Anal Bioanal Chem
DOI 10.1007/s00216-012-6326-7

ORIGINAL PAPER

Dechlorane Plus in eggs of two gull species (*Larus michahellis* and *Larus audouinii*) from the southwestern Mediterranean Sea

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Received: 18 May 2012 / Revised: 5 July 2012 / Accepted: 2 August 2012

Abstract Dechlorane Plus (DP) and some of its possible degradation products were measured in eggs from the yellow-legged gull (*Larus michahellis*) and Audouin's gull (*Larus audouinii*) from a protected area in the southwestern Mediterranean Sea. Statistically significant differences were found between both gull species, with yellow-legged gull eggs showing the highest average total DP concentration (209 pg/g wet weight). According to stable nitrogen and carbon isotope values, variations in DP concentrations in the gull species studied are explained by foraging behavior and diet rather than by the trophic position. Both DP stereoisomers were quantified in all the samples studied, and a slight enrichment of the *anti*-DP could have occurred in both species. The quantification of *anti*-[DP-1Cl] only in ~58 % of yellow-legged gulls support the hypothesis of a species-dependent factor influencing the bioaccumulation and/or biotransformation of Dechlorane-related compounds. This study reports on the first measurements of Dechlorane-related compounds in biota from the North African continent, contributing to the knowledge about DP environmental fate and distribution. In the light of our results, more research on differences in species-dependent bioaccumulation and biotransformation capabilities as well as ecological effects is encouraged in future Dechlorane-related compound studies.

Keywords Dechlorane Plus · Degradates · *syn*-DP · *anti*-DP · *Larus michahellis* · *Larus audouinii* · Eggs · Emerging contaminants · Chlorinated flame retardants · Stable isotopes

Introduction

Dechlorane Plus (DP, $C_{18}H_{12}Cl_{12}$, CAS-13560-89-9) has been used for over four decades now since it was first synthesized as a substitute for Dechlorane or Mirex ($C_{10}Cl_{12}$) in the late 1960s [1]. Developed as a flame retardant (FR), it is today in use as part of wire and cable coatings, electronic connectors, automotive lubricants, and plastic roofing materials, among others [2, 3]. Its production is carried out by means of a Diels–Alder reaction between two equivalents of hexachlorocyclopentadiene and one equivalent of 1,5-cyclooctadiene (2:1). This process yields two stereoisomers, *syn*-DP and *anti*-DP, which, in technical formulations, have been reported in ratios (*syn*/*anti*) between 1:3 and 1:2 [2, 3].

Today, DP is classified as a high-production-volume (HPV) chemical by the United States Environmental Protection Agency (US EPA); however, it is currently regarded as a low-production-volume chemical in the European Union (EU) [2]. Although DP is not yet subjected to bans or regulatory measures, this situation may rapidly change in the future, since the EU has listed DP as a plausible FR replacement for the very extended and today regulated Deca-BDE mixture [4].

DP has received the most attention among the chlorinated flame retardants after 2006, when Hoh et al. [1] reported for the first time their presence in air and sediment samples from the Great Lakes region of North America. The increasing number of papers mainly about its environmental

Published in the topical collection *Emerging Contaminants in Biota* with guest editors Yolanda Picó and Damià Barceló.

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Published online: 17 August 2012

occurrence and behavior has been reviewed recently, being well established that DP is a globally distributed contaminant and therefore susceptible to long-range transportation [2, 3]. Despite its size (654 Da) and high lipophilicity (log K_{ow}=9.3), it is bioavailable and bioaccumulative to a certain degree in biota and humans [2]. Furthermore, some studies have reported the existence in the environment of dechlorinated forms of DP, e.g., undecachloropentacyclooctadiene [DP-1Cl] and decachloropentacyclooctadiene [DP-2Cl], which have been postulated as possible abiotic and/or biotic DP degradation products [2, 3]. The environmental presence of other DP-related compounds such as 1,3- and 1,5-DPMA monoadducts has also been described as impurities of technical DP formulations but, in some cases, has been found at higher levels than DP in lake trout [5] and peregrine falcons [6]. Nonetheless, and despite the increasing number of new studies on Dechlorane-related compounds, the current knowledge on DP is still very limited when compared to other FRs such as polybrominated diphenylethers (PBDEs) or hexabromocyclododecane. For instance, DP toxicological data are very scarce, and most of them originate from a US EPA HPV Test Challenge report on some ecotoxicological effects in fish and potential effects on rabbit reproduction [7]. Recently, the first study on DP toxicity effects in chicken embryos was published [8]. No adverse effects on embryonic viability were found at DP contents much higher than those currently found in the environment. Regarding DP stereoisomers, distinct and even opposed outcomes for biomagnification potential have been described in different food webs [9–11]. Finally, while most studies have reported DP occurrence in North America and China, there is an important lack of data from other parts of the globe that are lagging behind, including Europe [2].

For a long time now, different bird species have been used as bioindicators for environmental contamination, especially when dealing with marine ecosystems [12]. Normally, at higher trophic levels, species are more susceptible to biomagnification of contaminants. Consequently, birds at the top of food webs may be suitable as geographic bioindicators of contamination [13]. The use of intrinsic biomarkers can help to decipher the trophodynamics of pollutants in marine ecosystems. The analysis of stable isotope ratios of C ($^{13}C/^{12}C$, $\delta^{13}C$) and N ($^{15}N/^{14}N$, $\delta^{15}N$) is recognized as a useful tool to investigate the trophic ecology of marine organisms [14]. While $\delta^{15}N$ is commonly used to establish the trophic position of consumers based on the relative enrichment of ^{15}N to ^{14}N across marine food webs, $\delta^{13}C$ can help to decipher seabird food sources since terrestrial, freshwater, and marine prey items show an increasing gradient in $\delta^{13}C$ [15, 16]. Consequently, several works have focused on the relationship between persistent organic pollutant levels and $\delta^{15}N$ values in marine wildlife to explore the biomagnification of these pollutants as well as their relationship with $\delta^{13}C$ to assess their main sources in marine predators [17, 18].

When seabirds are used as bioindicators for contamination in marine ecosystems, different sampling strategies can be selected. Geographic and trophic patterns of organochlorine compounds have been evaluated in pelagic seabirds from the NE Atlantic and the Mediterranean Sea using blood as a nondestructive sample [13]. As a different approach, eggs have been successfully used to evaluate the relationship between seabird pollutant burdens and stable isotope ratios [19, 20]. Eggs are easily collected in the field, and a negligible effect is expected at population level after removing a single unit in a bird species that lays clutches of several eggs, such as gulls [21]. In fact, eggs of herring gulls have been successfully used to assess the current use of flame retardants from the Laurentian Great Lakes [22].

The gull species considered in the present work, i.e., yellow-legged gull (*Larus michahellis*) and Audouin's gull (*Larus audouinii*), breed at the Mediterranean Chafarinas archipelago. Under Spain's sovereignty, these African islands are located only about 4 km away from the Moroccan coast in waters of the southwestern Mediterranean Sea. Because of its diverse fauna, some of which are rather unique and/or endangered, this archipelago was declared a protected natural area by the Spanish authorities in 1989 [23]. Both gull species breed sympatrically at several Mediterranean locations; however, the yellow-legged gull is a more common and abundant species than Audouin's gull, which is currently listed as “near threatened” due to its decreasing population trends under the International Union for Conservation of Nature Red List of Threatened Species [24]. The yellow-legged gull is a highly opportunistic species, and its diet includes marine, terrestrial, and human-derived resources (i.e., garbage and fishery discards). In contrast, Audouin's gull feeds almost exclusively on marine resources (pelagic prey and fishery discards). Their specific feeding strategies are believed to be mostly responsible for differences in their population trends [25, 26].

The main objective of this work was to investigate the distribution of DP and some possible degradates in eggs of yellow-legged and Audouin's gulls, assessing the influence of the trophic level and food sources on DP contents by means of stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$.

Materials and methods

Study area and sample collection

Gull eggs were collected during the breeding season of 2007. Nineteen individual eggs were collected per species. Both colonies breed at the archipelago of Chafarinas Islands, which is located in the southwestern Mediterranean

Sea (35° 10′–35° 11′N and 2° 24′–2° 27′W) and composed of three small islands (Fig. 1) that, combined, have a surface area of 0.525 km^2 [23]. Drop traps were used during early periods of incubation to trap one of the members of each breeding pair [27]. A number of biometric measurements including body mass and tarsus length of each trapped bird were obtained. In nests where at least one breeder was captured, one randomly selected egg was removed for analysis. Eggs were frozen and kept at −80 °C until analysis. The whole egg content was used for chemical analysis while the remaining eggshell was kept for further structural analysis.

Chemicals

All solvents used were of pesticide grade, including acetone, methanol, chloroform, and toluene, purchased from SDS (Peypin, France); n-hexane was purchased from Merck (Darmstadt, Germany). Silica gel (70-230 mesh) was also supplied by Merck and sodium sulfate anhydrous by J.T. Baker (Deventer, Netherlands). Standards of *syn*-DP, *anti*-DP, $^{13}C_{10}$-labeled-*syn*-DP, and $^{13}C_{10}$-labeled-*anti*-DP were purchased from Cambridge Isotope Laboratories Inc. (Andover, USA). $^{13}C_{12}$-labeled BDE-138 and -139 were purchased from Wellington Laboratories (Guelph, Canada) along with the standards of dechlorinated DP species *anti*-[DP-1Cl] and *anti*-[DP-2Cl], and the DP monoadduct 1,5-DPMA (Fig. 2).

Sample preparation and DP analysis

After homogenization and lyophilization of the whole egg content, from 2 to 3 g of each egg was used for residue analysis following the analytical protocol described in detail elsewhere [28]. In brief, each sample was spiked with $^{13}C_{10}$-DPs (*syn*- and *anti*-) and $^{13}C_{12}$-BDE-138. The extraction was based on a matrix solid-phase dispersion procedure. A subsequent cleanup was carried out on acidic and basic silica gel multilayer columns. A final fractionation of different analytes was achieved by means of Supelclean™ Supelco ENVI™-Carb cartridges. Of the three different fractions obtained, the first one contained the bulk of DPs along with other identified pollutants such as PBDEs, DDTs, and *ortho*-PCBs. Final extracts were rotary evaporated until ~1 mL, transferred to vials, and dried under a gentle nitrogen steam. Samples were reconstituted in a solution of $^{13}C_{12}$-BDE-139 in nonane as an internal standard for chromatographic analysis. The lipid content of each egg was determined gravimetrically as described elsewhere [29].

Quantification of the target analytes was performed by high-resolution gas chromatography low-resolution mass spectrometry using a 6890N Series gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, USA) operated in selected ion monitoring mode with electron capture negative ionization. The GC injection port was configured for 1 μL pulsed hot splitless injections (4 min) at a temperature of 260 °C. Gas chromatographic separation was achieved using a 15-m×0.20-mm×0.20-μm DB-5MS low-bleed column (J&W Scientific, USA). The oven temperature program was: 130 °C for 4.2 min; ramped to 200 °C at 30 °C/min; ramped to 275 °C at 5 °C/min; ramped to 300 °C at 40 °C/min, held for 10 min; and finally, ramped to 310 °C at 10 °C/min and held for 2 min. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min, and methane was used as reaction gas. The temperatures of the transfer line, source, and quadrupole were set at 300, 150, and 150 °C, respectively. The identification of target compounds was based on detection, at the corresponding

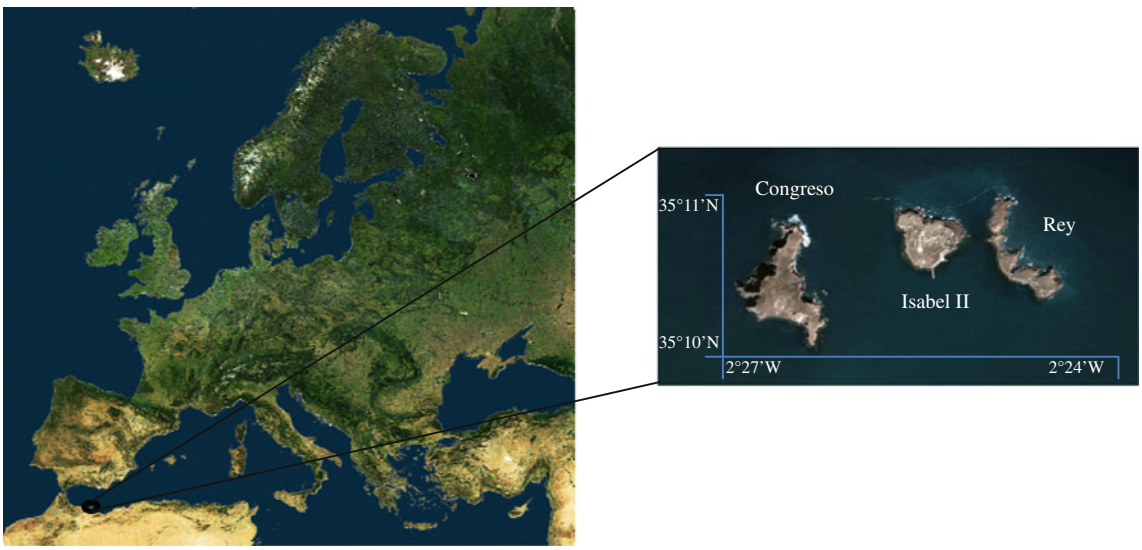

Fig. 1 Geographic location of the gull colonies. Three small islands, Congreso, Isabel II, and Rey, make up the Chafarinas archipelago

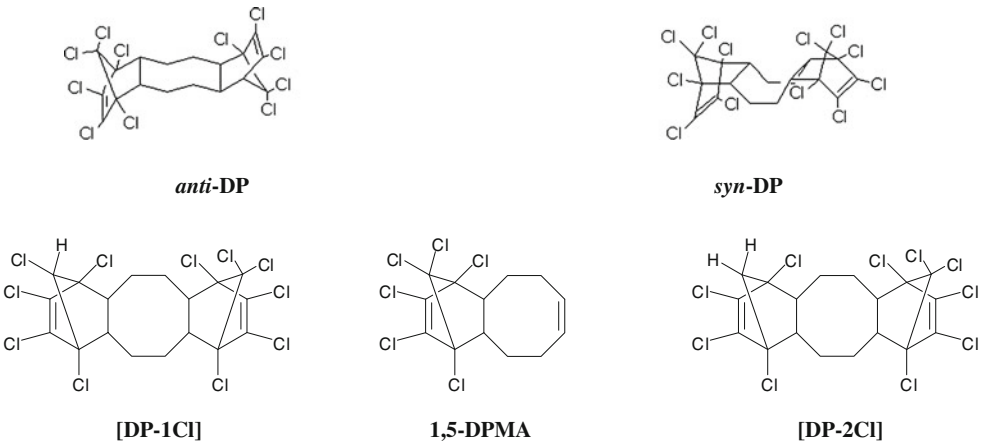

Fig. 2 Chemical structures of *anti*-DP, *syn*-DP, [DP-1Cl], [DP-2Cl], and 1,5-DPMA (Dechlorane Plus monoadduct)

retention time, of the following *m*/*z* ions: 652/654 (*syn*- and *anti*-DP), 662/664 ($^{13}C_{10}$-*syn*- and $^{13}C_{10}$-*anti*-DP), 618/620 (*anti*-[DP-2Cl]), 584/586 (*anti*-[DP-2Cl]), 344/346 (1,5-DPMA), and 79/81 ($^{13}C_{12}$-BDE-138 and $^{13}C_{12}$-BDE-139). Quantification was based on a seven-point calibration curve for each target analyte ranging from 1 to 200 pg/μL. $^{13}C_{12}$-BDE-139 was used as injection internal standard for 1,5-DPMA,$^{13}C_{12}$-BDE-138, and $^{13}C_{10}$-DPs (*syn*- and *anti*-), while the isotope dilution technique was used for the quantification of *anti*-[DP-2Cl] and *syn*-DP against $^{13}C_{10}$-*syn*-DP, and *anti*-[DP-1Cl] and *anti*-DP against $^{13}C_{10}$-*anti*-DP.

Sample preparation and stable isotope analysis

The carbon and nitrogen stable isotopes were determined at the Serveis Cientifico-Tecnics of the University of Barcelona (Barcelona, Spain). A subsample (1–2 g) of each lyophilized egg was placed in a glass centrifuge tube. Lipid extraction of the dried powdered samples was conducted following the procedure described elsewhere [30]. Basically, a mixture (approximately 10 mL) of chloroform/ methanol (2:1) was added to the tube, vortexed for 30 s, and centrifuged for 10 min at 400 rpm. The supernatant phase containing lipids was discarded. The procedure was repeated until the supernatant was completely clear and colorless. Then, samples were dried at 60 °C for 24 h. Sample analysis was carried out following the procedure described in Roscales et al. [31]. In short, from 0.36 to 0.4 of lipid-free egg samples were weighted to the nearest microgram in tin buckets and crimped for combustion. Stable isotope ratios were determined by elemental analysis–isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLOIII interface. Isotope ratios are conventionally expressed as parts per thousand according to the equation $\delta X=[(R \text{ sample}/R \text{ standard})-1]$, where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Vienna Peedee Velemnite and atmospheric nitrogen (AIR) were used as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively.

QA/QC

Quality criteria were based on the application of quality control and quality assurance measures, which included the analysis of blank samples covering the complete analytical procedure (one procedural blank in each set of four samples). Care was taken to minimize exposure to UV light throughout the whole analytical procedure. Dechlorination of DP in the injection liner of the chromatographic system was observed in consistent ratios always under 0.5 %. Quantification was carried out according to the following criteria: (a) ratio between the two monitored ions within ±15 % of the theoretical value and (b) limits of quantification (LOQs) corresponding to instrument signal-to-noise (*S*/*N*) of 10. Average LOQs were 4.30 pg/g (*syn*-DP), 9.09 pg/g (*anti*-DP), and 2.66 pg/g (*anti*-[DP-1Cl]). In the case of *anti*-[DP-2Cl] and 1,5-DPMA, average LOQs were estimated for the study samples in 3.01 and 54.9 pg/g, respectively. DP isomers were detected slightly above their LOQs in most procedural blanks. The measured levels were subtracted from the corresponding batch of samples associated to each blank. A recovery study was carried out by spiking a triplicate of the same sample with 5 ng of all target analytes. Satisfactory average recoveries were achieved for *syn*-DP (>87 %), *anti*-DP (>87 %), *anti*-[DP-1Cl] (>65 %), and *anti*-[DP-2Cl] (>84 %). A recovery <10 % was found for 1,5-DPMA, suggesting its loss under the acidic attack at the cleanup step; therefore, its presence could not be determined in the egg samples. The precision of the analytical procedure was evaluated by extracting and analyzing three times the same egg gull sample, obtaining RSDs lower than 12 % for all target analytes. The precision for the quantification method was checked by reanalyzing three different egg gull samples in three different days within the same week and with different calibration curves, obtaining RSDs lower than 7 % for all target analytes. The method was found to be linear for all DPs over a range of 1.0 to 1,000 pg/μL ($r^2 \geq$ 0.998). Average recoveries for surrogates were 101±26 %

($^{13}C_{12}$-BDE138), 100±19 % ($^{13}C_{10}$-*syn*-DP), and 94±23 % ($^{13}C_{10}$-*anti*-DP). Calibration curves were checked daily.

In the case of stable isotope analysis, three reference materials provided by the International Atomic Energy Agency were analyzed every 12 samples to compensate any shift over time and calibrate the equipment. Standards spanned the range of stable isotope signatures found in the gull egg samples. Standards replicate analysis showed accuracy lower than 0.1‰ and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively. RSD for $\delta^{13}C$ measurements in the reference materials was 0.64 and 0.94 % for $\delta^{15}N$ measurements.

Data analysis

Concentrations are expressed in both wet weight (w.w.) and lipid weight (l.w.) bases. Values in l.w. basis are provided for comparison purposes; however, no significant correlation was found between the content of lipids and DP in the eggs. Samples with concentrations below the LOQs were assigned a value of zero. Statistical analyses were carried out with SigmaPlot for Windows version 12.0 (Systat Software Inc, CA, USA) and IBM SPSS Statistics for Windows version 19 (SPSS Inc, IL, USA). Concentration data for DPs and variables derived from them were not normally distributed (Shapiro–Wilk test, $p<0.001$). Log transformation was applied to meet the criterion of normality when exploring possible relationships between variables. The relationship between gull egg DP content and species, $\delta^{15}N$, and $\delta^{13}C$ was tested simultaneously by means of a generalized linear model analysis (GLM, type III SS) including total DP as response variable, species as fixed factor, and both $\delta^{15}N$ and $\delta^{13}C$ as covariables. Additionally, Pearson's correlation analysis was used to evaluate intraspecific relationships between total DP and stable isotope ratios in gull eggs. When log transformation did not achieve normality, non-parametric tests were applied. A minimum significance level of $\alpha=0.05$ was set throughout this study.

Results and discussion

DP concentrations and stable isotope ratios

DP concentrations, expressed in both wet weight (w.w.) and lipid weight (l.w.) bases, are summarized in Table 1. It is noticeable that DP was detected in all samples. Statistically significant differences were found between both gull species (*t* test, $t=10.40$, $p<0.001$). In eggs of yellow-legged gulls, the average total DP concentration was 209 pg/g w.w. ranging from 39.5 to 433 pg/g, after the exclusion of one outlier sample (1,540 pg/g w.w. and 28,250 pg/g l.w.). Lower levels were found in Audouin's eggs with an average total DP concentration of 29.6 pg/g w.w. and ranging from 6.81 to 79.3 pg/g. Interspecies differences in DP concentrations found in this study are probably related to the trophic ecology of the gull species. However, other factors such as detoxification capabilities and geographic movements to regions with different levels of contamination could also influence DP burdens.

The diet of yellow-legged and Audouin's gulls has been thoroughly studied in the Chafarinas archipelago as well as in several Mediterranean breeding localities [32]. Both species are third-order consumers, and no marked differences in their trophic position should be expected based on previous dietary (both species feed mainly on fish on the Chafarinas Islands [25, 26]) and isotopic studies conducted during their breeding period [33]. The main difference in their trophic ecology is based on the highly opportunistic behavior of the yellow-legged gull compared to Audouin's gull. Previous dietary studies on the Chafarinas Islands showed that yellow-legged gulls exploit more frequently than Audouin's gulls alternative food sources to marine resources such as terrestrial prey (including Audouin's gull eggs and chicks) and refuse tips, the last one consumed exclusively by yellow-legged gulls [25, 26]. Differences in carbon and nitrogen stable isotope ratios between both species found in this work agree with these studies. Stable isotope ratios of carbon were significantly greater (*t* test, $t=5.44$, $p<0.001$) in Audouin's (mean ± SD, −18.62±0.36‰) than in yellow-legged gulls (−19.65±0.72‰) and ranged from −19.54 to −18.21‰ and −21.44 to −18.65‰, respectively. Since terrestrial prey and refuse tips show markedly lower $\delta^{13}C$ values than marine prey, $\delta^{13}C$ interspecies differences found here reflect the greater exploitation of these resources by the yellow-legged gull compared to Audouin's gull [32, 34]. Regarding $\delta^{15}N$, Audouin's gulls showed significantly (*t* test, $t=6.98$, $p<0.001$) higher values (12.21±0.24‰) than the yellow-legged gulls (10.89±0.77‰), but in contrast with $\delta^{13}C$, $\delta^{15}N$ values in gull eggs showed a great overlap between the species (range, 11.68–12.73 and 9.24–12.06 for Audouin's and yellow-legged gulls, respectively). Nonetheless, both C and N stable isotope ratios showed a greater variability in the yellow-legged than in Audouin's gulls, which agrees with the marked opportunistic character of the first one. Previous studies have reported an approximated increase of 3–4‰ of $\delta^{15}N$ per trophic level on marine food webs [14]. Therefore, the great overlap in the values of $\delta^{15}N$ for both species, along with the small difference between their average $\delta^{15}N$ values (1.32‰), suggests no noticeable differences in their trophic position.

A GLM analysis with total DP as response variable, species as fixed factor, and $\delta^{15}N$ and $\delta^{13}C$ as covariables showed that after accounting for the variability explained by the factor species ($F_{1,37}=31.75$, $p<0.001$), $\delta^{13}C$, but not $\delta^{15}N$, showed a significant effect ($F_{1,37}=4.74$, $p<0.05$, for

Table 1 Average, median, range, and detection frequencies of *syn*- and *anti*-DP, total DP, and *anti*-[DP-1Cl] concentrations and fractional abundance values ($f_{anti=[anti\text{-}DP]/([anti\text{-}DP]+[syn\text{-}DP])}$) in yellow-legged and Audouin's gull eggs from Chafarinas Islands

	Yellow-legged gull (n=18)				Audouin's gull (n=19)			
	Average	Median	Range	% > LOQ	Average	Median	Range	%>LOQ
syn-DP	40.8 (526)	35.5 (494)	6.91–90.7 (91.3–1270)	100	6.10 (80.5)	5.53 (79.7)	<LOQ–15.3 (<LOQ–338)	89.5
anti-DP	168 (2180)	146 (2060)	32.6–342 (431–4780)	100	23.5 (317)	19.2 (228)	6.67–64.0 55.8–1410	100
Total DP	209 (2700)	181 (2550)	39.5–433 (522–6050)	100	29.6 (398)	26.6 (324)	6.81–79.3 (57.0–1750)	100
anti-[DP-1Cl]	1.28 (17.3)	0.91 (9.92)	<LOQ–4.48 (<LOQ–65.3)	57.9	–	–	–	–
f_{anti}	0.81	0.81	0.73–0.85	–	0.82	0.79	0.70–1.00	–

Concentrations expressed in both picograms per gram w.w. and picograms per gram l.w. (in parenthesis)

$\delta^{13}C$, $F_{1,37}$=0.02, p=0.91) on DP concentrations and explained a greater variability (η^2=0.18 and η^2<0.01 for $\delta^{15}N$ and $\delta^{13}C$, respectively). These results indicate that there is a significant relationship at intraspecific levels between $\delta^{13}C$ and DP content in the gull eggs. DP concentrations in gull eggs decreased similarly with $\delta^{13}C$ within both species (Fig. 3), and separated Pearson's correlation analysis showed that this relationship was significant for yellow-legged gulls (r=−0.51, p=0.03) but not for Audouin's gulls (r=−0.29, p=0.12), which is probably due to the lower $\delta^{13}C$ variability found in Audouin's compared to the yellow-legged gull. Similarly, DP egg concentrations decreased with $\delta^{15}N$ in both species, but these relationships were not significant (r=−0.31, p=0.20 for Audouin's gull eggs; r=−0.18, p=0.46, for yellow-legged gull eggs). At interspecific level, stable isotope signatures showed similar relationships with DP concentrations than those described at intraspecific level. The yellow-legged gull showed significantly higher DP concentrations but significantly lower $\delta^{15}N$ and $\delta^{13}C$ compared to Audouin's gull. Overall, these results indicate that a relative increase in the consumption of terrestrial prey and/or refuse tips related to marine resources, as shown by the decrease in $\delta^{13}C$ at intra- and interspecific level, leads to a greater exposition to DP in the gulls. These findings are in line with a recent study in several gull colonies from Canada reporting greater levels of DP in those populations that feed on both terrestrial and marine/freshwater ecosystems [35]. A significant increase in the content of some organochlorine contaminants with $\delta^{15}N$ has been reported at intraspecific level for seabirds [31] due to the biomagnification properties of these pollutants. In the present study, the negative nonsignificant relationship between DP concentrations and $\delta^{15}N$ within both gull species as well as the inverse relationship between species, DP, and $\delta^{15}N$ values does not bring evidences for DP biomagnification. Our results are consistent with the most recent study conducted in eggs of different gull species from Canada. However, other authors have postulated a biomagnification potential for DP in terrestrial birds [36].

Regarding possible DP variations in gull eggs due to geographic movements, whereas the yellow-legged gull is a permanent resident of the Chafarinas archipelago, Audouin's gull migrates during winter and returns to the archipelago to breed. Indeed, an important shift in Audouin's gull diet has been described between the winter and breeding seasons [37]. However, it is generally assumed that gulls mainly rely on local food sources while breeding, without mobilization of stored resources [38]. Consequently, Audouin's gull migratory behavior is expected to have a minor influence on the DP burden in their eggs. Finally, since we are dealing with different species, distinct metabolic rates cannot be completely ruled out between yellow-

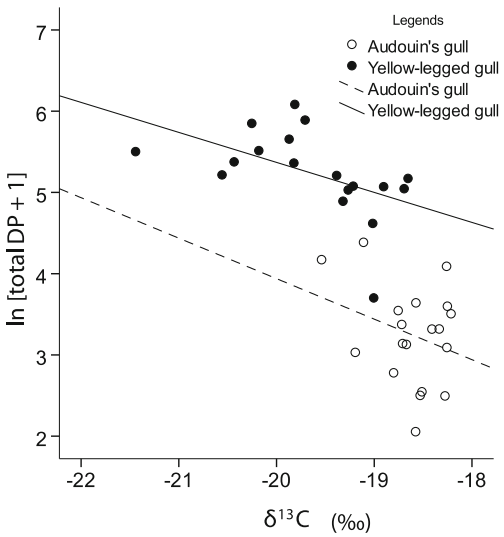

Fig. 3 Relationship between stable isotope signatures of C and total DP in yellow-legged and Audouin's gull eggs

legged and Audouin's gulls despite their close phylogenetic relationship.

To date, DP has only been reported in eggs from a few bird species from Canada and Spain. In a recent study, DP levels were measured in eggs of four gull species from several colonies spanning Pacific to Atlantic Canada [35], finding in general higher values in comparison to our study. DP burdens in herring gulls (*Larus argentatus*) from the Laurentian Great Lakes averaged from 500 to 5,500 pg/g w.w. Colonies from Canadian non-Great Lakes averaged values of 300 pg/g w.w. for California gulls (*Larus californicus*), 600 to 1,900 pg/g w.w. for herring gulls, and 2,400 pg/g w.w. for ring-billed gulls (*Larus delawarensis*). In contrast to freshwater ecosystems, DP concentrations found in colonies from marine environments averaged from nonquantified to 300 pg/g w.w. for glaucous-winged gulls (*Larus glaucescens*) and from 500 to 1,000 pg/g w.w for herring gulls. DP concentrations in white storks (*Ciconia ciconia*) from Spain were 105 pg/g w.w. for a rural colony and 401 pg/g w.w. for an urban colony [39]. It is worthy to note how the values found in this study for the gulls are relatively similar to those previously found for white storks, even though the gulls were sampled in the northernmost region of the African continent and the white storks were sampled at two inward locations of Spain. In the case of peregrine falcons (*Falco peregrinus*), geometric mean values of 36,400 pg/g l.w. in eggs of Canadian specimens and 1,780 pg/g l.w. in eggs of Spanish specimens were reported [6]. DP content in Spanish peregrines was found between those for Audouin's gulls (398 pg/g l.w.) and yellow-legged gulls (2,700 pg/g l.w.). Irrespective of the species, these great differences in DP burdens between Canadian, especially those from the Great Lakes, and Spanish birds are likely to respond to the existence of a DP manufacturing plant in the area of the Great Lakes. Furthermore, it may also partially reflect a higher use of this flame retardant in North America than in Europe and most likely than in Africa. However, the relative influence of each factor in the total DP burden, i.e., the species, year of sampling, and the geographic location, is difficult to distinguish. At this point, more data about DP production, use, and its geographic distribution are needed.

DP stereoisomer ratio

With the exception of two eggs of Audouin's Gulls where *syn*-DP was not found, both DP stereoisomers were quantified in the whole set of samples. To study the possible DP stereoselective enrichment in the egg samples, the *anti*-isomer fractional abundance (f_{anti}=[*anti*-DP]/([*anti*-DP]+[*syn*-DP])) was calculated (Table 1). Nonstatistically significant differences were found in f_{anti} values between both species (Mann–Whitney U=146, p=0.321). In the case of yellow-legged gulls, the average f_{anti} was 0.81, ranging from, 0.73 to 0.85, whereas for Audouin's gulls, the average f_{anti} value was 0.82, ranging from 0.70 to 1.00. Several values of f_{anti} for technical DP have been reported with a mean value of 0.68 (ranging from 0.59 to 0.8) according to Wang et al. [40]. Based on this, it seems that a slight enrichment of the *anti*-DP could have occurred in both species. This is in disagreement with the nonclear stereoisomer enrichment reported in white storks [39] and herring gulls [22] or the *syn*-DP biomagnification described in some aquatic food webs [9, 10, 41]. Furthermore, it is not consistent either with the proven fact that *anti*-DP degrades faster in the environment under UV light [42], or with the supposed ease of the same stereoisomer to undergo biological attack based on its less-hindered spatial conformation [1] relative to *syn*-DP. However, other studies have also shown a net *anti*-DP biomagnification in an aquatic food web [10]. Zhang et al. [43] have recently found significant differences in the tissue distribution of each DP stereoisomer. Having analyzed muscle, liver, and brain tissue of two fish species, the authors observed a clear *syn*-DP enrichment in liver while a higher *anti*-DP affinity was found for brain tissue. In the light of all these different behaviors, to explain different stereoisomer enrichments in biota, not only dissimilarities in bioavailability between *syn*- and *anti*-DP stereoisomers, but also differences in species-dependent bioaccumulation and biotransformation capabilities should be considered.

DP-related compounds

Anti-[DP-1Cl] was only quantified in ~58 % of the yellow-legged gull eggs. *Anti*-[DP-2Cl] and the 1,5-DPMA monoadduct were not found in any sample of both species. Comparing the values of this study with those reported in the literature for other bird eggs, the concentration and detection frequency of *anti*-[DP-1Cl] (Table 1) were higher than those in white storks (0.55 and 2.35 pg/g w.w. in 10 % of the samples of both urban and rural colonies, respectively) [39]. In the case of Spanish peregrine falcons, the concentration was up to one order of magnitude greater (180 pg/g l.w.) than that in yellow-legged gulls, but *anti*-[DP-1Cl] was found with a lower frequency (~23 %). As for Canadian peregrine falcons, both concentration and detection frequency were much higher (1,840 pg/g l.w. and 100 % of the samples) than in any other case [6]. It is interesting to note how the behavior found for this dechlorinated product is similar to that described for the parent compound. Preferential bioaccumulation of *anti*-[DP-1Cl] in comparison to the parent DP has also been observed in fish tissues [43]. However, it should be noted that this preferential bioaccumulation was tissue and species dependent.

Conclusions

This study reports on the first measurements of Dechlorane-related compounds in biota from a protected area in the southwestern Mediterranean Sea, contributing to the knowledge about DP environmental fate and distribution in still unexplored areas such as the North African continent. DP levels were significantly different in the yellow-legged gull (*L. michahellis*) and Audouin's gull (*L. audouinii*). Our results indicate that a relative increase in the consumption of terrestrial prey and/or refuse tips related to marine resources, as shown by the decrease in $\delta^{13}C$ at intra- and interspecific level, leads to a greater exposition to DP in the gulls. Overall, variations in DP concentrations in the gull species studied are explained by foraging sources rather than by trophic position.

The quantification of *anti*-[DP-1Cl] only in ~58 % of yellow-legged gulls supports the hypothesis of a species-dependent factor influencing the bioaccumulation and/or biotransformation of Dechlorane-related compounds. DP stereoisomer enrichment was similar in both species with f_{anti} values of 0.81 (yellow-legged gull) and 0.82 (Audouin's gull). This anti-stereoisomer enrichment is overtly different from what has been reported in other terrestrial and aquatic food webs. Thus far, Dechlorane-related compound bioaccumulation and biomagnification in food webs are not yet well understood. This, along with the facts that the use of DP may increase in the near future and that the current toxicological data are very limited, should encourage further studies on sources, environmental distribution, and fate of DP and related compounds.

Acknowledgments Part of this research was funded by OAPN from the Spanish Ministry of Environment. We thank Reserva Nacional de Caza de las Islas Chafarinas, its director, and staff for their support during the sampling campaign. The authors also thank Inmaculada Calvillo for her technical support in the laboratory.

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