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# Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies☆

Pere Colomer-Vidal [a], Albert Bertolero [b], Carles Alcaraz [c], Elba Garreta-Lara [a], Francisco Javier Santos [d], Silvia Lacorte [a,*]

[a] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Catalonia, Spain
[b] Associació Ornitològica Picampall de les Terres de l'Ebre, Amposta, Spain
[c] IRTA-Marine and Continental Waters, Sant Carles de la Ràpita, Spain
[d] Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Catalonia, Spain

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ABSTRACT

Gull eggs are excellent bioindicators of environmental pollution as reflect the contamination levels of coastal areas, especially of persistent and bioacumulative compounds such as perfluoroalkyl substances (PFAS). This study aims to evaluate the geographical distribution and 10-year temporal trends (2009–2018) of 17 PFAS in eggs of two gull species (*Larus michahellis* and *Larus audouinii*) from 5 main Spanish colonies. ∑PFAS ranged from 13.7 ± 5.9 to 164 ± 17 ng $g^{-1}$ wet weight and higher concentrations were observed in *L. audouinii* than in *L. michahellis*. Perfluorooctane sulfonate (PFOS) was the predominant compound in all samples, followed by perfluoroundecanoic acid (PFUnA) and perfluorotridecanoic acid (PFTriDA). Perfluorododecanoic acid (PFDoA), perfluorodecanoic acid (PFDA) and perfluorooctanoic acid (PFNA) were also found in all studied areas but at lower concentrations, while perfluorooctanoic acid (PFOA) was only detected in the Medes Islands. Principal Component Analysis revealed the co-occurrence of the 6 detected PFAS, and differentiated samples from Ebro Delta and Medes Islands, both located in the North-Eastern Mediterranean Sea, with high contribution of all PFAS, from Chafarinas and Atlantic Islands with lower concentration levels and variability. Also, different patterns were observed among colonies, suggesting the fish-based diet plays an important role in PFAS bioaccumulation. In all colonies, except for the Medes Islands, ∑PFAS decreased through the 10-year study period, with PFOS, PFUnA, and PFTriDA showing a significant concentration reduction in a colony-specific manner. This study demonstrates the usefulness and importance of continuous systematic long-term monitoring to determine the geographical distribution and temporal variations of PFAS in marine protected areas using gull eggs as bioindicators of environmental pollution.

## 1. Introduction

Coastal areas are pivotal to sustain fisheries, preserve marine biodiversity and maintain the marine ecological equilibrium, but still, they receive high loads of contaminants on a daily basis (Sánchez-Avila et al., 2013, 2012). Among other contaminants, Perfluoroalkyl Substances (PFAS) are of concern because of their global occurrence and potential adverse effects (Sinclair et al., 2020). PFAS are synthetic chemicals with unique properties regarding heat stability, resistance to degradation, and the ability to repel both water and oil and have been used in a myriad of industrial processes and consumer products such as in the production of fluoropolymers, adhesives, lubricants, cosmetics, cleaners, stain-resistant and non-stick coatings, food packaging, electronics, pesticide adjuvants, fire foams, paper, and photographic products (Podder et al., 2021). They reach coastal waters from points sources as wastewater treatment plant effluents and marine emissaries (Giesy and Kannan, 2002; Gómez et al., 2011), diffuse sources such as river discharges (Sánchez-Avila et al., 2010), or run-off from contaminated soils and sediments (Munoz et al., 2019; Zareitalabad et al., 2013). Once in the marine environment, PFAS do not hydrolyze, photodegrade or biodegrade, and are considered persistent, bioaccumulate in fish and are biomagnified along with the food webs, especially those compounds with a longer carbon length (Giesy and Kannan, 2002; Conder et al., 2008).

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* Corresponding author.
*E-mail address:* slbqam@cid.csic.es (S. Lacorte).

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Marine birds are apex predators exposed to PFAS depending on the habitat, diet, behavior, and life strategy (Kannan et al., 2001). Gulls are excellent bioindicators of environmental pollution as PFAS are accumulated through the diet on a yearly basis (Bertolero et al., 2015) and are transferred to the entire clutches (Vicente et al., 2015). Thus, gull eggs have been used to assess the contamination levels and patterns of PFAS in areas of ecological interest (Gebbink et al., 2009; Gewurtz et al., 2016) and permit to deduce PFAS exposure of other protected and non-protected species sharing a habitat (Vicente et al., 2012). In Spain, the biomonitoring strategy using eggs from the opportunistic yellow-legged gull (*Larus michahellis*) and the protected Audouin gull (*Larus audouinii*) has been carried out for dioxins and furans (Morales et al., 2012) and for polychlorinated biphenyls, organochlorine pesticides, polybromodiphenyl ethers and short-chain chloroparaffins (Zapata et al., 2018), and has shown colony-specific differences based on the exposure sources. These species are widespread in coastal ecosystems of the Mediterranean area and their biomonitoring represents a non-invasive method to determine the impact of contaminants. In addition, systematic annual biomonitoring is valuable to determine temporal patterns of contaminants (Sebastiano et al., 2020) and to diagnose the efficiency of applied actions to mitigate the pollution impact in vulnerable areas, which are breeding grounds of many species.

Within this context, the objectives of the present study were: (i) to determine the geographical distribution and time trends of 17 PFAS in *L. michahellis* and *L. audouinii* eggs over the period 2009 to 2018 in five main Spanish breeding colonies; and (ii) to evaluate the different PFAS patterns between *L. michahellis* and *L. audouinii* that breed sympatrically in the Ebro Delta Natural Park but with a very different diet. Overall, we describe the advantages of long-term monitoring schemes using gull eggs as sentinel species to determine the occurrence of PFAS and to assess the trends considering the conservation status of the studied areas. This study is in line with the Stockholm Convention that has recently included perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), its salts, and related compounds with the purpose to protect human health and the environment, identify contaminated areas and define management action to minimize the occurrence of these compounds (Stockholm Convention, 2019).

## 2. Material and methods

### 2.1. Study species and areas

The long-term biomonitoring was done using eggs from *L. michahellis* and *L. audouinii*. *L. michahellis* is a big sized marine gull with an increasing population along the western Mediterranean Sea, west Moroccan coast, and northeast Atlantic coast (BirdLife International, 2019). They have high feeding adaptability, from natural prey to fishing discards, rubbish tips, and nestlings from other birds (Ramos et al., 2009a, 2009b). *L. audouinii*, it is a medium-sized gull that breeds mostly in the western Mediterranean and winter in the northern and western coast of Africa and is included in the IUCN 2019 Red List as Least Concern (BirdLife International, 2019). It is mainly a piscivorous species, feeding preferably on clupeiformes and perciformes (Navarro et al., 2010), but also exploit fishery discards and nocturnal purse seine fisheries (Arcos and Oro, 2002). Both species inhabit colonies that are protected areas with the status of marine Natural or National Parks, thus areas with a high level of protection but nevertheless affected by chemical pollution. Four main colonies were studied including the Ebro Delta Natural Park (thereafter Ebro Delta, hosting *L. michahellis* and *L. audouinii* colonies), the Montgrí, Medes and Baix Ter Natural Park (thereafter Medes), the Chafarinas Islands National Hunting Refuge (thereafter Chafarinas), and the Atlantic Islands of Galicia National Park (thereafter Atlantic Islands), hosting main Spanish *L. michahellis* colonies. Table S1 shows the location and characteristics of each colony studied.

### 2.2. Sampling

The sampling protocol used in this work follows a previous strategy (Vicente et al., 2012). Each colony was divided into 3 sub-colonies to

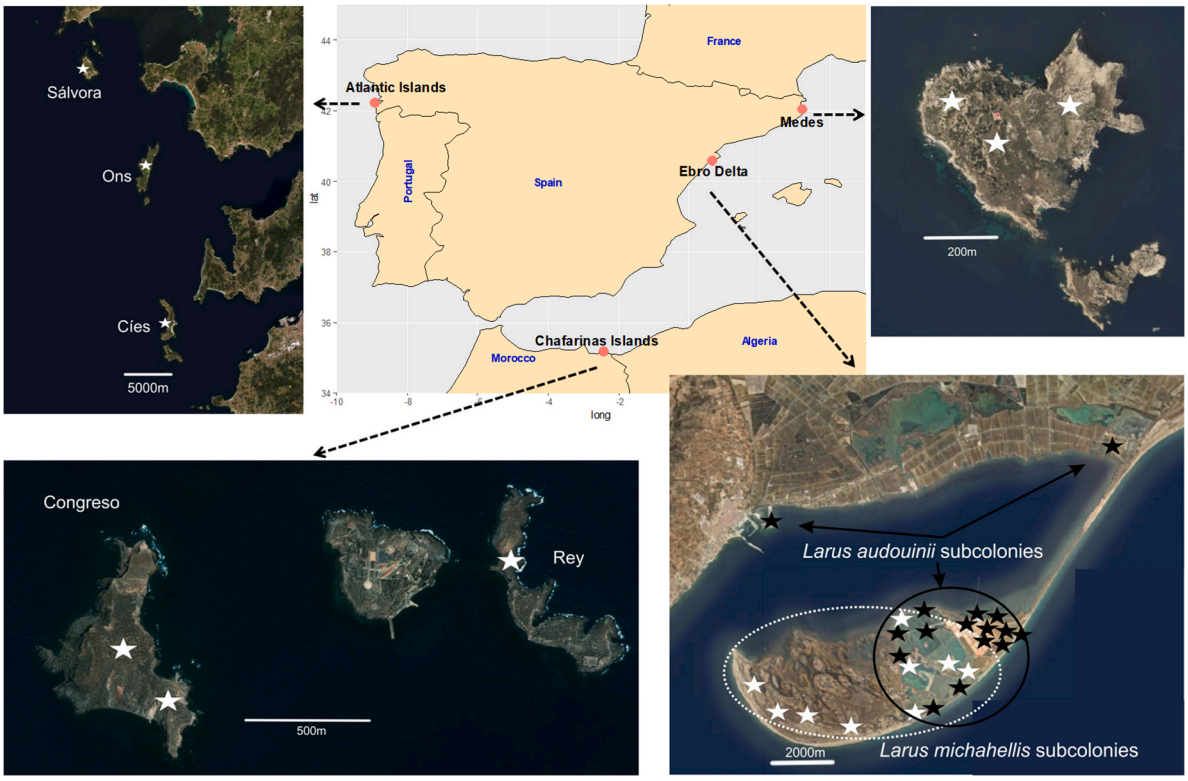


**Fig. 1.** Map showing the colonies and subcolonies studied (marked with a star) during the period 2009–2018.

have a complete coverage of the colony so that the sampling is representative (Fig. 1). The division of each sub-colonies depended on geographic limits but also on the availability of eggs which can vary yearly in each laying period as gulls move within the colony to make new nests (Payo-Payo et al., 2018). As gulls build a new nest each year and some breeding colonies hold hundreds or thousands of couples, the parents cannot be considered the same when comparing years. In each subcolony, 12 first-laid fresh eggs were randomly collected, resulting in a total of 36 eggs per colony and year. Each year the sampling was performed at the very beginning of the laying period at the end of March for *L. michahellis* and beginning of May for *L. audouinii* (it varies 1–3 days among years depending on the weather) in order to ensure collection of the first egg. The first laid egg of each nest represents the maximum pollutant transferred from female to eggs and allows the comparison of the results obtained among colonies and years (Bertolero et al., 2015; Vicente et al., 2015). The *L. michahellis* eggs had slightly different sizes being the higher ones Medes > Ebro > Chafarinas > Atlantic Islands and the smallest were form *L. audouinii* form the Ebro Delta. Information on the length, width and weight of the eggs is provided in Table S1 of the Supplementary Information. After sampling, the eggs were transported to the laboratory in a 28 L cool box and then cracked and opened and if embryonated they were discarded for analysis. The 12 eggs sampled from each sub-colony were pooled to create a representative composite sample (12 eggs per pool), and the resulting 3 pooled samples per colony and year were analyzed independently. In a previous study we demonstrated that single egg analysis and pooled samples provided similar results with overall small standard deviations on the concentrations detected (Vicente et al., 2012). By analyzing pooled samples, the analytical effort and cost of analysis is drastically reduced and this enables to have representative results in a "cost-efficient" way over the years. *L. michahellis* eggs were collected during the breeding season from 2009 to 2018, except in Chafarinas where it was not possible to obtain samples in the 2012–2014 period, and only one pooled sample could be collected in 2011 and two in the 2015–2018 period. Furthermore, only 12 eggs could be obtained in Medes during 2012 since the sampling was carried out at a late stage of the laying period and nests with only one egg were very few and among those collected, some were already embryonated. *L. audouinii* eggs were only collected in the Ebro Delta as it was the main breeding colony of this gull species in Spain when the study began in 2009. As the modal clutch size of both species is three eggs, this kind of sampling does not cause population damage to the colony. Sampling was done with yearly authorizations from each Natural and National Park.

### 2.3. Sample preparation and LC-MS/MS analysis

Seventeen PFAS (compounds and material used specified in Text S1) were extracted from wet samples adapting a previous established methodology where only 5 PFAS were studied (Vicente et al., 2012). Briefly, 1 g of sample (on a wet weight basis, ww) was placed in a 40 mL polypropylene falcon, spiked with the isotopically labeled internal standards (m-PFOS and m-PFOA) at 50 ng $g^{-1}$ and left overnight. Then, 9 mL of acetonitrile were added and the sample was thoroughly mixed using a vortex mixer and extracted in an ultrasonic bath (Selecta, Barcelona) for 10 min (3 times) at room temperature. Afterwards, the sample was centrifuged at 2500 rpm for 5 min, and the supernatant was transferred to a 40 mL glass vial specially fitted for the Turbovap (Caliper, USA) and evaporated to dryness under a stream of nitrogen (99.9% purity, supplied by a generator) at 40 °C. Following, 1.5 mL of acetonitrile was added to the dried extract and incubated in an ultrasonic bath for 10 min. The clean-up was performed by adding 25 mg of activated carbon and 50 μL of glacial acetic acid, the solution was vortexed for 1 min and centrifuged at 10000 rpm for 10 min at room temperature. The supernatant was collected, evaporated under a nitrogen stream and the extract was reconstituted with 500 μL of a mixture of acetonitrile/10 mM ammonium acetate (50/50, v/v).

The analysis of PFAS was carried out on an Acquity UPLC system coupled to a Waters Xevo TQD (triple quadrupole) mass spectrometer, equipped with an orthogonal Z-spray electrospray (ESI) source using negative ionization (Waters, Massachusetts, USA). To remove background PFAS contribution from the mobile phase and LC tubing, a reused XBridge $C_{18}$ column (50 × 4.6 mm, 3.5 μm particle size) was used as trap column, following a previous paper (Vicente et al., 2012). Chromatographic separation was carried out on an Acquity UPLC BEH $C_{18}$ column (100 × 2.1 mm, 1.7 μm particle size) (Waters) at a flow rate of 0.3 mL $min^{-1}$. The mobile phase composition was a mixture of (A) 10 mM ammonium acetate aqueous solution and (B) methanol/acetonitrile (80:20, v/v) buffered with 10 mM of ammonium acetate. The initial conditions of the gradient elution were the following: 50% A and 50% B (condition kept for 3 min) and increase to 100% B in 7 min. The injection volume was 5 μL. Nitrogen (Air Liquid) was used as drying gas and nebulizing gas at 30 and 750 L $h^{-1}$, respectively. The ESI capillary voltage was set at 2500 kV and source and desolvation temperature were set at 120 °C and 350 °C, respectively. For MS/MS analysis, argon (Air Liquid) was used as collision gas at a pressure of 0.19 mL $min^{-1}$. Experimental conditions for the selected compounds are shown in Table S2. Quantification of the target compounds was performed by internal standard method using m-PFOA to quantify all perfluorinated carboxylic acid (PFCAs) and m-PFOS to quantify perfluorinated sulfonic acids (PFSAs). Mass Lynx v.4.1 software was used to control the instrument setup, data acquisition, and processing.

### 2.4. Quality control and quality assurance

Table S2 summarizes the quality control parameters of the method used. A calibration curve with five points was built over a concentration range of 5–300 ng $mL^{-1}$ containing the isotopically labeled internal standards at 50 ng $mL^{-1}$. The response factor, linearity ($r^2 > 0.999$) and precision (typically RSD% < 15%) were routinely checked by injecting a standard at 100 ng $mL^{-1}$ every 12 injections along the sequence to ensure the quality of the results. Extraction efficiency was evaluated using chicken eggs spiked with native PFAS at a concentration of 10 and 100 ng $g^{-1}$ ww. Recoveries of the target compounds at 100 ng $g^{-1}$ spiking level ranged from 71 ± 12% to 116 ± 16%, somewhat higher than at 10 ng $g^{-1}$ spiking level, especially for long-chain PFAS (Table S2). Given the adequate extraction efficiency, no matrix effects were observed indicating the effectiveness of the clean-up step to eliminate lipids and other interferents. Chicken eggs spiked at 10 ng $g^{-1}$ were used to calculate the method detection limits (MDL) using a signal to noise ratio of 3 and ranged between 0.07 and 1.1 ng $g^{-1}$. To ensure any external contamination of PFAS during extraction and analysis, procedural blanks without any matrix and unspiked chicken eggs were processed as a sample. PFAS were not detected in any of the blank samples and this indicates that the extraction procedure does not add any external contamination to the sample. The absence of PFAS in these blanks is attributed to the fact that all material used for the extraction is made of polypropylene and the 40 mL and chromatographic glass vials are baked at 450 °C before use to eliminate any trace of contaminants. The solvents used, once opened, the cap was covered with aluminum foil to avoid contact with the Teflon septum of the cap. External contribution from the mobile phase or from the tubing was avoided by placing a trap-column between the mobile phase and the injector to retain potential PFAS present in the chromatographic system, and no carry-over was observed. Concentrations are reported as mean values ± standard deviation (SD) unless otherwise stated.

### 2.5. Statistical data treatment

All data treatments were applied considering the compounds detected: PFOS, PFNA, PFDA, PFUnA, PFDoA, and PFTriDA, while PFOA was excluded since it was only detected in Medes. PFTriDA in Chafarinas was the only case with values < MDL and for statistical analysis MDL were

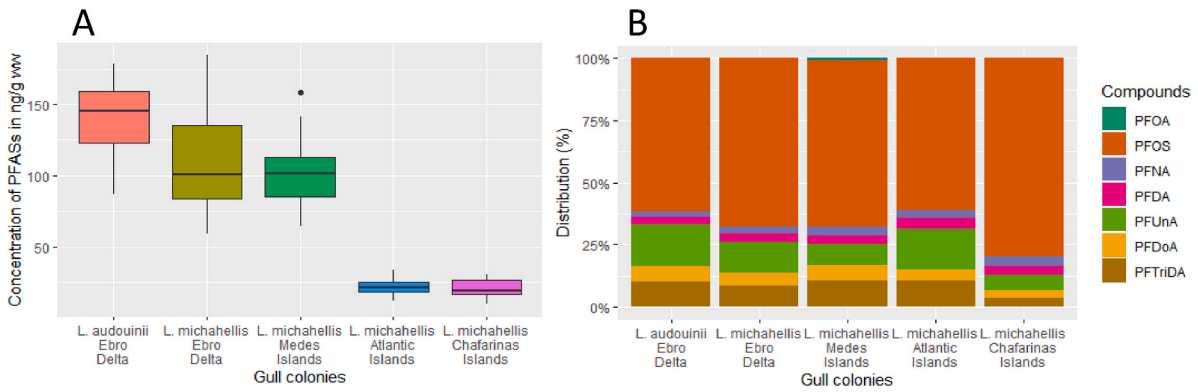


**Fig. 2.** (A) Boxplot on the concentration of ∑PFAS in the 5 colonies considering the median, 25th and 75th percentiles and maximum and minimum concentration in the 10-year studied (2009–2018); (B) profiles of the different PFAS detected considering the 10-year study period. Compounds are ordered from shorter to longer chain length.

substituted by the half values of the MDL. All data, including outliers, was retained for statistical analysis as very similar results were obtained when including or excluding outliers. Principal Component Analysis (PCA) was carried out using raw data to study the association patterns among PFAS considering 5 colonies × 3 samples per colony × 10 years × 6 compounds. The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was used to assess the usefulness of the PCA. KMO ranges from 0 to 1 and should be well above 0.5 if variables are sufficiently interdependent for PCA to be useful (Tabachnick and Fidell, 2000).

The temporal variation of PFAS concentrations in eggs was compared using repeated measures multivariate analysis of variance on original PFAS concentrations (RM-MANOVA) to include the geographic factor in the analysis (proximity between subcolonies). RM-MANOVA is used when several dependent variables are measured in each sampling unit instead of only one variable (Rovira et al., 2012). In this study, RM-MANOVA was used to compare the PFAS on *L. michahellis* colonies from Ebro, Medes and Atlantic Islands considering the 6 detected PFAS during the period 2009 to 2018. Chafarinas was not included because the data set was incomplete due to sampling problems (see section 2.1.) but PFAS concentrations were also compared in the four colonies excluding the period 2011–2014 (Supplementary information Text S2). RM-MANOVA also was used to compare the temporal variations in *L. michahellis* and *L. audouinii* species sharing habitat in the Ebro Delta. Estimated marginal means (EMM) were calculated to describe temporal trends, and differences among colonies and between species and are reported as mean ± standard error (SE), adjusted for the other variables. Significances were further explored with one-way RM-ANOVA and contrast tests were performed to determine the differences among colonies with regards to the concentrations of ∑PFAS and individual compounds. A P-value $< 0.05$ was considered statistically significant. Finally, we used partial eta squared ($\eta_p^2$), which is the proportion of variation explained for a certain variable, for measuring the importance of factors, in our case, the effect of the colony, year or colony per year. A higher partial $\eta_p^2$ value indicates a stronger effect (Alcaraz et al., 2008; Tabachnick and Fidell, 2000).

All statistical analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) for Windows, except the association of EMM with years (i.e., temporal trend) within each gull colony that was analyzed with Spearman's rank correlation coefficient ($\rho$) using R software version 3.4.4 (RStudio Team, 2020).

## 3. Results and discussion

### 3.1. Concentration levels and patterns among colonies

PFAS were detected in all gull eggs from the 5 studied colonies and throughout the years, suggesting that National and Natural Parks, despite being areas of a high level of protection, are still affected by environmental pollution caused by humans. Compounds detected were PFOS (linear and branched), PFNA, PFDA, PFUnA, PFDoA, PFTriDA, and PFOA. Fig. 2A shows the boxplot with, the median, 25th and 75th percentiles of PFAS concentrations in each colony considering the 10 years (2009–2018). The boxplots of the individual compounds are indicated in Figure S1. The concentrations of ∑PFAS ranged from 13.7 ± 5.9 to 164 ± 17 ng $g^{-1}$ ww. ∑PFAS mean concentrations varied among colonies following the order: *L. audouinii* Ebro Delta > *L. michahellis* Ebro Delta ≈ Medes > Chafarinas ≈ Atlantic. *L. audouinii* had higher levels than *L. michahellis* and this is attributed to the fish-based diet, as previously observed for dioxins, furans, and dioxin-like polychlorobiphenyls (Morales et al., 2012) and also for non-dioxin-like PCBs and organochlorine pesticides (Zapata et al., 2018). Among *L. michahellis*, the higher levels found in Ebro Delta and Medes colony compared to Chafarinas and Atlantic Islands can be attributed to the more confined and contaminated Mediterranean basin compared to the high dilution capacity of the Atlantic Ocean (Vicente et al., 2012).

PFOS was the dominant compound in both *L. michahellis* and *L. audouinii* eggs accounting for 61–81% of ∑PFAS and reaching the highest proportion in Chafarinas (Fig. 2B). The widespread presence of PFOS reflects its uptake through the diet and yearly maternal transfer to eggs (Lopez-Antia et al., 2021), although biodegradation or depuration of PFOS play an important role in the bioaccumulation of PFOS in gulls (Bertolero et al., 2015). Other PFAS were colony specific and had much lower concentrations (Fig. 2B), but generally, PFUnA and PFTriDA were the second predominant compounds that reflect their also high bioaccumulation potential. The patterns were as follow: in Ebro Delta (both species) showed PFUnA > PFTriDA > PFDoA > PFDA > PFNA; in Medes Islands' colony was PFTriDA > PFUnA > PFDoA > PFDA > PFNA > PFOA; in Chafarinas Islands was PFUnA > PFTriDA = PFDA = PFNA > PFDoA; and in Atlantic Islands was PFUnA > PFTriDA > PFDA > PFDoA > PFNA. Detected PFAS are long-chain compounds and are consistent with previous findings (Groffen et al., 2019; Sebastiano et al., 2020). Medes Islands was the only colony where PFOA was detected over the years although at low concentrations. PFOA may originate either from landfills leachates that contain high concentrations of PFOA and other PFCA (Duhem et al., 2005) as there are 4 landfills of 7.4–11 ha within a distance of 32–47 km from Medes Islands. PFOA could also originate from the use of fire-fighting foams as in fact, in 2012 a fire burned more than 14,000 ha in this area and one year later the concentration of PFOA increased in comparison to previous years (Table S3). The other analyzed compounds (PFBA, PFPeA, PFHxA, PFHpA, PFTeDA, PFHxDA, PFODA, PFBS, PFHxS, and PFDS) were not detected either because they are short-chain PFAS with low bioaccumulation potential or either because they are compounds of low production volumes.

The mean concentrations and standard deviation (n = 3) of

individual PFAS detected in each colony each year are summarized in Table S3. PFAS levels varied among colonies and this proves the usefulness of gull eggs as bioindicator of environmental contamination. Moreover, it also indicates that the sampling protocol based on the collection of the first egg, which is the one that accumulates the highest concentration of contaminants (Vicente et al., 2012) allows comparisons among colonies over the years. In our study, PFOS was detected at concentrations from 10.5 ± 4.2 to 101 ± 6 ng $g^{-1}$ ww in *L. michahellis* and from 48 ± 10 to 101 ± 15 ng $g^{-1}$ ww *L. audouinii*. These concentrations are within the range of other reported studies using gull eggs as biomonitors. PFOS was reported in herring gull (*Larus argentatus*) eggs from Germany ranging from 24.2 to 170 ng $g^{-1}$ ww (Rüdel et al., 2011) and from 91 ± 13 to 507 ± 47 ng $g^{-1}$ ww in the Great Lakes of North America (USA), explained by proximity to industrial and urban areas (Gebbink et al., 2009). Lower levels are found in remote areas, with PFOS at 20.0 ± 1.1 ng $g^{-1}$ ww in eggs of glaucous gull (*Larus hyperboreus*) from Nunavut (Braune and Letcher, 2013), and from 55.8 ± 24 to 72.6 ± 31 ng $g^{-1}$ ww in eggs of Ivory gulls (*Pagophila eburnea*) from Norwegian and Russian Arctic (Miljeteig et al., 2009). The high relative abundance of PFOA, PFNA, and PFDA in samples from these remote areas is attributed to fish consumption (Verreault et al., 2007) and atmospheric transport of precursors such as fluorotelomer alcohols (Gewurtz et al., 2016). Still, the prevalence of PFOS in gull eggs from different parts of the word is relevant because it demonstrates the ubiquity of this compound in industrialized, remote and protected areas, suggesting that marine environments are highly affected by this compound.

### 3.2. Principal Component Analysis and sources of PFAS in gulls

PCA revealed differences in PFAS patterns among colonies and species. The KMO measure of sampling adequacy (0.89) showed the usefulness of the PCA, with the first two components explaining 76.5 and 7.8% of the total variance, respectively (Fig. 3). The PCA on PFAS concentrations in *L. michahellis* and *L. audouinii* eggs showed that all compounds were interdependent and significantly correlated (Pearson's $r > 0.62$; $N = 133$; $P < 0.0001$, for all paired comparisons), where PFDoA, PFDA, PFNA and PFOS ($r > 0.68$; $N = 133$; $P < 0.0001$) had a similar contribution to first PCA axis scores, while PFUnA and PFTriDA ($r = 0.86$; $N = 133$; $P < 0.0001$) had a specific contribution in the PC2 axis. Although PC1 captures the highest amount of variance, the different colonies are separated along the PC2 axis suggesting that PC1 shows inter-colony variance while PC2 captures intra-colony and intra-year variance. Ebro Delta (both *L. michahellis* and *L. audouinii*) and Medes samples are distributed in the right PC1 axis indicating those gull eggs with the highest concentration of all PFAS. Atlantic Islands and Chafarinas colonies are at the bottom-left quadrant of the PCA plot and indicate the colonies with the lowest levels of all compounds. Across PC2 axis, samples towards the bottom quadrant (basically *L. audouinii* in Ebro delta and Atlantic Islands) indicate high PFUnA and PFTriDA contribution. In addition, PCA separated *L. michahellis* from *L. audouinii* that share habitat in the Ebro Delta. *L. audouinii* presented a high contribution of all PFAS, especially from PFUnA, and PFTriDA, compared to *L. michahellis*. PCA also showed that PFAS in eggs from Medes and Ebro Delta (both *L. michahellis* and *L. audouinii*) have a high dispersion in the multivariate space compared to gull eggs from Chafarinas and Atlantic Islands, which have a very similar PFAS pattern composition. Colonies with higher dispersion are the colonies showing time-related variations.

The levels and patterns of PFAS in eggs from the different Spanish colonies vary and this can be attributed to changes in the availability of the food resources that birds exploit. A previous study estimated the uptake and mass transfer of PFOS in gulls through a fish-based diet and demonstrated that diet produced a net yearly accumulation (Bertolero et al., 2015) although the uptake depends on the compounds (Lopez-Antia et al., 2021). In fact, stable isotopes analysis indicated that most marine colonies are exposed to PFAS via marine prey (Gebbink et al., 2009) despite PFAS levels in fish are generally low (Fernández-Sanjuan et al., 2010; Vassiliadou et al., 2015). We hypothesize that diet, trophic resource availability and feeding habits (reliance on marine or terrestrial food sources, refuse tips or availability of garbage) of gulls explains the degree of variability in concentrations in the different colonies and species. Table S1 shows the diet of gulls from different colonies. In the Ebro Delta colony, marine sources and American crayfish *Procambarus clarki* were predominant in the diet of *L. audouinii* while in *L. michahellis* fish and refuse tips account for 91% (Navarro et al., 2010; Ramos et al., 2009a), but rely also on garbage and terrestrial preys (Morera-Pujol et al., 2018; Ramos et al., 2009b). In Medes, both marine prey and garbage contributed almost equally to the diet (Ramos et al., 2009a). In Chafarinas, *L. michahellis* exploits epipelagic and benthic fish collected from trawler or purse site discards, and to a lesser extend refuse tips and terrestrial prey account for <5% of the food (González-Solís, 2003). In the Atlantic Islands, the diet is mainly based on fish such as *Micromesistius poutassou* and *Trachurus trachurus*, and in a complementary manner with pelagic crabs *Polybius henslowii* and mussels *Mytilus galloprovincialis* (Moreno et al., 2010). Patently, gulls are not unwise and when fish is available and predictable (fishery discards), they exploit this resource opposite to garbage. According to feeding habits, the *L. michahellis* colony with the highest dependence on garbage is Medes (50% garbage) followed by Chafarinas (10%) and Ebro Delta (9% garbage). In the PCA analysis, it is observed that these colonies have a higher contribution of PFOS than the colonies feeding primarily on fish.

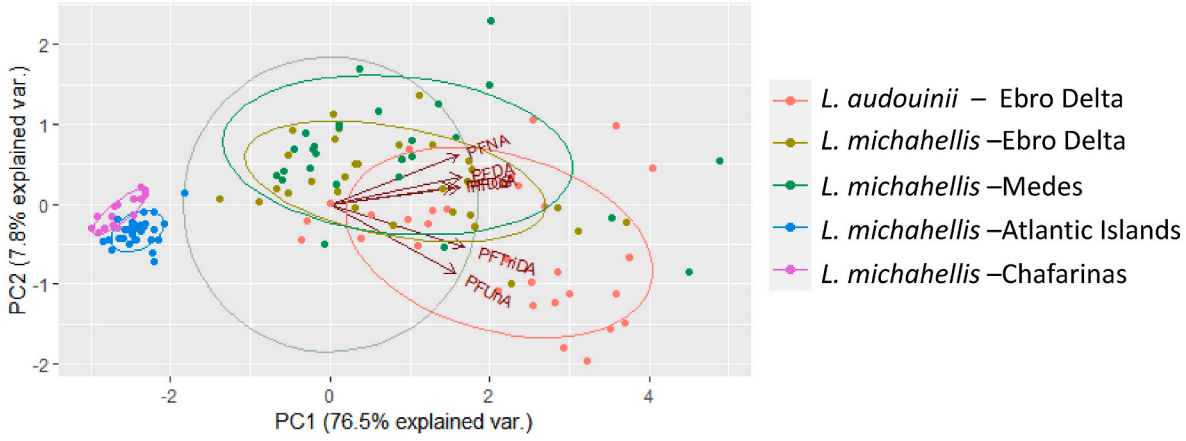


**Fig. 3.** Principal Component Analysis (PCA) on raw concentrations of PFAS in eggs of *L. michahellis* and *L. audouinii*. Factor loadings and scores of the PFAS compounds on the first two principal component axes are shown. The percent variation explained by PCA axes 1 and 2 is also shown.

Contrarily, *L. audouinii* from Ebro delta and *L. michahellis* from Atlantic Islands that rely on a fish-based diet have a higher contribution of PFUnA and PFTriA than colonies whose diet is based on other sources, which may suggest that these compounds are accumulated through the fish diet. This finding is also corroborated by the higher PFUnA and PFTriA contribution to the ∑PFAS in these 2 colonies (Figs. 2B and 3).

### 3.3. Time variations

PFAS variability over the 10-year period was assessed using RM-MANOVA to reveal significant differences among years and colonies. The concentration of individual PFAS and ∑PFAS in *L. michahellis* eggs (excluding Chafarinas Islands) significantly varied among years (RM-MANOVA: Wilks's $\lambda = 0.07$, $F_{54.0,\ 162.7} = 4.96$, $P < 0.0001$, $\eta_p^2 = 0.563$), and this temporal pattern differed among colonies (Year × Colony; Wilks's $\lambda < 0.001$, $F_{108.0,\ 184.8} = 4.84$, $P < 0.0001$, $\eta_p^2 = 0.723$). Univariate tests (RM-ANOVAs) for each compound confirmed this pattern (see $\eta_p^2$ values in Table 1) and significant differences were observed within years for ∑PFAS and individual compounds. In general, PFAS concentrations differences were mainly explained by colony > year × colony interaction > year (see $\eta_p^2$ in Table 1). It was observed that the concentrations differed in a significant way for all compounds during the 10 years ($\eta_p^2$ ranging from 0.409 to 0.737), indicating that PFAS time variability was colony specific. When considering the interaction within years and colonies, all compounds had a significant variation ($\eta_p^2$ ranging from 0669 to 0.801), except for PFDoA that did not vary among colonies ($\eta_p^2$ of 0.445). Finally, the highest significant variations were observed considering between subjects effects in all colonies, where $\eta_p^2$ was >0.99 for all compounds, indicating that the colony factor was higher than the time factor, and therefore, the colonies behave differently among years. The contrast test distinguished the colonies with significantly higher concentrations than the others. Among Ebro Delta and Medes Islands no differences were observed for ∑PFAS, PFNA and PFOS, but PFDA, PFDoA and PFTriDA had higher concentration in Medes and PFUnA had higher concentrations in Ebro Delta (Table 1). Similar results were obtained when the Chafarinas Islands colony was included in the analyses (Table SI4 and Text SI1).

When comparing *L. michahellis* and *L. audouinii* eggs in the Ebro Delta colony over the 10 year period, we found significant differences in PFAS concentrations among years (RM-MANOVA; Wilks's $\lambda = 0.04$, $F_{54.0,\ 162.7} = 5.90$, $P < 0.0001$, $\eta_p^2 = 0.602$), and the temporal pattern differed significantly among gull species (Year × Species; Wilks's $\lambda = 0.104$, $F_{54.0,\ 162.7} = 1.62$, $P = 0.007$, $\eta_p^2 = 0.314$). Univariate tests confirmed this pattern and showed that the species factor is more important than the temporal factor. Univariate test showed significant annual differences in PFAS concentrations (all $P < 0.005$ and $\eta_p^2 > 0.55$), as shown in Table 2. Considering the interaction year × species, the temporal variation was similar for PFNA, PFDA, PFUnA, PFDoA and PFTriA in both species, indicating that *L. michahellis* and *L. audouinii* varied similarly over the 10-year study period. Contrarily, PFOS ($PFOS_{Year\ \times\ Species}$: $P < 0.001$ and $\eta_p^2 = 0.557$) and ∑PFAS ($P < 0.05$ and $\eta_p^2 = 0.454$) showed significant differences suggesting that *L. audouinii* and *L. michahellis* varied differently during this period. *L. audouinii* eggs showed significantly higher concentrations of PFOS, PFDA, PFUnA, PFTriDA and ∑PFAS than those found in *L. michahellis* eggs ($P < 0.05$, between subjects effects in Table 2), while concentrations were not significantly different for PFNA and PFDoA ($P > 0.05$). Specifically, PFUnA and PFTriDA showed the highest $\eta_p^2$ values, indicating that the greatest differences between species were attributed to these two compounds. Even between species that are phylogenetically close and inhabiting the same area, these differences show that the bioaccumulation of these pollutants depends on the feeding ecology of each species. The highest contribution of PFUnA and PFTriDA to ∑PFAS in *L. audouinii* eggs could be attributed to the fish-based diet as suggested above, although degradation of the PFAS precursors in the atmosphere can be an additional source of PFCAs (Ellis et al., 2004).

### 3.4. Temporal trends in the studied colonies

The data obtained throughout this study provided evidence of decreasing trends of ∑PFAS in gull eggs from all colonies, except for Medes, over the period 2009–2018 and can be attributed to a

**Table 1**
RM-ANOVAs on the concentrations of PFAS in *L. michahellis* eggs collected in Medes (MI), Ebro Delta (ED), and Atlantic Islands (AI) from 2009 to 2018 indicating the F (Fischer) and *df* (degrees of freedom) and $\eta_p^2$ (partial eta-square). Significance levels; *ns*: not significant; *a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.005$; *d*: $P < 0.001$.

| Compound | Within samples | | | | | | Between samples | | |
|---|---|---|---|---|---|---|---|---|---|
| | Year | | | Year × Colony | | | Colony | | |
| | *F* | *df* | $\eta_p^2$ | *F* | *df* | $\eta_p^2$ | $F_{2,\ 4}$ | $\eta_p^2$ | Contrast tests |
| **PFNA** | $5.64^c$ | 5.67, 22.7 | 0.585 | $4.63^d$ | 11.3, 22.7 | 0.699 | $211^d$ | 0.991 | AI < ED ≈ MI |
| **PFOS** | $3.75^a$ | 3.95, 15.8 | 0.484 | $6.24^c$ | 7.89, 15.8 | 0.757 | $303^d$ | 0.993 | AI < ED = MI |
| **PFDA** | $8.65^d$ | 5.81, 23.2 | 0.684 | $7.73^d$ | 11.6, 23.2 | 0.794 | $210^d$ | 0.991 | AI < ED < MI |
| **PFUnA** | $11.21^d$ | 4.39, 17.6 | 0.737 | $8.07^d$ | 8.79, 17.6 | 0.801 | $91.9^d$ | 0.979 | AI < MI < ED |
| **PFDoA** | $2.77^a$ | 4.90, 19.6 | 0.409 | $1.61^{ns}$ | 9.80, 19.6 | 0.445 | $373^d$ | 0.995 | AI < ED < MI |
| **PFTriDA** | $8.59^d$ | 5.80, 23.2 | 0.682 | $6.82^d$ | 11.6, 23.2 | 0.773 | $310^d$ | 0.994 | AI < ED < MI |
| **∑PFAS** | $6.76^c$ | 3.45, 13.8 | 0.628 | $6.50^c$ | 6.89, 13.8 | 0.765 | $297^d$ | 0.993 | AI < ED = MI |

**Table 2**
RM-ANOVAs on the concentrations of PFAS in *L. michahellis* and *L. audouinii* eggs collected in Ebro Delta from 2009 to 2018. Significance levels; *ns*: not significant; *a*: $P < 0.05$; *b*: $P < 0.01$; *c*: $P < 0.005$; *d*: $P < 0.001$.

| Compound | Within samples | | | | | | Between samples | |
|---|---|---|---|---|---|---|---|---|
| | Year | | | Year × Species | | | Species | |
| | F | df | $\eta_p^2$ | F | df | $\eta_p^2$ | $F_{2,\ 4}$ | $\eta_p^2$ |
| **PFNA** | $8.86^d$ | 9.00, 36.0 | 0.689 | $0.42^{ns}$ | 9.00, 36.0 | 0.095 | $1.79^{ns}$ | 0.309 |
| **PFOS** | $10.3^d$ | 7.12, 28.5 | 0.720 | $5.03^d$ | 7.12, 28.5 | 0.557 | $9.58^a$ | 0.706 |
| **PFDA** | $7.58^d$ | 7.67, 30.7 | 0.655 | $2.27^{ns}$ | 7.67, 30.7 | 0.362 | $17.2^a$ | 0.812 |
| **PFUnA** | $11.1^d$ | 5.03, 20.1 | 0.735 | $1.41^{ns}$ | 5.03, 20.1 | 0.261 | $115^d$ | 0.967 |
| **PFDoA** | $4.91^c$ | 6.43, 25.7 | 0.551 | $1.38^{ns}$ | 6.43, 25.7 | 0.257 | $5.26^{ns}$ | 0.568 |
| **PFTriDA** | $10.3^d$ | 9.00, 36.0 | 0.721 | $1.34^{ns}$ | 9.00, 36.0 | 0.251 | $227^d$ | 0.983 |
| **∑PFAS** | $14.3^d$ | 7.08, 28.3 | 0.781 | $3.32^a$ | 7.08, 28.3 | 0.454 | $30.8^b$ | 0.885 |

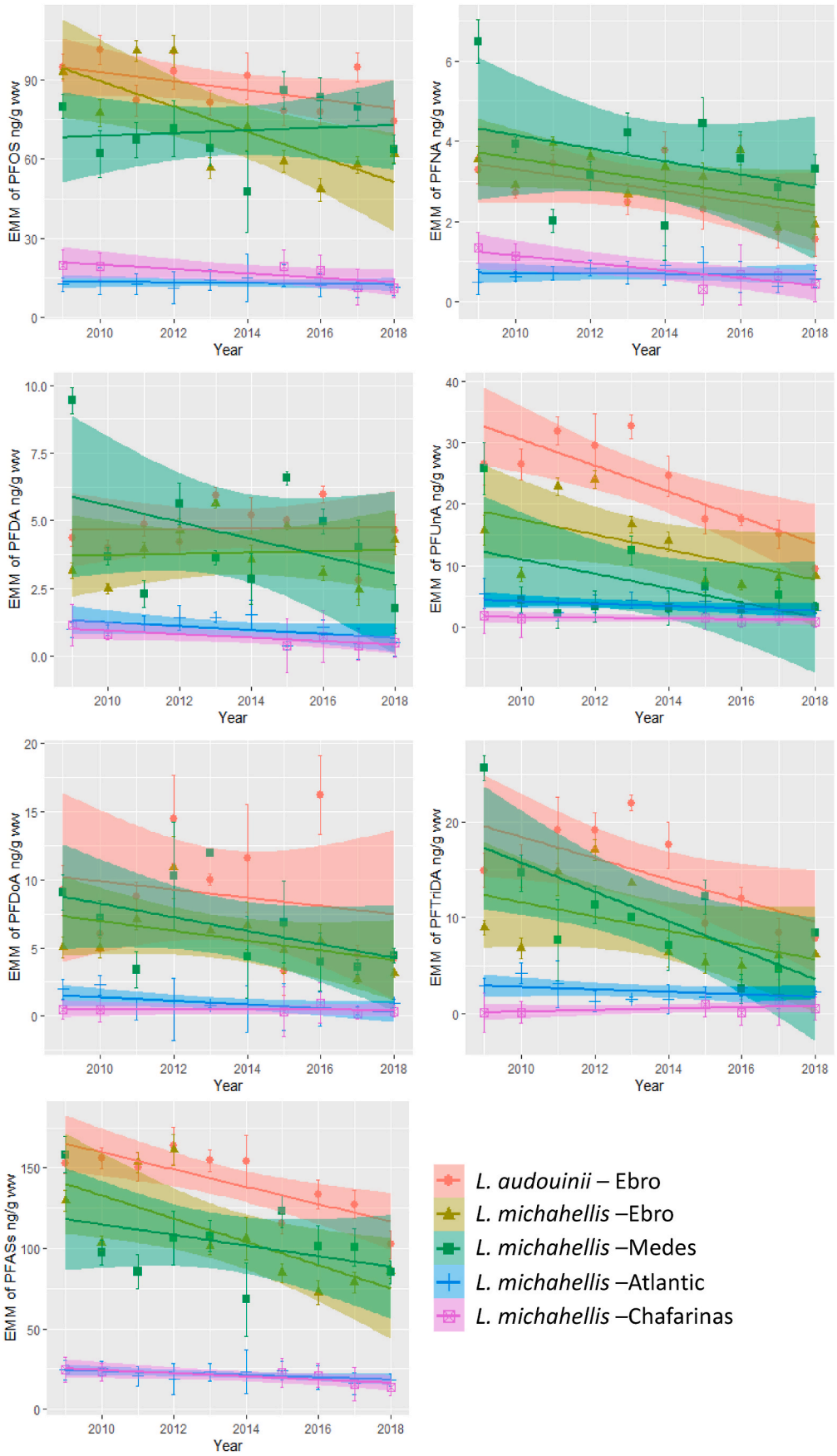


**Fig. 4.** Estimated marginal mean (EMM) and standard error ($n = 3$) of PFAS in eggs of *L. audouinii* and *L. michahellis* per locality. *L. michahellis* EEM from Chafarinas Islands were extracted from RM MANOVA excluding from 2011 to 2014 (see Table S4 in Supplementary information).

preliminary environmental response on the PFAS phase-out in 2002 from the industry and the restrictions of the Stockholm Convention in 2009 (Stockholm Convention, 2019).

Individual PFAS and ∑PFAS temporal trends over the period 2009–2018 are shown in Fig. 4. ∑PFAS, PFUnA and PFTriA, and in some colonies PFOS, show a linear decreasing trend along time. The rest of the compounds remained stable during the study period. However, in several cases, a curvilinear relationship was observed (i.e., PFDoA and PFTriDA in *L. audouinii* from Ebro Delta and PFNA, PFOS and ∑PFAS from Medes). Table 3 shows the Spearman's rank correlations for both *L. michahellis* and *L. audouinii* and indicates compounds showing a significant decreasing trend in each colony. For *L. audouinii* the decreasing trend was only significant for PFUnA and ∑PFAS, while for PFOS and PFTriDA a slight decrease in concentration over the years was observed, but it was not significant. In *L. michahellis* from Ebro Delta, a significant decreasing trend was observed for PFOS, PFUnA, PFTriDA and ∑PFAS, with the highest PFOS levels in the period 2009–2012 and halved thereafter, while PFUnA and PFTriDA had a peak in concentration in the

**Table 3**
Spearman's rank correlation coefficient ($\rho$), years ($n$) and significance ($P$ value) between the estimated marginal means of the PFAS concentrations (RM-MANOVA) and year for both *L. michahellis* and *L. audouinii* eggs from all colonies. Compounds with significant differences are highlighted in bold.

| Species | Colony | Compound | $\rho$ | $n$ | $P$ |
|---|---|---|---|---|---|
| *Larus michahellis* | Ebro Delta | PFNA | −0.47 | 10 | 0.178 |
| | | PFOS | **−0.66** | **10** | **0.044** |
| | | PFDA | 0.02 | 10 | 0.973 |
| | | PFUnA | **−0.65** | **10** | **0.049** |
| | | PFDoA | −0.5 | 10 | 0.143 |
| | | **PFTriDA** | **−0.7** | **10** | **0.031** |
| | | ∑PFAS | **−0.75** | **10** | **0.018** |
| | Medes Islands | PFNA | −0.27 | 10 | 0.448 |
| | | **PFOS** | 0.21 | 10 | 0.559 |
| | | PFDA | −0.29 | 10 | 0.427 |
| | | **PFUnA** | −0.24 | 10 | 0.514 |
| | | PFDoA | −0.41 | 10 | 0.247 |
| | | **PFTriDA** | **−0.66** | **10** | **0.044** |
| | | **∑PFAS** | −0.31 | 10 | 0.387 |
| | Chafarinas Islands | PFNA | −0.66 | 6 | 0.175 |
| | | **PFOS** | **−0.94** | **6** | **0.017** |
| | | PFDA | −0.6 | 6 | 0.242 |
| | | PFUnA | −0.6 | 6 | 0.242 |
| | | PFDoA | −0.37 | 6 | 0.497 |
| | | PFTriDA | 0.6 | 6 | 0.241 |
| | | **∑PFAS** | **−1** | **6** | **0.003** |
| | Atlantic Islands | PFNA | −0.15 | 10 | 0.682 |
| | | PFOS | −0.27 | 10 | 0.448 |
| | | PFDA | −0.35 | 10 | 0.331 |
| | | PFUnA | −0.44 | 10 | 0.204 |
| | | PFDoA | −0.35 | 10 | 0.331 |
| | | PFTriDA | −0.33 | 10 | 0.349 |
| | | **∑PFAS** | **−0.7** | **10** | **0.031** |
| *Larus audouinii* | Ebro Delta | PFNA | −0.42 | 10 | 0.232 |
| | | PFOS | −0.58 | 10 | 0.088 |
| | | PFDA | 0.2 | 10 | 0.584 |
| | | **PFUnA** | **−0.78** | **10** | **0.012** |
| | | PFDoA | −0.25 | 10 | 0.492 |
| | | PFTriDA | −0.64 | 10 | 0.054 |
| | | **∑PFAS** | **−0.71** | **10** | **0.028** |

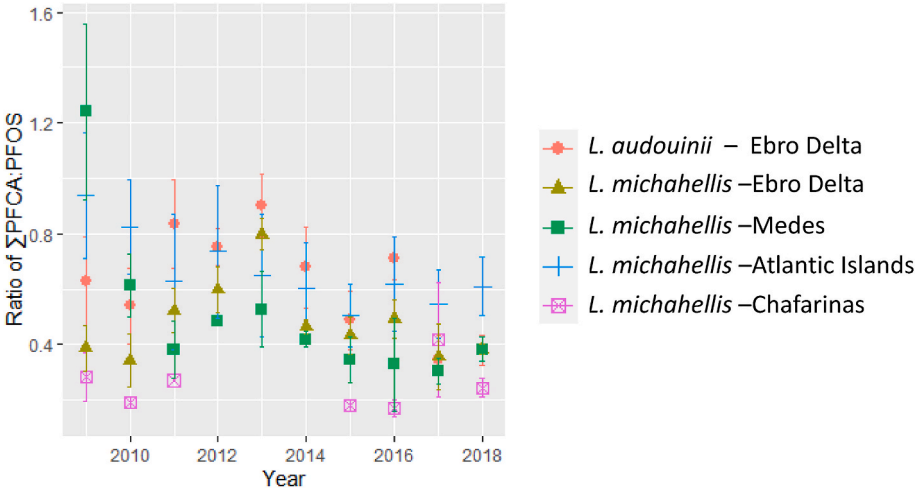


**Fig. 5.** Mean and standard deviation ($n = 3$) of ∑PFCA:PFOS ratios in eggs of *L. audouinii* and *L. michahellis* per colony during the period 2009–2018.

period 2011–2013 and followed by a general decrease towards the end of the study period in 2018 (Fig. 4, Table 3). In the Medes Islands, a significant decreasing trend was observed only for PFTriDA, while the other PFAS showed no trend although a peak in concentration was observed in the period 2015–2016. In contrast, in Chafarinas Islands, PFOS followed a decreasing trend, as well ∑PFAS due to the PFOS influence, while there were no differences along the years for the rest of the compounds, but rather showing a low-level but constant concentration. In the Atlantic Islands, ∑PFAS had a significant linear negative correlation over the years and no differences for individual compounds were observed during the study period.

Long term studies using birds as bioindicators reflect time-trends of PFAS according to the manufacturing and the phase out periods. During the period PFAS were in use, increasing trends are reported such as in white-tailed sea eagle *(Haliaeetus albicilla)* from marine colonies in Sweden from the 1960s to 2010 (Faxneld et al., 2016); in common murre (*Uria aalge*) from the Baltic Sea during 1968–2003 (Holmström et al., 2005); in herring gull (*Larus argentatus*) from Northern Norway during 1983–1993 (Verreault et al., 2007); in peregrine falcon (*Falco peregrinus*) from the southwest Swedish coast during 1974–2007 (Holmström et al., 2010); in herring gull (*Larus argentatus*) from the Baltic Sea during 1988–2008 (Rüdel et al., 2010). Our study is performed over the period 2009–2018, when PFOS and PFOA production was already phased out and the Stockholm Convention implemented, which may have urged the manufacturing and use of other PFCAs. Fig. 5 shows the ∑PFCA:PFOS ratios over the study period to illustrate changes in patterns along the years. In Medes and Atlantic Islands the ratio decreased while remained constant in Chafarinas. For both *L. michahellis* and *L. audouinii* colonies of the Ebro delta the ∑PFCA:PFOS increased during the period 2009–2013, and thereafter decreased. The decrease in the ∑PFCA:PFOS ratios over the last years (2013–2018) suggests a rapid decay in PFCA as PFOS remains constant in all colonies expect in *L. michahellis* of the Ebro delta and in Chafarinas where it decreases. Increased levels of PFCA in comparison to PFOS were observed in common eider (*Somateria mollissima*), European shag (*Phalacrocorax aristotelis*), and European herring gull (*Larus argentatus*) from Norway (Huber et al., 2015), and in gulls from the Great Lakes coastal areas (Gewurtz et al., 2016). In eggs of UK gannets (*Morus bassanus*) ∑PFSA first rose and then fell and ∑PFCA remained unchanged over the period from 1977 to 2014, although long-chain odd PFCA concentrations still increase (Pereira et al., 2021). Increasing temporal trends of PFNA, PFOS and F–53 B, a PFOS alternative, was observed in gull eggs (*Larus crassirostris*) eggs from South Korea islands during 2012–2018 (Wang et al., 2021). However, this trend was also species-related, as described in birds eggs from the Canadian Pacific coast during the period 1990 to 2010, where PFOS decreased in double-crested cormorant (*Phalacrocorax auritus*) and rhinoceros auklet (*Cerorhinca monocerata*), increased in Leach's storm petrel (*Oceanodroma leucorhoa*), and remain constant in great blue heron (*Ardea herodias*), while PFCA increased only in offshore species (Miller et al., 2015). Moreover, in Prince Leopold Island in Nunavut, ∑PFCA: PFOS ration increased in eggs of northern fulmar *(Fulmarus glacialis)* and thick-billed murre *(Uria lomvia)* from 1975 to 2011 (Braune and Letcher, 2013). All these studies reflect that gulls are still exposed to compounds massively produced and discharged to the environment in the past decades and that changes in the emissions rates of PFAS and their precursors are reflected in gull eggs' concentrations.

## 4. Conclusions

This study used gull eggs for the long-term biomonitoring of PFAS in Natural and National Parks in Spain to reflect the pollution level of a given habitat and identify the geographical differences and time trends. PFAS were detected in all colonies, despite being areas with a high degree of protection. Among 17 PFAS analyzed, only 7 compounds were detected, all of them long-chained PFAS. Differences among colonies depended on location (Mediterranean/Atlantic) and proximity to human settlements and industrial activities, whereas trophic resources availability and diet habits defined time and species-related variability. PFOS accounted for the main contaminant in the five colonies studied and its contribution to ∑PFASs was high in all gulls while PFUnA and PFTriDA were present in higher concentrations in Audouin gull and in gulls from Atlantic Islands relying on fish. Yearly variations of PFAS were colony and species dependant. Time trends suggested a significant decreasing concentration of ∑PFAS, exemplified by PFOS, PFUnA, and PFTriDA, after approximately a decade of the phase out of PFOS. Longer time-trend biomonitoring studies are needed to confirm the evolution of the presence of PFAS in the environment and evaluate the effectiveness of pollution mitigation actions in areas of ecological interest, in a way to protect coastal bird species.

## Credit author statement

Pere Colomer-Vidal made the analysis of contaminants in gull eggs, contributed to the sampling and was involved in the writing and discussion of the results. Albert Bertolero set up the conceptual basis and was in charge of the sampling procedure and involved in the statistical analysis and interpretation, and also in the writing and supervision. Carles Alcaraz contributed to the multivariate and statistical analysis. Elba Garreta-Lara developed the method and the quantitative analysis and the quality control performace. Francisco Javier Santos supervised the analytical part and was involved in the writing. Silvia Lacorte set up the conceptual basis, was responsible for the analytical part and discussion of results and was in charge of the writing and supervision of the paper.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118555.

## References

Alcaraz, C., Bisazza, A., García-Berthou, E., 2008. Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. Oecologia 155, 205–213. https://doi.org/10.1007/s00442-007-0899-4.

Arcos, J.M., Oro, D., 2002. Significance of nocturnal purse seine fisheries for seabirds: a case study off the Ebro Delta (NW Mediterranean). Mar. Biol. 141, 277–286. https://doi.org/10.1007/s00227-002-0828-3.

Bertolero, A., Vicente, J., Meyer, J., Lacorte, S., 2015. Accumulation and maternal transfer of perfluorooctane sulphonic acid in yellow-legged (Larus michahellis) and Audouin's gull (Larus audouinii) from the Ebro Delta Natural Park. Environ. Res. 137, 208–214. https://doi.org/10.1016/j.envres.2014.12.018.

BirdLife International, 2019. Species Factsheet: Larus Michahellis ([WWW Document]).

Braune, B.M., Letcher, R.J., 2013. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: temporal trends (1975-2011) and interspecies comparison. Environ. Sci. Technol. 47, 616–624. https://doi.org/10.1021/es303733d.

Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ. Sci. Technol. 42, 995–1003. https://doi.org/10.1021/es070895g.

Duhem, C., Vidal, E., Roche, P., Legrand, J., 2005. How is the diet of yellow-legged gull chicks influenced by parents' accessibility to landfills? Waterbirds 28, 46–52. https://doi.org/10.1675/1524-4695(2005)028[0046:hitdoy]2.0.co;2.

Ellis, D.A., Martin, J.W., De Silva, A.O., Mabury, S.A., Hurley, M.D., Sulbaek Andersen, M.P., Wallington, T.J., 2004. Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. Environ. Sci. Technol. 38, 3316–3321. https://doi.org/10.1021/es049860w.

Faxneld, S., Berger, U., Helander, B., Danielsson, S., Miller, A., Nyberg, E., Persson, J.O., Bignert, A., 2016. Temporal trends and geographical differences of perfluoroalkyl acids in baltic sea herring and white-tailed sea eagle eggs in Sweden. Environ. Sci. Technol. 50, 13070–13079. https://doi.org/10.1021/acs.est.6b03230.

Fernández-Sanjuan, M., Meyer, J., Damásio, J., Faria, M., Barata, C., Lacorte, S., 2010. Screening of perfluorinated chemicals (PFCs) in various aquatic organisms. Anal. Bioanal. Chem. 398, 1447–1456. https://doi.org/10.1007/s00216-010-4024-x.

Gebbink, W.A., Hebert, C.E., Letcher, R.J., 2009. Perfluorinated carboxylates and sulfonates and precursor compounds in herring gull eggs from colonies spanning the laurentian great Lakes of north America. Environ. Sci. Technol. 43, 7443–7449. https://doi.org/10.1021/es901755q.

Gewurtz, S.B., Martin, P.A., Letcher, R.J., Burgess, N.M., Champoux, L., Elliott, J.E., Weseloh, D.V.C., 2016. Spatio-temporal trends and monitoring design of perfluoroalkyl acids in the eggs of gull (Larid) species from across Canada and parts of the United States. Sci. Total Environ. 565, 440–450. https://doi.org/10.1016/j.scitotenv.2016.04.149.

Giesy, J.P., Kannan, K., 2002. Peer reviewed: perfluorochemical surfactants in the environment. Environ. Sci. Technol. 36, 146A–152A. https://doi.org/10.1021/es022253t.

Gómez, C., Vicente, J., Echavarri-Erasun, B., Porte, C., Lacorte, S., 2011. Occurrence of perfluorinated compounds in water, sediment and mussels from the Cantabrian Sea (North Spain). Mar. Pollut. Bull. 62, 948–955. https://doi.org/10.1016/j.marpolbul.2011.02.049.

González-Solís, J., 2003. Impact of fisheries on activity, diet and predatory interactions between yellow-legged and Audouin's gulls breeding at the Chafarinas Islands. Sci. Mar. 67, 83–88. https://doi.org/10.3989/scimar.2003.67s283.

Groffen, T., Lasters, R., Lopez-Antia, A., Prinsen, E., Bervoets, L., Eens, M., 2019. Limited reproductive impairment in a passerine bird species exposed along a perfluoroalkyl acid (PFAA) pollution gradient. Sci. Total Environ. 652, 718–728. https://doi.org/10.1016/j.scitotenv.2018.10.273.

Holmström, K.E., Järnberg, U., Bignert, A., 2005. Temporal trends of PFOS and PFOA in guillemot eggs from the Baltic Sea, 1968-2003. Environ. Sci. Technol. 39, 80–84. https://doi.org/10.1021/es049257d.

Holmström, K.E., Johansson, A.K., Bignert, A., Lindberg, P., Berger, U., 2010. Temporal trends of perfluorinated surfactants in Swedish peregrine falcon eggs (Falco peregrinus), 1974-2007. Environ. Sci. Technol. 44, 4083–4088. https://doi.org/10.1021/es100028f.

Huber, S., Warner, N.A., Nygård, T., Remberger, M., Harju, M., Uggerud, H.T., Kaj, L., Hanssen, L., 2015. A broad cocktail of environmental pollutants found in eggs of three seabird species from remote colonies in Norway. Environ. Toxicol. Chem. 34, 1296–1308. https://doi.org/10.1002/etc.2956.

Kannan, K., Franson, J.C., Bowerman, W.W., Hansen, K.J., Jones, P.D., Giesy, J.P., 2001. Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ. Sci. Technol. 35, 3065–3070. https://doi.org/10.1021/es001935i.

Lopez-Antia, A., Kavelaars, M.M., Müller, W., Bervoets, L., Eens, M., 2021. Understanding PFAAs exposure in a generalist seabird species breeding in the vicinity of a fluorochemical plant: influence of maternal transfer and diet. Environ. Pollut. 271, 116355. https://doi.org/10.1016/j.envpol.2020.116355.

Miljeteig, C., Strøm, H., Gavrilo, M.V., Volkov, A., Jenssen, B.M., Gabrielsen, G.W., 2009. High levels of contaminants in ivory gull Pagophila eburnea eggs from the Russian and Norwegian arctic. Environ. Sci. Technol. 43, 5521–5528. https://doi.org/10.1021/es900490n.

Miller, A., Elliott, J.E., Elliott, K.H., Lee, S., Cyr, F., 2015. Temporal trends of perfluoroalkyl substances (PFAS) in eggs of coastal and offshore birds: increasing PFAS levels associated with offshore bird species breeding on the Pacific coast of Canada and wintering near Asia. Environ. Toxicol. Chem. 34, 1799–1808. https://doi.org/10.1002/etc.2992.

Morales, L., Martrat, M.G., Olmos, J., Parera, J., Vicente, J., Bertolero, A., Ábalos, M., Lacorte, S., Santos, F.J., Abad, E., 2012. Persistent Organic Pollutants in gull eggs of two species (Larus michahellis and Larus audouinii) from the Ebro delta Natural Park. Chemosphere 88, 1306–1316. https://doi.org/10.1016/j.chemosphere.2012.03.106.

Moreno, R., Jover, L., Munilla, I., Velando, A., Sanpera, C., 2010. A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. Mar. Biol. 157, 545–553. https://doi.org/10.1007/s00227-009-1340-9.

Morera-Pujol, V., Ramos, R., Pérez-Méndez, N., Cerdà-Cuéllar, M., González-Solís, J., 2018. Multi-isotopic assessments of spatio-temporal diet variability: the case of two sympatric gulls in the western Mediterranean. Mar. Ecol. Prog. Ser. 606, 201–214.

Munoz, G., Budzinski, H., Babut, M., Lobry, J., Selleslagh, J., Tapie, N., Labadie, P., 2019. Temporal variations of perfluoroalkyl substances partitioning between surface water, suspended sediment, and biota in a macrotidal estuary. Chemosphere 233, 319–326. https://doi.org/10.1016/j.chemosphere.2019.05.281.

Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A., Forero, M.G., 2010. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. Mar. Biol. 157, 2453–2459. https://doi.org/10.1007/s00227-010-1509-2.

Payo-Payo, A., Sanz-Aguilar, A., Genovart, M., Bertolero, A., Piccardo, J., Camps, D., Ruiz-Olmo, J., Oro, D., 2018. Predator arrival elicits differential dispersal, change in age structure and reproductive performance in a prey population. Sci. Rep. 8, 1971–1977. https://doi.org/10.1038/s41598-018-20333-0.

Pereira, M.G., Lacorte, S., Walker, L.A., Shore, R.F., 2021. Contrasting long term temporal trends in perfluoroalkyl substances (PFAS) in eggs of the northern gannet (Morus bassanus) from two UK colonies. Sci. Total Environ. 754, 141900. https://doi.org/10.1016/j.scitotenv.2020.141900.

Podder, A., Sadmani, A.H.M., Reinhart, D., Chang, N.B., Goel, R., 2021. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: a transboundary review of their occurrences and toxicity effects. J. Hazard Mater. 419, 126361. https://doi.org/10.1016/j.jhazmat.2021.126361.

Ramos, R., Ramírez, F., Sanpera, C., Jover, L., Ruiz, X., 2009a. Feeding ecology of yellow-legged gulls Larus michahellis in the western Mediterranean: a comparative assessment using conventional and isotopic methods. Mar. Ecol. Prog. Ser. 377, 289–297. https://doi.org/10.3354/meps07792.

Ramos, R., Ramírez, F., Sanpera, C., Jover, L., Ruiz, X., 2009b. Diet of Yellow-legged Gull (Larus michahellis) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. J. Ornithol. 150, 265–272. https://doi.org/10.1007/s10336-008-0346-2.

Rovira, A., Alcaraz, C., Ibáñez, C., 2012. Spatial and temporal dynamics of suspended load at-a-cross-section: the lowermost Ebro River (Catalonia, Spain). Water Res. 46, 3671–3681. https://doi.org/10.1016/j.watres.2012.04.014.

RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. URL. http://www.rstudio.com/.

Rüdel, H., Müller, J., Jürling, H., Bartel-Steinbach, M., Koschorreck, J., 2011. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from representative German ecosystems. Environ. Sci. Pollut. Res. 18, 1457–1470. https://doi.org/10.1007/s11356-011-0501-9.

Rüdel, H., Müller, J., Jürling, H., Schröter-Kermani, C., 2010. Retrospective Monitoring of Perfluorinated Compounds in Archived Herring Gull Eggs. Interdiscip. Stud. Environ. Chem. — Environ. Specim. Bank Explor. Possibility Setting-Up ESBs Dev. Ctries, pp. 81–86.

Sánchez-Avila, J., Meyer, J., Lacorte, S., 2010. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ. Pollut. 158, 2833–2840. https://doi.org/10.1016/j.envpol.2010.06.022.

Sánchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: sources distribution and potential risk. Environ. Int. 46, 50–62. https://doi.org/10.1016/j.envint.2012.04.013.

Sánchez-Avila, J., Vicente, J., Echavarri-Erasun, B., Porte, C., Tauler, R., Lacorte, S., 2013. Sources, fluxes and risk of organic micropollutants to the Cantabrian Sea (Spain). Mar. Pollut. Bull. 72, 119–132. https://doi.org/10.1016/j.marpolbul.2013.04.010.

Sebastiano, M., Angelier, F., Blévin, P., Ribout, C., Sagerup, K., Descamps, S., Herzke, D., Moe, B., Barbraud, C., Bustnes, J.O., Gabrielsen, G.W., Chastel, O., 2020. Exposure to PFAS is associated with telomere length dynamics and demographic responses of an arctic top predator. Environ. Sci. Technol. 54, 10217–10226. https://doi.org/10.1021/acs.est.0c03099.

Sinclair, G.M., Long, S.M., Jones, O.A.H., 2020. What are the effects of PFAS exposure at environmentally relevant concentrations? Chemosphere 258, 127340. https://doi.org/10.1016/j.chemosphere.2020.127340.

Stockholm Convention, 2019. List Perfluorooctanoic Acid (PFOA), its Salts and PFOA-Related Compounds in Annex A with Specific Exemptions (Decision SC-9/12). Unep/Pops/Cop.9/12 2–4.

Tabachnick, B.G., Fidell, L.S., 2000. Computer-Assisted Research Design and Analysis, first ed. Allyn & Bacon, Inc., USA.

Vassiliadou, I., Costopoulou, D., Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Zafeiraki, E., Dassenakis, M., Leondiadis, L., 2015. Levels of perfluorinated compounds in raw and cooked Mediterranean finfish and shellfish. Chemosphere 127, 117–126. https://doi.org/10.1016/j.chemosphere.2014.12.081.

Verreault, J., Berger, U., Gabrielsen, G.W., 2007. Trends of perfluorinated alkyl substances in herring gull eggs from two coastal colonies in northern Norway: 1983-2003. Environ. Sci. Technol. 41, 6671–6677. https://doi.org/10.1021/es070723j.

Vicente, J., Bertolero, A., Meyer, J., Viana, P., Lacorte, S., 2012. Distribution of perfluorinated compounds in Yellow-legged gull eggs (Larus michahellis) from the Iberian Peninsula. Sci. Total Environ. 416, 468–475. https://doi.org/10.1016/j.scitotenv.2011.11.085.

Vicente, J., Sanpera, C., García-Tarrasón, M., Pérez, A., Lacorte, S., 2015. Perfluoroalkyl and polyfluoroalkyl substances in entire clutches of Audouin's gulls from the ebro delta. Chemosphere 119, S62–S68. https://doi.org/10.1016/j.chemosphere.2014.04.041.

Wang, W., Lee, J., Oh, J.-K., Lee, S.-J., Choi, S.-D., Oh, J.-E., 2021. Per- and polyfluoroalkyl substances and their alternatives in black-tailed gull (Larus

crassirostris) eggs from South Korea islands during 2012–2018. J. Hazard Mater. 411, 125036. https://doi.org/10.1016/j.jhazmat.2020.125036.

Zapata, P., Ballesteros-Cano, R., Colomer, P., Bertolero, A., Viana, P., Lacorte, S., Santos, F.J., 2018. Presence and impact of Stockholm Convention POPs in gull eggs from Spanish and Portuguese natural and national parks. Sci. Total Environ. 633, 704–715. https://doi.org/10.1016/j.scitotenv.2018.03.081.

Zareitalabad, P., Siemens, J., Hamer, M., Amelung, W., 2013. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater – a review on concentrations and distribution coefficients. Chemosphere 91, 725–732. https://doi.org/10.1016/j.chemosphere.2013.02.024.