GEOGRAPHIC AND TROPHIC PATTERNS OF PCBs AND DDTs IN MEDITERRANEAN AND NE ATLANTIC SEABIRDS: CAN POPs ADVERSELY AFFECT MEDITERRANEAN SEABIRDS?

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Introduction

Trophic ecology and geographic location are crucial factors explaining persistent organic pollutant (POPs) levels in marine vertebrates, but these factors are often difficult to disentangle[1]. On one hand, the trophic position of marine consumers is directly and positively associated to their contaminant burdens due to the biomagnification capability of POPs. However, partial upsets between trophic position and pollutant levels have been reported suggesting that specific dietary habits and metabolic capabilities also play a significant role in explaining consumer POP levels[2]. On the other hand, contaminant emissions and their distribution are not spatially uniform, which ultimately determines the exposition to POPs of marine organisms.

The Mediterranean Sea is a semi-closed marine region surrounded by highly industrialized countries and with a slow water exchange with the adjacent NE Atlantic. These characteristics have been related to the greater levels of POPs such as organochlorinated contaminants (OCs) that can be found in Mediterranean ecosystems compared to other oceanic regions[3]. Indeed, previous studies have reported levels of polychlorinated biphenyls (PCBs) and dichlorodiphenyl ethane plus its main metabolites (DDTs) of toxicological concern in Mediterranean organisms from different trophic positions[4,5].

With the aim of evaluating the influence of geographic distribution and trophic ecology of marine predators over their exposition to OCs as well as possible adverse effects associated to these pollutants in the Mediterranean basin, here we present a combination of two different approaches in marine contamination monitoring. On one hand, we analyzed PCBs, DDTs and stable-nitrogen isotope signatures ($\delta^{15}N$), as a proxy of trophic position[1], in blood of pelagic seabird species across different breeding localities from the northeast Atlantic and the Mediterranean. On the other hand, we explored the relationship between plasma OC levels and biochemical health and nutritional parameters in a Mediterranean gull species and thereby, we evaluated the potential health impact of chronic exposition to these contaminants in the Mediterranean basin.

Materials and methods

Sample collection: From 2003 to 2006, we sampled 0.2-0.5 mL of blood from the brachial vein of most petrels and shearwaters (9 species, O. Procellariiformes, listed in Fig.1) breeding in the NE Atlantic Ocean and the Mediterranean archipelagos (n=188). Blood was transferred into vials with 1 mL of absolute ethanol and preserved at -24 ºC until OC and isotopic analysis. In addition, in 2007, we sampled about 1 ml of blood from yellow-legged (*Larus michahellis*) breeding in Chafarinas Is., within the Mediterranean basin (n=36). Heparinized blood was centrifugated after extraction at 3000 rpm for 10 minutes to obtain the plasma, which was stored at -80 ºC until OC and biochemical analysis. All the birds were sampled during their breeding period and after egg laying.

Isotopic analysis: About 0.36-0.40 mg of dried blood was used to determine $\delta^{15}N$ signatures by elemental analysis-isotope ratio mass spectrometry (EA-IRMS) by means of a ThermoFinnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer *via* a CONFLOIII interface. Precision and accuracy for $\delta^{15}N$ measurements were 1.3% and ≤ 0.2‰, respectively.

***OC analysis*:** From 0.2 to 0.05 g of dried blood (Procellariiform species) or 0.5g of plasma (yellow-legged gull) were used for OC determination. 20 *ortho*-PCB congeners, *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE were determined. Sample treatment consisted basically of 3 successive extraction steps with hexane and concentrated sulfuric purification. PCB and DDT concentrations were determined by high-resolution gas chromatography with micro-electron capture detection (HRGC-μECD, Agilent Technologies, model 6890N). Further details about Q-Q parameters can be found in Roscales et al. 2009[5].

Biochemical analysis. A suite of biochemical parameters (plasma protein, albumin, glucose, cholesterol, triglycerides, phospholipids, zinc, phosphorus, calcium, magnesium, uric acid, glutamic oxalacetic transaminase AST, glutamic pyruvic transaminase ALT, creatine kinase CK and alkaline phosphatase ALP) were determined by standardized colorimetric, colorimetric-enzymatic and kinetic tests as described in detail in Aguire et al.[6].

Results and discussion:

Our results show that both breeding locality and species have a significant influence explaining OC levels in seabirds. Within the Atlantic, minimum mean ΣPCB and ΣDDT levels (39.4 and 3.2ng/g w.w, respectively) were found in Cape Verde little shearwater. Madeiran storm-petrel from Azores showed the maximum mean concentrations (1073 and 319 ng/g w.w). In the case of Mediterranean seabirds, Scopoli's shearwater from Balearic Is. showed the lowest mean OC levels (168 and 69.57 ng/g for ΣPCB and ΣDDTs) and European storm-petrel from Terreros Is. (SE Spain) the greatest (6843 and 5816 ng/g). Interspecies differences in OC levels were significant within all the breeding localities and showed similar inter-species patterns (Fig.1, ANOVA-test within localities, all P<0.001). However, stable-nitrogen signatures of seabirds showed a noticeable overlap and we did not find a significant relationship between species $\delta^{15}N$ and OC levels within each breeding place. These results suggest no marked differences in the trophic position of the studied species[7], with the exception of little shearwater species from the Atlantic, which seems to exploit a lower trophic position than the rest of species and accordingly showed the lowest OC levels (Fig.1). Overall, the rest of seabirds, that is *Calonectris* shearwaters and petrel species, showed overlapped $\delta^{15}N$ signatures but PCB and DDT burden were significantly greater in petrels than in shearwater (Fig.1), reflecting that OC levels are not determined by the trophic position alone.

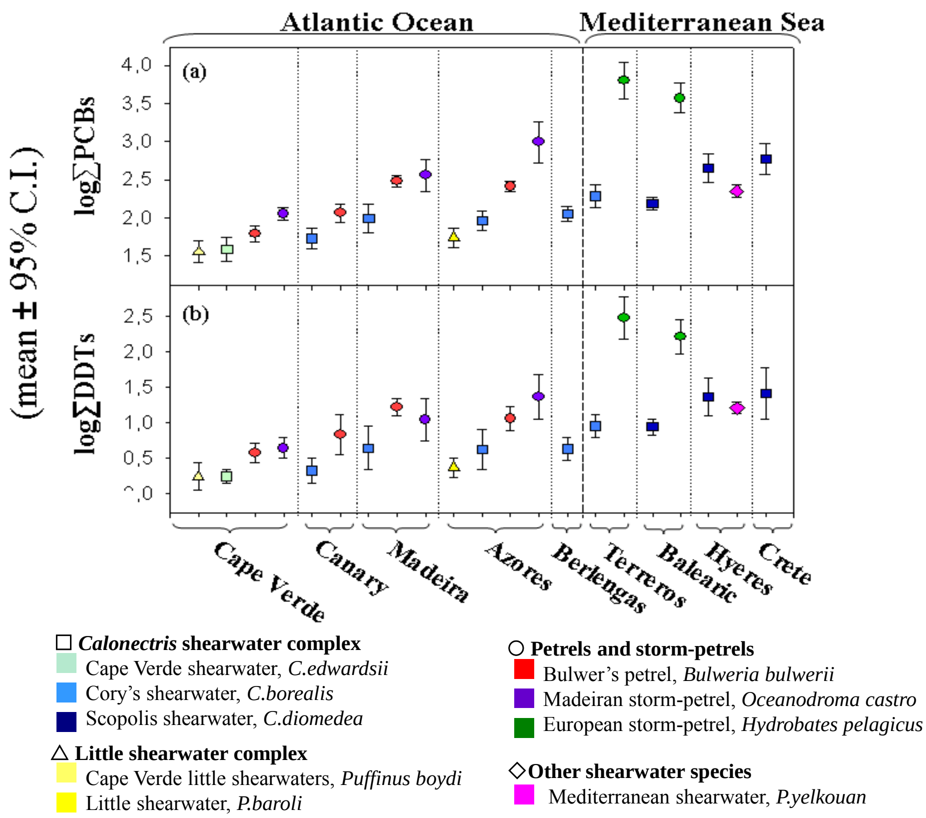

Fig.1. OC levels in Mediterranean and Atlantic pelagic seabirds depending on their breeding locality.

Although most petrel and shearwater species included in this study mainly feed on pelagic resources, they exploit different prey types. For instance, the Atlantic petrel species studied here, feed mainly at night on mesopelagic (200-1000 m depth) vertical migratory preys (mainly Myctophid fish) and *Calonectris* shearwaters are diurnal epipelagic feeders[8] (0-200 m). Since no marked differences in the metabolic capabilities of the seabird species here included are expected[9,10], feeding specialization among species emerge as the most probable hypothesis to explain the differences found in OC burdens and the lack of correlation between species OC levels and trophic position. In fact, previous studies in the Atlantic and the Pacific oceans have reported higher burdens of OCs in some mesopelagic and deep sea fishes, including Myctophids[11], compared to those from the upper water layers. In addition, the smaller size of petrels compared to *Calonectris* shearwaters probably involve major energetic requirements and feeding rates in the case of petrels, which also could contribute to elevate their OC burdens. In sum, interspecies OC patterns found in this study suggest that multispecies strategies in marine monitoring are potentially useful to study the vertical distribution of these contaminants. Moreover, a multispecies approach provides a more realistic interval of risk levels than single species based strategies.

Our results revealed similar spatial patterns of OCs in pelagic seabirds regardless of the species. Significant OC patterns were found within the Atlantic and Mediterranean as well as between these basins (Fig.1, ANOVA-test within species or species complexes, all $p<0.001$). In the case of Atlantic seabirds, those breeding in the northern localities (Madeira and Azores) showed significant greater OC levels than seabirds from the southern (Canary and Cape Verde). These patterns in seabird OCs are probably associated with long-range pollutant transport from North American costs over mid-north Atlantic regions. In addition, the major presence of *p,p'*-DDT that we found in the case of seabirds from the southern Macaronesian archipelagos (DDE/DDT<1 in 47.4% and 31.6 of the cases from Cape Verde and Canary Is., respectively) suggests recent inputs of DDTs, which could be related to the use of this pesticide in the Sub-Saharan African countries[1]. Within the Mediterranean, OC levels tended to increase from west (Spanish coast, Terreros Is.) to east (Crete). Moreover, Mediterranean seabird populations showed significant greater concentrations of OCs and a greater presence of high-chlorinated PCBs compared to their Atlantic counterparts. This pattern is consistent with the historically great input of OCs and the confined character of the Mediterranean basin compared to the Atlantic Ocean.

Previous studies on seabirds have reported adverse ecological effects on the reproductive performance and behavior of gulls related to their whole blood PCB and *p,p'*-DDE burdens[12,13]. The mean ∑PCB levels reported in the present study were lower than those reported for gulls, except in the case of storm-petrel species and some shearwater populations (Hyeres and Crete) from the Mediterranean. The rest of Mediterranean seabird populations showed a range of OC levels that strongly overlap with those reported for gulls. The greater levels found in the Mediterranean compared to the Atlantic shearwaters as well as the high OC levels found in storm-petrels probably represent an extra stressor element for these populations. In these regards, the evaluation of OC burdens and biochemical health parameters in the plasma of Mediterranean gulls could bring new lights into the possible effects of these pollutants.

Plasmatic levels of ∑PCBs and ∑DDTs in the yellow-legged gull (71.8 and 50.9 ng/g w.w, respectively) breeding in the Mediterranean were below the range of OC levels reported in other seabird studies and associated to direct toxicological effects. However, Mediterranean gull species showed mean PCB and *p,p'*-DDE levels that were markedly greater than those believed to be of toxicological concern in black-footed albatross[14]. Similarly, DDE levels found in gulls exceed the plasmatic level (27.8 ng/g w.w.) associated to population effects in fish eating bald eagles[15]. Despite of these results, sensibility to pollutants vary widely across bird species, even within close species, and thus, cause-effect relationships cannot be directly established for yellow-legged gulls. In fact, few health and nutritional plasma biochemical parameters showed significant relationships with gull plasma OC levels.

Albumin concentrations and AST activity were negatively and significant correlated with PCB and DDT levels in yellow-legged gull plasma (Lineal Regression Analysis, all $P<0.05$, r^2 ranged from 0.20 to 0.31). Albumin is considered a highly sensitive and specific indicator of hepathocyte damage and liver chronic damage[16]. Previous studies found a significant decrease in albumin concentrations associated to long-term OC exposition in wild bird species (including gulls) as well as in controlled bird diet exposition (revised in Fox et al.

2007[16]). AST enzyme in birds is also considered a good indicator of hepatocellular disease and hepatic or kidney damages[17]. A significant decrease in AST activity has been detected in gulls from the Great Lakes chronically exposed to PCBs and DDTs[16] and even in humans after long exposition periods to PCBs[18] (at least five years). Therefore, the general picture obtained regarding plasmatic health parameters and OC levels suggest some possible adverse hepatic effects associated with the chronic exposition of Mediterranean yellow-legged gulls to organochlorines.

In sum, the present study highlights the lasting and unequal impacts of past human activities in the marine environment, especially in confined regions such as the Mediterranean Sea. Although our results suggest that some adverse effects could be associated with the chronic exposition of Mediterranean predators to OCs, further studies focusing on ecotoxicological effects and specific biomarkers related to POPs exposition in Mediterranean species are needed to accurately evaluate the risk derived from these contaminants.

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