of mineral oil, 60,000 tons of mercury, 3800 tons of lead and 36,000 tons of phosphates are dumped into the Mediterranean each year (www.wissenschaftsparlament.eu). As regards to PFCs, Sánchez-Avila et al. (2010) has estimated a daily input of 190 g of PFCs to the NW Mediterranean based on 8 wastewater discharge emmisaries and 6 Catalan rivers and Loos et al. (2008) estimated a mass load contribution around 300 g PFOA per hour or 2.6 tons per year to the Adriatic Sea. Differing from those conditions, the Atlantic coast is characterized by a more severe climate which is influenced by the temperatures of the surface waters, water currents and winds. Because of the ocean's great extension and the important oceanic currents, PFC pollution in the Atlantic coast is lower than in the Mediterranean (Yamashita et al., 2005; Gómez et al., 2011). Differences among northern Mediterranean colonies (Medes, Ebre and Columbretes) with southern Mediterranean (Dragonera, Grosa Island and Chafarinas) and Atlantic (Berlengas and Cies) might be in part due to differences in waterbodies generated by sea currents. The Catalan–Balearic sea in Northern Mediterranean carries northern cold waters from the Gulf of Lion southward along the continental slope in the Balearic Sea. This current bifurcates in the northern end of the Eivissa Channel. On the other hand, the SW Mediterranean is connected by a stream of Atlantic water that flows eastward from the Strait of Gibraltar to the Sicilian channel through the Algerian current (Millot, 1999). Temporary eddies leave the Algerian current and supply the Balearic Islands with Atlantic water (López-Jurado, 1990; Millot, 1999), thereby expanding northward the influence of Atlantic water. Maybe this high influence of the Atlantic water in the southern Mediterranean reduces the concentration of PFOS in Dragonera, Grosa Island and Chafarinas (Fig. 1). However, more studies are needed to evaluate the concentration of PFOS within the Mediterranean.

In addition to sea currents, anthropogenic pressures play an important role in the accumulation of PFCs in Yellow-legged gull eggs, as suggested by Gebbink et al. (2009). Within the Iberian Peninsula, northern Mediterranean colonies (Fig. 1) which have the highest PFOS levels are settled in areas characterized by a dynamic industrial sector with textile, metalurgic and chemical factories as the main activities and also represent a highly populated area. On the other hand, Dragonera, Grosa Island, Chafarinas, Berlengas and Cies do not receive direct industrial and urban discharges, but rather agricultural run-off. Similar to this study, Giesy and Kannan (2001) detected lower PFCs levels in wildlife from remote areas (Arctic and the North Pacific Oceans) than in urban and industrial regions. In the USA, Gebbink et al. (2009) found that the accumulation of PFCs in Herring gull eggs

Table 3
Egg characterization from each colony.

Colony	Weight (g)		Length (mm)		Width (mm)		Thickness (mm)		Dried eggshell weight (g)		Eggshell thickness index I = Wt (mg)/L (mm)*Wd (mm)		Volume (mL) V = 0.000477*L (mm)*Wd² (mm)		Desiccation index Di = (Wt egg (g) − Wtshell (g))/V (mL)	
	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM
Medes Is.	91.2	0.9	71.2	0.4	49.7	0.3	0.368	0.005	6.2	0.1	1.74	0.02	84.0	1.2	1.015	0.0071
Ebro Delta	89.7	1.3	70.7	0.5	48.8	0.3	0.385	0.005	6.2	0.1	1.78	0.02	80.4	1.2	1.039	0.002
Columbretes	85.5	3.1	69.9	0.9	48.1	0.6	0.377	0.006	6.1	0.3	1.81	0.07	77.4	2.9	1.026	0.012
Dragonera	85.6	2.5	69.0	0.9	47.2	0.6	0.454	0.014	5.5	0.2	1.67	0.04	73.7	2.2	1.092	0.021
Grosa	89.2	1.3	70.3	0.6	48.8	0.3	0.370	0.005	6.1	0.1	1.77	0.03	80.1	1.3	1.038	0.004
Chafarinas	82.7	1.3	69.2	0.6	48.7	0.2	0.395	0.008	6.0	0.11	1.78	0.02	78.3	1.2	0.982	0.010
Berlengas	82.8	0.8	68.4	0.3	47.7	0.2	NA	NA	NA	NA	NA	NA	74.2	0.7	NA	NA
Cies	77.6	1.1	67.7	0.4	46.3	0.3	0.368	0.005	5.5	0.1	1.74	0.02	69.4	1.0	1.039	0.006

(*Larus argentatus*) was highly lake- and/or colony-dependant, showing higher concentrations in eggs from colonies in close proximity to highly urbanized and industrialized sites in Lakes Erie and Ontario.

Feeding habits account for the main input and accumulation of PFCs in birds. Gebbink et al. (2011) studied dietary tracers [δ^{15}N and δ^{13}C stable isotopes (SIs)] and revealed that PFCs exposure is colony dependant. Dietary tracers are useful to determine the feeding habits of each colony. Yellow-legged gulls from the Iberian Peninsula feed basically on both aquatic (marine and freshwater) and terrestrial prey, including fish discards and refuse tips, but when the colonies are close or in the mainland, waste becomes an important food source. Ramos et al. (2009) studied the feeding habits of Yellow-legged gulls chicks in the western Mediterranean colonies and found that younger chicks fed on invertebrates while older chicks used refuse dumps as well as fishery discards. On the other hand, *Larus audouinii* and *Larus cachinnans* from Chafarinas feed on pelagic and benthic preys, but also on fish, and on terrestrial preys and refuse tips (González-Solís et al., 1997). SI signatures suggested that gulls from most marine colony sites are exposed to PFCs via marine prey. On the other hand, for the freshwater sites, egg SIs suggested both aquatic and terrestrial prey consumption as the source for PFC exposure depending on the colony and differences between marine and terrestrial biota were observed (Holmström et al., 2010).

Finally, distance to mainland is another factor that influences feeding ecology. Among Yellow-legged gull colonies studied, Columbretes is the most separated colony from mainland (≈54 km, Table 1) which cause a fish-based diet rather than waste-based (Duhem et al., 2005; Ramos et al., 2009). The high PFOS levels in this colony (40.5 ng/g-ww) were attributed to its geographical location and to the specific food habits, since Columbretes is a Natural Park with no anthropogenic pressure.

Table 4 gives evidence of the worldwide spread distribution of PFCs by comparing PFOS concentration in eggs of several bird species, including the present results of Yellow-legged gull from the Iberian Peninsula. Levels of the present study were similar or lower than most of the results found in literature, most of them referring to the northern hemisphere. The lowest PFOS concentration was detected in two Antarctic penguin species (*Pygoscelis adeliae* and *Pygoscelis papua*) (Schiavone et al., 2009) that presented 2–3 orders of magnitude lower concentration than in other sites, including the Norwegian Arctic (Verreault et al., 2005). Overall, interspecies ecological and feeding habits play an important role in PFC accumulation in birds and therefore bird eggs become a very useful matrix to determine the impact of pollutants within an area.

3.3. Effects of PFOS on egg parameters

It is recognized that some anthropogenic substances released into the environment can affect avian egg production. Exposure of female birds to DDTs and PCBs, for example, is known to reduce eggshell thickness and egg size (Lundholm, 1997; Ratcliffe, 1967; Verboven et al., 2008). Other studies indicated that high levels of hexachlorobenzene, oxyclordane, DDE, and PCBs correlate with a worse condition of gull chicks (Bustnes et al., 2000, 2008). A decrease in shell thickness due to DDT exposure has been described as the major cause of population decline in raptors from Spain (Martinez-Lopez et al., 2007). Also, effects in embryonic development have been described (Cortinovis et al., 2008; Hurk et al., 2007; Verboven et al., 2008) as well as at population and community level (González-Solís et al., 2002). Therefore, risk assessment tools have been proposed to determine the effects of the POPs in birds (Strause et al., 2007).

As regards to PFCs, little is known on the effects they may cause on birds. Newsted et al. (2006, 2007) reported that PFCs induce liver weight gain in the Mallard (*Anas platyrhynchos*) and the Northern Bobwhite (*Bobwhite virginianus*), a decrease in body weight and a decrease in the length of testes. In field studies, Hoff et al. (2005) indicated that there is a significant increase in the activity of alanine aminotransferase, which is a biomarker of liver damage. Also, a decrease in serum cholesterol and triglycerides levels were correlated with increased levels of PFOS, which suggests that PFOS affect the metabolism of lipids in the exposed organisms. In addition, negative association between concentrations of PFOS in eggs and hatching success was reported at PFOS concentrations as low as 150 ng/g-ww (Custer et al., in press). Finally, impaired hatching success and sublethal toxicological effects from PFOS exposure in the Swedish Peregrine falcon (*Falco peregrinus*) were not ruled out (Holmström et al., 2010). Newsted et al. (2005) described Toxicity Reference Values (TRVs) and Predicted No Effect Concentrations (PNECs) for PFOS

Table 4
Levels of PFOS in eggs of several bird species, ordered according to the sample location.

Specie	Common name	Location	Levels (ng/g ww)	Reference
Falco peregrinus	Swedish peregrine falcon	Sweden	40–220	Holmström et al., 2010
Phalacrocorax aristotelis Somateria mollissima	Common shag	Norway	28.9	Herzke et al., 2009
	Common eider		14.6–29	
Larus hyperboreus	Glaucous gull	Norwegian Arctic	51.7–196	Verreault et al., 2005
Phalacrocorax carbo	Cormorant	Germany	14–1451	Rüdel et al., 2011
Corvus frugilegus	Rook		0.6–16.5	
Uria aalge	Guillemot	North western Europe	3.2–760	Löfstrand et al., 2008
Nycticorax nycticorax	Night heron	South China	22.6–343	Wang et al., 2008
Ardea alba,	Great egret		14.4–27.7	
Egretta garzetta	Little egret		31.6–87.2	
Egretta garzetta	Little egret	Korea	30.4–1205	Yoo et al., 2008
Ardea herodias	Great blue heron	Minnesota	34–1848	Custer et al., 2010
Ardea herodias	Great blue heron	North America	45.9–9453	Custer et al., 2009
Pandion haliaetus	Osprey	North America	115–291	Rattner et al., 2004
Phalacrocorax auritus	Double-crested Cormorant	North America	170	Giesy and Kannan, 2001
Larus argentatus	European Herring gull		73	
Larus delawarensis	Ring-billed gull	North America	67	Kannan et al., 2001
Larus argentatus	European Herring gull	North America	484–507	Gebbink et al., 2009
Larus glaucescens	White-headed gull	Canada	7.15–599	Gebbink et al., 2011
L. californicus	California gull			
L. delawarensis	Ring-billed gull			
L. argentatus	European Herring gull			
Pygoscelis papua Pygoscelis adeliae	Gentoo Penguin	Antarctica	0.29	Schiavone et al., 2009
	Adélie Penguin		0.38	
Larus michahellis	Yellow-legged gull	Iberian Peninsula	10.1–54.0	Present study

based on two avian species (*Anas platyrhynchos* and *Colinus virginianus*). For PFOS, TRV for eggs was of 1.7 μg/mL and PNEC was of 1.0 μg/mL. However, an uncertainty value of 6 was used in order to protect other avian species because the analysis was carried out on only two bird species (Newsted et al., 2005).

In this study, differences among egg parameters (Table 3) were evaluated to determine the potential effects of PFOS during egg development. In a first instance, differences among colonies were significant for all egg measurements (length: $F_{7,233}=6.28$, $p<0.001$; width: $F_{7,233}=13.21$, $p<0.001$; weight: $F_{7,233}=2.67$, $p=0.011$; volume: $F_{7,233}=15.66$, $p<0.001$; thickness: $F_{6,186}=14.61$, $p<0.001$; eggshell weight: $F_{6,186}=7.89$, $p<0.001$), as well for desiccation index ($F_{6,186}=11.83$, $p<0.001$), but not for eggshell thickness index ($F_{6,186}=1.94$, $p=0.077$). Thickness, dried thickness weight and the two indexes were not available for the eggs from Berlengas islands because these parameters were not measured when sampling. Finally, there was no significant correlation between the eggshell thickness index or the desiccation index and the PFOS concentration (Spearman rank correlation, both $p>0.05$), indicating that the levels of PFOS in Yellow-legged gull do not affect shell parameters and thus, have no impact at this stage.

4. Conclusions

Gull eggs have been proposed as a biomonitoring matrix to determine the presence of contaminants (Stockholm Convention, United Nations Environmental Programme (UNEP) and the Oslo Paris Convention (OSPAR)). Our target species, the Yellow-legged gull, is omnivorous and also an opportunistic species, sedentary within each colony and very common along the Iberian Peninsula coast. Its characteristic biology makes this species interesting as a pollution indicator. PFOS was the only compound detected in whole egg samples and its concentration was higher in the most industrialized sites of the Catalan coast (Medes, Ebro and Columbretes) compared to the southern Mediterranean (Dragonera, Mar Menor and Chafarinas) or Atlantic colonies (Berlengas and Cies). Nonetheless, the concentrations detected did not induce any negative effect upon egg shell parameters. Except for eggshell thickness index, significant differences were found for egg weight, length, width, eggshell weight, volume and Dessication index between colonies. However, negative effects at other stages of development or in the long term cannot be ruled out given the high accumulation potential of PFC in Yellow-legged gull eggs.

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References

Bermejo A, Molina B, Cantos FJ, Mouriño J. Gaviotas reidora, sombría y patiamarilla en España. Población en 2007-2009 y método de censo. Ed. Blas Molina. Madrid: SEO/BirdLife; November 2009. pp 33–49. ISBN 878-84-937351-2-8.

Bustnes JO, Erikstad KE, Bakken V, Mehlum F, Skaare JU. Feeding ecology and the concentration of organochlorines in glaucous gulls. Ecotoxicology 2000;9:179–86.

Bustnes JO, Fauchald P, Tveraa T, Helberg M, Skaare JU. The potential impact of environmental variation on the concentrations and ecological effects of pollutants in a marine avian top predator. Environ Int 2008;34:193–201.

Cortinovis S, Galassi S, Melone G, Saino N, Porte C, Bettinetti R. Organochlorine contamination in the Great Crested Grebe (*Podiceps cristatus*): effects on eggshell thickness and egg steroid levels. Chemosphere 2008;73:320–5.

Custer TW, Kannan K, Tao L, Saxena AR, Route B. Perfluorinated compounds and polybrominated diphenyl ethers in great blue heron eggs from Indiana Dunes National Lakeshore. Indiana J Great Lakes Res 2009;35:401–5.

Custer TW, Kannan K, Tao L, Yun SH, Trowbridge A. Perfluorinated compounds and polybrominated diphenyl ethers in great blue heron eggs from three colonies on the Mississippi River, Minnesota. Waterbirds 2010;33:86–95.

Custer CM, Custer TW, Schoenfuss HL, Poganski BH, Solem L. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. Reprod Toxicol in press.

Duhem C, Vidal E, Roche P, Legrand J. How is the diet of Yellow-legged Gull chicks influenced by parents' accessibility to landfills? Waterbirds 2005;28:46–52.

Fernández-Sanjuan M, Meyer J, Damásio J, Faria M, Barata C, Lacorte S. Screening of perfluorinated chemicals (PFCs) in various aquatic organisms. Anal Bioanal Chem 2010;398:1447–56.

Furness RW, Camphuysen K. Seabirds as monitors of the marine environment. ICES J Mar Sci 1997;54:726–37.

Gebbink WA, Hebert CE, Letcher RJ. Perfluorinated carboxylates and sulfonates and precursor compounds in herring gull eggs from colonies spanning the Laurentian Great Lakes of North America. Environ Sci Technol 2009;43:7443–9.

Gebbink WA, Letcher RJ, Burgess NM, Champoux L, Elliott JE, Hebert CE, et al. Perfluoroalkyl carboxylates and sulfonates and precursors in relation to dietary source tracers in the eggs of four species of gulls (Larids) from breeding sites spanning Atlantic to Pacific Canada. Environ Int 2011;37:1175–82.

Giesy JP, Kannan K. Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 2001;35:1339–42.

Gómara B, González MJ, Baos R, Hiraldo F, Abad E, Rivera J, et al. Unexpected high PCB and total DDT levels in the breeding population of red kite (*Milvus milvus*) from Doñana National Park, south-western Spain. Environ Int 2008;34:73–8.

Gómez C, Vicente J, Echavarri-Erasun B, Porte C, Lacorte S. Occurrence of perfluorinated compounds in water, sediment and mussels from the Cantabrian Sea (North Spain). Mar Pollut Bull 2011;62:948–55.

Gonzalez MJ, Fernandez MA, Hernandez LM. Levels of chlorinated insecticides, total PCBs and PCB congeners in Spanish gull eggs. Arch Environ Contam Toxicol 1991;20:343–8.

González-Solís J, Oro D, Jover L, Ruiz X, Pedrocchi V. Trophic niche width and overlap of two sympatric gulls in the southwestern Mediterranean. Oecologia 1997;112:75–80.

González-Solís J, Sanpera C, Ruiz X. Metals and selenium as bioindicators of geographic and trophic segregation in giant petrels Macronectes spp. Mar Ecol Prog Ser 2002;244:257–64.

Guitart R, Clavero R, Mateo R, Máñez M. Levels of persistent organochlorine residues in eggs of greater flamingos from the Guadalquivir marshes (Doñana), Spain. J Environ Sci Health B 2005;40:753–60.

Haukås M, Berger U, Hop H, Gulliksen B, Gabrielsen GW. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. Environ Pollut 2007;148:360–71.

Helander B, Olsson A, Bignert A, Asplund L, Litzén K. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. Ambio 2002;31:386–403.

Herzke D, Nygard T, Berger U, Huber S, Rov N. Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: a suburban to remote pollutant gradient. Sci Total Environ 2009;408:340–8.

Hoff PT, Van de Vijver K, Dauwe T, Covaci A, Maervoet J, Eens M, et al. Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings. Chemosphere 2005;61:1558–69.

Holmström KE, Johansson A-K, Bignert A, Lindberg P, Berger U. Temporal trends of perfluorinated surfactants in Swedish peregrine falcon eggs (*Falco peregrinus*), 1974–2007. Environ Sci Technol 2010;44:4083–8.

Hoyt DF. Practical methods of estimating volume and fresh weight of bird eggs. The Auk 1979;96:73–7.

http://www.wissenschaftsparlament.eu/forums/2010-water-science-economy/1155/mediterreanean-sea-pollution.

Hurk P, Wiley PE, Lavoie ET, Grasman KA, Bowerman W. Activity patterns of biotransformation enzymes in juvenile chickens after in ovo dosage of PCB126. Comp Biochem Physiol C 2007;146:301-3077.

Jiménez B, Merino R, Abad E, Rivera J, Olie K. Evaluation of organochlorine compounds (PCDDs, PCDFs, PCBs and DDTs) in two raptor species inhabiting a Mediterranean island in Spain. Environ Sci Pollut Res 2007;14:61–8.

Kannan K, Hilscherova K, Imagawa T, Yamisshita N, Williams LL, Giesy JP. Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ Sci Technol 2001;35:3065–70.

Kannan K, Choi JW, Iseki N, Senthilkumar K, Kim DH, Masunga S, et al. Concentrations of perfluorinated acids in livers of birds from Japan and Korea. Chemosphere 2002;49:225–31.

Löfstrand K, Jörundsdóttir H, Tomy G, Svavarsson J, Weihe P, Nygård T, et al. Spatial trends of polyfluorinated compounds in guillemot (*Uria aalge*) eggs from North-Western Europe. Chemosphere 2008;72:1475–80.

Loos R, Locoro G, Huber T, Wollgast J, Christoph EH, De Jager A, et al. Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy. Chemosphere 2008;71:306–13.

López-Jurado JL. Masas de agua alrededor de las Islas Baleares. Bol Inst Esp Oceanogr 1990;6:3-20.

Lundholm CE. DDE-induced eggshell thinning in birds: Effects of p, p′-DDE on the calcium and prostaglandin metabolism of the eggshell gland. Comp Biochem Physiol C Pharmacol Toxicol 1997;118:113–28.

Martinez-Lopez E, Maria-Mojica P, Martinez JE, Calvo JF, Wright J, Shore RF, et al. Organochlorine residues in booted eagle (*Hieraaetus pennatus*) and goshawk (*Accipiter gentilis*) eggs from southeastern Spain. Environ Toxicol Chem 2007;26:2373–8.

Meyer J, Jaspers VLB, Eens M, de Coen W. The relationship between perfluorinated chemical levels in the feathers and livers of birds from different trophic levels. Sci Total Environ 2009;407:5894–900.

Millot C. Circulation in the Western Mediterranean Sea. Oceanol Acta 1987;10:143–9.

Millot C. Circulation in the Western Mediterranean Sea. J Mar Syst 1999;20:423–42.

Moreno R, Jover Ll, Munilla I, Velando A, Sanpera C. A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. Mar Biol 2010;157:545–53.

Newsted JL, Coady KK, Beach SA, Butenhoff JL, Gallagher S, Giesy JP. Avian toxicity reference values for perfluorooctane sulfonate. Environ Sci Technol 2005;39: 9357–62.

Newsted JL, Beach SA, Gallagher SP, Giesy JP. Pharmacokinetics and acute lethality of perfluorooctanesulfonate (PFOS) to juvenile mallard and northern bobwhite. Arch Environ Contam Toxicol 2006;50:411–20.

Newsted JL, Coady KK, Beach SA, Butenhoff JL, Gallagher S, Giesy JP. Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. Environ Toxicol Pharmacol 2007;23:1–9.

Oro D. Living in a ghetto within a local population: an empirical example of an ideal despotic distribution. Ecology 2008;89:838–46.

Pastor D, Ruiz X, Barcelo D, Albaiges J. Dioxins, furans and AHH-active PCB congeners in eggs of 2 gull species from the western Mediterranean. Chemosphere 1995a;31: 3397–411.

Pastor D, Jover L, Ruiz X, Albaiges J. Monitoring organochlorine pollution in Audouin's Gull eggs: the relevance of sampling procedures. Sci Total Environ 1995b;162: 215–23.

Pinot J-M, López-Jurado JL, Riera M. The CANALES experiment. Interanual, seasonal, and mesoscale variability of the circulation in the Balearic Channels. Prog Oceanogr 2002;55:335–70.

Pusch K. Gull eggs – food of high organic pollutant content? J Environ Monit 2005;7: 635–9.

Ramos R, Ramirez F, Sanpera C, Jover L, Ruiz X. Diet of Yellow-legged Gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. J Ornithol 2009;150:265–72.

Ratcliffe DA. Decrease in eggshell weight in certain birds of prey. Nature 1967;215: 208–10.

Rattner BA, McGowan PC, Golden NH, Hatfield JS, Toschik PC, Lukei RF, et al. Contaminant exposure and reproductive success of ospreys (*Pandion haliaetus*) nesting in Chesapeake Bay regions of concern. Arch Environ Contam Toxicol 2004;47:126–40.

Rüdel H, Müller J, Jürling H, Bartel-Steinbach M, Koschorreck J. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from representative German ecosystems. Environ Sci Pollut Res 2011;18: 1457–70.

Sánchez-Avila J, Bonet J, Velasco G, Lacorte S. Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant. Sci Total Environ 2009;407:4157–67.

Sánchez-Avila J, Meyer J, Lacorte S. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ Pollut 2010;158:2833–40.

Schiavone A, Corsolini S, Kannan K, Tao L, Trivelpiece W, Torres D, et al. Perfluorinated contaminants in fur seal pups and penguin eggs from South Shetland, Antarctica. Sci Total Environ 2009;407:3899–904.

Strause KD, Zwiernik MJ, Im SH, Bradley PW, Moseley PP, Kay DP, et al. Risk assessment of great horned owls (*Bubo virginianus*) exposed to polychlorinated biphenyls and DDT along the Kalamazoo River, Michigan, USA. Environ Toxicol Chem 2007;26: 1386–98.

Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. Maternally derived testosterone and 17β-estradiol in the eggs of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Comp Biochem Physiol C 2008;148:143–51.

Verreault J, Houde M, Gabrielsen GW, Berger U, Haukås M, Letcher RJ, et al. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Environ Sci Technol 2005;39(19): 7439–45.

Verreault J, Berger U, Gabrielsen GW. Trends of perfluorinated alkyl substances in herring gull eggs from two coastal colonies in northern Norway: 1983–2003. Environ Sci Technol 2007;41:6671–7.

Wang Y, Yeung LWY, Taniyasu S, Yamashita N, Lam JCW, Lam PKS. Perfluorooctane sulfonate and other fluorochemicals in waterbird eggs from South China. Environ Sci Technol 2008;42:8146–51.

Yamashita N, Kannan K, Taniyasu S, Horii Y, Petrick G, Gamo T. A global survey of perfluorinated acids in oceans. Mar Pollut Bull 2005;51:658–68.

Yoo H, Kannan K, Kim SK, Lee KT, Newsted JL, Giesy JP. Perfluoroalkyl acids in the egg yolk of birds from Lake Shihwa, Korea. Environ Sci Technol 2008;42:5821–7.