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Influence of trophic ecology on the accumulation of dioxins and furans (PCDD/Fs), non-*ortho* polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in Mediterranean gulls (*Larus michahellis and L. audouinii*): A three-isotope approach☆

Jose L. Roscales [a], [*], Alba Vicente [a], Juan Muñoz-Arnanz [a], Laura Morales [b], Esteban Abad [b], Jose I. Aguirre [c], Begoña Jiménez [a]

[a] *Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, IQOG-CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*
[b] *Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain*
[c] *Department of Zoology and Physic Anthropology, Faculty of Biology, University Complutense of Madrid, José Antonio Novais 2, Ciudad Universitaria, 28040 Madrid, Spain*

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ABSTRACT

The impact of pollution caused by severe anthropogenic pressure in the Mediterranean Sea, an important biodiversity hotspot, requires continuous research efforts. Sources of highly toxic chemicals such as Persistent Organic Pollutants (POPs) are misunderstood in representative Mediterranean species, which limits our capability to establish proper conservation strategies. In the present study, eggs of Audouin's and yellow-legged gulls (*Larus audouinii* and *L. michahellis*) were used to investigate the trophic sources, as measured by $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, of legacy POPs, in particular, polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and non-*ortho* polychlorinated biphenyls (no-PCBs), as well as recently-regulated POPs, e.g., polybrominated diphenyl ethers (PBDEs). Special attention was paid to the usefulness of rarely-explored $\delta^{34}S$ ratios in explaining POP exposure in wildlife, and $\delta^{34}S$ was the isotopic ratio that best explained POP variations among gulls in most cases, thus demonstrating its usefulness for understanding POP exposure in wildlife. Significant relationships between stable isotope signatures and POP concentrations revealed increasing levels of no-PCBs and low halogenated PCDD/Fs and PBDEs in Mediterranean gulls as the consumption of marine resources increases. In contrast, highly chlorinated and brominated congeners appeared to preferentially accumulate in gulls feeding primarily on refuse from dump sites and terrestrial food webs. The use of suitable dietary tracers in the study of POPs in yellow-legged gulls revealed the importance of dump sites as a source of POPs in Mediterranean seabirds, which has not previously been reported. In contrast, the preferential accumulation through marine food webs of low chlorinated PCCD/Fs and no-PCBs, which show the highest toxic equivalents factors (TEFs), led to a significantly greater toxicological concern in Audouin's as compared to yellow-legged gulls. Audouin's gull exposure to POPs appears primarily related to the pelagic food webs commonly exploited by fisheries, highlighting the need for further research given the potential impact on human consumption.

1. Introduction

Severe impacts of marine pollution have been identified within the Mediterranean basin (Danovaro, 2003; Albaigés, 2005; Zorita et al. 2007; Coll et al. 2010). Persistent organic pollutants (POPs) in particular have been recognized for many years as a significant threat to Mediterranean wildlife, especially for top predators, given that POPs biomagnify through food webs (Jiménez et al. 2000; Albaigés, 2005; Roscales et al. 2010; Fossi et al. 2013). Among

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[*] Corresponding author. Dept. of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

E-mail address: jlroscales@iqog.csic.es (J.L. Roscales).

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them, 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are ranked among the most toxic POPs, along with the non-*ortho* substituted polychlorinated biphenyls (no-PCBs). Several studies have shown that PCDD/F and PCB levels in the environment have declined since the 1980's in several developed regions (Alcock and Jones, 1996; Srogi, 2008) including the Mediterranean basin (Munschy et al. 2008; Augusto et al. 2015). However, significant levels, probably resulting from chronic exposure, are still reported in some Mediterranean regions, and this justifies continuous research on these pollutants (e.g. Di Leo et al. 2014; Storelli and Zizzo, 2014).

In contrast to legacy organochlorines, regulations on the use of emerging POPs, such as polybrominated diphenyl ethers (PBDEs), are more recent (Stockholm Convention, 2006), and research on their environmental presence is still imperative (Law et al. 2014). PBDEs were widely applied as flame retardants until *penta*- and *octa*-PBDE formulations were banned in most industrialized countries as of 2004 (Jinhui et al. 2015). Although several studies have related this ban with decreasing temporal trends in the presence of PBDEs associated with these formulations (e.g., Miller et al. 2014), general global temporal trends cannot be assumed. During the last decade, both increasing and decreasing temporal patterns have been described in wildlife and other environmental matrixes in developed regions such as Europe and North America (Chen and Hale, 2010; Law et al. 2014; Miller et al. 2015). Although PBDE-209, the main component of *deca*-PBDE formulation, is not yet included in the POPs list under the Stockholm Convention, bans on *deca*-PBDE production have recently been placed in developed countries (Jinhui et al. 2015). However, levels of PBDE-209, are thought to have been generally increasing in the environment during the last decade (Law et al. 2014). In agreement with those findings, studies on air and wildlife suggest that levels of PBDE-209 are increasing in the Mediterranean region (Muñoz-Arnanz et al. 2011; Roscales et al. 2013).

Many studies have demonstrated the usefulness of seabirds as bioindicators of marine pollution (Burger and Gochfeld, 2004; Roscales et al. 2010). In particular, yellow-legged and Audouin's gulls (*Larus michahellis* and *L. audouinii*) have been widely used to monitor POPs in the Mediterranean Sea (Pastor et al. 1995; Morales et al. 2012; Lacorte et al. 2014). However, to our knowledge, no studies on the trophic factors driving POP exposure in these species have been conducted yet, and never on an individual-level basis. This is particularly relevant because diet constitutes the main source of POPs in birds, and dietary habits of yellow-legged and Audouin's gulls vary markedly between species as well as across their distribution (González-Solís et al. 1997a; Ramos et al. 2011). Trophodynamics of POPs in gulls can be addressed by using dietary tracers such as stable isotope ratios of carbon, nitrogen, and sulfur (δ^{13}C, δ^{15}N and δ^{34}S), because ratios in consumers reflect those of their prey in a predictable manner (Kelly, 2000). δ^{15}N has commonly been used to delineate biomagnification and bioaccumulation of POPs (Hop et al. 2002). δ^{13}C provides information about the origin of resources exploited by consumers (e.g., δ^{13}C-terrestrial < δ^{13}C-marine ecosystems) and thus, the potential trophic sources of POPs (Chen et al. 2012). δ^{34}S has been proven to be very useful for addressing trophic preferences and pollutant sources in gulls, because sulfur ratios provide better discrimination than δ^{13}C when several land-based and aquatic resources, such as refuse, terrestrial, continental water, and marine items, are involved (Moreno et al. 2010; Ramos et al. 2013). Although relatively common in trophic studies, to our knowledge, δ^{34}S has not yet been evaluated to address trophodynamics of POPs in birds.

In the present study, stable isotope ratios of C, N, and S, and concentrations of PCDDs, PCDFs, no-PCBs, and PBDEs were measured in individual eggs of yellow-legged and Audouin's gulls breeding sympatrically in the Chafarinas Islands, SW Mediterranean. We aimed to investigate the influence of trophic ecology of gulls on their exposure to POPs, and to provide a more comprehensive view of their role as potential bioindicators in the Mediterranean basin than in previous studies. Because POP studies in seabirds have not previously explored the potential of δ^{34}S, special attention was paid to the usefulness of adding S ratios to the commonly-used δ^{13}C and δ^{15}N. In addition, temporal variations and potential toxicological implications related to POPs in Mediterranean gulls are evaluated.

2. Materials and methods

2.1. Sampling design and studied species

A total of 19 and 18 eggs from Audouin's and yellow-legged gulls, respectively, were analyzed in the present study. Samples were collected during the 2007 breeding season in the Chafarinas Islands (35°10′ − 35°11′N and 2°24′− 2°27′W), which are located close to the North African coast of Morocco within the Alboran Sea, SW Mediterranean (supplementary material, Fig. S1). The breeding season of these gulls spans about four months, and eggs were collected during the first days of the laying period, in early March and early April, for yellow-legged and Audouin's gulls, respectively. The collection of eggs used in this study was previously used to assess emerging contaminants such as Dechlorane plus and further details on sampling procedure can be found in Muñoz-Arnanz et al. (2012).

In the Mediterranean, the yellow-legged gull is considered a superabundant and highly-opportunistic predator that can feed on a wide array of terrestrial and marine resources, as well as on food items derived from human activities (Ramos et al. 2009). In contrast, Audouin's gull is a scarce and endemic Mediterranean species that mainly feeds on epipelagic fish prey and fishery discards (Arcos et al. 2001). Outside of the breeding season, the yellow-legged gull is considered to be a non-migratory and dispersive species (Sol et al. 1995). Audouin's gulls are known to visit NW Atlantic waters, although a portion of the population remains in the Mediterranean (Sanpera et al. 2007). Nonetheless, both species are considered income breeders (Ruiz et al. 1998; Ramírez et al. 2010). Therefore, isotope ratios and pollutant levels in eggs are expected to reflect gull diets and pollutant sources mainly from their breeding area.

2.2. Stable isotope analysis (SIA)

About 0.36 mg (weighed to the nearest μg) of dried lipid-free egg were placed into tin cups for δ^{15}N and δ^{13}C analysis (see further details on sample preparation in Muñoz-Arnanz et al. 2012). Additionally, 3.60 mg of sample were used to determine δ^{34}S values. Analyses were carried out in the Serveis Científico-Técnics of the University of Barcelona. Stable isotope ratios were determined by means of elemental analysis-isotope ratio mass spectrometry (EA-IRMS) using a Carlo-Erba Flash 1112 series elemental analyzer coupled to a Finnigan Delta C isotope ratio mass spectrometer by means of a Thermo Finnigan MAT ConFlo III interface. Stable isotope ratios were expressed as δ values in parts per thousand (‰) relative to atmospheric nitrogen (AIR; δ^{15}N), Vienna Pee Dee Belemnite (VPDB; δ^{13}C), and Vienna Cañon Diablo Troilite (VCDT; δ^{34}S). Three reference materials (International Atomic Energy Agency, IAEA) were analyzed every 12 samples to calibrate the equipment and correct potential shifts over time (detailed data in the supporting information, Table S1).

2.3. Chemicals and residue analysis

We used pesticide residue analysis grade acetone and toluene (SDS, Peypin, France) and n-hexane (Merck, Darmstadt, Germany). Sodium sulfate anhydrous and silica gel (70–230 mesh) were supplied by Merck and J.T.Baker (Deventer, Netherlands), respectively. Supelclean™ ENVI™-Carb tubes used for fractionation were supplied by Supelco (Bellefonte, Pennsylvania, USA). Native and labeled standards of PBDEs, PCDFs, PCDDs, and no-PCBs were purchased from Wellington Laboratories (Guelph, Ontario, Canada). A detailed list of all the standards used is provided in the supplementary material (Table S2).

Approximately 0.5 g of lyophilized egg were used to determine the lipid content gravimetrically with 100 mL of acetone:hexane (1:1). From 2 to 3 g of sample were used for residue analysis based on previously-described methods (Merino et al. 2005). Briefly, samples were spiked with $^{13}C_{12}$-labeled standards (Supplementary Material Table S2), and extraction and purification were based on a matrix solid phase dispersion (MSPD) procedure, followed by a clean-up with multilayer acid and basic silica columns. Fractionation of target analytes was performed with Supelclean™ ENVI-Carb™ tubes.

Identification and quantification of PBDEs were performed by high resolution gas chromatography-low resolution mass spectrometry (HRGC-LRMS) using a 6890N gas chromatograph equipped with a DB-5MS low bleed column (15 m × 0.20 mm × 0.20 μm film thickness; J&W Scientific, Folsom, CA, USA) coupled with a 5975N quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). The mass spectrometer operated in selected ion monitoring mode (SIM) with electron capture negative ionization (ECNI). Further details on operating conditions can be found in the supplementary material (Supplement 1). The identification of target compounds was based on the detection at the corresponding retention time of at least two *m/z* ions of the product ion cluster of each congener (supplementary material, Table S3). Quantification was based on a linear seven-calibration curve (from 1 to 200 pg/μL) using $^{13}C_{12}$-PBDE-139 as the internal standard.

PCDD/Fs and no-PCBs were analyzed by HRGC coupled to high resolution mass spectrometry (HRMS). All the analyses were performed on a TRACE™ Ultra Gas Chromatograph (Thermo Fisher Scientific, Milan, Italy) fitted with a DB-5ms fused silica column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) for PCDD/Fs, and a DB-XLB fused silica column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) for no-PCBs, coupled to a high resolution mass spectrometer (DFS, Thermo Fisher Scientific) operated in positive electron ionization (EI+) source operating in SIM mode at 10,000 resolving power (10% valley definition). Further operational details can be found in Morales et al. 2014. Quantification of PCDD/Fs and no-PCBs were carried out by the isotopic dilution technique.

2.4. Quality assurance/quality control (QA/QC)

QA/QC criteria included the analysis of blank samples covering the complete analytical procedure. In those cases where some analytes showed levels above the limit of detection (LOD) in the blanks, they were subtracted from those found in the corresponding sample batch. Identification and quantification of PBDEs was ensured by: (a) similar GC retention times (±0.1 min) as those of standard compounds, (b) a ratio between the monitorized ions of ±15% of the mean values obtained for the calibration standards, and (c) a signal-to-noise ratio of 10 as the limit of quantification (LOQ). Average LOQs for PBDEs in egg samples ranged from 8 to 50 pg/g on a wet weight basis (ww). In the case of PCDD/Fs and no-PCBs determination, QA/QC criteria were applied as previously described in Morales et al. 2012. Average LODs ranged from 0.05 to 0.19 for PCDD/Fs and from 0.13 to 0.44 pg/g ww for no-PCBs. Recovery for labeled PBDEs, no-PCBs, and PCDD/Fs were satisfactory with average values over 75% in all cases (supplementary material, Table S4).

2.5. Data analysis

Statistical analyses were carried out using IBM SPSS Statistics 21 for Windows (SPSS Inc, Chicago, IL, USA). Concentrations are expressed in terms of wet weight since non-significant correlations were found between the lipid content of eggs and pollutant concentrations. Pollutant concentrations bellow the LOD were substituted by ½ LOD because our dataset meets the conditions for substitution without significant bias in the results (see Ogden, 2010): the percent of non-detects is between 1 and 50 for all the considered variables, number of observations between 20 and 100, and lognormal distribution, with geometric standard deviation (GSD) between 1.2 and 4. Normal distribution of stable isotope values and pollutant concentrations was checked by means of Q–Q plots and the Shapiro–Wilk test. Pollutant concentrations were log transformed (ln) to satisfy the assumptions of a normal distribution and homogeneous variance. Mean egg lipid content and moisture (%) is provided for further conversions (Table 1). In the case of PCDD/Fs and no-PCBs, toxic 2,3,7,8-TCDD equivalents (TEQs) were estimated based on the bird toxic equivalency factors (TEFs) reported in 1998 by the World Health Organization (Van den Berg et al. 1998). In order to evaluate interspecies patterns of the POPs under study, Principal Component Analysis (PCA) based on contaminant concentrations in gull eggs was conducted separately for organochlorines (PCDD/Fs and no-PCBs) and brominated compounds (PBDEs). The selected procedure with SPSS software was Categorical Principal Component Analysis (CATPCA) because both the object punctuations and variable saturations can be presented. When all the variables included in the CATPCA are continuous and numerical, and when the principal variable is selected as the normalization method, which is the case in the present study, the procedure is analogous to the common PCA (Meulman and Heiser, 2010). Moreover, the CATPCA procedure can include supplementary variables ($\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$) that are fitted into the procedure solution but are not considered for the extraction of components. A backwards stepwise linear modeling procedure (LM) was used to assess the relationship between pollutant concentrations (response variables) and stable isotope ratios, as well as to evaluate the best predictor among $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$. To reduce the number of analyses, POP concentrations were grouped according to their halogen substituents (bromine or chlorine). Direct interspecies comparisons for the studied variables were performed using Student's t-test.

3. Results and discussion

3.1. POP concentrations and interspecies patterns

Among PBDEs, PBDE-17, -28 and 66 levels were below the LOD in all eggs of both species. PBDE-85 and -184 levels were above the LOQ in only one sample of Audouin's and yellow-legged gull, respectively. In the case of PCDD/Fs, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8- and 1,2,3,4,7,8-HxCDD were found below the LOD in all samples. In addition, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8,9-HpCDF, OCDF, and 1,2,3,7,8,9,-HxCDD levels were below the LOD in more than 50% of the samples for both species. Therefore, none of these analytes were considered for statistical comparisons and total mean concentrations.

Total PBDEs (ΣPBDEs) did not differ significantly between Audouin's and yellow-legged gull eggs (Table 1). In contrast,

Table 1
Median, mean, standard deviation (SD), minimum and maximum PBDE concentrations (pg/g ww), lipid and moisture content (%), and stable isotope ratios (‰) of S, N, and C in eggs of Audouin's and yellow-legged gulls.

	Audouin's gull (n = 18)						Yellow-legged gull (n = 19)						Interspecies Student's t-test*
	% <LOQ	Median	Mean	SD	Min	Max	% <LOQ	Median	Mean	SD	Min	Max	
PBDE-17	100	–	–	–	–	–	100	–	–	–	–	–	–
PBDE-28	89	–	–	–	<LOQ	5.227	100	–	–	–	–	–	–
PBDE-47	0	5280	5970	3650	1570	17,100	0	2840	3150	1830	658.8	7990	$t = 3.28$, $p < 0.002$**
PBDE-66	100	–	–	–	–	–	100	–	–	–	–	–	–
PBDE-100	0	1130	1730	1500	400	5880	6	627	692	387	0	1420	$t = 2.73$, $p = 0.01$
PBDE-99	0	766	1210	1790	176	8370	6	741	1060	935	12.35	3700	$t = 0.40$, $p = 0.69$
PBDE-85	100	–	–	–	–	–	94	–	–	–	<LOQ	80.8	–
PBDE-154	0	224	296	188	80.8	696	0	232	232	120	80.91	521	$t = 1.06$, $p = 0.30$
PBDE-153	0	297	412	571	73.0	2730	0	503	486	213	184.1	956	$t = -2.06$, $p = 0.05$
PBDE-184	100	–	–	–	–	–	94	–	–	–	<LOQ	16.5	–
PBDE-183	63	–	–	–	<LOQ	135	0	130	179	147	18.53	534	$t = -8.32$, $p < 0.002$**
PBDE-191	37	11.7	14.1	10.9	<LOQ	53.6	28	11.9	12.9	7.45	<LOQ	35.6	$t = 0.33$, $p = 0.74$
PBDE-197	79	–	–	–	<LOQ	247	11	85.1	130	148	<LOQ	649	$t = -6.57$, $p < 0.002$**
PBDE-196	89	–	–	–	<LOQ	28.2	17	44.3	99.5	184	<LOQ	807	$t = -9.14$, $p < 0.002$**
PBDE-209	68	–	–	–	<LOQ	562	33	116	475	597	<LOQ	1940	$t = -4.90$, $p < 0.002$**
ΣPBDEs		**8050**	**9720**	**6230**	**2330**	**25,400**		**5290**	**6520**	**3510**	**1940**	**15,000**	$t = 2.01$, $p = 0.05$
Lipids (%)	–	8.61	8.30	2.04	4.96	11.9	–	7.66	7.78	1.80	4.21	10.6	$t = -1.41$, $p = 0.17$
Moisture (%)	–	72.6	72.3	3.34	64.9	76.7	–	73.8	73.6	2.51	67.9	76.7	
$\delta^{34}S$ (‰)	–	18.3	18.2	0.67	17.0	19.7	–	14.6	14.1	2.72	9.50	19.2	$t = 6.25$, $p < 0.002$**
$\delta^{15}N$ (‰)	–	12.3	12.2	0.24	11.7	12.7	–	10.9	10.9	0.78	9.21	12.1	$t = 7.08$, $p < 0.002$**
$\delta^{13}C$ (‰)	–	−18.6	−18.6	0.36	−19.5	−18.2	–	−19.8	−19.7	0.73	−21.4	−18.7	$t = 5.55$, $p < 0.002$**

*- p values for significant differences have been set to $p < 0.002$ according to the Bonferroni adjustment.
**-Significant differences between yellow-legged and Audouin's gull eggs.

significant interspecific patterns emerged when comparing PBDE congeners separately, as reflected by PCA based on PBDE levels in the gull eggs (Fig. 1a). PCA resulted in two principal components (PC) with eigenvalues over Kaiser's criterion of 1 (Field, 2009). An inflexion point in the scree plot justified retaining two PC. PC1 and PC2 explained 44% and 29% of the initial variance, respectively. The Kaiser-Meyer-Olkin measure (KMO = 0.74) verified the sampling adequacy for the analysis (Hutcheson and Sofroniou, 1999), and all variables accounted for factor loadings greater than 0.3 in at least one of the retained components. Visualization of the PC scores (Fig. 1a) segregated the studied samples along PC1 by species. This was a result of the lower levels of *hepta*-, *octa*-, and *deca*-PBDEs, but greater levels of *tetra*- and *penta*-PBDEs found in Audouin's compared with yellow-legged gull eggs (see Table 1 for interspecific Student's t-test comparisons). Due to interspecies differences in the accumulation of PBDE congeners, relative contributions (%) of PBDE congeners to ∑PBDEs varied among gull species (supplementary material, Fig. S2). Although PBDE-47 was by far the most abundant congener (mean of 63% in Audouin's gulls and 48% in yellow-legged gulls), the mean contribution of *tetra*-plus *penta*-PBDEs was noticeably greater in Audouin's (91%) than in yellow-legged gulls (73%). Among the heavier PBDEs, *hexa*-brominated congeners accounted for 8% and 13% in Audouin's and yellow-legged gulls, respectively. While *hepta*-to *deca*-PBDEs represented less than 1.5%

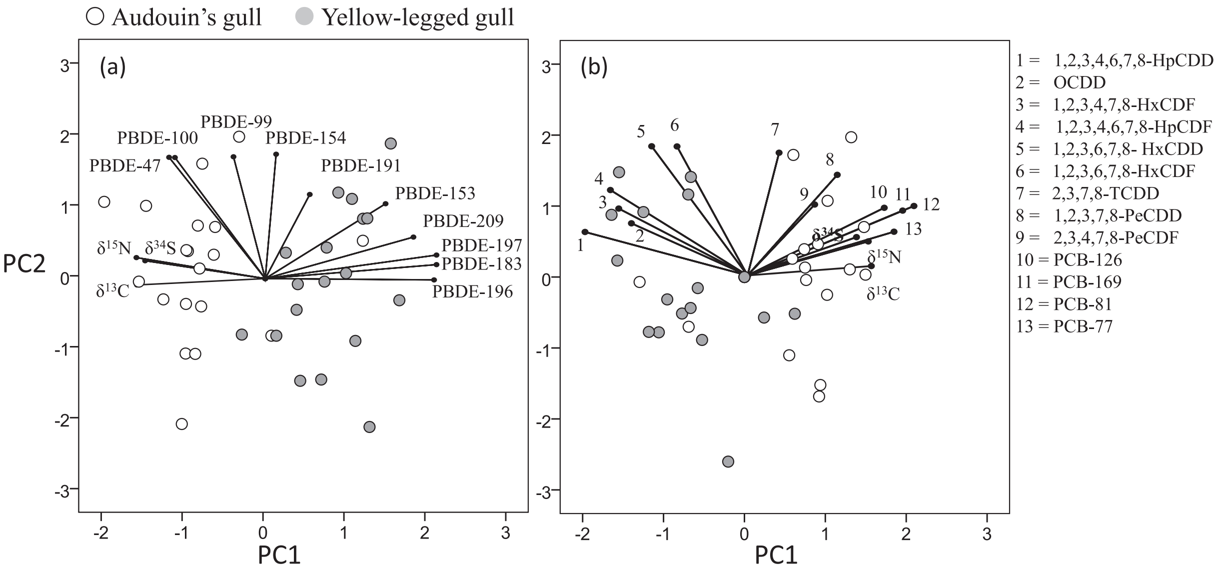

Fig. 1. Output of principal component analysis (PCA) based on (a) PBDE and (b) PCDD/F and non-ortho PCB concentrations in Audouin's and yellow-legged gull eggs. Stable isotope signatures ($\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$) were included in both cases as supplementary variables. Variable saturations on PC1 and PC2 are shown (lines) for all the variables. Line directions indicate increasing values for variables. Angles between lines represent qualitative relationships between variables (0° maximum positive correlation; 90° no relationship; 180° maximum negative correlation)

of ∑PBDEs in Audouin's gull eggs, they contributed 13% of ∑PBDEs in yellow-legged gulls, with PBDE-209 being the most abundant congener.

Total concentrations of furans (∑PCDFs), dioxins (∑PCDDs), and no-PCBs (∑no-PCBs) in gull eggs did not differ significantly between species (Table 2). As in the case of PBDEs, significant interspecific patterns emerged when separately comparing each of the PCDD/Fs and no-PCBs congeners, as reflected by the results of PCA based on their concentrations (Fig. 1b). Two out of four factors were retained according to Kaiser's criterion and the scree plot. The KMO measure (0.70) was satisfactory, and PC1 and PC2 explained 39% and 21% of the initial variance, respectively. Most samples were segregated by species along PC1. *Tetra*- and *penta*-substituted PCDD/Fs, as well as all no-PCBs tended to show higher concentrations in Audouin's gulls, while the opposite pattern was found for *hexa*-, *hepta*-, and *octa*-substituted PCDD/Fs (see Table 2 for interspecies Student's t-test). These interspecies patterns resulted in noticeable interspecies differences in the contribution of dioxins and furans to ∑PCDD/Fs (supplementary material, Fig. S2). OCDD showed the greatest mean contributions in both species (17% and 29% in Audouin's and yellow-legged gull eggs, respectively). However, while OCDD was the most abundant in 5 out of 19 Audouin's gull eggs, it was the prevalent compound in 100% of yellow-legged gull eggs. In the case of Audouin's gull, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD showed mean contributions of 14 and 16%, respectively, which was almost similar to OCDD, followed by 2,3,7,8-TCDD (8%). In contrast, the pattern in yellow-legged gull eggs was 1,2,3,4,6,7,8-HpCDD (12%) > 1,2,3,6,7,8-HxCDD (9%) > 1,2,3,7,8-PeCDD (7%). Regarding PCB contributions to ∑no-PCBs, PCB-126 was by far the most abundant congener in both gull species (means ≥ 70%). While mean abundances of PCB-81 and -77 were greater in Audouin's than in yellow-legged gull eggs (2 and 18%, 1 and 9%, respectively), PCB-169 showed a similar mean contribution in both species (11%). Yellow-legged and Audouin's gulls are phylogenetically close species. Therefore, although we cannot completely rule out some physiological differences between them, interspecies patterns in pollutant concentrations described here are more likely to be related to dietary habits than to metabolic differences between the species.

3.2. *Foraging ecology and POP exposure*

The mean stable isotope ratios of N, C, and S were significantly greater in Audouin's than in yellow-legged gull eggs (Table 1). Interestingly, $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values showed noticeably wider variations in yellow-legged than in Audouin's gulls (Fig. 2), in line with the respective generalist and specialist character of the species. The stable isotope results in gull eggs agree well with dietary information available from previous studies (González-Solís et al. 1997a, 1997b; González-Solís, 2003). Audouin's gulls breeding in the Chafarinas mainly feed on marine resources from trawling discards or caught in the open sea (mean > 90% biomass fresh weight percentage, fw). Stable isotope ratios of C and S are greater as the consumption of marine resources increases (Ramos et al. 2009; Moreno et al. 2010). Therefore, the high values and small range found for $\delta^{13}C$ and $\delta^{34}S$ in Audouin's gull eggs, together with the available dietary information, indicate that female Audouin's gulls mainly fed on marine prey during egg formation. The diet of yellow-legged gulls in the study area includes terrestrial prey (up to 2% biomass fw percentage) as well as a great proportion of refuse (up to 44% fw) from nearby dumps. Ramos et al. (2009) showed that refuse consumption results in significantly lower $\delta^{13}C$ and $\delta^{34}S$ ratios in Mediterranean yellow-legged gulls compared with consumption of natural terrestrial and marine resources. Refuse also tends to cause the lowest $\delta^{15}N$ ratios as a result of a short food chain. The lowest values of $\delta^{34}S$, which provides better

Table 2
Median, mean, standard deviation (SD), minimum and maximum PCDD, PCDF, and no-PCB concentrations (pg/g ww) in eggs of Audouin's and yellow-legged gulls.

	Audouin's gull (n = 18)						Yellow-legged gull (n = 19)						Interspecies Student's t-test
	%<LOD	Median	Mean	SD	Min	Max	%<LOD	Median	Mean	SD	Min	Max	
2,3,7,8-TCDF	53	–	–	–	<LOD	0.09	76	–	–	–	<LOD	0.16	–
1,2,3,7,8-PeCDF	76	–	–	–	<LOD	0.24	100	–	–	–	–	–	–
2,3,4,7,8-PeCDF	12	0.32	0.30	0.12	<LOD	0.68	18	0.13	0.16	0.12	<LOD	0.51	$t = -3.29$, $p < 0.002^{**}$
1,2,3,4,7,8-HxCDF	65	–	–	–	<LOD	0.08	24	0.08	0.09	0.07	<LOD	0.34	$t = -2.50$, $p = 0.02$
1,2,3,6,7,8-HxCDF	41	0.08	0.09	0.06	<LOD	0.18	35	0.09	0.11	0.07	<LOD	0.22	$t = -0.89$, $p = 0.38$
2,3,4,6,7,8-HxCDF	65	–	–	–	<LOD	0.12	82	–	–	–	<LOD	0.09	–
1,2,3,7,8,9-HxCDF	100	–	–	–	–	–	100	–	–	–	–	–	–
1,2,3,4,6,7,8-HpCDF	94	–	–	–	<LOD	0.11	41	0.08	0.10	0.07	<LOD	0.27	–
1,2,3,4,7,8,9-HpCDF	100	–	–	–	–	–	100	–	–	–	–	–	–
OCDF	88	–	–	–	<LOD	0.29	82	–	–	–	<LOD	0.58	–
ΣPCDF		**0.81**	**0.83**	**0.42**	**0.50**	**1.46**		**0.75**	**0.81**	**0.38**	**0.43**	**2.25**	$t = -0.83$, $p = 0.42$
2,3,7,8-TCDD	18	0.19	0.18	0.09	<LOD	0.40	12	0.13	0.12	0.04	<LOD	0.19	$t = -2.06$, $p = 0.05$
1,2,3,7,8-PeCDD	12	0.33	0.35	0.15	<LOD	0.73	29	0.19	0.17	0.07	<LOD	0.27	$t = -3.89$, $p < 0.002^{**}$
1,2,3,4,7,8-HxCDD	100	–	–	–	–	–	100	–	–	–	–	0.06	–
1,2,3,6,7,8-HxCDD	18	0.16	0.17	0.09	<LOD	0.30	18	0.23	0.21	0.09	<LOD	0.34	$t = -0.74$ $p = 0.08$
1,2,3,7,8,9,-HxCDD	94	–	–	–	<LOD	0.13	65	–	–	–	<LOD	0.18	–
1,2,3,4,6,7,8-HpCDD	24	0.09	0.16	0.18	<LOD	0.45	12	0.30	0.29	0.12	<LOD	0.47	$t = -4.07$, $p < 0.002^{**}$
OCDD	13	0.31	0.36	0.21	<LOD	0.87	0	0.65	0.75	0.42	0.24	1.78	$t = -3.61$, $p < 0.002^{**}$
ΣPCDD		**1.24**	**1.27**	**0.55**	**0.43**	**1.88**		**1.67**	**1.66**	**0.57**	**0.64**	**2.51**	$t = -2.03$, $p = 0.05$
ΣPCDD_F		**1.98**	**2.10**	**0.91**	**0.95**	**2.95**		**2.38**	**2.47**	**0.82**	**1.07**	**4.41**	$t = -1.15$, $p = 0.25$
PCB81	0	2.53	2.65	1.73	0.07	6.25	0	0.98	0.98	0.45	0.24	2.02	$t = 3.56$, $p < 0.002^{**}$
PCB77	0	17.1	19.2	11.5	1.36	42.2	0	7.22	9.37	6.52	3.32	27.3	$t = 2.59$, $p = 0.01$
PCB126	0	149	156	83.6	0.21	316	0	72.8	78.4	24.0	50.3	138	$t = 0.50$, $p = 0.62$
PCB169	0	25.9	24.1	12.2	0.03	46.4	0	11.1	11.4	3.09	7.31	18.4	$t = 1.53$, $p = 0.14$
Σno-PCBs		**202**	**202**	**105**	**2.00**	**387**		**91.2**	**100**	**29.0**	**63.7**	**164**	$t = 0.87$, $p = 0.39$
ΣTEQ PCDDFs		0.91	0.95	0.32	0.15	1.53		0.48	0.49	0.17	0.10	0.77	$t = 3.78$, $p < 0.002^{**}$
ΣTEQ-no-PCBs		16.5	16.9	8.89	0.11	33.2		7.60	8.41	2.51	5.34	14.2	$t = 7.21$, $p < 0.002^{**}$
ΣTEQ		**17.9**	**17.9**	**8.91**	**1.38**	**34.7**		**8.15**	**8.91**	**2.60**	**5.59**	**14.9**	$t = 7.30$, $p < 0.002^{**}$

*- p values for significant differences have been set to $p < 0.002$ according to the Bonferroni adjustment.
**-Significant differences between yellow-legged and Audouin's gull eggs.

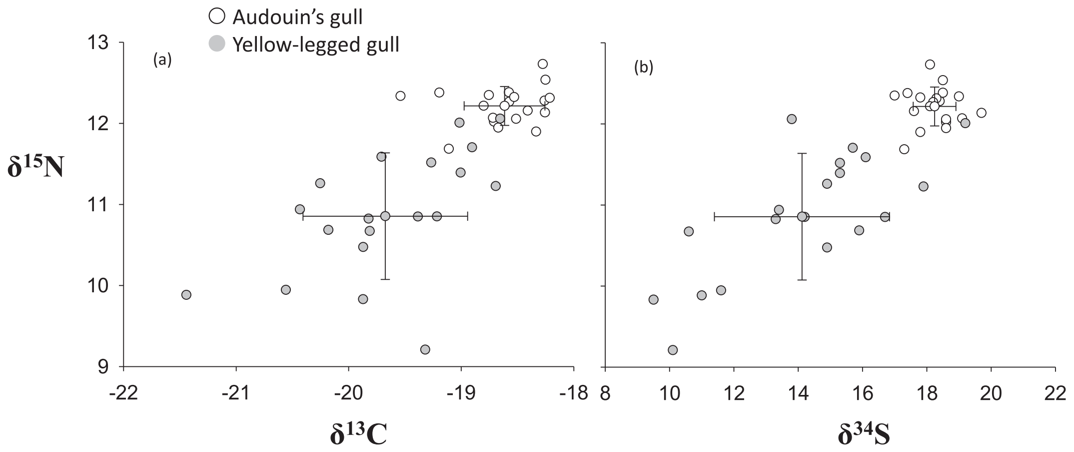

Fig. 2. Stable isotope ratios (a) δ^{15}N vs. δ^{13}C and (b) δ^{15}N vs. δ^{34}S in individual Audouin's and yellow-legged gull eggs. Mean ± SD is also shown for each species

discrimination between refuse and other potential dietary items, in yellow-legged gulls reported here overlap with those found in colonies where this species feeds on a significant proportion of refuse (>40% fw) (Ramos et al., 2009). On the contrary, the highest δ^{13}C, δ^{15}N, and δ^{34}S values found in yellow-legged gull eggs overlapped with those found in Audouin's gull eggs. The higher ranges of δ^{13}C, δ^{15}N, and especially δ^{34}S found in yellow-legged gull eggs indicate that some females largely exploited refuse while others could feed preferentially on marine prey during the prelaying period.

Concentrations of *tetra*- and *penta*-PBDES, as well as *tetra*- and *penta*-substituted PCDD/Fs and no-PCBs, tended to increase with values of δ^{15}N, δ^{13}C, and δ^{34}S in gull eggs, as shown by PCA analysis (Fig. 1). The remaining pollutants studied showed decreasing concentrations with stable isotope ratios. In the case of PBDEs, LM showed a significant positive relationship between stable isotope ratios and *tetra*-PBDEs, and significant negative relationships for *hepta*-, *octa*-, and *deca*-PBDEs (R^2 ranged from 0.21 to 0.56, all $p < 0.05$, LM details in Table S5a). Final models included only δ^{34}S in the case of *tetra*-, *hepta*-, and *deca*-PBDEs, and both δ^{34}S and δ^{13}C for *octa*-PBDEs. *Tetra*- and *penta*-PCDD/Fs and *tetra*-no-PCBs showed a positive significant relationship with stable isotope ratios, while *hepta*-PCDD/Fs showed a negative significant relationship (R^2 ranged from 0.14 to 0.30, all $p < 0.05$, see Table S5b). Final models for PCDD/Fs and no-PCBs included only δ^{34}S in all cases. Overall, the significance of δ^{34}S and δ^{13}C reveal that the origins of the resources consumed by gulls are the main source of variations in their exposure to the studied POPs. Our results show an increase in the presence of less substituted PCDD/Fs and PBDEs in Mediterranean gulls as the consumption of marine resources increases. In contrast, highly chlorinated and brominated congeners ($\geq$*hexa*-) are preferentially accumulated in gulls through refuse and terrestrial food webs. In addition, our three-isotope modeling results showed that δ^{34}S was the isotopic ratio that best explained POP variations among gull eggs in most cases.

Although ΣPCDD/F and Σno-PCB concentrations did not differ significantly between species, TEQ values were significantly greater in Audouin's than in yellow-legged gulls (Table 2). As described above, a marine diet provides significantly higher amounts of lighter PCDD/Fs and no-PCBs: 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, PCB-77, and PCB-81. These congeners have the highest TEF values and thus explain the significant interspecific pattern in TEQ values. In fact, total TEQ values (ΣTEQ) were positively- and significantly-correlated with stable isotope signatures of C, N, and S in gull eggs ($r = 0.58$, $r = 0.61$, and $r = 0.71$, respectively; all $p < 0.001$). These relationships reflect increasing TEQ values with the consumption of marine resources (summarized in Fig. S3). Consequently, Mediterranean seabirds inhabiting similar regions can suffer different toxic exposure levels to dioxin-like compounds depending on their dietary habits.

Several factors can contribute to a differential accumulation of PBDE and PCDD/F congeners between gulls feeding on terrestrial and marine ecosystems. Fate and bioavailability of POPs, as well as their bioaccumulation, biomagnification, and biotransformation, are both congener and food web type dependent. The bioaccumulation and biomagnification capacity of PBDE congeners strongly depends on their Log K_{OW} in aquatic food webs. According to Kelly et al. (2007), congeners with Log K_{OW} values between ~5.9 and ~7.2 (such as the *tetra*- and *penta*-PBDEs included in this study) are prone to the greatest biomagnification factors (BMFs) in aquatic food webs. In contrast, BMFs decrease when Log K_{OW} exceeds ~7.2 (e.g., *octa*-to *deca*-PBDEs). Bioavailability and bioconcentration at the base of the food web also decrease for heavy molecular weight and large molecular size PBDEs (Eljarrat et al. 2004). In addition, some studies have found an active biotransformation and elimination of highly brominated PBDEs (e.g., debromination of PBDE-209 by fish) across aquatic food webs (Roberts et al. 2011). Accordingly, previous studies have reported a greater accumulation of highly brominated PBDEs in birds feeding on terrestrial food webs compared with those consuming aquatic resources (Chen et al. 2010, 2012). In the case of terrestrial food webs, both Log K_{OW} and Log K_{OA} are thought to explain the biomagnification potential of POPs, and thus, the estimated BMFs of PBDEs differ from those reported in marine systems (Kelly et al. 2007). Some studies in birds from terrestrial food webs have reported greater BMFs for some *hepta*-to *deca*-PBDEs (e.g., PBDE-183 and -209, respectively) compared with *tetra*- and *penta*-PBDEs (e.g., PBDE-47 and -99) (Voorspoels et al. 2007; Yu et al. 2011). In addition, anthropogenic-based food resources, such as refuse, have been previously identified as a significant exposure pathway to highly brominated PBDE in terrestrial predators from North America (Newsome et al. 2010). Therefore, disparate bioavailability and biomagnification characteristics of PBDEs between marine and terrestrial food chains, as well as the influence of anthropogenic food resources consumed by gulls in dumps, can explain the PBDE patterns found in Mediterranean gulls.

Compared with PBDEs, fewer studies on the trophodynamics of PCDD/Fs have been conducted to date, especially across full terrestrial food chains. Nonetheless, the bioavailability and bioconcentration capacity of highly chlorinated PCDD/Fs in aquatic ecosystems, especially *octa*-PCDD/Fs, is thought to be markedly lower than that of lighter congeners such as *tetra*-PCDD/Fs (Khairy et al. 2014). Moreover, significantly-greater BMFs and trophic magnification factors (TMFs) have been reported for *tetra*- and *penta*-PCDD/Fs compared with *hexa*-to *octa*-PCDD/Fs, which in fact, have been suggested to undergo biodilution across aquatic food webs (Broman et al. 1992; Wan et al. 2005; Ruus et al. 2006; Khairy et al. 2014). To our knowledge, no evidence of an active elimination of highly chlorinated PCDD/Fs has been reported in the case of terrestrial food webs. Consequently, highly chlorinated PCDD/Fs tend to show significantly-lower contributions to total burdens in fish-eating birds than in terrestrial species, in which these compounds, particularly OCDD, usually predominate (e.g., Braune and Simon, 2003; Jiménez et al. 2007; Custer et al. 2010). As in the case of PBDEs, the accumulation patterns of PCDD/Fs in Mediterranean gull eggs reported here agree with those expected from their differential bioavailability and biomagnification potential in marine and terrestrial food webs.

3.3. *Exposure to POPs and health implications for Mediterranean gulls*

Pooled samples of eggs of yellow-legged gulls collected at the Chafarinas Islands from 2010 to 2011 (Lacorte et al. 2014), up to four years later than those used in this study, showed mean Σ_8PBDE levels (16.4 ng/g ww) one order of magnitude greater than those reported here. Levels of Σ_8PBDEs found in pooled eggs of yellow-legged and Audouin's gulls sampled at Ebro Delta Natural Park (NW Mediterranean, Spain) in 2010 (Morales et al. 2012) were also markedly greater (38.3 and 35.9 ng/g ww, respectively) than levels found here in eggs sampled in 2007. Although in this case geographic differences in PBDE sources could be influencing inter-study comparisons, Morales et al. (2012) reported mean levels of *tetra*-, *penta*-, and *octa*-PBDEs slightly below those found in the present study. In contrast, gulls sampled at Ebro Delta showed levels of PBDE-209 two orders of magnitude greater than those found in the Chafarinas. Similarly, PBDE-209 was the congener found at the highest concentration in all yellow-legged gull eggs from the Chafarinas Islands in 2010–2011 (levels for each PBDE congener were not provided separately in Lacorte et al. 2014). This was not the case in the present study for PBDE-209, which showed levels and contributions clearly below those found for *tetra*- and *penta*-PBDEs. Therefore, in line with previous studies from other marine regions (Gauthier et al. 2008; Fliedner et al. 2012), our inter-study comparison suggests that the presence of PBDE-209 could be undergoing a marked temporal increase in both terrestrial and aquatic ecosystems within the Mediterranean basin. Adverse health effects related to PBDE-209 and other congeners, resulting from its debromination, have been described for wildlife and humans (McDonald, 2002; Fernie et al. 2005; Letcher et al. 2014). Therefore, levels of PBDE-209 in Mediterranean ecosystems could lead to potential undesired adverse effects if levels continue to rise. As shown in the present study, risk related to PBDE-209 exposure can increase when refuse and other terrestrial resources are included in the diet of Mediterranean wildlife. Proper studies on temporal trends and trophodynamics of PBDEs, especially PBDE-209, in Mediterranean ecosystems are recommended to confirm the temporal variation described here, and to identify vulnerable areas and species in order to prevent potential threats to the health of these ecosystems.

A wide range of threshold levels for adverse effects related to PCDD/Fs and no-PCBs in bird eggs can be found in the literature. Mean ΣTEQ values found in yellow-legged and Audouin's gull eggs (Table 2) exceeded the no-observed-effect-level (NOEL) reported for other gulls and waterbird species: 7 pg/g TEQ ww for bald eagles, *Haliaeetus leucocephalus*, (Giesy et al. 1995); 7.5 pg/g TEQ ww for Caspian terns, *Sterna caspia*, and 10 pg/g TEQ ww for herring gulls, *Larus* sp., (Giesy et al. 1994). However, ΣTEQ values reported here were well below the lowest-observed-effect-levels (LOELs) in bird eggs (210 and 130 pg/g TEQ ww for bald eagles (Hart et al. 1996) and coast ospreys, *Pandion haliaetus* (Elliott et al. 2001), respectively). Lacorte et al. (2014) reported ΣPCDD/F concentrations in pooled samples of eggs of yellow-legged gulls from the Chafarinas sampled in 2010–2011, showing mean levels (2.45 pg/g ww) similar to those reported here. The apparent lack of a significant temporal increase in ΣPCDD/Fs, together with TEQ values well below toxic threshold levels, do not suggest that PCDD/Fs and no-PCBs can compromise reproductive success or lead to other toxic effects in yellow-legged and Audouin's gulls inhabiting the Alboran Sea region.

4. Conclusions

The combined study of dietary tracers (δ^{15}N, δ^{13}C, and δ^{34}S), and chlorinated and brominated POPs in gull eggs presented here provides a comprehensive view of the trophic sources of these pollutants in Mediterranean consumers. Our results show how different exploitation of refuse, terrestrial, and marine resources lead to different accumulation patterns of PCDD/Fs, no-PCBs, and PBDEs in yellow-legged and Audouin's gulls. The presence of highly chlorinated and brominated congeners in gull eggs increased with the consumption of land based resources, mainly refuse from dumping sites. In contrast, levels of PCDD/Fs and PBDEs with a low substitution degree tended to increase with the consumption of marine resources, which in turn also resulted in higher TEQ values. Based on our results, yellow-legged gulls and Audouin's gulls may reflect how anthropogenic pressure is affecting the Mediterranean basin in terms of their contamination with POPs. In particular, yellow-legged gulls were found to be good bioindicators of the influence that anthropogenic activities such as dumping sites have as a source of POPs in Mediterranean species. In contrast, Audouin's gull better reflects the presence of POPs in pelagic food webs exploited by fisheries. Because fish constitute an important component in the Mediterranean diet, further studies on the role of this species as a potential sentinel for human health are recommended. Regardless, dietary tracers are mandatory for understanding POP levels and profiles in these species, because dietary preferences can vary widely within a single colony, as shown in the present study. In this sense, our results show that δ^{34}S is a valuable predictor for evaluating the trophodynamics of POPs in gulls, which enhances the usefulness of this dietary tracer for the assessment of POP sources when both land-based and marine resources are involved.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.01.078.

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