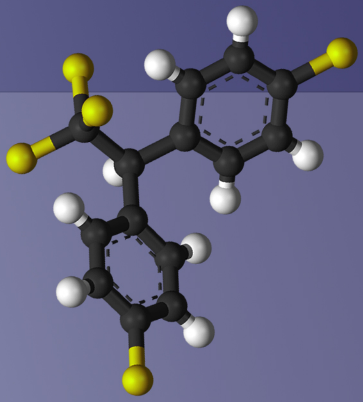
Linking ecology and Environmental chemistry: Pelagic seabirds as indicators of marine contamination

Entre la ecología y la química ambiental: Las aves pelágicas como indicadores de la contaminación marina

Jose Luis Roscales García



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Universitat de Barcelona

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PhD Thesis

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ENTRE LA ECOLOGÍA Y LA QUÍMICA AMBIENTAL: LAS AVES PELÁGICAS COMO INDICADORES DE LA CONTAMINACIÓN MARINA

LINKING ECOLOGY AND ENVIRONMENTAL CHEMISTRY: PELAGIC SEABIRDS AS INDICATORS OF MARINE CONTAMINATION

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A mis padres

A mi hermano

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Jose

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LINKING ECOLOGY AND ENVIRONMENTAL CHEMISTRY: PELAGIC SEABIRDS AS INDICATORS OF MARINE CONTAMINATION

Jose L. Roscales

Abstract: Exposure to contaminants such as Persistent Organic Pollutants (POPs) is currently regarded as a serious anthropogenic threat to marine wildlife and their food webs since it may lead to a decline in health, reproductive success and global populations of marine predators. To monitor ecosystems health and contamination levels we need indicator species. However, to interpret contamination levels it is crucial to understand how these species interact with the ecosystems trough the study of their trophic niche and their spatiotemporal dynamics. This knowledge will also be essential to design effective conservation strategies for threatened species. In the present thesis, some ecological factors, mainly feeding ecology, breeding locality and movements, and their influence in shaping the isotopic signatures and the contaminant burdens were examined in most Procellariiformes breeding in the Northeast Atlantic Ocean and the Mediterranean Sea. In particular, the isotopic signatures of Carbon and Nitrogen as well as PAH, PCB and DDT burdens in seabirds were determined and the relationship between the ecological factors mentioned above and the variability in contamination burdens is discussed. The suitability of pelagic seabirds to monitor contamination from pelagic environments is also evaluated.

BACKGROUND

Over the past decade, the role of human activity in causing changes in marine environments at the local, regional and global level has been widely recognised (Barange 2003). Marine resource overexploitation (mainly associated with fishery activities), climate change and habitat destruction, which includes marine pollution, are ranked as the most important pressures involving marine biodiversity changes and affecting ecosystems functioning (Halpern *et al.* 2008). Therefore, understanding these changes represent a major issue to face up the challenges of marine environment conservation (Kappel 2005).

The United Nations Convention on the Law of the Sea defined marine pollution as “the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of the sea water and reduction of amenities”. Therefore, marine pollution includes several threats that affect not only marine ecosystems and wildlife but also human health.

Beyond the direct toxic effect on marine organisms, marine pollution and the rest of processes damaging marine environments interact resulting in complex and unpredictable adverse effects on marine populations and whole ecosystems. For instance, studies on Arctic marine regions have reported that climate change can reinforce the detrimental effects of chemical contaminant impact and push ecosystems and species, especially those placed at high trophic position, over their limits (Jenssen 2006).

In this global context, by raising awareness about human impacts on wildlife and natural ecosystems, governments and societies around the world see now protection of the marine environment and control of marine pollution as an ever more pressing necessity (Islam & Tanaka 2004). Aiming for a better global evaluation and understanding of pollution threats in the marine environment, the new European Marine Strategy Directive (EMS) identified as one of the most important challenge for scientists the development of monitoring networks and suitable methodologies which could be applied to different marine ecoregions (Sheppard 2006). This global approach comprises several tools such as the use of ecological indicators to assess the impact of human activities and to measure the response of marine ecosystems to anthropogenic disturbances (US EPA 2000).

An ecological indicator has been defined as a measurable characteristic related to the structure, composition or functioning of ecological systems (US EPA 2002). These ecological indicators can be aggregated into ecological attributes with reporting categories, such as biotic condition (e.g. trophic structure, community composition and organism condition), chemical and physical characteristics (e.g. trace inorganic and organic chemicals and physical parameters), and ecological processes (e.g. energy and material flow) (Harwell *et al.* 1999; US EPA 2002). Most applications of ecological indicators, especially in the environmental chemistry, have focused at the species level shaping the concept of indicator or biomonitor species (Niemi & McDonald 2004). Indicator species are primarily used either to assess the condition or the relative condition (relative to a specific standard) of the environment and to diagnose the cause of environmental changes and stress (Furness *et al.* 1993; Fleischman *et al.* 2001; Dale & Beyler 2001).

The use of indicator species is firmly established in ecology, environmental toxicology, pollution control and wildlife management and previous studies have reported valuable examples of it in the marine environment. For example, seabirds have been largely used to evaluate marine ecosystems status. That is, seabirds have been used to decipher spatial and temporal trends of marine pollution as well as to the study of fish stocks, climate change and regimen shifts in marine ecosystems (Aebischer *et al.* 1990; McDonald *et al.* 2000; Thompson & Ollason 2001; Braune *et al.* 2002; Barrett 2002; Piatt *et al.* 2007). Besides seabird, several indicator species have been used to monitor the health of marine ecosystems. That are the cases of jellyfish blooms as consequence of absence of predators and climate change, the widespread decline of health and reproductive success in turtles and marine mammals due to contamination by Persistent Organic Pollutants (POPs), or spatial and temporal variations in marine pollution as shown by mammals and fish species (Mössner & Ballschmiter 1997; Marine Mammal Comission 1999; Keller *et al.* 2004; Stern *et al.* 2005; Purcell *et al.* 2007).

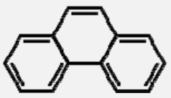
MARINE CONTAMINATION AND INDICATOR SPECIES

The negative direct and indirect effects of contamination over sea life have been widely described. Oceans act as a final repository for many land-based source contaminants. Consequently, the massive use of harmful pesticides, the increasing industrial chemical runoffs as well as dramatic accidental episodes such as oil spills, have been established as the direct cause of death of thousands of marine organisms, especially those placed at high trophic positions (Gilbertson *et al.* 1991; Ross *et al.* 1996; Marine Mammal Comission 1999; Wiese & Robertson 2004; Kappel 2005; Livingstone *et al.* 2009). This loss of individuals involves population declines of several marine species, which eventually affect the whole ecosystems. Moreover, the high persistence of several contaminants in the environment involves several long-term and indirect effects over marine predators, including fluctuations of their populations. Long-term or chronic effects of pollution over marine wildlife are due to the constant and accumulative exposure to contaminants which involves, for example, marked reductions in the breeding success of marine mammals and birds or changes in food web structures that limit food availability (Peakall & Lincer 1996; Peterson *et al.* 2003; Kappel 2005).

One of the most widespread and long-established application of indicator species has been their use to evaluate marine contamination as well as to understand contaminant dynamics in the marine environment (Furness *et al.* 1993; Rice 2003; Depledge & Galoway 2005). Among marine contaminants, Persistent Organic Pollutants (POPs) have attracted much scientific and social interest because this wide family of pollutants includes diverse chemical substances that persist in the environment, bioaccumulate and pose a risk to human health and the environment (Islam & Tanaka 2004; Muir & Howard 2006). International action on this contaminants culminated in the signing of the Stockholm Convention on POPs in 2001 (UNEP 2001) to aim for the elimination and restriction on their production and use. Recently, the Conference of the Parties (COP, 2009) added nine new POPs to those included previously in Stockholm.

Within POPs, Polycyclic Aromatic Hydrocarbons (PAHs, Box 1) and Organochlorine Contaminants (OCs), which includes polychlorinated biphenyls (PCBs, Box 2) and dichlorodiphenyl ethane (DDT, Box 3), are globally distributed in marine food webs. These pollutants are known to have a wide array of biochemical, physiological and ecological adverse effects in the marine environment as well as carcinogenic and mutagenic properties (Walker & Livingstone 1992; Hellou 1996; World Health Organization 1998; Jones & Voogt 1999). Although some of them were banned in most parts of the world long ago, they still constitute an element of concern nowadays because their persistent and lipophilic nature allows them to continuously affect to marine biota and bioaccumulate in most marine organisms (Meador *et al.* 1995; Harding *et al.* 1997; Livingstone *et al.* 2009). In fact, in the case of OCs, these contaminants biomagnify throughout marine food webs (Hop *et al.* 2002), which results in great concentrations with their subsequent toxicological effects in marine predators placed at high trophic positions, mainly in fishes, seabirds and marine mammals (Walker 1992; Tanabe & Tatsukawa 1992).

Box 1. ***What are the*** polycyclic aromatic hydrocarbons (PAHs)



E.g. Phenanthrene structure

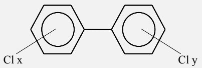
PAHs are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Chemical properties and persistence of PAHs are related to the number of rings and their aromaticity

Some properties: They have a relatively low solubility in water, but are highly lipophilic and are liquids or solids at normal conditions being susceptible to chemical and biochemical transformation.

Some legislation: The U.S. EPA indicated 16 PAHs as priority compounds due to their great toxicity. Although PAHs have not been included in the Stockholm Convention, they are regulated in their production and emission trough different European policies.

Origin and main sources: PAHs are formed mainly as a result of pyrolytic processes**.** Main sources are incomplete combustion of organic materials during industrial and other human activities as well as natural processes such as carbonization. In the case of marine environment, we must add the accidental or intentional discharges from oil tankers, ships and fuel extraction activities.

Box 2. ***What are the*** polychlorinated biphenyls (PCBs)



General structure of PCBs

They are a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl, which is a molecule composed of two benzene rings. Depending on the number and position of chlorine atoms there are 209 possible related compounds (congeners).

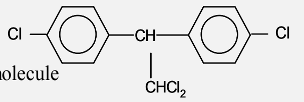
Some properties: PCBs are industrial contaminants which were globally used as commercial mixtures (Aroclor, Kaneclor…) due to their physical properties including their great chemical and thermical stability. They have been widely used as hydraulic fluids, dielectric fluids in transformers and capacitors, and plasticizers in paints.

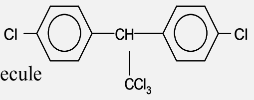
Some legislation: Their use and production has been progressively restricted and banned from the 70s until now through different policies (e.g. 76/403/CE; 76/769/CE; 85/467/CE; 96/56/CE)

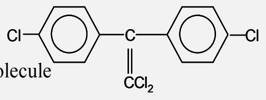
Origin and main sources: Human activities constitute the only source of PCBs in the environment. Commonly, major sources of these contaminants are (or have been) manufacturing wastes and the careless disposal or dumping of the liquids referred to above.

Once introduced into the environment, OCs and PAHs can circulate around the globe on air and water currents affecting wildlife not only at the points of their origin but also at remote oceanic regions (Walker *et al.* 2006; Nizzetto *et al.* 2008). Therefore, the spatial distribution of these contaminants as well as their possible sources in the marine

Box 3. ***What are the*** dichlorodiphenyl ethane (DDT) and its metabolites


DDT molecule


DDE molecule


DDD molecule

Some properties: The DDT is an organochlorine insecticide with a long and controversial history. It was massively used in the 60s for the control of agricultural pests and vectors of disease (e.g. malarial mosquitoes). It is nearly insoluble in water but has a good solubility in most organic solvents, fats and oils. Dichlorodiphenyl dichloroethylene (DDE) and dichlorodiphenyl dichloroethane (DDD) are its main metabolites. Although DDT is greatly persistent and toxic, DDE is even more. This contaminants and their dramatic environmental impacts during the 60s are joined to the history of conservation biology since they were related in the classic "*Silent Spring*" from Rachel Carson.

Some legislation: DDT was banned in most part of the world in the 70s (E.g. Spain,, B.O.E 22-3-71). The Stockholm Convention, signed by 160 countries, includes this insecticide in the list of chemicals restricted on their use and production.

Origin and main sources: Past massive spills referred to above. Although DDT is actually banned in most part of the world, some undeveloped countries still use the insecticide to control malaria outbreaks (Walker *et al.* 2003).

environment have been the subject of extensive research over the last thirty years (e.g. Muirb *et al.* 1999; Aguilar *et al.* 2002; Cifuentes *et al.* 2003). In relation to this general interest in contamination assessment, environmental chemists have made a substantial progress in determining the fate and concentrations of contaminants in the marine environment. Sensitive analytical methods (Box 4) have been developed for detecting specific chemicals in biological tissues at low detection levels. These approaches provide useful and valuable background information about the pollutant concentrations and profiles in a large number of marine species.

Typically, marine contamination studies have been mainly based on species from the macrofauna, especially aquatic macroinvertebrates, fish and birds. The taxonomic status of indicator species is largely associated with the studied family of pollutants (e.g. PAHs have been commonly monitored in macroinvertebrates while PCBs tend to be studied in vertebrates placed at higher trophic positions) as well as with the particular objectives to develop (e.g. molluscs to assess the contamination status of harbours and seabirds such as gulls for general coastal environments) (Cifuentes *et al.* 2003; Perugini *et al.* 2007b; Livingstone *et al.* 2009). As a result several marine organisms such as crustaceans, molluscs, fishes, seabirds and marine mammals have been proposed as indicator species of contamination (Aguilar *et al.* 2002; Burger & Gochfeld 2004; Soriano *et al.* 2006; Perugini *et al.* 2007a).

At the same time, ecologists have developed new methodologies and have conducted field studies designed to understand the marine organism trophic relationships and movements as well as to detect the impact of pollutants among marine biota and ecosystems. However, few studies, especially concerning organic contaminants, deal with both the ecology and contamination status of the studied species. In fact, while contaminant burdens in marine indicator species serve as a measure to evaluate their exposure and possible adverse effects, several shortcomings involve it when the objective is to use one species as surrogate for many others. That is, when we want to use the contaminant status of a target species as indicator to evaluate the status of other species, communities or ecosystems within the same area or from other regions the usefulness of most indicator species is unclear. Similarly, when the objective lies in understanding the dynamic and sources of marine pollutants, those approaches including indicator species has been commonly questioned (Hilty & Merenlender 2000; Burger & Gochfeld 2001; Gray 2002; Niemi & McDonald 2004).

Box 4. ***Analytical methods*** in environmental chemistry

The analytical methods commonly applied in environmental chemistry to determine pollutant concentrations involve two main steps: sample preparation and instrumental analysis. The sample preparation methodology strongly depends on the characteristics of the matrix under study and the target analytes. The instrumental methodologies for the determination of environmental pollutants such as POPs are commonly based on the use of chromatographic techniques coupled to specific selective detectors depending on the analytes to be determined. In the present thesis **High Resolution Gas Chromatography with micro-Electron Capture Detection (HRGC-µECD)** was applied to the identification and quantification of PCBs and DDTs**.** The Electron Capture Detector is one of the most sensitive detectors to analyze electronegative compounds such as chlorinated, fluorinated, or brominated molecules. **High pressure liquid chromatography (HPLC) coupled to fluorimetric detector** was applied to the identification and quantification of PAHs.

HRGC-µECD

HPLC

The controversy of using marine species as indicators is strongly associated with the partial unknown about the ecology and physiology of the target species. The lack of knowledge regarding species ecology makes particularly difficult to interpret the dynamic of marine pollutants in wildlife since it restricts the understanding of the factors that shape organism exposition to marine contaminants. For example, several contamination studies assume ecological features of the considered species from previous studies about related species or the same species but from different regions. However, on one hand dietary preferences of marine organisms can change within their geographic distribution but also within a single region due to specific feeding grounds or dietary shifts, which involvs different exposure to contaminants (e.g. González-Solís *et al.* 2000a). Indeed, similar trophic positions in predator species do not involve similar dietary preferences (Hobson *et al.* 1994). On the other hand, migratory patterns and movements in the marine environment of most organisms are not well known which makes the interpretation of their contaminant levels particularly difficult (Hobbs *et al.* 2001; Braune *et al.* 2002; Stern *et al.* 2005). Moreover, other factors such as sex or age can also involve specific resources exploitation and thus, specific exposure ways to marine contaminants. Therefore, an extensive knowledge of the feeding ecology and its consistence across the distribution of marine organisms as well as their spatiotemporal dynamics are needed to understand the contamination levels of marine organisms and their value as indicators of marine pollution.

LINKING ECOLOGY AND CONTAMINATION IN WILDLIFE

In general, the diet represents the principal route of exposure to contaminants, especially when we focus on vertebrates placed at high trophic positions such as marine mammals or seabirds. Therefore, it is obvious that an extensive knowledge of the trophic ecology of marine organisms is required to correctly interpret the meaning of the observed pollutant levels (Burger & Gochfeld 2001; Fisk *et al.* 2001). On the other hand, contaminant emissions, discharges and their distribution in the marine environment are not spatially uniform. Moreover, long range transport dynamic of several contaminants also result in specific spatial patterns across worldwide oceanic basins (Bard 1999; Walker *et al.* 2006). The inhomogeneous distribution of contaminants in the marine environment has been clearly reflected in contamination burdens of marine invertebrate and vertebrate species (Walker & Livingstone 1992; Mössner & Ballschmiter 1997; Pol *et al.* 2004). However, the relative contribution of trophic ecology and pollutant geographic distributions in shaping contaminant burdens of most marine predators is poorly understood (Jarman *et al.* 1996; Ricca *et al.* 2008). That is, when comparing contaminant burdens of distant populations of a single or related species, whether or not contaminant level differences are due to geographic pattern of pollution or specific dietary habits of studied species is uncertain.

The influence of trophic ecology: The importance of trophic ecology when studying contaminant levels and their accumulation in marine organisms, especially in marine predators, has been largely recognized in several studies (Fisk *et al.* 2001). One of the most widespread approaches in contaminant studies is the use of marine top predators as indicators of the contaminant status of ecosystems as well as a surrogate for other marine species. This tendency is based on the assumption that marine predators can provide an index of the levels of contamination in environmental marine monitoring since they are exposed to greater burdens due to the biomagnification process (Walker & Livingstone 1992). Most studies assume that comparable contaminant burdens are expected in other species placed at similar trophic positions. However, on one hand, since the accumulation of contaminants is not determined by trophic level alone, partial upsets between the trophic position and the contaminant levels have been reported in marine predators (Tanabe & Tatsukawa 1992; Muir *et al.* 2003; Riget *et al.* 2004; Buckman *et al.* 2004; Elliott 2005; Elliott *et al.* 2009). That is, when marine food webs or communities (e.g. marine mammal or seabirds from a specific region) have been explored, some species show different contaminant concentrations than those expected from their trophic position (Fisk *et al.* 2001; Buckman *et al.* 2004; Elliott 2005; Wan *et al.* 2007). These unexpected interspecific differences in contaminant levels have been attributed to particular dietary habits but also to different metabolic capabilities of marine organisms. On the other hand, we also must consider intraspecific sources of variability in contaminant burdens. Factors such as sex and age, involve specific feeding ecology and excretion opportunities for several marine organisms.

The trophic ecology of marine organisms, mainly related to a particular dietary composition and feeding ground, has been identified as a possible cause which may explain unexpected interspecies differences in organism contaminant burdens. Species with high opportunistic or scavenging roles in marine ecosystems have shown grater pollutant levels than those expected from their trophic position (Muir *et al.* 2003; Buckman *et al.* 2004). Similarly, some marine consumers feeding on mesopelagic (100-1000 m of depth in the water column) resources or on bottom dwelling prey have shown greater contaminant levels than those with similar trophic position but feeding on surface prey (Thompson *et al.* 1998; Brown *et al.* 1998; Takahashi *et al.* 1998; Takahashi *et al.* 2000). These examples illustrate the strong link between species feeding ecology and their exposure to contamination and emphasize the need of multi-species approaches to accurately evaluate marine pollution hazards.

Concerning intraspecific variation in contaminant levels foraging habits segregation (dietary preferences or feeding grounds) between males and females also has been identified as a cause of different exposure to pollution between sexes (González-Solís *et al.* 2002; Wearmouth & Sims 2008). Males and females from several marine predators show different feeding strategies, which could involve sex-specific risks associated to marine contamination (González-Solís *et al.* 2000b; Sims *et al.* 2001; Becker *et al.* 2002; Martínez-Abraín *et al.* 2006; Breed *et al.* 2006). In the same way, age also has been identified as an intraspecific cause of variation in the presence of contaminants in marine organisms, mainly in vertebrates. Previous studies have reported specific age accumulation among fish, birds and marine mammals: adult seabird species feeding their offspring with prey items with specific nutritional requirements involve different contaminant burdens between chicks and their parents (Hodum & Hobson 2000; Nisbet *et al.* 2002); marine mammals which have reached the reproductive senescence show greater pollution levels than reproductive adults and juveniles (Aguilar & Borrell 1994; Ross *et al.* 2000); studies on fish species from the Arctic have shown that globally adult fish show greater pollutant levels than juvenile or immature fish (Harding *et al.* 1997).

The influence of detoxification capability: Although metabolic and excretion capabilities are not strictly ecologic features of marine organisms, they also must be considered when comparing contaminant levels. Previous studies have reported the influence of these factors over contaminant burdens. At intraspecific level, gender differences in contaminant levels have been identified as a partial cause of the major opportunities of females to excrete contaminants. For example, in the case of seabirds the excretion of pollutants through their deposition into the eggs or in the case of marine mammals through the maternal milk, have resulted in lower levels of contaminants in females compared to males (Donaldson & Braune 1999; Ross *et al.* 2000; Becker *et al.* 2002; Borrell *et al.* 2009). At interspecific level, metabolic capabilities of marine organisms have been found to explain lower contaminant burdens in several species. One clear example is the lower levels of PAHs found in most vertebrate marine predators related to those found in their prey (mainly invertebrate species) which suggest a biodilution of this contaminants throughout marine food webs (Wan *et al.* 2007). This interspecific trend is due to the major capabilities of most vertebrates compared with invertebrates to metabolize and eliminate PAHs (Walker 2002).

The influence of marine distribution and movements: Several marine predators exploit trophic resources among vast geographic scales, including coastal and open water ecosystems. Moreover, many species show wide breeding areas and undergo long-distance migrations. Therefore, since the spatial distribution of marine contaminants is not uniform, marine organism exposition to marine pollutants not only is markedly influenced by their feeding habits but also by their spatiotemporal dynamics. For example, studies of sympatrically breeding marine predators in the Pacific, such as albatrosses, have shown that contamination differences in these species are primarily due to a regional segregation in their foraging areas, ruling out point sources or trophic position as possible explanations (Finkelstein *et al.* 2006). In fact, some studies have suggested that trophic levels *per se* may be less important explaining contaminant burdens of marine predators than the exploitation of locations where there are ongoing major sources of contaminants (Elliott & Norstrom 1998; Elliott *et al.* 2009).

Well known examples of the unequal distribution of marine pollutants are the greater levels found in confined seas such as the Mediterranean basin related to other open oceanic systems (Martí *et al.* 2001; Albaigés 2005; Migon 2005). Similarly, coastal and remote pelagic environments show different sources and inputs of contaminants. These spatial trends are associated with the major exploitation, use and thus, direct influence, of human activities in coastal regions as well as with the transport processes and persistence of contaminants in open waters. As consequence, studies of marine pollution have showed specific contamination profiles and levels in marine organism that exploit offshore and inshore resources (Jarman *et al.* 1996; Yamada *et al.* 1997; Vorkamp *et al.* 2004; Islam & Tanaka 2004; Ricca *et al.* 2008). Nonetheless, the number of studies which focus on coastal compared to pelagic environments is considerably greater. This shift in contamination studies probably reflect the great interest in coastal resources but also the practical and technical difficulties in obtaining samples from remote marine regions (Yamashita *et al.* 2007).

Other global spatial trend in marine contamination concerns to the irregular latitudinal distribution of pollutants. On one hand, oceanic waters from the northern hemisphere tend to show greater pollutant levels than those from the southern probably due to the major development of northern countries (O'Shea & Brownel 1994; Yamada *et al.* 1997; Finkelstein *et al.* 2006). On the other hand, previous studies have revealed the occurrence of regional shifts in marine pollutant concentrations and profiles in extreme oceanic latitudes, which were considered as pristine regions until the 80s, as well as in some marine current systems. In this case, long-range pollutant transport dynamics have been suggested as the main cause (McDonald *et al.* 2000; Braune *et al.* 2001; Blais 2005).

Because the strong geographic patterns in marine pollution, it seems relatively obvious that not only the foraging grounds but also the migratory movements of marine organisms could result in marked variations in their exposition to marine contaminants. Indeed, recent studies suggest that migratory movements not only are a relevant issue in understanding species exposition but also in explaining the transport of pollutant between different regions, which is known as biovector transport. Biovector transport concern mainly to gregarious animals that biomagnify certain contaminants and then migrate and congregate to other regions becoming the predominant transport pathway for pollutants. Seabirds such as the northern fulmar (*Fulmarus glacialis*) or different arctic penguin species have been proposed as active biovectors for PCBs, DDTs and infectious pathogens (Daszak *et al.* 2000; Blais *et al.* 2004; Blais *et al.* 2007), which represents an important issue for public health and environmental science.

However migratory movements of several marine species are not well understood which limits our capability to interpret their interaction with the marine environment. This difficulty is particularly relevant when we analyse contaminant levels in tissues that undergo a long time scale accumulation such as fat tissues. A partial solution for these shortcomings could be the use of tissues that reflect short time-scale accumulation and indicate contaminant levels due to recent exposure. Similarly, the combination of methodologies that allow us to know the spatiotemporal dynamics of marine predators (e.g. Ramos *et al.* 2009a) with the study of their pollutant levels constitutes a remarkable research direction.

In this global context, the need of reference pollutant levels across wide geographic scales emerges as a crucial need. Multi-locality approaches including, for example, a single species sampled in several localities, are particularly valuable since they comprise a better understanding of spatial trends in marine pollution than those focusing on a single population. In this regards, species which show huge distributions emerge as useful candidates for marine monitoring studies because they offer the opportunity to evaluate marine contamination across different biogeographic regions. Nonetheless, a previous evaluation of possible trophic ecology difference among distant population should be done before direct comparison.

THE STUDY OF THE ECOLOGY OF MARINE PREDATORS: TRADITIONAL APPROACHES AND NEW METHODOLOGIES

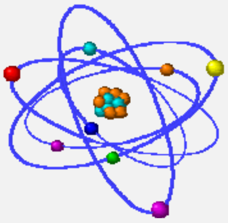
Significant progress has been made during the last twenty years in the study of the ecology of marine organisms, especially in those species highly mobile and thus, with huge foraging grounds. On one hand, this advance has been possible due to the accumulative effort of marine scientist in understanding marine species trophic relationships and movements as well as the development of new methodologies to their study. In the other hand, the growing interest in the conservation of marine environments and resources has enhanced the need of understanding marine species ecology and spatiotemporal dynamics.

Traditional methodologies: Traditional approaches used to study the interaction between marine predators and their ecosystems have been often found limited. Overall, these methodologies mostly offer a biased vision of marine predator ecology. For instance, the examinant ion of stomach contents or pellets suffer from several methodological shortcomings, including reduced time integration, bias towards hard-body prey and confusion in the parental allocation of food between self and offspring in the case of birds (Duffy & Jackson 1986; Hobson *et al.* 1994; González-Solís *et al.* 1997; Bowen 1997; Lesage *et al.* 2001). Moreover, in the case of several species, the sampling design depends on the availability of death specimens which do not allow a systematic sampling procedure. Finally, several marine predators are only accessible to scientists during the breeding period and thus, several studies focus only on a piece of the life cycle of marine species.

Movements of marine predators have been tracked mainly by direct observation of their presence and abundances and by using extrinsic markers, such as ringing and banding individuals (Hobson & Norris 2008). Marking techniques are based on the mark-recapture techniques which hope to relocate those marked individuals elsewhere. However, the recovery rates of most marine predators are extremely low and show a lower applicability compared to terrestrial animals (Hobson & Norris 2008). Moreover, studies of movements of marine predators using traditional techniques require a great monitoring effort through a long time to obtain reliable information about animal migratory movements (e.g. Smith *et al.* 1999; Gregr *et al.* 2000).

In this general context, trophic ecology as well as migratory routes and wintering grounds of most marine predators have been relatively unknown so far. However, new methodologies have been developed and applied since the 80s with the aim to mitigate most of the biases and constraints related to traditional approaches. Among them, the use of intrinsic biomarkers and the technologic development of useful extrinsic markers such as stable isotopes (Box 5) and tracking devices, respectively, have increased our knowledge of spatiotemporal dynamics and trophic ecology of marine animals.

Box 5. ***What are*** the stable isotopes



Several chemical elements show variations in their atomic weight as a consequence of having a different number of neutrons in their nucleus. In spite of this, their physical and chemical characteristics do not change substantially. Each class of weight of each element is known as isotope, and they are called stable as they do not decay over time (Hoefs 2004). There are many elements with multiple stable isotopic forms, but only those related with the biosphere (plants, animals), the hydrosphere (water), and the atmosphere (gaseous) are used in ecological research, i.e. those of carbon, nitrogen, sulphur, hydrogen and oxygen (West et al. 2006).

Commonly, stable isotopes most wide used for the study of marine wildlife are carbon and nitrogen. The most abundant form of carbon is the ^{12}C isotope (98.90%), but there is also a heavy isotope, ^{13}C which is less represented (1.10%). Nitrogen also presents two isotopic forms, ^{14}N, being the most common isotope (99.63%), and ^{15}N which occurs in a minor proportion (0.37%).

Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios (Sparkman 2000). This technique provides an extremely accuracy in estimating the ratio of the heavier to lighter isotope in an unknown sample relative to an international standard. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following delta notation:

$$\delta X = [(Rsample/Rstandard) - 1] \times 1000$$

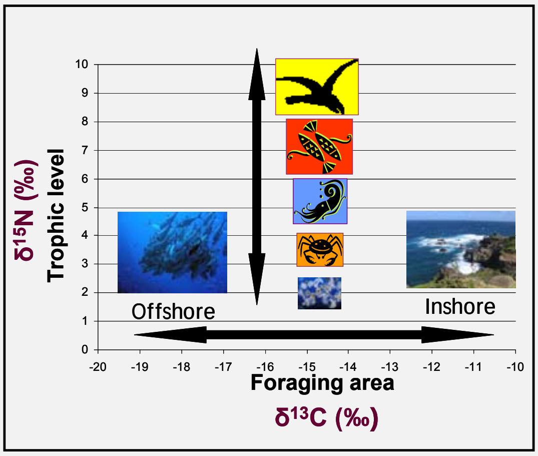
where X (‰) is ^{13}C or ^{15}N and R are the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ related to the standard values. Standard for ^{13}C is Pee Dee Belemnite (PDB) and for ^{15}N is atmospheric nitrogen (AIR). Because these international standards are arbitrary, some isotope ratios are positive (i.e. more enriched in the heavier isotope relative to the standard) and others are negative (i.e. more depleted in the heavier isotope relative to the standard).

New methodologies; intrinsic biomarkers and tracking devices: There is an increasing interest in using intrinsic markers such as Stable Isotope Analysis (SIA) in the study of animal ecology. This interest is probably derived from the fact that SIA can overcome or minimize some of the problems of traditional approaches commonly used in the study of animal ecology and movements. Moreover, isotopic approaches in animal ecology can be used as one means to depict species' niche (Cherel *et al.* 2007; Newsome *et al.* 2007; Layman *et al.* 2007) and it allows to identify and link breeding and wintering areas in all marine organisms (Gómez-Díaz & González-Solís 2007; Semmens *et al.* 2007; Rooker *et al.* 2008; Caut *et al.* 2009), as no other intrinsic markers (i.e., biometric or genetic) can identify them (Hobson 2008).

Stable isotope analyses of carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) have a broad array of applications in ecology (Kelly 2000; Fry 2006). Their use is based on the statement "you are what you eat" but also on "you are what you swim in" (Box 6) (Hobson 1999). That is, isotopes ratios in consumer tissues reflect those of their prey in a predictable manner (DeNiro & Epstein 1978; 1981; Hobson & Clark 1992a; 1992b). Concretely, Nitrogen isotope ratios ($\delta^{15}N$) can be used to infer trophic position whereas stable-carbon isotope analysis ($\delta^{13}C$) can indicate inshore or benthic compared to offshore or more pelagic feeding habits of marine organisms (Kelly 2000). Moreover, the natural abundance of stable isotopes of Carbon and Nitrogen (baseline levels) show marked spatial patterns in the marine environment since they vary among large

Box 6. ***How can we*** use stable isotopes of Caron and Nitrogen in the study of wildlife

The utility of stable isotopes in animal ecology relies on their distribution in foods (diet) and how they are incorporated into the tissues of consumers. DeNiro and Epstein (1978,1981) were the first to provide evidence that the carbon and nitrogen-isotope composition of a consumer was a direct reflection of its diet. However, consumer's tissue show specific differences, enrichment or depletion, in their isotope signatures related to those found in the diet. Enrichment happens when the heavier stable isotope is accumulated in the product (relative to the substrate, e.g. the diet in the case of consumers), while the lighter isotope is preferably eliminated. On the other hand, depletion occurs when the lighter isotope is favored. Processes of enrichment and depletion are known in general as fractionation or discrimination.



_ **δ^{13}C** _ Differential fractionation of stable isotopes of carbon during photosynthesis causes C4 plants and C3 plants to have distinct carbon-isotope signatures. In addition, marine C3 plants have stable isotope ratios of carbon that are intermediate between C4 and terrestrial C3 plants. Further, within marine environment, inshore carbon sources can sometimes be distinguished from pelagic sources because phytoplankton has lighter δ^{13}C values than many inshore plants (e.g., seagrasses, average δ^{13}C = –10‰, range = –15 to –3‰) (Kelly 2000). Therefore, stable-carbon isotope signatures in the study of tropho-dynamic of marine predators have been commonly used to difference between coastal (inshore) or pelagic (offshore) dietary preferences (Kelly 2000).

**δ^{15}N** In the case of stable-nitrogen isotope signatures, the heavy isotope of nitrogen (^{15}N) is preferentially incorporated into the tissues of the consumer from the diet, which results in a systematic enrichment in nitrogen-isotope ratio (δ^{15}N) with each trophic level. This process is comparable to the biomagnification of organic pollutants. Consequently, stable isotopes of nitrogen have been used primarily to assess the position of marine organisms in their food chains (Kelly 2000).

In addition the natural abundances of stable isotopes (baseline levels) in the marine environment show marked spatial patterns which also are reproduced in wildlife isotopic signatures. Several studies have assessed animals' origin or movements in the marine environment (Hobson & Schell 1998;Cherel, Hobson & Weimerskirch 2000;Kurle & Worthy 2002;Quillfeldt, McGill & Furness 2005;Cherel, Hobson & Weimerskirch 2005) using geographic variations in oceanic stable isotopes base line levels. The most common geographic trend for stable isotopes in some oceans is the latitudinal decline in δ^{13}C values documented in marine plankton and thus, in consumers tissues too (Rau, Sweeney & Kaplan 1982;Goericke & Fry 1994;Chamberlain *et al.* 1997;Burton & Koch 1999). Occasionally, some longitudinal variation have also been observed (France *et al.* 1998)

geographic scales, and thus, isotope ratios in consumer tissues also reflect their spatial variability (Hobson 1999). Therefore, on one hand, variation in isotopic signatures of marine predators has been widely used in a number of studies to decipher the trophic structure of marine food webs (Bode *et al.* 2007), trophic relationship within fish, bird and mammal communities (Lesage *et al.* 2001; Jennings *et al.* 2002; Forero *et al.* 2004) as well as to more specific questions of spatial and temporal variability in diets (Hodum & Hobson 2000). On the other hand, spatial variations in isotope baseline levels have been successfully applied to assess migratory movements and geographic origin of fish, marine mammals and seabirds (Hobson 1999; Rubenstein & Hobson 2004; Gómez-Díaz & González-Solís 2007). However, although the usefulness of SIA has been widely documented, some controversial issues still limit the applicability of this methodology in the study of marine wildlife.

As commented above, feeding ecology and geographic location are two major factors influencing isotopic signatures of animals. However, the relative importance of spatial patterns in isotopic baseline levels and animal trophic relationships in shaping isotopic signatures of top predators and their food webs remains poorly understood (Jaquemet & McQuaid 2008; West *et al.* 2008). This lack of knowledge is particularly noticeable in the marine environment, where the complexity of the biochemical and oceanographic processes make the isotopic dynamics of marine food webs comparatively more difficult to decipher than those in terrestrial ecosystems (Michener & Schell 1994). In sum, it is still unclear whether the isotopic variability determined by differences in the trophic ecology of marine predators could be override by differences in isotopic baseline levels among food webs. Moreover, biogeographic trends of isotopic baseline levels in several marine areas such as tropical and subtropical north Atlantic regions remains poorly explored. The ignorance of isotopic spatial trends limits the applicability of SIA, for example, to understand trophic relationships of species that show vast distribution areas. Therefore, to expand our knowledge of marine isotopic landscapes across large geographic scales as well as to evaluate the relative influence of the factors influencing isotopic signatures of marine organisms, constitute an important way to optimize the effectiveness of this methodology in the study of marine ecosystems (West *et al.* 2008).

Another important issue in the use of SIA to explore animal ecology is the selection of an appropriate tissue for the analysis. Since tissues turn over at different rates, they integrate trophic information over different temporal scales and, if the animal migrate, also over different spatial scales (Rubenstein & Hobson 2004). The spatiotemporal frame integrated by an animal tissue depends on its growth and metabolic replacement (Hobson & Clark 1992a; Hobson & Clark 1992b). Moreover we must distinguish between metabolic active and inert tissues. Metabolic active tissues show different information depending on the sampling moment since this tissues turnover continually. Within this kind of tissues those which show a high turnover rate will integrate isotopic signatures incorporated on the relative recent past (e.g. plasma and liver integrate information from hours to days), while those that are slowly replaced will integrate isotopic forms from a longer period of time (e.g. weeks in the case of muscle and whole blood, months or years for bone collagen). Metabolically inert tissues integrate isotopic forms at the time of their growth and once they became inert their isotopic signatures remain unchanged. Therefore, in this case the sampling moment is independent of the isotopic information integrated in the tissue. Most of these tissues are keratin based (e.g. claws, hair, feathers, and nail) and although some of them can continue to grow over extended periods, stable isotope ratios are locked into the keratin structure during growth. Moreover, these tissues show some specific advantages which relay in their non destructive and low invasive character since they do not involve killing the target animal and are relatively easy to sample minimizing animal disturbances, both necessary conditions especially when working with threatened species. Therefore, depending on the target species and objectives when studying ecology of marine organisms, we can choose a tissue as one means depicting wide spatiotemporal information or a specific period in the life cycle of the animal, which is particularly relevant in the case of migratory studies.

Technology developments have provided to scientists extrinsic marker which improve remote-sensing techniques for the study of animal movements. Among them, radio transmitters and satellite technology devices have attracted much scientific interest since they allow knowing the position of a marked animal with a great accuracy compared to other techniques. Radio telemetry works well for large animals and those that move short distances, but not as well for smaller and long distance migrants such as seabirds. In these cases satellite technology such as geolocators and Global Location Sensing (GLS) devices are more useful and allow a major accuracy than telemetry techniques. Geolocation devices can now be used to follow medium-sized and even small animals (Stutchbury *et al.* 2009) over long periods. This devices have been successfully applied to delineate migratory routes, wintering grounds as well as feeding areas of diverse marine predators such as turtles, sharks, marine mammals or seabirds (Southall *et al.* 2005; Takahashi *et al.* 2008; Zbinden *et al.* 2008; Lowry *et al.* 2009; Ramos *et al.* 2009a). Furthermore, the combination of GLS devices and stable isotopes analysis seems to be a reliable approach to improve our knowledge of marine isotopic landscapes commented above. That is, if we track the movements of marine animals in the ocean as well as the stable isotope signatures derived from that location, we could draw more accurately than ever the biogeographic patterns of stable isotopes in marine environments (e.g. Ramos *et al.* 2009a).

Therefore, new methodologies such as extrinsic and intrinsic markers open up several ways forwards to understand marine predator's ecology and movements. However, their development and implantation still depends on scientific studies which show their real applicability in unexplored isotopic regions and marine species.

SEABIRDS IN THE MARINE ENVIRONMENT: ON THEIR ROLE AS INDICATORS OF CONTAMINATION

Seabirds have long attracted the attention of humans. The development of their study has followed the development of other aspects of marine biology, ecology and ornithology. Indeed, seabird populations have probably the longest history of detailed monitoring at their colonies of any marine organism (Tasker & Reid 1997). Previous studies have suggest seabird as appropriate marine monitors of pollution, fish stocks, food webs structure and global threats such as climate change (Furness & Camphuysen 1997; Thompson *et al.* 1998; Burger & Gochfeld 2004; Piatt *et al.* 2007). However, despite of the interest in seabirds and their suitability as marine indicators, their value is still unclear in several species because our knowledge on their ecology remains patchy, especially in some marine regions such as the Mediterranean Sea (Forero & Hobson 2003).

Overall, seabirds as monitor organisms of marine environment health show specific advantages over other marine predators that could resolve partially some of the problems that have been previously exposed (summarized in Box 7). On one hand, part of these advantages comes from the particular life history of seabirds. Most seabird species are placed at high trophic positions within marine food webs and thus, great contaminant levels can be expected. Seabirds breed on specific locations among wide geographic ranges and feed on particular marine areas (Brooke 2004) which make it possible to compare contaminant levels from specific locations among large geographic scales as no other marine predator. In addition, most species are colonial breeders which allow sampling enough individuals for ecology and contamination studies. The pelagic character of several taxonomic groups is also an important feature since they can be used to study remote pelagic environments and contaminant background levels from our oceans. However, few works have dealt with pollution concerning open sea regions and their seabird species. This bias concerning pelagic seabirds could be related to the ethical, practical and technical difficulties in obtaining samples (Elliott 2005; Yamashita *et al.* 2007). That is, most pelagic seabirds are threatened species and breed on isolated islands which limit the access to free-living seabirds. Moreover, tissues commonly used (e.g. fat, liver or muscle) for contaminant analysis involve killing the animal or methodologies which do not allow systematic sampling procedures such as carcasses collection.

Box 7. ***Seabirds as Sentinels*** of Environmental Pollution

Advantages
✓ **Top predators, high trophic position** → Great levels from biomagnification
✓ **Long-lived** → Time for bioaccumulation
✓ **Philopatric** → Allows for sampling of individuals from year to year
✓ **Widespread distribution** → Large geographic comparisons
✓ **Often colonial** → Large sample size concentrated in one place
✓ **Conspicuous** → Easy to find for sampling
✓ **Large in size** → Do not require pooling
✓ **Many species are abundant**
✓ **Integrate over time and space**
✓ **Mixed-species colonies** → Different trophic niches
✓ **Some species are sedentary**
✓ **Gather food over large areas** → Adults integrate wide geographic scales

Disadvantages
✕ **Gather food over large areas** → Adults do not reflect point source
✕ **Most species are migratory** → Difficult to know exposition from the wintering grounds
✕ **Need to understand molt and migration patterns**
✕ **Some species are threatened or endangered** → Too few to collect

(from Burger and Gochfeld 2004)

On the other hand, the features that make seabirds valuable indicator organisms are associated with the great applicability of the new methodologies for the study of animal ecology (e.g. stable isotopes and GLS devices). In fact, when reviewing bibliography more isotopic or tracking studies have been conducted with seabirds compared with other marine predators. This trend is probably associated with the useful presence of feathers. Feathers maintain the isotopic forms assimilated during their growth as other inert tissues with the particularity that each feather grew in a specific moment. This moment is determined by the moulting pattern of bird species. As result, one individual presents feathers grown at different periods of its live cycle such as breeding and wintering periods. Therefore, when moulting patterns are known, isotopic signature of feathers can be used to assess spatiotemporal differences in seabird trophic ecology as well as to check baseline levels from remote regions (when wintering areas are known). However, feathers are not only especially useful for SIA but also for contaminant studies. Heavy metals also are trapped in feather structures once they have grown. It has made it possible to compare heavy metal levels among seabird breeding areas as well as between wintering and breeding grounds (e.g. Ramos *et al.* 2009b). Moreover, recent studies also have validated the use of feathers to monitor organic pollutants such as PCBs, which open up several new possibilities for marine environment monitoring (Jaspers *et al.* 2007; Van den Steen *et al.* 2007).

Besides feathers, several studies have focused on the use of other non destructive seabird tissues to monitor marine pollution or study their ecology. Eggs have been commonly used to assess contaminant status of seabirds and through stable isotopes analysis, also to evaluate trophic resources exploited by them (e.g. Buckman *et al.* 2004). Moreover, studies covering large marine geographic scales and assessing spatiotemporal trends of several contaminants have been conducted mainly using seabird eggs (Braune *et al.* 2002; Albanis *et al.* 2003; Pereira *et al.* 2009). However, this methodology cannot be applied in species which show a limited and small clutch size since it could involve adverse affects in their population dynamic. Moreover, pollutant levels and isotopic signatures in eggs can be influenced by migratory movements and capital-income strategies of seabirds (Yates *et al.* 2009), confounding geographic patterns of contaminants and stable isotope signatures. Besides, eggs only can be sampled during the breeding period and reflect information from females and thus, this tissue could not represent the general seabird population during the year. In consequence, other tissues such as blood or preen gland oil have been explored to monitor marine pollution.

Environmental chemistry studies have validated the suitability of blood or preen gland oil to evaluate contaminant burdens in seabirds (Henriksen *et al.* 1998; Bustnes *et al.* 2001; Burger & Gochfeld 2004; Yamashita *et al.* 2007). They have suggested that these tissues provide information regarding short-term exposure to contamination although other factors such as bird condition can influence their contaminant levels.

Moreover, blood also has been used to assess trophic ecology of seabirds by means of SIA (e.g. Forero *et al.* 2004) and contamination studies have found significant relationships between contaminant levels (e.g. PCBs, DDTs, heavy metals) and stable isotopes signatures of carbon and nitrogen in this tissue (Finkelstein *et al.* 2006; Elliott *et al.* 2009). In fact, some studies have found that under stable environmental conditions, including stable food sources, OCs or heavy metal concentrations seem to be stable in blood not only within short-term periods (e.g. within the incubation period) but also among long-term periods (e.g. between breeding seasons) (2001; Bustnes *et al.* 2005; Anderson *et al.* 2009).

The potential of these techniques in the assessment of environmental contamination by means of seabirds increases considerably when combining with GLS devices. That is, we can know the position of seabird individuals with accuracy by means of tracking devices and evaluate the consistence of their trophic niche throughout SIA. In addition, by means of non-destructive methodologies, we can determine pollutant concentrations reflecting delimited exposition periods without killing the animal. Therefore, combining all these approaches we could evaluate the influence of geographic patterns of pollutants and dietary habits of marine predators with a great accuracy. In fact, satellite tracking combined with stable isotope and contaminant analysis in seabird blood has been recently applied to determine geographical differences in contaminant levels in the Pacific Ocean (Finkelstein *et al.* 2006).

Overall, the main aim of the present PhD Thesis is to contribute with new insights into the understanding of the ecological factors shaping the isotopic signatures and the dynamic of selected contaminants in marine wildlife. It constitutes a consistent way to evaluate the suitability of seabirds as indicators of the marine contamination but also to identify possible risks for them, bringing new lights into their conservation strategies. In this research we focus on the study of the trophic ecology and distribution of marine predators as two crucial factors influencing their exposure to Persistent Organic Pollutants and determining their isotopic signatures.

Most Procellariiformes breeding within the northeast Atlantic Ocean and the Mediterranean Sea (Box 8) have been selected as target species in the present study. Procellariiformes is an order of seabirds that comprises albatrosses, petrels and shearwaters. They are almost exclusively pelagic (feeding in the open ocean) and only come to land to breed. Moreover, most species are on the top of the marine food webs and thus, not only their habitat but also their feeding ecology prone them to high exposure to organic pollutants. Procellariiformes have a cosmopolitan distribution across the world's oceans and several species show wide breeding ranges. They are colonial, mostly nesting on remote predator-free islands. The larger species nest on the surface, while smaller species nest in natural cavities and burrows. Moreover, they exhibit strong philopatry, returning to their natal colony to breed and returning to the same nesting site over many years. Therefore, the characteristics of Procellariiformes make of these seabird species exceptional candidates as marine monitors (Box 7).

Procellariiformes have had a long relationship with humans and have attracted much public interest. For instance, they have been the subject of numerous cultural depictions, particularly albatrosses (Tasker & Reid 1997). Another important consideration about Procellariiformes is that these seabirds are one of the most endangered bird taxa, with many species threatened due to introduced predators in their breeding colonies, marine pollution and the danger of fisheries by-catch (Brooke 2004). Scientists, conservationists, fishermen and governments around the world are working to reduce the threats posed to them. These efforts have led to the signing of the Agreement on the Conservation of Albatrosses and Petrels, a legally binding international treaty signed in 2001. At the start of this doctoral thesis, no data were available about levels of POPs such as PCBs, DDTs or PAHs in free-living pelagic seabirds from the mid-northeast Atlantic Ocean and the Mediterranean Sea. Nonetheless, some pelagic species were studied and evaluated as possible indicators for heavy metal contamination. Moreover, the knowledge of the trophic ecology of several species from these regions remained unknown (e.g. Feas and Bulwer’s petrel). In sum, the life history of Procellariiformes, their public interest, the lack of knowledge of their ecology and contaminant status as well as their threatened character, enhance the need of study their ecology and contamination to face up the challenge of their conservation.

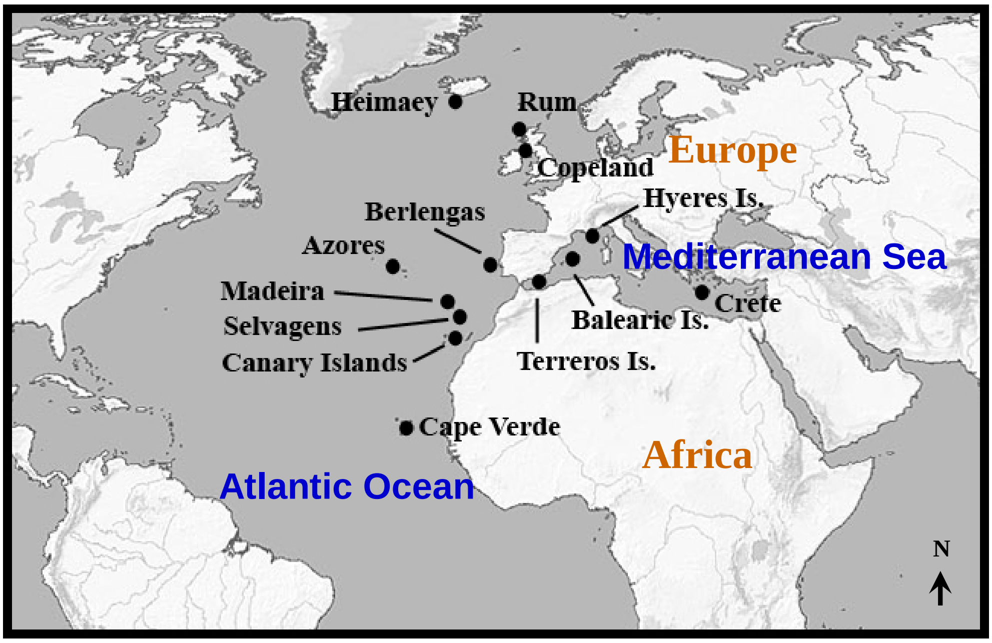


FIGURE 1. Black dots indicate the breeding localities sampled in this thesis

In this context, the global outline of the thesis involve the exploration of isotopic signatures and movements of most Procellariiformes breeding in the northeast Atlantic Ocean and the Mediterranean Sea (Figure 1), as well as the analysis of their PAH, PCB and DDT burdens. The results are structured in four chapters depending on the specific objectives of the study. In the Chapter 1, we evaluate the variability of seabird $\delta^{13}C$ and $\delta^{15}N$ signatures in the first primary feather (mostly grown at the end of the breeding period) across several Atlantic populations and species, from Cape Verde (20º N) to Iceland (60º N). Moreover, in this study we combined the isotopic information with the use of GLS devices to delineate the moulting grounds of the primary feather and thus, to understand the spatial information that this tissue offers. The specific aims of this study were (*1*) **to explore the relative influence of feeding ecology and geographic location over isotopic signatures of marine predators combining the spatial information of GLS with the isotopic signatures of feathers**; (*2*) **to increase our knowledge about the isotopic landscape of the northeast Atlantic Ocean and** (*3*) **to understand the trophic structure of northeast pelagic seabird communities and evaluate the consistence of species trophic niches across their breeding range**.

Across chapters 2, 3 and 4, the concentrations of PAHs, PCBs and DDTs are evaluated in the target species. Within these sections we explore the variability of POP burdens in seabirds attending mainly to their distribution and trophic ecology inferred from stable isotopes analysis. Moreover, other sources of variability in seabird contaminant levels such as sex or age also were inspected. Overall, we attempt to assess the influence of all these factors drawing the dynamic and burdens of POPs in pelagic seabird communities as a way to evaluate their potential as marine indicators. These three chapters involve common objectives that are (*4*) **to provide new data of POP**

levels in pelagic seabird species from the Atlantic and the Mediterranean Sea resulting from chronic marine pollution, i.e. not associated to any specific spill; (*5*) **to evaluate POP burdens of marine predators with the purpose to assess the relative contribution of their geographic origin and their specific trophic niche**; (*6*) **to outline the geographic patterns of OCs and PAHs and their possible sources in the northeast Atlantic Ocean and the Mediterranean Sea throughout seabird contaminant burdens and profiles**; (*7*) **to relate interspecific and intraspecific variations of OCs and PAHs to the trophic ecology of marine predators as shown by stable isotope signatures of carbon and nitrogen, paying special attention to the biomagnification process**.

Box 8. Sampled species

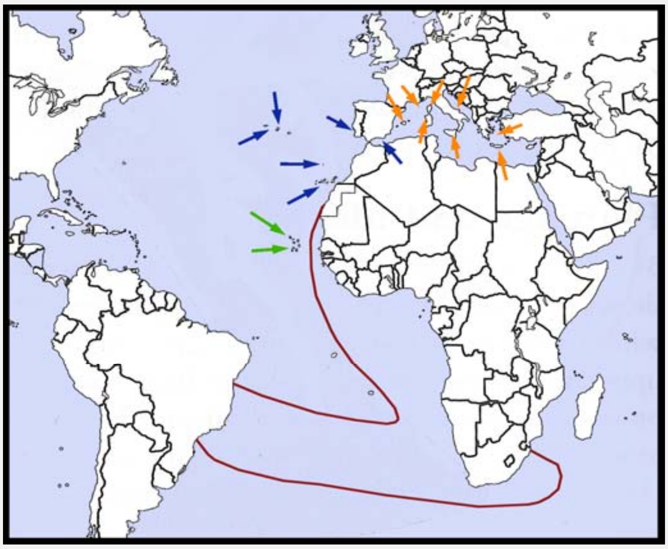
From Box 8a to 8d we show the species which have been studied in this thesis. Each box show photos of the seabirds and a map. Maps show the most relevant breeding places of seabirds (arrows) and their distribution during the non-breeding period (within drawn lines). Seabirds' photos show a colored frame which correspond with the colors used in the maps. The information we show here has been obtained from Cramp and Simons 1977 and Brooke 2004. Further information of specific studies on these seabirds is included among the thesis' chapters.

Box 8a. *Calonectris* shearwaters

Cory's shearwater
Calonectris borealis

Scopoli's shearwater
Calonectris diomedea

Cape Verde shearwater
Calonectris edwardsii



The Scopoli's (654 ± 62 g), the Cory's (male 900 ± 70; female 779 ± 70 g), and the Cape Verde (lightly smaller than the rest) shearwaters are three closely related forms until recently considered subspecies of the same species *Calonectris diomedea*. Although their taxonomic status is still being debated, recent studies suggest the three forms should be regarded as separate rather than a single species. The three forms have mostly disjoint distributions, across the Mediterranean Sea, the NE Atlantic (except Cape Verde) and the Cape Verde archipelago although they show common wintering areas. These shearwaters show very similar morphology and ecology.

The Scopoli's, Cory's and Cape Verde shearwaters are epipelagic feeders and their diet is reasonably homogeneous mostly composed by epipelagic fish and, to a lesser extent, by cephalopods, crustaceans and plankton.

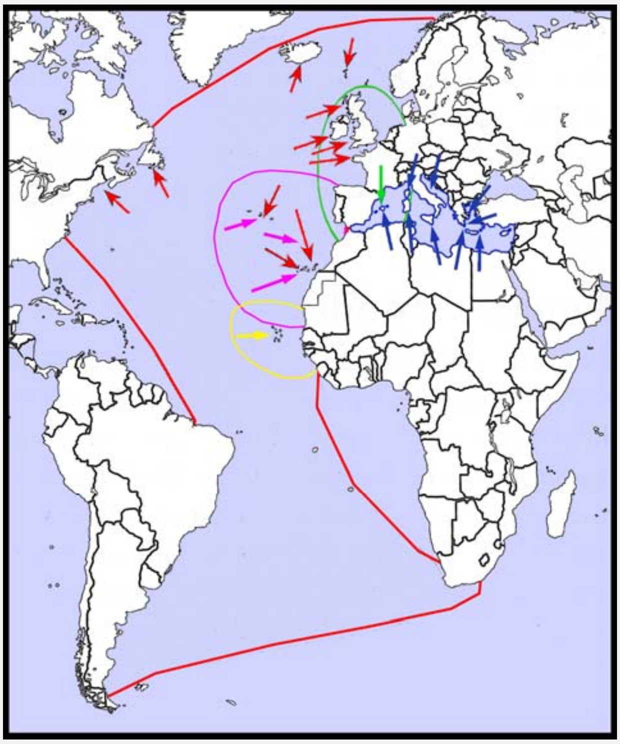
Box 8b. *Puffinus* shearwaters

Mediterranean (416 ± 29 g), Balearic (male 537 ± 25; female 506 ± 26 g) and Manx's (male 479 ± 23; female 466 ± 25 g) shearwaters are bigger than **Little shearwaters (male 160 ± 12; female 151 ± 9 g)** which is the smallest shearwater group included in this study.

Mediterranean shearwater
Puffinus yelkouan

Balearic shearwater
Puffinus mauretanicus

Manx's shearwater
Puffinus puffinus



Little shearwater
Puffinus assimilis baroli

Cape Verde little shearwater
Puffinus assimilis boydi

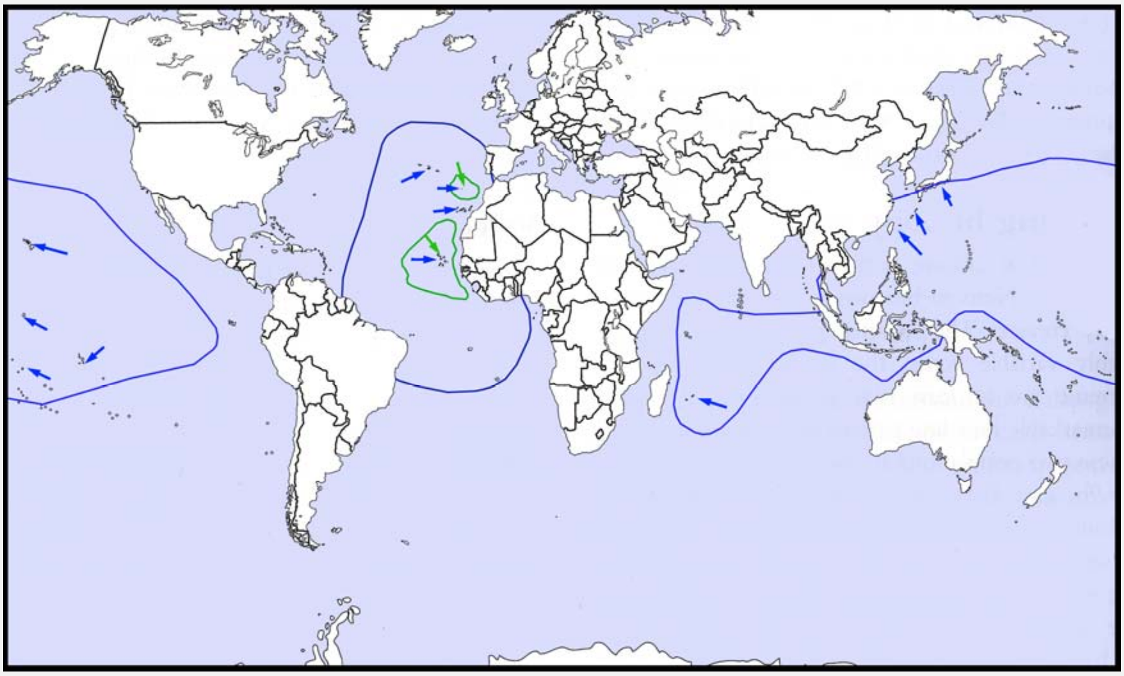
Manx's, Mediterranean and Balearic shearwaters are epipelagic feeders and they fed mainly on small shoaling fish (Clupeids) and squid. They commonly follow fishery boats and their discards is an important food resource for these shearwaters, specially in the case of Balearic shearwaters. Moreover, the Balearic shearwater population (endemic from Balearic islands) is currently in critic danger as indicated by the IUCN Red List.

Little shearwaters also are epipelagic feeders. Although there is not extensive information about their diet, it is composed mainly in small fish species and larvae, crustaceans and squid. The Spanish population is considered at moderate risk.

Box 8c. Petrel species

Bulwer's petrel
Bulweria bulwerii

Fea's petrel
Pterodroma feae



Bulwer's petrel (male 107 ± 11; female 99.9 ± 10 g) is considered a mesopelagic feeder. This species feed mainly at night on daily vertical migratory mesopelagic fish species (Myctophids). followed by lower proportions of cephalopods, crustaceans and plankton. The Spanish population of this species is considered as vulnerable as indicated by the Spanish Red List (Libro Rojo 2004).

Fea's petrel (311 ± 20 g) is a species that has been little studied. Its diet is almost unknown. Probably feed on fish and squid.

Box 8d. Storm-petrel species

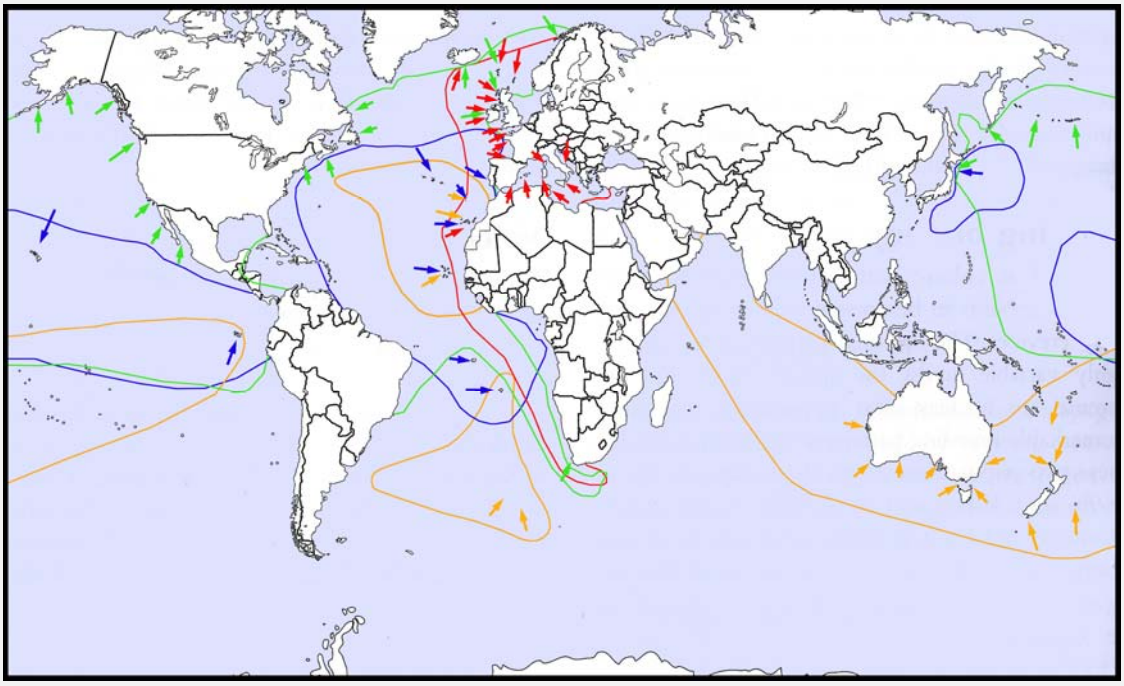
In this group we found the smallest species included in the present study.

Madeiran storm-petrel
Oceanodroma castro

White-faced storm-petrel
Pelagodroma marina

European storm-petrel
Hydrobates pelagicus

Leach's storm-petrel
Oceanodroma leucorhoa



In the case of Azores breeding population, the **Madeiran storm-petrel** shows two forms that breed simpatrically. They have been recently proposed as separated species. The cool season storm-petrel (*Oceanodroma castro*; **mean weight 49 ± 4 g**) show an egg-laying period which goes from October to December while the hot season storm-petrels (*Oceanodroma monteiroi*; mean **44 ± 4 g**) lay their eggs between May and early July. The diet of this petrels is also composed on mesopelagic preys as in the case of Bulwer's petrel described above. The Spanish population of Madeiran storm-petrel is considered at risk.

White-faced storm-petrel from Macaronesian archipelagos (*Pelagodroma marina hypoleuca;* **53 ± 6 g**) and the **Leach's storm-petrel (male 45 ± 4; female 45 ± 3 g)** have been suggested to feed mainly on epipelagic crustacea, small fish and other surface plankton species during the day but also on mesopelagic vertical migratory fish species at night.

The **European storm-petrel (20 ± 1 g)** is considered as a vulnerable species in the Spanish Red List. The migratory pattern of Mediterranean populations remains unknown and it is unclear if they leave this basin during winter. It is known that this storm-petrel species feeds on a wide variety of marine resources (fish, crustacean and cephalopods) from offshore and inshore areas. Additionally, the European storm-petrel also commonly feeds on miscellaneous marine fatty droplets and scavenges on fisheries discards and dead marine mammals such as whales.

Informe del Director

El doctorando Jose Luis Roscales García presenta en su tesis doctoral titulada "Entre la ecología y la química ambiental: las aves pelágicas como indicadores de la contaminación marina" una serie de trabajos de gran calidad científica, publicados o en revisión en revistas científicas internacionales de gran prestigio e incluidas en el *Science Citation Index*. Paso a detallar a continuación la contribución científica que ha realizado el doctorando en cada uno de los artículos, así como su factor de impacto (*Thompson Institute for Scientific Information*):

- **Trophic versus geographic structure in stable isotope signatures of pelagic seabirds breeding in the NE Atlantic**
  J.L. Roscales, E. Gómez-Díaz, V. Neves, J. González-Solís (2010)

  En revisión en *Marine Ecology Progress Series*
  Factor de impacto (2008): 2,63
  Diseño del trabajo: **J.L.R.**, E.G.-D., J.G.-S.
  Muestreo y análisis de muestras: **J.L.R.**, E.G.-D., V.N., J.G.-S.
  Redacción científica: **J.L.R.,** E.G.-D., J.G.-S.

- **Geographical PCB and DDT patterns in shearwaters breeding across the NE Atlantic and the Mediterranean**
  J.L. Roscales, J. Muñoz-Arnanz, J. González-Solís, B. Jiménez (2010)

  Environmental Science and Technology 44 (7): 2328-2334
  Factor de impacto (2008): 4,46
  Diseño del trabajo: **J.L.R.**, J.G.-S., B.J.
  Muestreo y análisis de muestras: **J.L.R.**, J.G.-S., J.M.-A.
  Redacción científica: **J.L.R.**, J.G.-S., B.J.

- **Polycyclic aromatic hydrocarbons (PAHs) in Atlantic and Mediterranean seabirds**
  J.L. Roscales, J. González-Solís, P. Calabuig, B. Jiménez (2010)

  En revisión en *Environment International*
  Factor de impacto (2008): 3,52
  Diseño del trabajo: **J.L.R.**, J.G.-S., B.J.
  Muestreo y análisis de muestras: **J.L.R.**, J.G.-S., P.C.
  Redacción científica: **J.L.R.**, J.G.-S., B.J.

- **Geographic and trophic patterns of OCs in pelagic seabirds from the NE Atlantic and the Mediterranean: a multi-species and multi-locality approach**
  J.L. Roscales, J. Muñoz-Arnanz, J. González-Solís, B. Jiménez (2010)

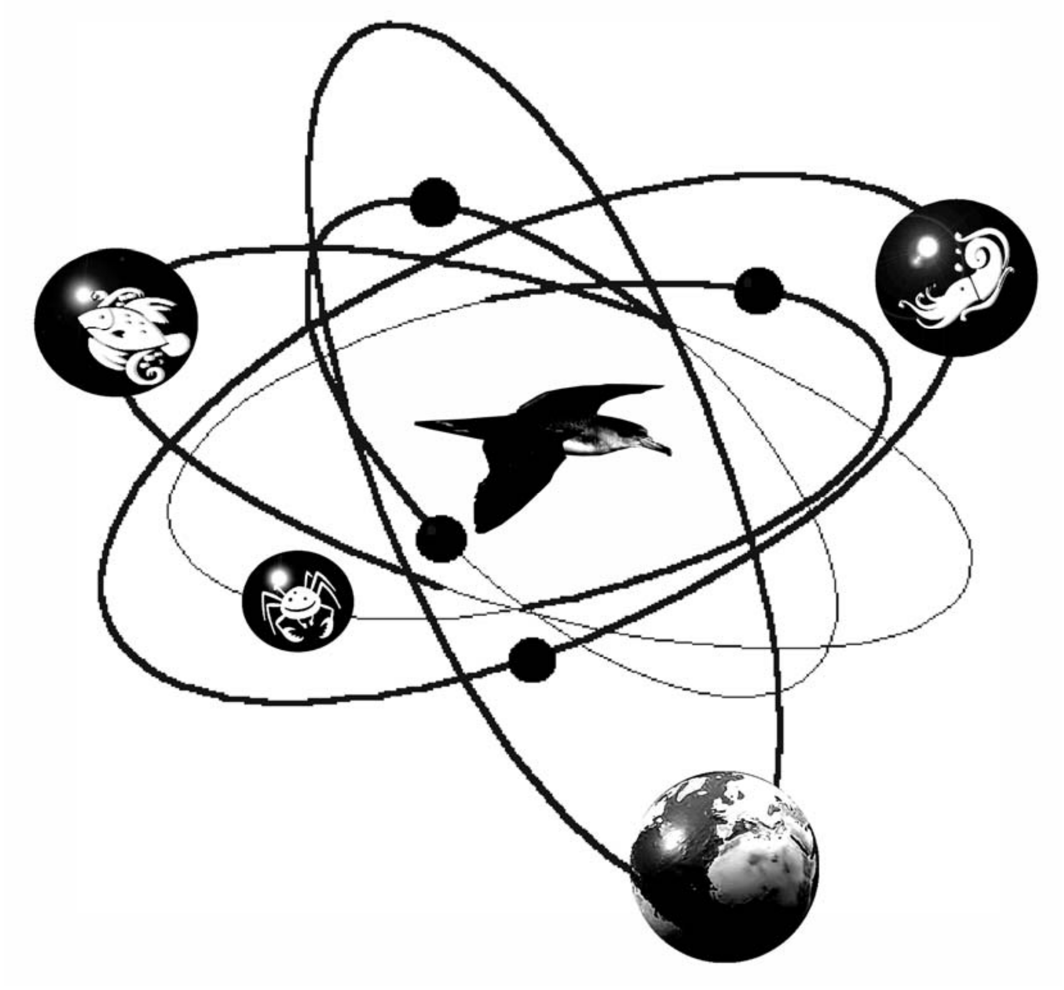
  En revisión en *Environmental Science and Technology*
  Factor de impacto (2008): 4,46
  Diseño del trabajo: **J.L.R.**, J.G.-S., B.J.
  Muestreo y análisis de muestras: **J.L.R.**, J.G.-S., J.M.-A.
  Redacción científica: **J.L.R.**, J.G.-S., B.J.

CHAPTER 1

Trophic versus geographic structure in stable isotope signatures of pelagic seabirds breeding in the northeast Atlantic

JOSE L. ROSCALES [†], ELENA GÓMEZ-DÍAZ [†], VERÓNICA NEVES [‡] AND JACOB GONZÁLEZ-SOLÍS [†]

Original paper under review in Marine Ecology Progress Series



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Estructuración trófica versus geográfica en los ratios de isotopos estables de Carbono y Nitrógeno en aves pelagicas del Atlántico noreste

JOSE L. ROSCALES, ELENA GÓMEZ-DÍAZ, VERÓNICA NEVES AND JACOB GONZÁLEZ-SOLÍS

RESUMEN

Dos de los factores más importantes que influencian los ratios isotópicos en los tejidos animales conciernen a su ecología trófica y origen geográfico. Por un lado, la proporción entre los isótopos $^{15}N/^{13}N$ ($\delta^{15}N$) depende fuertemente de la posición trófica del animal. Concretamente, cuanto más alta es la posición trófica, mayor es el ratio isotópico. En el caso de los isótopos estables de carbono, una dieta basada principalmente en presas costeras, por ejemplo, conlleva menores valores de $\delta^{13}C$ que una dieta basada en presas más pelágicas, es decir, especies capturadas en mar abierto. Por otro lado, los niveles basales de los ratios isotópicos de Carbono y Nitrógeno muestran una fuerte variación geográfica en nuestros océanos, que también se refleja en los ratios isotópicos de la fauna marina. Estas variaciones geográficas se han utilizado ampliamente para el estudio de los movimientos migratorios de la fauna. Sin embargo, la contribución relativa de las variaciones geográficas y la ecología trófica modelando los ratios isotópicos de la fauna está poco estudiada, especialmente en el caso de los ecosistemas marinos.

Con el objetivo de entender la influencia de estos factores sobre las firmas isotópicas en aves marinas, este estudio ha combinado el análisis de los ratios isotópicos en plumas con la aplicación de técnicas de seguimiento mediante geolocalización. Específicamente, entre 2001 y 2007, se han muestreado y determinado las firmas isotópicas de Carbono y Nitrógeno en la primera pluma primaria (n=609) de la mayor parte de las especies (11 especies) de aves pelágicas (O. Procellariiformes) que crían (16 localidades) a lo largo del noreste Atlántico, desde Cabo Verde (20º N) hasta Islandia (60ºN). Además, se han usado geolocalizadores para delimitar las áreas donde mudan la primera pluma primaria en 6 especies, en este caso también en varias colonias de cría para cada especie. De esta forma, se consigue relacionar los valores isotópicos encontrados en las plumas con regiones geográficas concretas.

En el caso de las especies de aves marinas que se muestrearon a lo largo de diferentes años no se han encontrado diferencias interanuales significativas en las firmas isotópicas. Esto indica que el efecto del año de muestreo resulta prácticamente insignificante en este estudio para explicar posibles variaciones en los ratios isotópicos de las plumas.

Las firmas isotópicas de Carbono y Nitrógeno variaron significativamente entre las especies y las localidades de cría. Sin embargo, las variaciones interespecíficas fueron mucho más acusadas que las debidas a la localización geográfica. De hecho, los patrones de variación que se encontraron para los valores de $\delta^{15}N$ and $\delta^{13}C$ carecieron de una estructuración geográfica dentro de la zona central del Atlántico noreste, que comprende los archipiélagos Macaronésicos y se corresponde con las regiones tropical y subtropical templada del Atlántico norte. En concreto, al combinar las firmas de ambos isótopos, las muestras se agruparon principalmente en base a la especie, independientemente del origen geográfico de la misa. Además, los ratios isotópicos obtenidos mostraron muy poco solapamiento entre las distintas especies, especialmente en el caso del Carbono, lo que sugiere que cada especie explota recursos tróficos específicos y de forma consistente a lo largo de toda su distribución.

El principal patrón geográfico que se ha encontrado en este estudio se basa en los valores significativamente mas altos de $\delta^{15}N$ (de un 3 a un 6‰) que reflejaron las poblaciones de aves pelágicas del Atlántico Norte (Islandia, Escocia e Irlanda del Norte), ya sea a nivel intra- o interespecífico. Estas diferencias tan marcadas sugieren un cambio en los niveles basales de $\delta^{15}N$ entre las zonas tropical y subtropical templadas respecto a la región templada del norte.

En conclusión, este estudio indica que la estructuración trófica de los petreles y pardelas del Atlántico noreste esta aparentemente marcada por una alta consistencia en el tiempo y en el espacio de los nichos tróficos de las especies estudiadas. Asimismo, los resultados ponen de manifiesto el análisis de isótopos estables como una metodología muy limitada para el estudio de los movimientos migratorios a escala regional (Zonas tropical y subtropical templada) pero altamente útil para el estudio de los movimientos a mayor escala geográfica (entre las regiones Norte y Tropical y Subtropical templada) en el medio marino.

Trophic versus geographic structure in stable isotope signatures of pelagic seabirds breeding in the northeast Atlantic

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ABSTRACT: Feeding ecology and geographic location are two major factors influencing animal stable isotope signatures but their relative contribution is poorly understood. To evaluate how these factors shape the isotopic signatures of seabirds, we analysed $\delta^{13}C$ and $\delta^{15}N$ in the first primary feather (P1) and tracked the post breeding movements of birds from several localities of the northeast Atlantic. From 2002 to 2007 we determined isotopic signatures in feathers of adult birds from 11 Procellariiform species (n=609) across 16 localities, from Cape Verde (20º N) to Iceland (60º N). Post breeding areas (where the P1 feather is thought to be grown) were determined using light level geolocation for 6 out of the 11 species. We found a negligible interannual variability in stable isotope signatures. Patterns of variation in $\delta^{15}N$ and $\delta^{13}C$ of seabird feathers seemed to be geographically unstructured within the mid-north Atlantic (Macaronesian archipelagos). That is, when combining both $\delta^{15}N$ and $\delta^{13}C$, samples mostly grouped regardless of the breeding location but according to the seabird species. In addition, interspecific isotopic overlap, especially in carbon signatures, was low suggesting that each species exploited exclusive trophic resources consistently across their geographic range. However, species breeding in North Temperate regions (Iceland, Scotland and Northern Ireland), showed enriched $\delta^{15}N$ compared to the same or similar species breeding on Tropical and Subtropical regions, suggesting some differences in baseline levels between these regions. In conclusion, this study illustrates a noticeable trophic segregation of the Procellariiform community breeding in the northeast Atlantic. Likewise, our results show that the isotopic approach has limited applicability to study movements of marine organisms at a regional scale, but is potentially useful to study long distance migrations among large geographic regions in the marine environment.

KEY WORDS: feeding ecology; isotopic gradients; migratory movements; Procellariiformes; trophic niche

INTRODUCTION

Stable isotope analyses (SIA) of carbon and nitrogen have been extensively used in animals as dietary tracers at different ecological levels, from individuals to communities. Isotope ratios in consumer tissues reflect those of their prey in a predictable manner (DeNiro and Epstein 1978; 1981; Hobson and Clark 1992), and thus, most studies assume the trophic ecology to be the major component shaping isotopic values of animal tissues. Recent studies emphasize the importance of the isotopic approaches in animal ecology because they can be used to depict species' niche (Cherel et al. 2007; Newsome et al. 2007; Layman et al. 2007). However, isotopic composition of both terrestrial and marine environments shows a marked spatial variability involving specific baseline geographic patterns which also strongly influence animal isotopic signatures. In fact, since these differences in baseline isotopic signatures are reflected in consumer tissues, they have been widely used to trace movements and geographic origin of wildlife (Rubenstein and Hobson 2004). Nonetheless, the relative importance of biogeographic trends and trophic relationships shaping isotopic signatures of top predators and their food webs remains poorly understood, particularly in the marine environment, where the complexity of the biochemical and oceanographic processes make the isotopic dynamics of marine food webs comparatively more difficult to decipher than terrestrial ones (Michener and Schell 1994).

On one hand, SIA provides valuable information on the feeding ecology minimising some of the biases intrinsic to the diet studies (Barrett et al. 2007), and it is being applied in an increasing number of studies on the trophic structure of marine food webs (Hobson and Welch 1992) , trophic relationship in bird and mammals communities (Lesage et al. 2001; Forero et al. 2004) as well as to more specific questions of spatial and temporal variability in diets (Cherel et al. 2007). On the other hand, several studies have assessed animals' origin or movements in the marine environment using geographic variation in oceanic stable isotope baseline levels (Quillfeldt et al. 2005; Ramos et al. 2009a). The most common geographic trend for stable isotopes in some oceans is the latitudinal decline in $\delta^{13}C$ values documented in marine plankton and, thus, in consumers tissues too (Goericke and Fry 1994; Cherel and Hobson 2007). Occasionally, some longitudinal variation has also been observed (France et al. 1998). Overall, there is an increasing interest in using intrinsic markers such as stable isotopes to identify and link breeding and wintering areas in all marine organisms (Gómez-Díaz and González-Solís 2007; Rooker et al. 2008; Caut et al. 2009), as other intrinsic markers (i.e., biometric or genetic) show a limited capacity to identify them (Hobson 2008).

However, in spite of the wide application and interest in SIA among scientists it is often unclear whether the isotopic signatures resulting from the trophic ecology can be overridden by differences in the isotopic baseline levels among food webs. For example, two relatively close populations from the same petrel species exploiting neighbouring water masses showed substantially distinct isotopic signatures, even when their scavenging role in the ecosystem is essentially maintained throughout the entire distribution of the species (Forero et al. 2005). Therefore, interpreting stable isotopic data when geographic variability is uncertain can be misleading. That is, our lack of knowledge in the biogeographic trends of the isotopic signatures hampers the applicability of SIA, especially in species showing huge distribution areas, wide foraging ranges or long-scale migratory movements (Jaquemet and McQuaid 2008). Being able to interpret animal isotopic signature's correctly is crucial to expand our understanding of isotopic landscapes across large geographic ranges and to increase the applicability of this methodology in the marine environments (West et al. 2008).

Seabirds offer an excellent opportunity to study the influence of feeding ecology and baseline isotopic levels on the spatial dynamics of stable isotope signatures in the marine environment. They are readily accessible on their breeding grounds and show wide breeding areas and rich species composition (Brooke 2004). Moreover, SIA on feathers can be exceptionally useful when moulting patterns are known because fully grown feathers become inert, integrating the isotopic forms assimilated through the diet when and where they were grown (Bearhop et al. 2002). In addition, it is now possible to track seabird movements over long periods of time. Therefore, if we track the movements of seabirds as well as the stable isotope signatures of feathers with known moulting patterns, we can connect feather isotopic information to specific geographic areas (Ramos et al. 2009a) and thus, address the biogeographic patterns of stable isotopes in the marine environment. In the present study, we analysed the isotope signatures of C and N in the first primary feathers of 11 petrel and shearwater species breeding throughout 16 northeast Atlantic localities and used global location sensing (GLS) devices to assess the moulting area of the first primary feather. We first checked the existence of latitudinal and longitudinal isotopic gradients or abrupt changes as well as possible spatial structures in $\delta^{15}N$ and $\delta^{13}C$ values. Secondly, we analysed the differences in carbon and nitrogen isotopic signatures at inter- and intraspecific levels. In brief, if biogeographic trends in isotopic baseline levels predominate over the influence of the feeding ecology, isotopic signatures of the species sampled at different localities should correlate with the geographic distance or the geographic coordinates across the species ranges (latitude and longitude). If the reverse is true, no biogeographic trends should emerge and the isotopic signatures of seabird species should hold over their entire breeding range, regardless of the breeding localities.

MATERIALS AND METHODS

Study species, area and field procedure: We selected 11 out of a total of 14 Procellariiform species nesting on the northeast Atlantic archipelagos, from Cape Verde (20º 15' 16") to Iceland (60º 50' 49"). The only three species not included in our analysis are *Fulmarus glacialis*, breeding at north-temperate latitudes and two endemic species, *Pterodroma madeira* breeding only in mainland Madeira and *Oceanodroma monteiroi*, breeding only in a couple of the Azorean islets. Most petrels studied are relatively abundant and have a wide distribution in the northeast Atlantic. Cory's shearwater (*Calonectris diomedea borealis*), Bulwer's petrel (*Bulweria bulwerii*), Madeiran storm-petrel (*Oceanodroma castro*), white-faced storm-petrel (*Pelagodroma marina*) and little shearwater (*Puffinus baroli*) breed throughout mid-north Atlantic (Macaronesian islands). Fea's petrel (*Pterodroma feae*) nests in Madeira and Cape Verde Islands. Cape Verde little shearwater (*Puffinus boydi*) and Cape Verde shearwater (*Calonectris edwardsii*) are endemic of the Cape Verde archipelago. Leach's storm-petrel (*Oceanodroma leucorhoa*) breeds close to the Arctic Circle, while Manx's shearwater (*Puffinus puffinus*) and European storm-petrel (*Hydrobates pelagicus*) have the widest latitudinal distributions breeding from close to the Arctic Circle down to the Canary Islands.

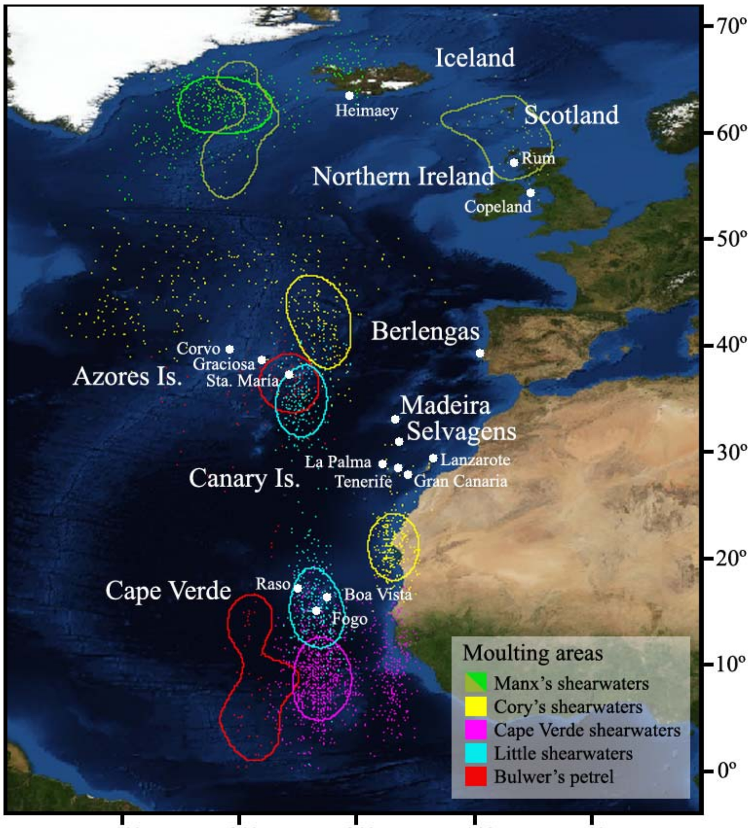


FIGURE 1. Sampled breeding colonies (white dots) and moulting areas (within drawn lines) of petrels and shearwaters from the northeast Atlantic Ocean. Moulting areas from north to south: Manx's shearwaters from Iceland in light green (N=10 in 2006) and from Scotland in dark green (N=4 in 2006 and N=2 in 2007); Cory's shearwaters in light yellow (N=9 in 2002), Bulwer's petrels in red (N=3 in 2007) and little shearwaters in blue (N=4 in 2007) from Azores; Cory's shearwaters from Canary Islands in light yellow (N=6 in 2002) and finally, Cape Verde shearwaters in magenta (N=24 in 2006), Cape Verde little shearwater in blue (N=7 in 2007) and Bulwer's petrel in red (N=5 in 2007) from Cape Verde.

From 2002 to 2007 we collected the first (innermost) primary feather (P1) from adult birds from 16 localities across the northeast Atlantic (Figure 1). Adults moult primary feathers annually. Manx, Cory's and Cape Verde shearwaters moult P1 at the end of the breeding period, when they are still feeding their chick (Monteiro and Furness 1996; Ginn and Melville 2000; Ramos et al. 2009a; 2009c). European storm-petrel moults P1 after laying (Cramp and Simmons 1977; Monteiro et al. 1996; Brooke 2004; Arroyo et al. 2004). Little and Cape Verde little shearwaters, Madeiran storm-petrel and Bulwer's petrel moult their P1 at the end of the breeding period just before migration (Cramp and Simmons 1977; Bolton et al. 2008; personal observation). In the case of Leach's storm-petrel previous studies have found that this species moults the P1 feather after birds depart the colonies (Hedd and Montevecchi 2006). Moulting patterns of Fea's petrel and white-faced storm-petrel have been scarcely explored. However, their morphological and ecological similarities with the rest of mid-north Atlantic petrel community and our personal observations in the field suggest that most individuals also have moulted their P1 just before dispersion in subtropical waters (Spear and Ainley 2007). Therefore, with the exception of Leach's storm-petrel, sampled feathers mostly should reflect isotopic composition from breeding areas. To test inter-annual variability in stable isotopes signatures some localities were re-sampled in different years. All feathers were conserved in polyethylene bags until analysis of stable isotopes.

To assess the areas where P1 feathers were grown we used global location sensing (GLS) devices based on recording light levels, which can be deployed on a bird all year-round and will give 2 positions per day with an accuracy of (mean ± standard deviation) 186 ± 114 Km (Phillips et al. 2004). Overall, we obtained tracking from 74 GLS devices, including Cory's shearwaters (n=15) breeding in Azores (n=9 in 2002) and Canary Islands (n=6 in 2002), Cape Verde (n=24 in 2006) and Cape Verde little (n=7 in 2007) shearwaters breeding in Cape Verde, Bulwer's petrels (n=8) breeding in Azores (n=3 in 2007) and Cape Verde (n=5 in 2007), little shearwaters breeding in Azores (n=4 in 2007) and Manx's shearwaters (n=16) breeding in Scotland (n=4 in 2006 and n=2 in 2007) and Iceland (n=10 in 2006) (Figure 1). The moulting areas of seabirds were inferred from the positions of the birds during the month prior to their migration, in which most seabirds are thought to moult P1 (see above).

Isotopic analyses: All feathers were cleaned in a solution of Na OH (0.25 M) and oven dried at 60ºC. To homogenize samples for isotope analysis large feathers were ground to powder using a cryogenic impact grinder (Freezer/mill 6750 -Spex Certiprep-) operating at liquid nitrogen temperature, whereas small feathers were meticulously cut with stainless steel scissors into tiny fragments. Weighed sub-samples (0.36 mg) of feathers were placed into tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectometry) by means of a ThermoFinnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer *via* a CONFLOIII interface (Serveis Científico-Tècnics, University of Barcelona). Stable isotope ratios were expressed in conventional notation as parts per thousand (‰), according to the following equation: $\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$ where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ and $^{13}C/^{12}C$. The standards used for ^{15}N and ^{13}C were atmospheric nitrogen (AIR), and Peedee Belemnite (PDB), respectively. Precision and accuracy for $\delta^{13}C$ measurements was ≤ 0.1‰ and ≤ 0.3‰ for $\delta^{15}N$.

Data analysis: Normality of $\delta^{13}C$ and $\delta^{15}N$ was validated by checking Q-Q plots and by the Shapiro-Wilk test. Moulting areas of the NE Atlantic petrels and shearwaters where assessed by means of 50% density Kernel on the locations obtained from GLS devices during the month prior to the onset of migration. To investigate the existence of biogeographical patterns in the stable isotope signatures in feathers, we used the position of the breeding colony as a proxy of the area where the first primary feather was grown. Since we could only track birds from a few populations and species, we used this criterion to guarantee homogeneous treatment for all sampled species. This is unlikely to introduce a strong bias, since populations included in this study are separated by several thousand kilometres and in some cases, located in different oceanographic regimes, whereas moulting areas of the tracked birds only exceeded their colony sites by a few hundreds of kilometres (Figure 1). To check for possible isotopic biogeographic gradients we correlated stable isotope signatures of feathers with latitude and longitude coordinates for each sampled locality using linear and quadratic regressions. Besides, to assess the existence of a spatial pattern in the isotopic variation across the breeding range of each species, we tested the correlation between geographic distances and isotopic differences (calculated as Euclidean distances of colony pairs). In this case, we first applied Multidimensional Scaling of dissimilarity data by PROXCAL analysis using SPSS 12.0. The method tries to find the underlying structure in a set of proximity measures among objects arranged in a two-dimensional space. Then we applied Procrustes analysis (least-squares orthogonal mapping), a non parametric approach for comparing any two kinds of non-linearized data sets (Jackson 1995; Peres-Neto and Jackson 2001). The method is based on matching corresponding points (landmarks) from each of the two data sets, geographic or isotopic coordinates of each colony, and provides a measure of fit (m^{12}), which decreases with an increasing correlation. The significance test of the m^{12} statistic was determined by employing a randomization approach to one of the data sets (Protest).

We checked possible differences in stable isotopic signatures among breeding colonies within archipelagos for each species (e.g. Cory's shearwater in four of the Canary Is.) by means of Student's t-test or one-way ANOVA. Since we found a negligible effect of breeding colony (see results) over isotopic signatures of seabirds within archipelagos, we grouped breeding colonies of each species shaping the factor breeding locality (Table 1) to evaluate isotopic variability among species and their distributions. Because our sampled design is unbalanced and shows some empty cells (the sampled species do not all breed in all the localities sampled), analyses of variability of carbon and nitrogen stable isotopes signatures were performed applying separate Generalized Linear Models (GLM, Type IV SS for unbalanced designs, SPSS v.15) using each stable-isotope signature as response variable. Factors used as explanatory variables for the model were species (11 levels), breeding locality (9 levels) and their interaction. Finally, since the model showed a significant interaction between species and breeding locality, we compared isotopic signatures within-species means among breeding localities (e.g. Cory's shearwater from Azores Is., Canary Is., Madeira, etc.) as well as among species within each breeding locality using the one-way ANOVA test. Inter-annual differences within a locality sampled in successive breeding periods were tested using t-test (for two consecutive years) or ANOVA (more than two years). Stable-carbon and nitrogen isotope signatures of sampled feathers from northeast Atlantic Procellariiformes are summarized in Table 1. Results are shown as means ± standard error, except otherwise indicated.

Table 1. Mean (‰) and coefficient of variation (SD/Mean) for stable isotope signatures of N and C in the first primary feather from petrel and shearwater species breeding in the Northeast Atlantic Ocean.

Spp	**Locality**	**Colony**	***n***	**δ^{13}C**		**δ^{15}N**		**Sampled year**
				Mean	CV	Mean	CV	
Cory's shearwater								
	Azores Is.	Sta. María	69	-16,23	0,02	13,16	0,05	2002 & 03
	Azores Is.	Graciosa	15	-16,06	0,03	12,63	0,06	2003
	Azores Is.	Corvo	13	-16,15	0,02	12,52	0,08	2003
	Canary Is.	Gran Canaria	48	-14,09	0,05	13,20	0,05	2002 & 03
	Canary Is.	Lanzarote	16	-14,30	0,08	12,92	0,06	2003
	Canary Is.	La Palma	25	-14,16	0,08	12,56	0,10	2006
	Canary Is.	Tenerife	9	-13,87	0,07	12,87	0,02	2003
	Madeira	Madeira	15	-16,06	0,04	12,91	0,07	2004
	Selvagens	Selvagens	8	-15,25	0,04	12,58	0,03	2004
	Berlengas	Berlengas	16	-16,37	0,04	13,31	0,05	2004
Cape Verde shearwater								
	Cape Verde	Boa Vista	28	-15,73	0,04	12,28	0,09	2002
Bulwer's petrel								
	Azores Is.	Sta. María	10	-16,44	0,03	11,79	0,05	2003
	Canary Is.	Lanzarote	10	-16,12	0,02	11,90	0,05	2003
	Canary Is.	La Palma	3	-16,37	0,01	11,63	0,01	2006
	Canary Is.	Gran Canaria	4	-15,81	0,02	11,42	0,05	2003
	Madeira	Madeira	12	-16,27	0,02	12,10	0,07	2004 & 05
	Selvagens	Selvagens	11	-16,41	0,02	11,36	0,03	2003
	Cape Verde	Raso	10	-16,17	0,01	11,84	0,08	2007
European storm-petrel								
	Canary Is.	Lanzarote	11	-16,01	0,07	13,15	0,02	2003
	Iceland	Heimaey	10	-16,53	0,05	15,15	0,10	2006
	N. Ireland	Copeland	15	-16,51	0,05	15,32	0,08	2005
Madeiran storm-petrel								
	Azores Is.	Sta. María	71	-16,67	0,03	12,21	0,05	2003
	Azores Is.	Graciosa	35	-16,68	0,03	12,36	0,06	2002 & 03
	Canary Is.	Lanzarote	8	-17,18	0,03	12,60	0,04	2003
	Madeira	Madeira	4	-17,12	0,03	12,32	0,07	2005
	Selvagens	Selvagens	23	-17,06	0,03	12,13	0,06	2003, 04 & 05
	Berlengas	Berlengas	8	-16,87	0,04	12,58	0,07	2004
	Cape Verde	Raso	10	-16,59	0,02	12,58	0,09	2006

Table 1. (Continuation)

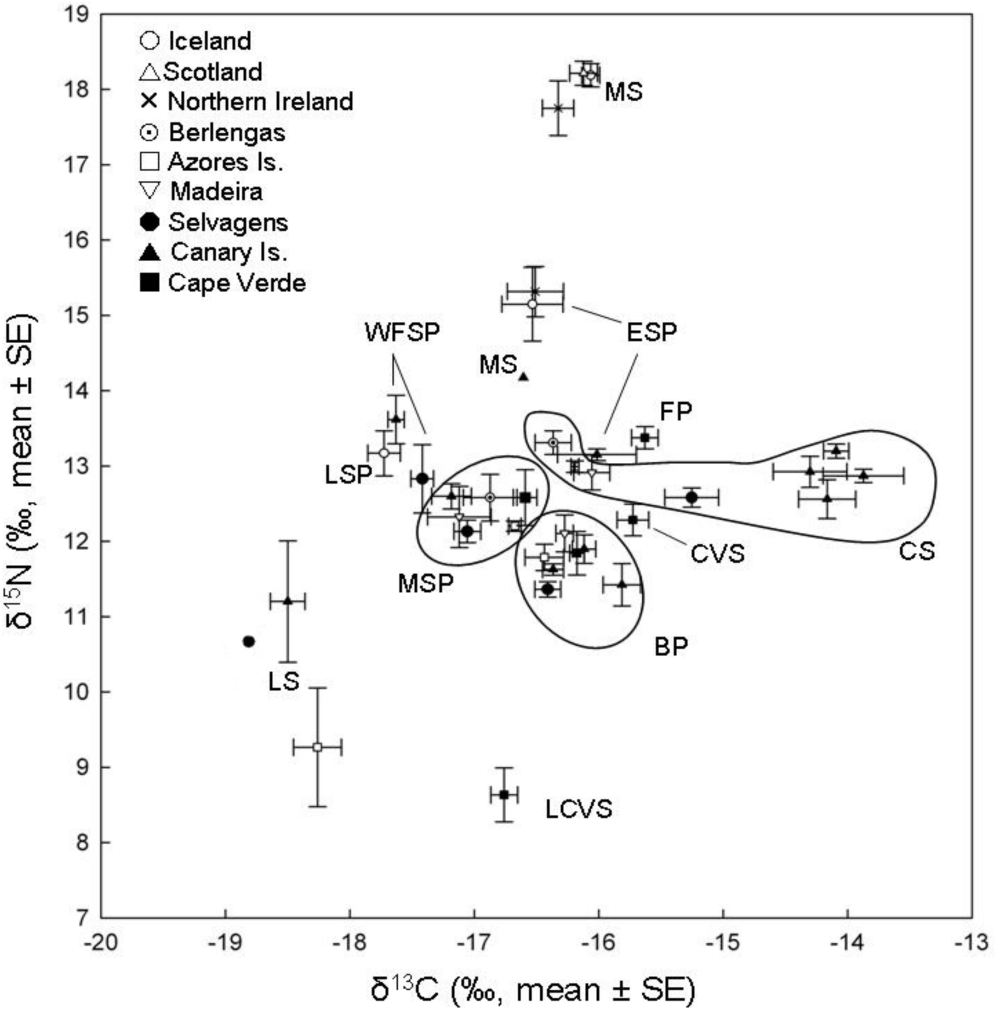
Spp	Locality	Colony	*n*	$\delta^{13}C$		$\delta^{15}N$		Sampled year
				Mean	CV	Mean	CV	
Little shearwater								
	Azores Is.	Sta. María	9	-18,26	0,03	9,27	0,25	2003
	Canary Is.	Lanzarote	4	-17,18	0,02	12,60	0,13	2007
	Selvagens	Selvagens	2	-18,81	0,01	10,67	0,07	2004
Cape Verde little shearwater								
	Cape Verde	Raso	10	-16,76	0,02	8,63	0,13	2005
White-faced storm-petrel								
	Canary Is.	Lanzarote	6	-17,63	0,01	13,62	0,06	2007
	Selvagens	Selvagens	10	-17,42	0,02	12,83	0,11	2006
Fea's petrel								
	Cape Verde	Fogo	10	-15,63	0,02	13,38	0,03	2007
Manx's shearwater								
	Canary Is.	La Palma	2	-16,60	0,08	14,17	0,18	2006
	Iceland	Heimaey	10	-16,06	0,01	18,19	0,03	2006
	N. Ireland	Copeland	10	-16,33	0,02	17,75	0,06	2005
	Scotland	Rum	9	-16,12	0,02	18,21	0,03	2006
Leach's storm-petrel								
	Iceland	Heimaey	10	-17,72	0,02	13,17	0,07	2006

RESULTS

Shapiro-Wilk test and Q-Q plots revealed a normal distribution for $\delta^{13}C$ and $\delta^{15}N$ among breeding colonies and species (all $P>0.05$).

Inter-annual stable isotopes variability: To test the potential effect of inter-annual variability in stable isotope signatures, we examined inter-year differences for colonies sampled in successive breeding periods (summarized in Table ESM1 in the Electronic Supporting Material). Cory's shearwaters from Gran Canaria (Canary Is.) and Sta. Maria (Azores Is.) sampled in 2002 and 2003 did not show differences between years within each colony, respectively. Similarly, we did not find significant differences in the isotopic signatures of Bulwer's petrels from Madeira sampled in 2004 and 2005 neither in the Madeiran storm-petrel from Selvagens sampled in three successive years (2003-2005). Only $\delta^{15}N$ values in feathers of Madeiran storm-petrel from Graciosa (Azores) showed significant differences between years (mean $\delta^{15}N$ value increased 0.5‰ from 2002 to 2003).

Biogeographical structure in δ^{13}C and δ ^{15}N signatures: Species showed clear differences in their isotopic signatures across their breeding range (Figure 2). However, regression models based on stable isotopes composition in P1 feather showed significant spatial gradients only within the Cory's shearwater. In this case, we found an inverse relationship between δ^{13}C and latitude ($F_{1,234}$=373.99, P<0.001; Figure SM1 in the Supporting Material). Stable carbon signatures from Cory's feathers (Mean ± SE) tended to decrease from south to north (-14.09‰ ± 0.10 in Gran Canaria, Canary Is.; -16.15‰ ± 0.10 in Corvo, Azores). Cape Verde shearwaters showed unexpected low values (-15.73‰ ± 0.13). Regression model including Cape Verde and Cory's shearwaters showed a significant relationship between δ^{13}C and latitude, but R^2 value decreased considerably (R^2 linear=0.16, R^2 quadratic=0.53; P<0.001). No significant associations were found between δ^{13}C and longitude or between δ^{15}N and geographical coordinates for any of the species studied.



CS Cory's shearwater; **CVS** Cape Verde shearwater; **BP** Bulwer's petrel; **ESP** European storm-petrel; **MSP** Madeiran storm petrel; **LS** little shearwater; **CVLS** Cape Verde little shearwater; **WFSP** White-faced storm-petrel; **FP** Fea's petrel; **MS** Manx's shearwater; **LSP** Leach's storm-petrel

Figure 2. Carbon and nitrogen stable isotope signatures in feathers (P1) of Procellariiformes breeding throughout northeast Atlantic Ocean. Most species showed small overlap in their isotope signatures, regardless of the breeding colony. Sampled colonies with N=2 (Manx's shearwater from Canary Is. and little shearwater from Selvagens) appears without SE.

To test the existence of spatial patterns of isotopic variation across the breeding range of each seabird species analyzed we examined the correlation between geographic and isotopic distances of colony pairs. For all five species-complex analyzed (Cory's and the Cape Verde shearwaters, Manx's shearwater, Bulwer's petrel, little shearwater and Madeiran storm-petrel), Procrustes analyses revealed no significant correlation between geographic and isotopic distances among colonies, although for *Calonectris spp* it was marginally non-significant (*Calonectris spp*: $m^{1,2}$ = 0.68, P = 0.06; *B. bulwerii*: $m^{1,2}$ = 0.91, P = 0.82: *P.baroli/boydi*: $m^{1,2}$ = 0.15, P = 0.14; *P.puffinus*: $m^{1,2}$ = 0.48, P = 0.22; *O.castro*: $m^{1,2}$ = 0.62, P = 0.23).

Inter-specific and inter-locality variability in stable isotope signatures: The isotopic landscape resulting from combining $\delta^{13}C$ and $\delta^{15}N$ values showed that most species displayed small overlap in their isotopic signatures, regardless of their breeding locality (Figure 2). That is, overall $\delta^{13}C$ and $\delta^{15}N$ values mostly grouped by species, and not by geographic proximity, except for north Atlantic localities, which clearly segregated from mid-north Macaronesian localities by their greater $\delta^{15}N$ signatures with the exception of Leach's storm-petrel, which showed isotopic signatures within the range of the Macaronesian breeders.

Stable-carbon isotope signatures in P1 feather ranged from -18.81 ± 0.13‰ (little shearwater from Selvagens Is.) to -13.87 ± 0.32‰ (Cory's shearwater from Tenerife, Canary Is.). Stable-nitrogen ranged from the upper values in Manx's shearwaters from Scotland (18.21 ± 0.16 ‰) to the lower values in Cape Verde little shearwaters (8.63 ± 0.36‰) although most sampled colonies ranged within narrower $\delta^{15}N$ values, from 13.31 ‰ to 11.36 ‰ (Figure 2). The GLMs explained up to 60.4% and 80.3% of the initial variance for $\delta^{15}N$ and $\delta^{13}C$, respectively. All the included factors, species ($F_{10,609}$=44.10 for $\delta^{15}N$; $F_{10,609}$=48.87 for $\delta^{13}C$; both $P<0.001$), breeding locality ($F_{8,609}$=10.38 and $F_{8,609}$=3.60 for $\delta^{15}N$ and $\delta^{13}C$ respectively; both $P<0.001$) and their interaction ($F_{31,609}$=2.09, $P<0.05$ for $\delta^{15}N$; $F_{31,609}$=18.71, $P<0.001$ for $\delta^{13}C$), showed a significant effect in both models and thus, none was removed. These results indicate that stable-nitrogen values vary mainly among species but to a lesser extent also among localities and further that these variations are not uniform but depend on the interaction between species and locality. The estimates of fixed effects showed that interaction in the case of nitrogen was mainly due to the lower $\delta^{15}N$ values found in European storm-petrel from Canary Is. compared with those from the Northernmost Atlantic. In the case of carbon interaction was mainly due to the lower $\delta^{13}C$ values found in Cory's shearwater breeding on Canary Is. and Selvagens compared to the remaining studied colonies (Figure 2).

At an intraspecific level both isotopes showed very low variability. Within breeding localities (Table 1) we did not find significant differences in $\delta^{13}C$ among neighbouring breeding colonies and only Cory's shearwater colonies from Azores showed significant differences in their δ^{15}N signature because of the greater values from Sta. Maria Island ($F_{2,97}$=7.11, P<0.05). Among breeding localities we found significant differences in δ^{13}C only for two species, the Cory's shearwater and the Madeiran storm-petrel (Table 2b). Post-hoc test (Bonferroni) showed that in the case of Cory's shearwaters these isotopic differences where due to the significant greater δ^{13}C values from Canary Is. and Selvagens. Madeiran storm-petrels showed significant differences in δ^{13}C between archipelagos due to higher values in Azores than in Selvagens. In the case of stable-nitrogen isotope signatures we only found significant differences among breeding localities in European storm-petrels and Manx's shearwaters (Table 2b) due to the greater values in the north Atlantic compared to mid-north localities. Contrarily, at interspecific level both stable-carbon and nitrogen signatures showed a great variability and differences in δ^{13}C and δ^{15}N (one-way ANOVA) among species within each breeding locality resulted significant in most cases (Table 2a).

TABLE 2a. Summary of ANOVA tests checking δ^{13}C and δ^{15}N differences among species within each breeding locality. Pairs of means differing significantly (p<0.05) by post-hoc *t*-test are linked with an 'x'.

Isotope	**Breeding locality**	**Inter-species comparison within breeding localities (one-way ANOVA)**		
		$F_{[df,\ n]}$ values	p values	Post-hoc *t*-test (Bonferroni)
δ^{13}C	**Cape Verde**	$F_{4,68}$=63.38	<0.001	CVLS x FP, BP, CVS MSP x FP, CVS
δ^{15}N		$F_{4,68}$=13.18	<0.001	CVLS x FP, BP, CVS, MSP BP x FP
δ^{13}C	**Canary Is.**	$F_{6,146}$=55.33	<0.001	CS x BP, MSP, ESP, LS, MS, WFSP ESP x LS, WFSP BP x WFSP
δ^{15}N		$F_{6,146}$=8.65	<0.001	LS x CS, ESP, MS, WFSP BP x CS, WFSP, MS
δ^{13}C	**Madeira**	$F_{2,31}$=8.53	0.001	CS x MSP
δ^{15}N		$F_{2,31}$=2.99	0.67	
δ^{13}C	**Selvagens**	$F_{4,54}$=8.54	<0.001	CS x LS, BP, MSP, WFSP BP x LS, MSP, WFSP LS x MSP, WFSP
δ^{15}N		$F_{4,54}$=6.55	<0.001	BP x CS, WFSP
δ^{13}C	**Azores**	$F_{3,222}$=67.27	<0.001	LS x CS, BP, MSP MSP x CS
δ^{15}N		$F_{3,222}$=63.38	<0.001	LS x CS, BP, MSP CS x BP, MSP
δ^{13}C	**Berlengas**	$F_{1,24}$=67.27	0.61	
δ^{15}N		$F_{1,24}$=5.56	0.02	CS x MSP
δ^{13}C	**Northern Ireland**	$F_{1,25}$=0.38	0.56	
δ^{15}N		$F_{1,25}$=23.26	<0.001	MS x ESP
δ^{13}C	**Iceland**	$F_{2,30}$=26.62	<0.001	LSP x MS, ESP
δ^{15}N		$F_{2,30}$=53.89	<0.001	LSP x MS, ESP ESP x MS

TABLE 2b. Summary of ANOVA tests checking $\delta^{13}C$ and $\delta^{15}N$ differences among breeding localities within each seabird species. Pairs of means differing significantly ($p<0.05$) by post-hoc *t*-test are linked with 'x'.

Isotope	**Species**	**Inter-locality comparisons within species (one-way ANOVA)**		
		$F_{[df, n]}$ values	p values	Post-hoc *t*-test (Bonferroni)
$\delta^{13}C$	**Cory's shearwater**	$F_{4,234}=129.22$	<0.001	CA x MA, SL, AZ, BE SL x CA, AZ, BE
$\delta^{15}N$		$F_{4,234}=1.32$	0.26	
$\delta^{13}C$	**Bulwer's petrel**	$F_{4,60}=2.58$	0.06	
$\delta^{15}N$		$F_{4,60}=2.58$	0.13	
$\delta^{13}C$	**European storm-petrel**	$F_{2,35}=2.58$	0.32	
$\delta^{15}N$		$F_{2,35}=12.19$	<0.001	CA x IC, NI
$\delta^{13}C$	**Madeiran storm-petrel**	$F_{5,159}=3.85$	0.03	AZ x SL
$\delta^{15}N$		$F_{5,159}=1.26$	0.27	
$\delta^{13}C$	**Little shearwater**	$F_{2,15}=1.13$	0.35	
$\delta^{15}N$		$F_{2,15}=1.30$	0.31	
$\delta^{13}C$	**White-faced storm-petrel**	$F_{1,16}=2.67$	0.13	
$\delta^{15}N$		$F_{1,16}=1.51$	0.24	
$\delta^{13}C$	**Manx shearwater**	$F_{3,31}=1.43$	0.27	
$\delta^{15}N$		$F_{3,31}=11.76$	<0.001	CA x IC, NI, SC

Seabird species: **CS** Cory's shearwater; **CVS** Cape Verde shearwater; **BP** Bulwer's petrel; **ESP** European storm-petrel; **MSP** Madeiran storm petrel; **LS** Little shearwater; **CVLS** Little Cape Verde shearwater; **WFSP** White-faced storm-petrel; **FP** Fea's petrel; **MS** Manx's shearwater; **LSP** Leach's storm-petrel.

Breeding localities: **CV** Cape Verde, **CA** Canary Is., **MA** Madeira, **SL**, Selvagens, **AZ** Azores, **BE** Berlengas, **NI** Northern Ireland, **SC** Scotland, **IC** Iceland.

DISCUSSION

Our study reveals an isotopic landscape where most petrel and shearwater species showed small overlap in their isotopic signatures. Most populations from the same species grouped according to their isotopic values regardless of their breeding origin (Figure 2). This result suggests that $\delta^{15}N$ and $\delta^{13}C$ signatures are primarily influenced by the feeding ecology of each species rather than by local values or biogeographical trends in the isotopic baseline levels. Since we sampled different populations from 2002 to 2007 our results could be confounded by some interannual variability in isotopic signatures. However, no changes in isotopic signatures are expected among years if feeding ecology remains consistent over time (Hodum and Hobson 2000). Indeed, previous studies have rejected inter-annual and inter-seasonal $\delta^{15}N$ and $\delta^{13}C$ as a source of relevant differences because temporal variations are considerably lower compared to inter-species or inter-locality differences (Quillfeldt et al. 2005; Forero et al. 2005; Ramos et al. 2009b). Our results also suggest that interannual variability in baseline isotopic signatures is low. Cory's shearwaters from Azores and Canary Is., Bulwer's petrel from Madeira and Madeiran storm-petrel from Selvagens, which were all sampled over two breeding periods, did not show significant differences in their isotopic signatures among years. We found significant interannual differences for $\delta^{15}N$ only in the case of Madeiran storm-petrel sampled in Graciosa (Azores) in 2003 and 2004, but these were negligible (see Table ESM1 in the Electronic Supporting Material) when compared to the interspecific differences. Overall, petrels breeding in the northeast Atlantic are trophically well structured, i.e. segregation particularly in carbon signatures suggests that each species exploited specific trophic resources consistently throughout time and space and with little overlap among species. Still, a few spatial differences and some intraspecific variability in the isotopic values have been found.

Spatial patterns in stable isotope signatures: In the present study geographic distance among breeding colonies did not correlate with differences in stable isotope values for any of the species sampled throughout the mid-north Atlantic archipelagos, although in the case of Cory's shearwater the correlation was marginally non significant. Moreover, neither latitudinal nor longitudinal gradients within each species explained differences in $\delta^{13}C$ and $\delta^{15}N$ signatures among breeding colonies, except between $\delta^{13}C$ and latitude in the case of Cory's shearwaters (Electronic supporting material, Figure ESM1). This exception, however, is probably explained by the proximity of some shearwaters populations to the productive upwelling areas on the African continental shelf, which typically shows more positive carbon signatures. That is, shearwaters from the southernmost archipelagos (e.g. Canary Is.) are moulting the analysed feathers (P1) on these areas (Figure 1), resulting in a spurious correlation between $\delta^{13}C$ signatures and latitude. Despite the fact that our study included breeding colonies from Cape Verde to Iceland, differences in stable isotope signatures among localities were only apparent in the $\delta^{15}N$ signatures between oceanographic provinces from north Temperate region (NECS, northeast Atlantic Shelves Province, breeding localities from N. Ireland and Scotland; SARC, Atlantic Subarctic Province, Iceland) and those from Tropical and Subtropical Temperate region (NAST, north Atlantic Subtropical Gyral Province, breeding localities form Canary to Azores Is.; NATR north Atlantic Tropical Gyral Province, Cape Verde) (Longhurst 1998) (Figure 1). In fact, we obtained larger $\delta^{15}N$ variability (over 9‰) than previous seabirds communities studies (Hobson et al. 1994; Thompson et al. 1999), mainly due to the greater $\delta^{15}N$ signatures of northernmost Atlantic compared to tropical and subtropical populations. For example, nitrogen isotopic signatures differed significantly among breeding localities only in the case of the European storm-petrel and the Manx's shearwater, the only two species studied that nest in both the northernmost and Macaronesian archipelagos.

Although potential differences in diet cannot be completely excluded, given the relative intraspecific homogeneity in isotopic signatures for all the species breeding from Cape Verde to Azores, the most plausible explanation for the enrichment in the nitrogen signatures in the northernmost Atlantic species is a change in the baseline isotopic levels rather than in the trophic position. This hypothesis is also sustained by the $\delta^{15}N$ values reported in the case of Leach's storm-petrel. This petrel species was the only north Atlantic breeder for which $\delta^{15}N$ signatures did not differ from those of Macaronesian breeders. This apparent divergence is probably due to the moulting pattern and migratory movements of this species. Since the moulting of wing feathers of Leach's storm-petrel starts after departure from the breeding colonies, isotopic values in P1 of this species probably reflect lower nitrogen isotopic signatures from the mid-north Atlantic (including the Macaronesian region) where the storm-petrel spends the non-breeding period (Hedd and Montevecchi 2006). These conclusions are consistent with previous studies which found a general trend in seabirds and marine mammals for enriched nitrogen signatures with latitude, from north Temperate to Arctic regions (Kelly 2000). Furthermore, marine chemistry studies also suggest differences in baseline levels between north and mid-north Atlantic. That is, different sources of N such us runoff, atmospheric deposition, subsurface NO_3^-, and N_2-fixation, determine the baseline $\delta^{15}N$ of marine biota. Overall, oceanic regions where N_2-fixation is the major source of N, $\delta^{15}N$ are depleted in ^{15}N when compared to areas where NO_3^- is the main source (Montoya et al. 2002). In the Tropical and Subtropical Temperate north Atlantic regions N_2-fixation is the major source of N (Montoya et al. 2002; McClelland et al. 2003). Contrarily, in the north Atlantic Temperate region there is a lower presence of N_2-fixation organisms (Tyrrell et al. 2003) and a major role of nitrate transported from deeper layers to the surface (Donald et al. 2001; Joint et al. 2001). As a result, previous studies have found a lower $\delta^{15}N$ in zooplankton and particulate organic matter (POM) from the Tropical and Subtropical regions (including the Macaronesian archipelagos) when compared with those from the north Atlantic Temperate region (Waser et al. 2000; Montoya et al. 2002; Mino et al. 2002).

Alternatively, dietary differences between north and mid-north Atlantic populations may also explain the observed differences in nitrogen signatures. North Temperate Manx's shearwater and European storm-petrel populations mainly feed on Clupeids (herrings *Clupea harengus* and sprats *Sprattus sprattus*, juveniles in the case of storm-petrels) (D'Elbée and Hémery 1997; Brooke 2004). To our knowledge there is no information available about the diet of these species in the mid-north Atlantic archipelagos. However, since Clupeids are abundant in both regions we think differences in diet are unlikely to explain the observed differences.

In sum, our results suggest no marked geographic structure in $\delta^{13}C$ and $\delta^{15}N$ within the mid-north Atlantic region. However, our results point out abrupt changes between regions, such in the case of north Temperate compared to Tropical and

Subtropical Temperate regions enhancing the potential of this pattern to assess migratory movements. Therefore, although geographical differences in baseline isotopic signatures do not seem to be the most important source of isotopic variability, they need to be considered when comparing distant populations, not only in terrestrial but also in the marine environment.

Trophic structure and foraging locations of the northeast Atlantic seabirds: Petrels and shearwaters are known to be pelagic foragers and their diet is mainly comprised of fish, cephalopods and crustaceans with different proportion and composition among species (Brooke 2004). While similar isotopic values among species or individuals cannot necessarily be interpreted as evidence for similar diets, differences among these values (when no different baseline levels occurs) do indicate different feeding patterns and provide evidences for trophic segregation (Hobson et al. 1994). Procellariiformes from mid-north Atlantic showed a noticeable overlap in their $\delta^{15}N$ signatures, as found in a number of isotopic (Forero et al. 2004) and dietary studies on seabird communities (Diamond 1983). In our study, most sampled colonies (66%) were grouped in a narrow range (about 3‰) of $\delta^{15}N$ signatures, which included all mid-north Atlantic breeders except the two little shearwater species (Figure 2). Forero *et al.* (2004) pointed out few dietary alternatives at each trophic level as the cause of overlap in stable nitrogen signatures of seabirds from Patagonia. This is also likely to occur in petrels breeding on mid-north Atlantic archipelagos, since this is an oligotrophic area, and thus, no rich pelagic communities are expected (Longhurst 1998).

In contrast with nitrogen, $\delta^{13}C$ signatures provided an axis of variability where the overlap among species was low. Differences in size among species are probably associated to different flight and diving capabilities, which ultimately shape their trophic niche. Despite this, we could not recognize obvious general patterns correlating stable isotope signatures with the biological traits of the studied species. In some cases, however, feeding locations can explain some differences in the isotopic signatures. This is the case for the *Calonectris* shearwaters breeding on Canary, Selvagens and Cape Verde Is., which showed significantly greater $\delta^{13}C$ signatures than any other species and any other Cory’s shearwater population breeding on mid-north Atlantic colonies. Since petrels usually breed in isolated archipelagos often far from any continental shelf, one axis of segregation is their capability to reach remote productive areas. As shown by the GLS devices (Figure 1), most Cory’s shearwaters breeding on Canaries fed along the productive African shelf associated to the Canary current, resulting in a relatively enriched $\delta^{13}C$ values in their feathers (Kelly 2000). In contrast, Cory’s shearwaters breeding on Azores fed on pelagic waters and showed depleted $\delta^{13}C$ values. Probably due to the larger distance to the African coast, Cory’s shearwaters breeding on Selvagens and Cape Verde shearwaters make an intermediate use of the African shelf, resulting in intermediate $\delta^{13}C$ values.

The lower $\delta^{15}N$ signatures of shearwaters breeding in Cape Verde compared to the rest of mid-north Atlantic shearwaters are not explained by biogeographic changes in baseline levels, but likely resulted from a divergence in their trophic niche related to the local adaptation to the different preys. For example, the Cape Verde shearwater is about 50% lighter in mass than the Cory's shearwater and this difference probably limit their potential preys to smaller sizes than the related Cory's shearwater, resulting in lower nitrogen signatures (Badalamenti et al. 2002; Cherel and Hobson 2005). Alternatively, biogeographic differences between the mid-north Atlantic Subtropical Gyral Province (NAST) and the north Atlantic Gyral Province (NATR) where Cape Verde is situated, could be behind the differences in $\delta^{15}N$ signatures. However, two species of petrels also breeding in both areas, the Madeiran storm-petrel and the Bulwer's petrel, did not show any shift in their isotopic signatures between the two oceanic provinces.

Our results also revealed some insights into the poorly known feeding ecology of some petrels and shearwaters from northeast Atlantic, such as the little shearwater and the Fea's petrel. Considering that generally, $\delta^{15}N$ signatures enrich from 3‰ to 5‰ for each trophic level across marine food webs (Hobson and Welch 1992), isotopic signatures of little shearwaters reported here suggest that these species feed on a trophic level below the other mid-north Atlantic species. We do not have details on the diet of these two species but a lower trophic level is consistent with previous studies reporting significant lower Hg levels in little than in Cory's shearwaters breeding on Azores (Monteiro and Furness 1997). This is because Hg and trophic level are usually positively correlated since Hg biomagnifies trough the food chain (Bearhop et al. 2000). Diet and movements of Fea's petrel are largely unknown, but its $\delta^{15}N$ signature suggests that this species feeds on higher trophic level preys than Cape Verde and Cory's shearwaters and probably over African shore, if we consider $\delta^{13}C$ signatures.

This study highlights the potential of combining isotopic and tracking approaches to reveal a meaningful view on the specific and interspecific trophic ecology of pelagic seabirds, but also to expose the relative influence of geographical variability in baseline isotopic levels when dealing with large-scale and multi-locality studies. In summary, our results suggest that within large regions isotopic variability was biogeographically unstructured. There were some geographic differences, but unrelated to distance among archipelagos, oceanographic traits, or geographic coordinates. In contrast, all populations from each species grouped together according to their $\delta^{15}N$ and $\delta^{13}C$ signatures and interspecific overlap in seabird $\delta^{13}C$ signatures was relative low, confirming trophic niche as the prime source of isotopic variability. Nevertheless, we found a clear enrichment in N signatures in the north Temperate Atlantic compared to the mid-north Atlantic Subtropical and Tropical regions, which can be reasonable attributed to baseline differences in isotopic signatures between regions. Therefore, in contrast with terrestrial ecosystems, this study points out the limited applicability of isotope gradients to study migratory movements of marine organisms at a regional scale. Nevertheless, it suggests stable isotope analyses can be a powerful tool to study long distance migrations between large geographic regions in the marine environment.

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SUPPORTING INFORMATION AVAILABLE

Table SM1 including mean values of $\delta^{13}C$ and $\delta^{15}N$ depending on the sampling year and Figure SM1 exposing the relationship between $\delta^{13}C$ and breeding colonies' latitude of Cory's shearwaters

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SUPPLEMENTARY MATERIAL

Manuscript title: Trophic versus geographic structure in stable isotope signatures of pelagic seabirds breeding in the Northeast Atlantic.

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Number of pages: 3
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Including materials:

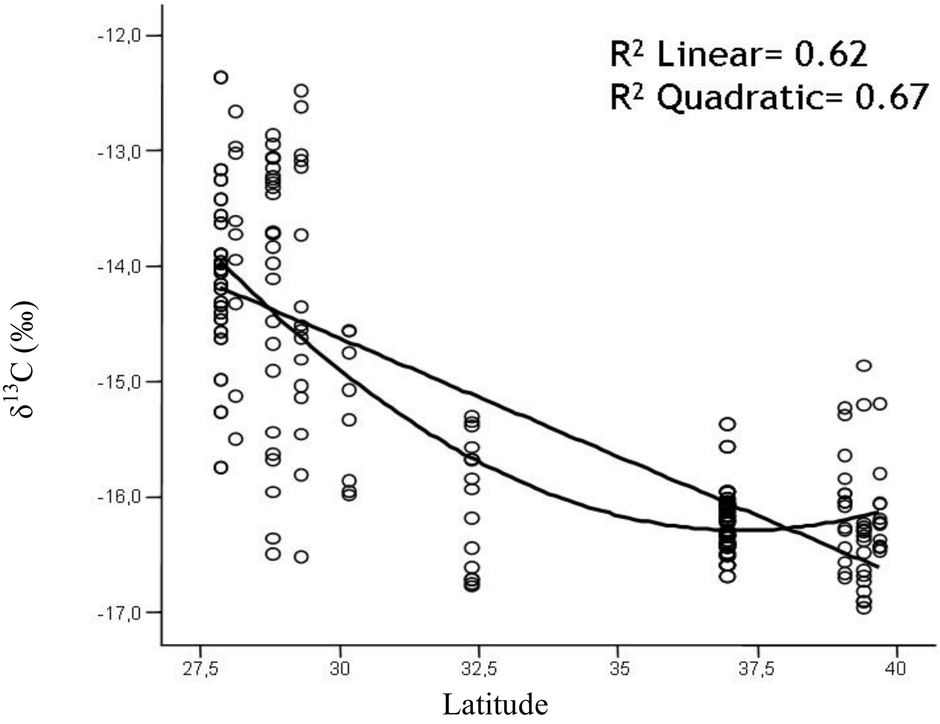
Table SM1: Values of δ^{13}C and δ^{15}N in NE Atlantic seabird feathers depending on the sampling year and summary of ANOVA tests checking interannual differences.

Figure SM1: Relationship between δ^{13}C and breeding colonies' latitude of Cory's shearwaters.

Table SM1. Mean ± SE of $\delta^{13}C$ and $\delta^{15}N$ in the first primary feather of seabirds from the NE Atlantic Ocean depending on the sampling year and summarized Student's and ANOVA tests checking inter-annual isotopic signatures differences.

Species	Breeding colony	Isotope	2002	2003	2004	2005	Sstatistics
Cory's shearwater	Sta. María (Azores)	$\delta^{15}N$	13.2 ± 0.6	13.1 ± 0.6			$t_{1,69} = -0.01$; $p = 0.99$
		$\delta^{13}C$	-16.2 ± 2.3	-16.2 ± 2.4			$t_{1,69} = -0.12$; $p = 0.91$
	Gran Canaria (Canary)	$\delta^{15}N$	13.1 ± 0.6	13.4 ± 0.7			$t_{1,48} = -1.27$; $p = 0.21$
		$\delta^{13}C$	-14.1 ± 0.7	-14.1 ± 0.8			$t_{1,48} = 0.35$; $p = 0.73$
Bulwer's petrel	Madeira	$\delta^{15}N$			12.2 ± 0.9	11.8 ± 0.4	$t_{1,12} = 0.77$; $p = 0.46$
		$\delta^{13}C$			-16.2 ± 0.3	-16.3 ± 0.2	$t_{1,12} = 0.50$; $p = 0.63$
Madeiran storm-petrel	Graciosa (Azores)	$\delta^{15}N$	12.1 ± 0.7	12.6 ± 0.8			$t_{1,35} = -2.23$; $p = 0.03$
		$\delta^{13}C$	-16.7 ± 0.5	-16.6 ± 0.6			$t_{1,35} = -0.66$; $p = 0.52$
	Selvagens	$\delta^{15}N$		12.3 ± 0.7	12.4 ± 0.6	12.0 ± 0.7	$F_{2,23} = 0.87$; $p = 0.44$
		$\delta^{13}C$		-17.1 ± 0.3	-17.4 ± 0.5	-16.9 ± 0.5	$F_{2,23} = 2.38$; $p = 0.12$

Figure SM1. Relationship (linear and quadratic) between δ^{13}C signatures in feathers of Cory's shearwaters and the latitude of their breeding colonies in degrees.



CHAPTER 2

Geographical PCB and DDT patterns in shearwaters breeding across the NE Atlantic and the Mediterranean archipelagos

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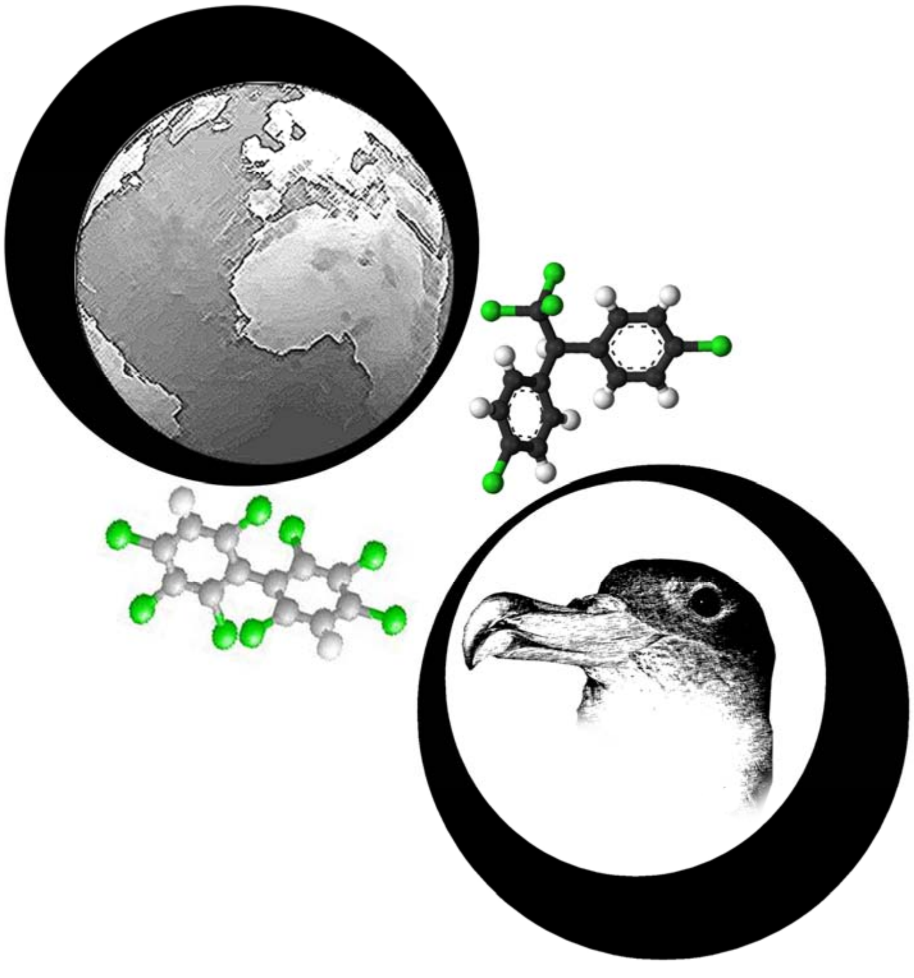

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Patrones geográficos de PCBs y DDTs en pardelas (*Calonectris* sp.) del Atlántico noreste y del Mediterráneo.

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RESUMEN

Diversos estudios han destacado el papel de las aves marinas como organismos bioindicadores de los ecosistemas marinos. Sin embargo, su utilidad para evaluar la presencia de contaminantes organoclorados en el medio marino ha sido escasamente explorada, especialmente en el caso de las aves pelágicas. El transporte oceánico a larga distancia de estos compuestos podría conllevar la aparición de altos niveles de los mismos a escala global en el mar abierto por lo que investigar estos aspectos resulta de particular importancia. Actualmente, técnicas mínimamente invasivas, como las basadas en el empleo de sangre, permiten evaluar la exposición reciente a contaminantes como PCBs o DDTs así como el análisis de lo isótopos estables de nitrógeno ($\delta^{15}N$) y carbono ($\delta^{13}C$). La combinación de estas técnicas permite explorar la relación entre la ecología trófica de los individuos y su carga de contaminantes, ofreciendo así una visión más completa de los factores que explican los niveles de contaminación en los ecosistemas marinos.

Con el fin de evaluar el potencial de las aves pelágicas de amplia distribución como especies indicadoras, se han analizado los niveles de PCBs, DDTs, $\delta^{15}N$ y $\delta^{13}C$ en sangre de individuos adultos de pardela cenicienta y de Cabo Verde (*Calonectris* sp.) de diferentes colonias abarcando el rango geográfico completo de dichas especies. Concretamente, dentro del Atlántico noreste, y de sur a norte, se han muestreado distintitas colonias de Cabo Verde, Canarias, Madeira, Azores y Berlengas. En el caso del Mediterráneo, este estudio incluye colonias de Terreros, Baleares, Hyeres y Creta. Este diseño experimental permite evaluar la influencia de la localización geográfica de la colonia de cría así como de la ecología trófica de las pardelas como factores explicativos de los niveles de contaminación en estas aves.

Los niveles de PCBs y DDTs en sangre de pardela mostraron variaciones significativas debido a la colonia de cría. A gran escala, las pardelas muestreadas en colonias Mediterráneas mostraron niveles totales de OCs significativamente más altos que las capturadas en el Atlántico. Además, los niveles de PCBs y DDTs en sangre

aumentaban de sur a norte dentro del Atlántico, y de oeste a este en el caso del Mediterráneo. La presencia de los distintos PCBs analizados también mostró variaciones a lo largo del área de cría de las pardelas. Concretamente, los PCBs de mayor grado de cloración resultaron más abundantes en el caso de los individuos del Mediterráneo comparados con los del Atlántico. Igualmente, se detectaron variaciones en la presencia de DDT y sus metabolitos, destacando la mayor abundancia de DDT respecto a DDE y DDD en el caso de las pardelas de Cabo Verde y Canarias, hecho que sugiere un uso reciente de este pesticida. En cuanto a los isótopos estables se encontró una relación positiva y significativa entre $\delta^{15}N$ y los niveles de OCs en sangre dentro de la mayoría de las colonias de pardelas muestreadas. Sin embargo, está relación no se encontró al combinar todos los datos independientemente de la colonia, indicando un origen geográfico y no trófico para las diferencias en los niveles de PCBs y DDTs entre colonias.

Los patrones geográficos encontrados en este estudio son consistentes con las características de las regiones oceanográficas estudiadas. Los altos niveles y el mayor grado de cloración de PCBs encontrados en sangre de las pardelas cenicientas del Mediterráneo está posiblemente ligado al abundante vertido de estos contaminantes por parte de los países industrializados de Europa y el confinamiento natural de esta cuenca oceánica. En el caso del Atlántico, los perfiles y niveles de OCs sugieren principalmente fuentes difusas para estos contaminantes. De hecho, los altos niveles encontrados en las colonias del norte (Azores y Madeira) respecto a las de Sur (Canarias y Cabo Verde) de la Macaronesia se podrían deber a la influencia de la corriente del golfo sobre esta región, ya que ésta puede transportar los contaminantes desde las costas de Norteamérica a la zona central del Atlántico. Además, los perfiles de DDTs encontrados en el caso de las pardelas de Cabo Verde y Canarias, que sugieren un uso reciente de DDT en estas regiones, se podrían explicar por el uso del mismo para el control de la malaria en los países del África subsahariana.

La relación que se ha encontrado entre la posición trófica de los individuos ($\delta^{15}N$) y sus niveles de OCs en este estudio probablemente refleja la capacidad de biomagnificación de estos contaminantes. Esta relación refleja la especialización de los individuos hacia el consumo de presas de determinados niveles tróficos como un factor clave para explicar los niveles de contaminación en aves pelágicas. Igualmente, los resultados obtenidos sugieren que dicha especialización supone que el proceso de biomagnificación no sólo pueda detectarse a lo largo de las cadenas tróficas sino también a nivel intraespecífico. En conjunto, este estudio revela la gran utilidad de las aves pelágicas para biomonitorizar las diferencias geográficas de OCs en el medio marino a diferentes escalas.

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Geographical PCB and DDT Patterns in Shearwaters (*Calonectris* sp.) Breeding Across the NE Atlantic and the Mediterranean Archipelagos

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Although seabirds have been proposed as useful biomonitors for organochlorine contaminants (OCs) in marine environments, their suitability is still unclear. To understand the geographic variability and the influence of seabird trophic ecology in OC levels, we analyzed PCBs, DDTs, $\delta^{13}C$, and $\delta^{15}N$ in the blood of adult *Calonectris* shearwaters throughout a vast geographic range within the northeast Atlantic Ocean (from Cape Verde to Azores) and the Mediterranean Sea (from the Alboran Sea to Crete). OC concentrations were greater in birds from the Mediterranean than in those from the Atlantic colonies, showing higher and lower chlorinated PCB profiles, respectively. This large-scale pattern may reflect the influence of historical European runoffs in the Mediterranean basin and diffused sources for OCs in remote Atlantic islands. Spatial patterns also emerged within the Atlantic basin, probably associated with pollutant long-range transport and recent inputs of DDT in the food webs of shearwaters from Cape Verde and the Canary islands. Moreover, a positive association of OC concentrations with $\delta^{15}N$ within each locality points out diet specialization as a major factor explaining differences in OCs at the intraspecific level. Overall, this study highlights wide range breeding seabirds, such as *Calonectris* shearwaters, as suitable organisms for biomonitoring large geographic trends of organochlorine contamination in the marine environment.

Introduction

Oceans act as a final repository for many land-based source contaminants. Among them, organochlorine contaminants (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyl ethane (DDT), and its main metabolites, have attracted much scientific interest due to their global distribution and wide array of adverse effects (*1, 2*). Although banned in most parts of the world since the 1970s, OCs still constitute an element of concern, because they are easily transported through air and water currents. Furthermore, their persistence and lipophilicity cause them to bioaccumulate and biomagnify through marine food webs worldwide (*3, 4*). Therefore, the spatial distribution of OCs and their possible sources in the marine environment have been the subject of extensive research (*5*−*7*).

Seabirds have been suggested among marine biota as useful biomonitors for OCs as a result of specific advantages that they have in comparison to other marine organisms (*8*). Most seabird species are placed at high trophic positions within marine food webs, and, thus, high contamination levels can be expected. Moreover seabirds show vast breeding ranges, allowing comparisons in pollutant levels across large geographic scales. Within a specific region, however, seabirds can forage up to several hundreds of km from their breeding colonies (*9*) integrating the chronic baseline levels of that region. To decipher the dynamics of OCs in the marine environment, several studies have combined OC analyses with spatial and trophic information using several approaches (*10, 11*). Among them, isotopic analyses are particularly valuable, because they provide information on the feeding ecology, while minimizing some of the common biases of the conventional diet studies (*12*). Nitrogen isotope ratios ($^{15}N/^{14}N$; $\delta^{15}N$) can be used to infer trophic position, whereas stable-carbon isotope analysis ($^{13}C/^{12}C$; $\delta^{13}C$) can indicate inshore or benthic compared to offshore or more pelagic feeding (*13*). In addition to geography or feeding ecology, further factors, such as sex, marked dietary switches, or specific excretion routes (e.g., egg laying), can also play a role in determining seabird OC levels (*10, 14, 15*).

Most seabird studies have revealed marked spatial patterns in OC concentrations and profiles (*6, 10, 11, 16*). These spatial differences have been commonly associated with different sources for these pollutants. For example, PCB profiles dominated by high chlorinated compounds have been widely related to specific point sources, such as direct industrial runoffs, while low chlorinated compounds, which are commonly associated with pollutant transport by atmospheric or oceanic currents, are related to diffuse sources (*16*). Large-scale geographic studies have been conducted mainly using seabird eggs (*10, 17*). However, this approach can be obscured by migratory movements and capital-income breeding strategies (*18*). Alternatively, minimally invasive procedures, such as blood sampling, could better reflect recent OC exposure (*19*). Previous studies have validated the suitability of blood to evaluate PCB and DDT burdens in seabirds (*20, 21*) and have reported significant relationships between blood OC levels and stable isotope signatures (*11, 22*). In fact, some studies have found that under stable environmental conditions, including stable food sources, OC concentrations seem to be stable in the blood not only for short-term periods (e.g., within the incubation period) but also over long-term periods (e.g., between breeding seasons) (*20, 23*).

In the present study, PCB and DDT levels and $\delta^{15}N$ and $\delta^{13}C$ signatures were analyzed in blood of three closely related taxa of shearwaters, which were, until recently, considered a single species: the Scopoli's (*Calonectris diomedea*), the Cory's (*C.borealis*), and the Cape Verde (*C.edwardsii*) shearwaters. Shearwaters are at the top of the food webs, mostly feeding on species that are also consumed by humans, such as several epipelagic fish and squid species, thus making shearwaters potentially useful as sentinels of marine contamination. Moreover, their wide distribution offers an excellent opportunity to investigate biogeographic patterns of OCs across a vast geographic area. In this context, we aimed (1) to explore the geographic patterns in PCB and DDT levels of shearwaters throughout the NE Atlantic Ocean

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and the Mediterranean Sea; (2) to examine profiles of PCBs and DDTs in shearwaters in order to assess their potential sources and (3) to understand the effects of the trophic ecology on PCB and DDT levels, as shown by stable isotope signatures of carbon and nitrogen, potentially influenced by sexual, intercolony, and individual variations in the trophic ecology of *Calonectris* shearwaters.

Material and Methods

Species, Study Area, and Sampling Procedure. The three shearwater taxa included in the present study, the Scopoli's, the Cory's, and the Cape Verde shearwaters, have mostly disjointed distributions across the Mediterranean Sea, the NE Atlantic (except Cape Verde), and the Cape Verde archipelago, respectively. Although their taxonomic status is still being debated, recent studies suggest that the three taxa should be regarded as separate rather than as a single species (*24*). However, since the three taxa show very similar morphology, ecology, and physiology (*9, 25*), we will consider them as a single statistical unit.

This study was conducted covering the whole breeding range of the three shearwater taxa. Adult shearwater sampling was carried out during their breeding season, concretely among incubation and chick rearing periods, from 2003 to 2006 (Table 1). Each breeding colony was sampled within a single year. The study covers 9 breeding colonies (Supporting Information; Figure S1): 5 throughout the northeast Atlantic Ocean including, from south to north, Cape Verde, Canary, Madeira, Azores, and the Berlengas islands, and 4 among the Mediterranean including, from west to east, the Terreros, Balearic, Hyeres, and Crete islands. Blood (0.5 mL) from the brachial vein was sampled and transferred into a vial with 1 mL of absolute ethanol and preserved at −24 °C until analysis. Bill depth, bill depth at the nostril, and maximum head length to the nearest ±0.1 mm were measured using a digital caliper in all individuals except on Hyeres islands. Biometrical measurements were used to sex shearwaters by means of a discriminant analysis (further details are described in the Supporting Information).

Chemical Analysis

A subsample from the blood fixed with absolute ethanol was used for stable isotope analysis. Once subsamples were dried, we placed from 0.36 to 0.40 mg of blood (weighed to the nearest μg) into tin buckets for combustion. Isotopic analyses were carried out by elemental analysis-isotope ratio mass spectrometry (EA-IRMS), and stable isotope ratios were expressed in conventional notation as parts per thousand (‰).

The following organochlorine compounds were analyzed: *ortho* PCB congeners #28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #183, #170, #189, #194, and DDTs, including *p,p'*-DDT and its two main metabolites, *p,p'*-DDD and *p,p'*-DDE. OC concentrations were determined following the procedure described by Otero et al. (*26*) with some modifications. From 0.2 to 0.1 g of dried blood was used for OC determination. 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 microelectron capture detection. Further details of the analytical procedure for the determination of stable isotope ratios and OC concentrations are described in the Supporting Information.

Data Analysis

SPSS 15.0 for Windows was used for statistical analysis. We validated the normal distribution of δ^{15}N, δ^{13}C, and pollutant levels (previously log transformed) among breeding colonies

TABLE 1. Concentrations of Σ_{20}PCBs and Σ_3DDTs (ng g^{-1} d.w.), Stable Isotope Signatures of Carbon and Nitrogen (‰), and Linear Regression Analysis Statistics in *Calonectris* Shearwater Blood

breeding colony	year	*n*	ΣPCBs AM[a] ± SE (min − max)	ΣPCBs GM[b]	ΣDDTs AM ± SE (min − max)	ΣDDTs GM	δ^{15}N AM ± SE (min − max)	δ^{13}C AM ± SE (min − max)	[ΣOCs] − δ^{15}N R^2, P
					Atlantic				
Cape Verde	2006	10	43.25 ± 7.31 (17.74 − 97.35)	38.42	7.691 ± 3.595 (1.85 − 39.80)	4.89	12.2 ± 0.4 (10.2 − 13.2)	−16.8 ± 0.2 (−17.9 − −16.2)	$R^2 = 0.02$, $P = 0.96$
Canary Is.	2003	10	59.22 ± 9.37 (40.94 − 114.8)	53.93	13.13 ± 4.43 (2.906 − 42.59)	8.38	12.1 ± 0.2 (11.0 − 12.8)	−16.9 ± 0.2 (−17.9 − −16.0)	$R^2 = 0.40$, $P < 0.05$
Madeira Is.	2005	10	125.3 ± 41.0 (51.40 − 488.9)	98.34	38.18 ± 19.28 (11.03 − 208.7)	22.06	11.2 ± 0.1 (10.9 − 11.9)	−18.8 ± 0.2 (−19.4 − −17.7)	$R^2 = 0.53$, $P < 0.05$
Azores Is.	2003	10	99.33 ± 46.93 (41.22 − 217.7)	91.21	42.09 ± 21.48 (12.49 − 234.2)	25.13	9.97 ± 0.16 (9.21 − 10.9)	−18.9 ± 0.1 (−19.4 − −18.5)	$R^2 = 0.51$, $P < 0.05$
Berlengas Is.	2004	10	119.4 ± 11.9 (69.98 − 175.4)	113.9	25.29 ± 3.66 (10.17 − 49.33)	22.97	13.0 ± 0.1 (12.6 − 13.4)	−18.0 ± 0.1 (−18,2 − −17.2)	$R^2 = 0.34$, $P = 0.06$
total			89.31 ± 71.34	73.31	25.28 ± 42.42	13.91	11.7 ± 1.2	−17.9 ± 1.0	
					Mediterranean				
Terreros Is.	2004	10	212.7 ± 34.5 (91.68 − 463.5)	191.5	100.1 ± 24.9 (25.50 − 248.1)	76.98	12.0 ± 0.1 (11.4 − 12.3)	−18.2 ± 0.1 (−18.5 − −18.0)	$R^2 = 0.67$, $P < 0.05$
Balearic Is.	2004	10	158.2 ± 13.4 (102.1 − 227.7)	153.3	69.57 ± 14.96 (30.98 − 191.8)	59.61	10.8 ± 0.1 (10.3 − 11.3)	−18.5 ± 0.1 (−18.7 − −18.3)	$R^2 = 0.52$, $P < 0.05$
Hyeres Is.	2006	8	510.6 ± 107.8 (216.2 − 1189)	448.5	303.2 ± 96.8 (96.26 − 911.6)	233.6	10.0 ± 0.1 (9.50 − 10.4)	−18.1 ± 0.1 (−18.3 − −17.8)	$R^2 = 0.24$, $P = 0.24$
Crete	2005	9	678.9 ± 102.5 (145.1 − 1124)	593.0	895.3 ± 203.6 (89.45 − 1720)	641.4	9.99 ± 0.13 (9.47 − 10.5)	−17.7 ± 0.2 (−18.3 − −16.6)	$R^2 = 0.34$, $P = 0.07$
total			375.8 ± 300.1	285.3	329.2 ± 461.7	152.0	10.8 ± 0.9	−18.1 ± 0.4	

[a] Arithmetic mean. [b] Geometric mean.

and sexes by checking Q-Q plots and by the Shapiro-Wilk test (all $P > 0.05$). In the case of values under the detection limit, they were set to zero. Analysis of the variability of PCBs and DDTs was performed applying generalized linear models (GLM) using total PCBs (Σ_{20}PCBs, the sum of the 20 individual PCBs) and total DDTs (Σ_3DDTs, the sum *p,p'*-DDT, and metabolites) as response variables. We tested the main effects and interactions of breeding colony, sex, and stable isotopes for both classes of pollutants. The initial model included all the breeding colonies except Hyeres, since in this case shearwater sex was unknown. The final selected model was built following a forward stepwise procedure, which included only the significant effects retained. We found that sex was a nonsignificant factor in the model, and, thus, we conducted the GLM procedure again including Hyeres breeding colony but not shearwater sex factor. Differences among breeding colonies were examined using posthoc pair-comparisons (Bonferroni adjustment). The relationship between OC levels and δ^{15}N was further examined within each breeding locality by means of linear regression analysis. To assess the relative exposure to PCBs among the breeding range of Cory's shearwater, we grouped PCB concentrations depending on their chlorination degree (≤tetra-, penta-, hexa-, and ≥heptachlorinated PCBs) and compared their concentrations among breeding colonies using one-way ANOVA. Moreover, we examined fingerprints of shearwaters (calculated as the average percent of each PCB or DDT in respect to Σ_{20}PCBs and Σ_3DDTs, respectively) to assess the geographic patterns and sources of these pollutants. In the case of DDTs we also explored the relationship *p,p'*-DDE/*p,p'*-DDT to decipher a possible recent use of DDT in the studied area (*27, 28*). Finally, a principal component analysis (PCA) was applied to the normalized contributions of all PCBs and DDTs to check whether OC profiles segregate shearwaters depending on their breeding locality or other possible factors. Concentrations are expressed in ng.g^{-1} on a dry weight basis (d.w.).

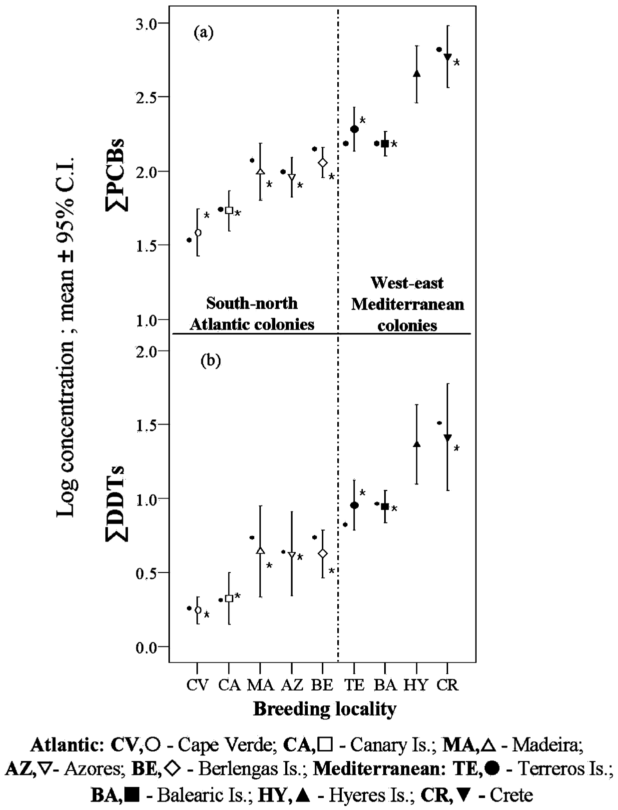

FIGURE 1. PCB (a) and DDT (b) levels in Scopoli's, Cory's, and Cape Verde shearwaters at different localities. Asterisks and dots indicate mean values of males and females, respectively.

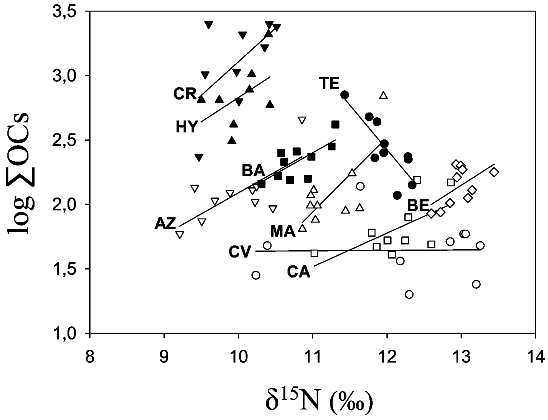

FIGURE 2. Relationship between δ^{15}N signatures and ΣOC levels (ΣOCs = Σ_{20}PCBs + Σ_3DDTs) in *Calonectris* shearwaters. Linear regressions are shown for each breeding colony separately and identified with the abbreviations of the locality. Symbol and abbreviation legends are included in Figure 1.

Results

Considering ΣPCB and ΣDDT levels (Table 1), the GLM analysis explained up to 77.1% and 65.7%, respectively, of the initial variance and included two main explanatory variables for both contaminant families: breeding colony ($F_{7,79} = 29.60$ for ΣPCBs; $F_{7,79} = 12.76$ for ΣDDTs; both $P < 0.0001$) and δ^{15}N ($F_{1,79} = 8.44$, $F_{1,79} = 8.26$ for ΣPCBs and ΣDDTs, respectively; $P < 0.01$). Seabird sex ($F_{1,79} = 0.01$ and $P = 0.95$ for ΣPCBs; $F_{1,79} = 0.07$ and $P = 0.79$ for ΣDDTs) and δ^{13}C ($F_{1,79} = 0.49$, $P = 0.48$; $F_{1,79} = 2.87$, $P = 0.10$; for ΣPCBs and ΣDDTs, respectively) did not show a significant effect explaining both PCB and DDT levels within *Calonectris* shearwaters. Since sex was not significant, we conducted the GLM analysis again including the breeding colony of Hyeres (France), where the sex of shearwaters was unknown. The resulting models explained up to 79.4% and 65.7% of the total variance of ΣPCBs and ΣDDTs, respectively. Breeding colony ($F_{8,87} = 29.87$ for ΣPCBs, $F_{8,87} = 14.37$ for ΣDDTs: both $P < 0.0001$) and δ^{15}N ($F_{1,87} = 9.51$ and $P = 0.003$, $F_{1,87} = 6.79$ and $P = 0.011$ for ΣPCBs and ΣDDTs, respectively) showed again a significant effect. Differences among colonies were due to the greater PCB and DDT levels found in the Mediterranean compared to the Atlantic colonies (Figure 1). Specifically, posthoc pair comparisons revealed that Crete and Hyeres, showing the greatest ΣPCB (Figure 1a) and ΣDDT (Figure 1b) concentrations, differed significantly for all pair comparisons. Conversely, Canary Is. and Cape Verde showed the lowest concentrations and also differed significantly from most breeding colonies. Stable isotope signatures of carbon and nitrogen also showed spatial differences (Table 1). Regarding stable-carbon isotope signatures, shearwaters from Cape Verde plus Canary Is. showed marked enriched signatures compared with the rest of shearwater colonies, and those from Madeira and Azores showed the lowest mean δ^{13}C values, reflecting different food sources among shearwater colonies. The mean δ^{15}N values ranged from 13.0 ± 0.2‰ in shearwaters from Berlengas to 10.0 ± 0.5‰ in those from Azores. Moreover, δ^{15}N values in shearwater blood were significantly correlated with total OC levels within most breeding colonies (Table 1) but not among the whole data set ($R^2 = 0.16$, $P = 0.06$). The relationships between [ΣOCs]-δ^{15}N (Figure 2) were positive in all the breeding localities except within Terreros Is. and Cape Verde, where we found a negative and flat relationship, respectively.

PCB congeners differed significantly among breeding colonies (Table S1). Hexachlorinated PCBs accounted for

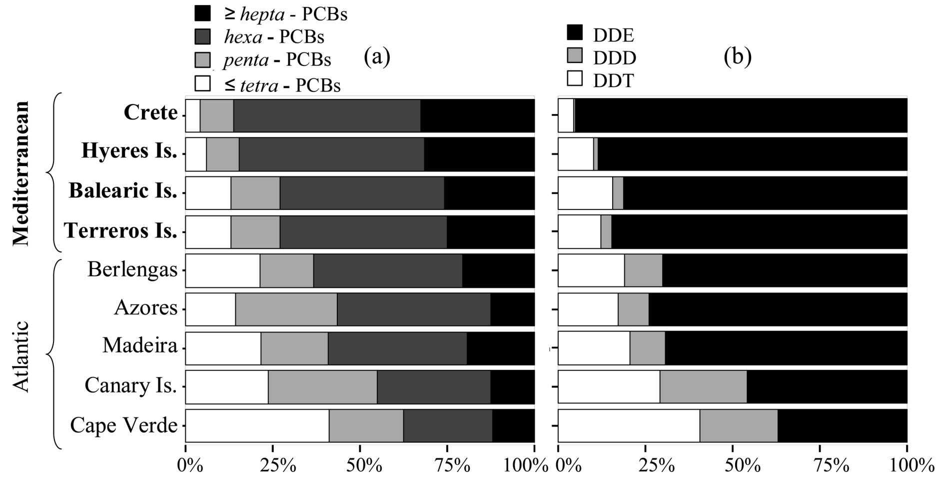

FIGURE 3. Fingerprints of PCBs and DDTs in the blood of *Calonectris* shearwaters among their breeding colonies.

the greatest mean concentrations (in all colonies except Cape Verde). Hexa- as well as penta- and ≥ heptachlorinated PCBs differed significantly among localities (statistics summarized in Table S1) due to the low levels in shearwaters breeding in the Atlantic compared to those breeding in the Mediterranean. Posthoc test comparisons revealed that significant differences arose mainly from the low values shown by shearwaters from Cape Verde and Canary Is. compared to the high concentrations from shearwaters breeding on Hyeres and Crete. Spatial differences in ≤ tetrachlorinated PCBs were marginally nonsignificant. PCB profiles (Figure 3a) in shearwaters were dominated by hexachlorinated PCBs (in all colonies except Cape Verde). The higher chlorinated congeners (hexa- and ≥ heptachlorinated PCBs) were more abundant in shearwaters from the Mediterranean than from the Atlantic colonies. Conversely, the low chlorinated congeners (≤tetra- and pentachlorinated PCBs) predominated in the profiles of Atlantic breeders. The presence of each PCB congener also varied among localities, but overall the most abundant congener in shearwater blood was CB153 (5.4 − 33.1%; average 22.54%) followed by CB180 (0.4 − 28.3%; average 12.0%).

Concerning DDTs, we also found significant differences among breeding colonies. Shearwaters from the Mediterranean showed significantly greater concentrations of *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD in the blood than those from the Atlantic (Table S1). Among DDTs, *p,p'*-DDE was the most abundant (Figure 3b) in all colonies except in Cape Verde, where *p,p'*-DDT (40.7%) was the predominant compound, and in the Canary Is., where the difference between *p,p'*-DDE (46.1%) and *p,p'*-DDT (30.0%) in shearwater blood was slight compared with the rest of the breeding colonies. In fact, shearwaters from Cape Verde and Canary Is. showed *p,p'*-DDE/*p,p'*-DDT ratios (2.0 and 2.8, respectively) markedly lower than the rest of shearwater colonies (Supporting Information; Table S1). The *p,p'*-DDD was the less abundant compound in all cases. The principal component analysis (PCA) on the relative abundance of all analyzed OCs (Figure 4) reflects the geographic differences of OCs among breeding colonies. PC1 and PC2 accounted for 42.7% and 13.5% of the variance, respectively. In the graphic representation of the two main principal components, Cory's, Scopoli's, and Cape Verde shearwaters appear mostly segregated. The component matrix indicated that PC1 was mainly negatively associated with higher chlorinated PCBs and *p,p'*-DDE and positively with lower chlorinated PCBs and *p,p'*-DDT. Therefore, PC1 represented a gradient of congener chlorination and an abundance of *p,p'*-DDT metabolite segregating between Atlantic and Mediterranean breeders. PC2 was dominated by CB114, CB153, CB194, CB180, and CB118, segregating within Atlantic (Azores, Canary, and Cape Verde from Maderia and Berlengas) and within Mediterranean breeders (Crete from the rest of the localities).

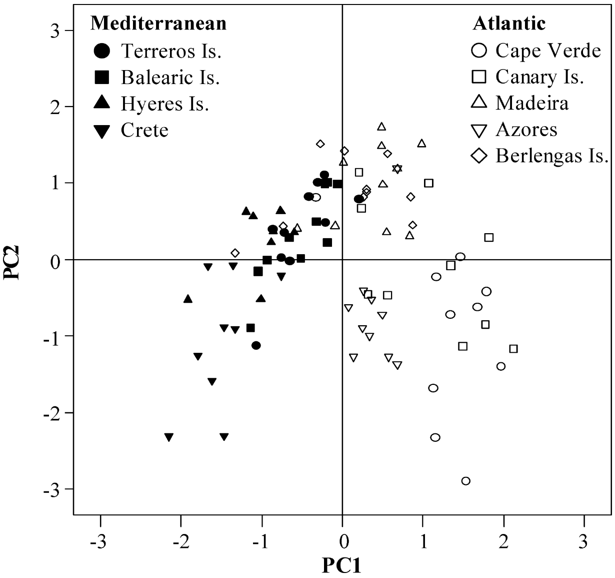

FIGURE 4. Principal components (PC1 and PC2) extracted from PCBs and DDTs in the blood from *Calonectris* shearwaters.

Discussion

To our knowledge, this is the first study dealing with OC contaminants in seabirds covering an extensive area across the northeast Atlantic and the Mediterranean. Our results showed clear geographic gradients in PCB and DDT levels increasing from the southern to northern Atlantic and from the western to eastern Mediterranean colonies (Figure 1). Further, differences in OC profiles were so different among colonies that a PCA analysis segregated most colonies, showing a relatively small overlap among them (Figure 4). Moreover, we found a significant and positive relationship of $\sum$OC levels with δ^{15}N signatures within most breeding localities (Figure 2).

The broad geographic coverage of our sampling design offers the opportunity to compare contamination levels among distant localities, but it would have been difficult to carry it out in a single year. Therefore, different localities were sampled during the period of 2003−2006. Potential changes in contamination among years may interfere with comparisons among localities, but the main geographic patterns also emerged among samples collected in a single year, suggesting that interyear differences in OCs were a minor source of variability in this study. In addition, temporal differences mainly emerge when comparing between distant time periods, such as interdecade comparisons, whereas interannual differences in OC levels are usually little or nonsignificant (*7, 20, 29, 30*). In fact, interannual coefficients of variation (CV) of PCB and DDT levels found in the literature were low compared to those obtained among localities in the present study, suggesting our differences can mainly be attributed to spatial variability in contamination rather than to the sampling year (this study CV PCBs = 0.99, CV DDTs = 1.74 among localities; Northern Atlantic fulmar: CV PCBs = 0.32, CV DDTs = 0.36 between 1998 and 2003 (*31*); Northern Atlantic gannets: CV PCBs = 0.08 between 1998 and 2004 (*17*); Mediterranean stripped dolphins: CV PCBs = 0.12, CV DDTs = 0.56 between 2000−2002 (*7*)).

Geographic differences in PCB and DDT levels mainly arose from the low OC levels found in the Atlantic compared to the Mediterranean shearwaters. Within each basin, ΣPCB and ΣDDT levels increased from the south to the north in the Atlantic shearwaters and from the west to the east in the Mediterranean ones (Figure 1). Several factors could be contributing to these patterns, but the two major factors are probably related to diet differences among colonies and to geographic differences in baseline levels. However, the diet of the Scopoli's, Cory's, and Cape Verde shearwaters is fairly homogeneous, mostly composed of epipelagic fish and, to a lesser extent, of cephalopods, crustaceans, and plankton (*32, 33*). Moreover, δ^{15}N was significantly correlated with ∑OC levels only after controlling for breeding colony, i.e., reflecting a relationship between OCs and trophic levels at the intralocality level but not among localities (Figure 2). The lack of correlation between ∑OC levels and δ^{15}N among the breeding localities suggests that differences in δ^{15}N among colonies do not arise from differences in trophic levels but from differences in baseline signatures among water masses (*34*). Therefore, intercolony differences in pollutant levels seem to reflect geographic differences in OC levels rather than differences in trophic ecology among localities. Our results are in line with other studies pointing out the Mediterranean Sea as a confined water mass, which results in greater levels of contamination compared to other oceanic waters (*35*).

Geographical differences in OCs among the Atlantic and Mediterranean waters are further supported by differences in PCB and DDT profiles. In the case of PCBs, highly chlorinated congeners (hexa-, ≥ heptachlorinated PCBs) were the most abundant in shearwaters breeding in the Mediterranean. Conversely, Atlantic PCB profiles were dominated by lower chlorinated congeners (≤tetra- and pentachlorinated PCBs) (Figure 3). This result is probably related to a differential transport and persistence among congeners. Lower chlorinated PCBs are more volatile and easily transported to remote locations, whereas highly chlorinated congeners are more persistent and not easily metabolized by marine biota (*36, 37*). Therefore, assuming biotransformation efficiencies across *Calonectris* shearwaters do not change, their PCB profiles suggest that Atlantic shearwaters are mainly exposed to PCBs originating from distant sources, whereas in the case of Mediterranean breeders, higher chlorinated compounds serve as further evidence of point sources for PCBs. This pattern is consistent with a confined sea with historically greater inputs of PCBs from the highly industrialized countries of Europe, such as is the case for the Mediterranean Sea, compared to the more isolated oceanic archipelagos in the Atlantic Ocean. Within the Atlantic basin, differences in PCB levels and profiles among shearwater colonies from Macaronesian archipelagos could be related to the influence of pollutant sources and waters flowing from North America above the Central North Atlantic Ocean, including the Azores region (*38, 39*), resulting in greater levels of pollutants in northern (Azores and Madeira) compared to southern Macaronesian colonies (Cape Verde and Canary Is.). In addition, greater OC levels in shearwater blood from Berlengas Is. could be related to the influence of Mediterranean waters along the southern Portuguese coast (*40*).

In this study, the high *p,p'*-DDE/*p,p'*-DDT ratios in shearwater from the Mediterranean indicate a major historical influence of DDTs in the Mediterranean countries. Although the mean ratio values were >1 in all sampled colonies (both Atlantic and Mediterranean), indicating a greater abundance of *p,p'*-DDE, this ratio was markedly lower in shearwaters from Canary Is. and Cape Verde. In fact, 7 out of 10 Cape Verde shearwaters and 5 out of 10 Cory's shearwaters from Canary Is. showed *p,p'*-DDE/*p,p'*-DDT ratios lower than 1. The remaining 67 shearwaters showed ratios far greater than 1, indicating that shearwaters from Canary Is. and Cape Verde have been more recently exposed to DDTs. This exposure is likely related to the location of the foraging grounds of the shearwaters breeding on these two southernmost Macaronesic archipelagos. This hypothesis is supported by the differences in *Calonectris* shearwater δ^{13}C signatures (Table 1), which, however, did not show a significant effect over OC levels, reflected the differential exploitation of inshore and offshore resources across their breeding range. That is, previous tracking studies showed that *Caloenctris* shearwaters from Cape Verde and Canary Is. feed mainly over the Sub-Saharan African shelf (*41, 42*), therefore showing greater δ^{13}C values typical of a neritic feeding habit. In contrast, *Calonectris* shearwaters breeding in Azores and Madeira showed the lowest mean δ^{13}C signatures, since they feed on pelagic waters that are far away from any coastal systems (*42*). Thus, unexpected shearwater DDT profiles from Cape Verde and Canary Is. colonies are probably related to their feeding habits over the African shelf, since Sub-Saharan countries still use DDT to control malaria outbreaks (*43*).

δ^{15}N showed a significant effect over PCB and DDT concentrations when controlling for the breeding locality. In fact, all the colonies showed a consistently positive relationship between δ^{15}N and OC levels, except Terreros Is. and Cape Verde. Since δ^{15}N signatures increase with trophic levels, relationships between δ^{15}N signatures and OCs probably reflect the well-known biomagnification properties of PCBs and DDTs. Exceptions, such as Terreros Is. and Cape Verde, are probably related to a differential use of feeding grounds with different baseline δ^{15}N levels, obscuring the bioaccumulation processes. For example, shearwaters from Terreros have been suggested to feed on both Mediterranean and Atlantic waters, with contrasting δ^{15}N baseline levels (*44*). The relationship between nitrogen signatures and OCs found in this study indicates that biomagnification processes not only become apparent across species but also can be detected at the intraspecific level, reflecting the relevance of dietary preferences among seabird individuals. Therefore, our results confirm the utility of combining stable isotope data with pollutant levels when evaluating the relative effect of feeding ecology over large regions in order to provide new insights into the dynamics of contaminants.

The present study emphasizes the suitability of seabirds with wide breeding ranges, such as the *Calonectris* shearwaters, as useful organisms to biomonitor the levels of OCs in pelagic ecosystems. As far as we know, no previous studies have related adverse effects to whole blood OC levels in Procellariiform species. Since species sensitivity to pollutants varies widely and stress factors do not impact upon different species equally, interspecific comparisons are difficult and scarcely representative (*2, 45*). Nonetheless, previous seabird studies have reported some evidence of adverse ecological effects associated to blood OC levels in gulls (*46*). Similarly, studies of Albatross species have indicated possible toxic effects associated with elevated OC concentrations (*11*). In this context, although we cannot assess the possible effects of OCs in shearwater populations, the greater levels found in the Mediterranean compared to Atlantic shearwaters represent an extra stressor element for the Mediterranean populations. Therefore, continued monitoring of OCs in *Calonectris* shearwaters seems an appropriate research direction in order to evaluate their sources and trends across the North Atlantic and Mediterranean subtropical region and their possible impact on the populations.

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Supporting Information Available

Details of analytical methods, sexual determination and its relevance in the present study, Figure S1 (breeding localities map), and Table S1 (PCB congener and DDT metabolites concentrations in Calonectris shearwaters). This material is available free of charge via the Internet at http://pubs.acs.org.

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SUPPORTING INFORMATION

Geographical PCB and DDT Patterns in Shearwaters (*Calonectris* sp.) Breeding Across the NE Atlantic and the Mediterranean Archipelagos

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INCLUDING MATERIALS

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SEXUAL DETERMINATION

Since *Calonectris* shearwaters show a substantial sexual size dimorphism, we used biometrical measurements of birds molecularly sexed in previous studies conducted in the same breeding colonies (*1,2*) to perform a discriminant analyses. Then, we used the obtained discriminant functions to sex our sampled birds. Discriminant functions (*D*) were based on the morphological measurements of bill depth (BD), bill depth at nostril (BDN) and maximum head length (MHL). Since each species show some body size differences, we calculated three separate discriminate functions (*3*): the Cory's (n=141; $D = 0.439\text{MHL} - 1.427\text{BDN} + 0.772\text{BD} - 36.943$, $p<0.001$), the Scopoli's (n=62; $D = 0.178\text{MHL} + 0.084\text{BDN} + 0.612\text{BD} - 33.915$, $p<0.001$) and the Cape Verde (n=24; $D = 0.374\text{MHL} + 0.069\text{BDN} + 0.027\text{BD} - 41.453$, $p<0.001$) shearwater functions. In all functions a positive score indicates male sex. Obtained discriminant functions correctly classified 87.2%, 83.9% and 75.8% and cross-validation correctly classified 86.9%, 82.6% and 70.8% for Cory's, Scopoli's and Cape Verde shearwaters, respectively. Sex of shearwaters from this study was further confirmed by molecular sexing in the 25.3% of the studied cases and by call dimorphism as well as checking the size of the partner in the nest (*4*). Shearwaters from Hyeres Is. were not sexed since we had not morphological measures.

Sex was explored to explain variations in blood OC concentrations at intra-colony level. Although males and females did not show significant differences in OCs, some inferences can be made from our results. Differences in OC burdens between sexes have previously been related to specific sexual foraging strategies or different opportunities of males and females to excrete pollutants, mainly associated with the additional route of females to excrete OCs by transferring them into the eggs (*5*). However, no sexual differences have been found in this study, suggesting similar ecology for both sexes, which agree with our isotopic results and previous studies (*6*). In addition, our results suggest that egg laying does not have a lasting effect on OC concentrations in blood from these species.

CARBON AND NITROGEN STABLE ISOTOPE ANALYSIS

In the laboratory, we sub-sampled 1-2 mg of blood previously homogenised by vortex stirred for 1 min. Sub-samples were dried at 60ºC for 24 h to remove ethanol. From 0.36 to 0.4 mg of homogenized blood were weighed to the nearest µg and placed into tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a ThermoFinnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer *via* a CONFLOIII interface. Stable isotope ratios were expressed in conventional notation as parts per thousand (‰), according to the following equation: $\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$ where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ and $^{13}C/^{12}C$. The standards for ^{15}N and ^{13}C were atmospheric nitrogen (AIR), and Peedee Belemnite (PDB), respectively. Precision and accuracy for $\delta^{13}C$ measurements was ≤ 0.1‰ and ≤ 0.3‰ for $\delta^{15}N$.

PCBS AND DDTS ANALYSIS

The following organochlorine compounds were analyzed: *ortho* PCB congeners #28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #183, #170, #189, #194 and DDTs, including *p,p'*DDT and its two main metabolites, *p,p'*DDD and *p,p'*DDE.

Elimination of ethanol from the blood samples (~0.5 mL) was carried out using a Speed-Vac (Jouan, RC.10.10) at 400 rev min^{-1} and 25° C coupled to a freeze-drier (Telstar, Cryodos-80) at 10 Pa and -80ºC for about 3h, until achieving constant weight of samples. Powdered residues were extracted with 3 mL of n-hexane and 2 mL of concentrated sulfuric acid. The tube was vortex stirred for 30 s and centrifuged at 4000 rpm for 10 minutes. The supernatant n-hexane phase was removed and the remaining sulphuric acid solution re-extracted twice with 2 mL of n-hexane. All hexane extracts were combined and the resulting 7 mL of n-hexane were purified by vortex stirring with 2 mL of sulfuric acid. Then the n-hexane phase was concentrated under a gentle stream of nitrogen.

PCB and DDT concentrations were determined by high resolution gas chromatography with micro-electron capture detection (HRGC-µECD, Agilent Technologies, model 6890N, Palo Alto, CA, USA). Before chromatographic analysis, 1,2,3,4-tetrachloro naphthalene (TCN) and PCB 209 were added as internal standards to correct for instrument variability. Samples were injected in hot splitless mode (1µl, 270ºC, splitless time 1.0 min). A DB-5 capillary column (J&W Scientific, USA; 60m, i.d. 0.25 mm, film thickness 0.25 µm) was used for chromatographic separation. The column temperature was programmed from 80ºC (2 min) to 185ºC (3 min) at a rate of 30ºC min^{-1}, then to 230ºC (10 min) at 1.5ºC min^{-1} and then to 270ºC (10 min). The detector temperature was set at 300ºC. Nitrogen was used as carrier gas (1.5 mL min^{-1}, constant flow) and as make-up gas (10 mL min^{-1}). Organochlorine compounds were identified on the basis of their relative retention times to the TCN and PCB 209 standards on the chromatographic column. Quantification was done within the linear range of the detector's seven-level calibration curve using HP ChemStation Plus program (Hewlett-Packard Co., Palo Alto, CA, USA). For every batch of 7 blood samples a blank sample was included. When results for blank samples were over the LODs they were subtract from the corresponding sample batch. Since sample preparation included an extra-step to remove ethanol from blood samples, five samples were previously spiked with PCB 209 to evaluate the method recovery. The recovery study showed high percentages with values ranging from 77 to 96%.

FIGURE S1. Geographic distribution of the studied breeding colonies. Black points indicate the localities were shearwaters were sampled.

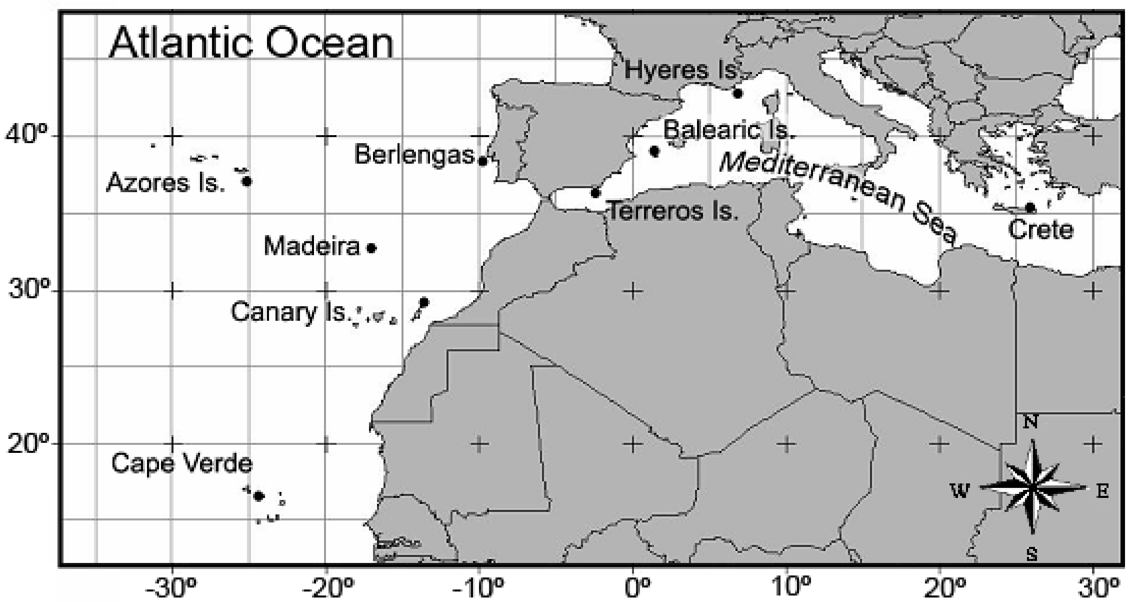

TABLE S1. Means and standard errors of PCBs depending on their chlorination degree and *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD (ng g^{-1} d.w.) in the blood of adult Scopoli's (*C.diomedea*), Cory's (*C. borealis*) and Cape Verde (*C.edwardsii*) shearwaters among their breeding range.

Breeding colony	**≤ *tetra*-PCBs**	***penta*-PCBs**	***hexa*-PCBs**	**≥ *hepta*-PCBs**	***p,p'*-DDT**	***p,p'*-DDE**	***p,p'*-DDD**	**DDE/DDT**
Atlantic								
Cape Verde	15.5 ± 2.7	8.23 ± 1.1	13.4 ± 3.4	6.07 ± 2.44	1.83 ± 0.17	4.81 ± 3.47	1.05 ± 0.17	2.01 ± 1.23
Canary Is.	11.9 ± 2.1	16.1 ± 1.9	23.1 ± 5.2	8.16 ± 2.36	2.54 ± 0.67	9.04 ± 3.81	1.54 ± 0.22	2.84 ± 0.90
Madeira	19.1 ± 1.6	20.4 ± 1.6	58.6 ± 24.5	27.3 ± 10.9	8.48 ± 4.55	27.4 ± 14.3	2.26 ± 0.53	3.64 ± 0.37
Azores Is.	12.7 ± 1.7	28.1 ± 4.0	45.5 ± 8.6	13.0 ± 2.3	7.84 ± 4.43	31.6 ± 16.1	2.70 ± 1.02	4.56 ± 0.44
Mediterranean								
Berlengas	21.1 ± 2.5	16.6 ± 1.7	53.9 ± 7.6	27.8 ± 4.6	4.81 ± 0.85	18.3 ± 2.9	2.15 ± 0.49	4.07 ± 0.59
Terreros Is.	23.1 ± 2.8	26.8 ± 3.1	104 ± 18	58.9 ± 11.3	10.2 ± 1.6	87.7 ± 23.3	2.16 ± 0.27	7.68 ± 0.96
Balearic Is.	18.6 ± 2.9	20.6 ± 1.7	75.4 ± 7.8	43.7 ± 4.2	9.32 ± 1.07	58.5 ± 14.1	1.74 ± 0.21	5.93 ± 0.82
Hyeres Is.	23.8 ± 4.5	43.6 ± 6.9	269 ± 60	174 ± 40	30.3 ± 9.4	270 ± 89	2.99 ± 0.78	9.28 ± 0.93
Crete	19.9 ± 2.3	56.6 ± 7.2	361 ± 58	240 ± 38	41.8 ± 14.7	848 ± 193	4.74 ± 1.59	25.48 ± 3.82
ANOVA Inter-colony	$F_{8,87} = 2.76$ P=0.05	$F_{8,87} = 17.1$ P<0.001	$F_{8,87} = 33.3$ P<0.001	$F_{8,87} = 42.6$ P<0.001	$F_{8,87} = 16.4$ P<0.001	$F_{8,87} = 25.4$ P<0.001	$F_{8,87} = 3.18$ P=0.004	$F_{8,87} = 24.8$ P<0.001

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CHAPTER 3

Polycyclic aromatic hydrocarbons (PAHs) in Atlantic and Mediterranean pelagic seabirds: interspecies and spatial trends

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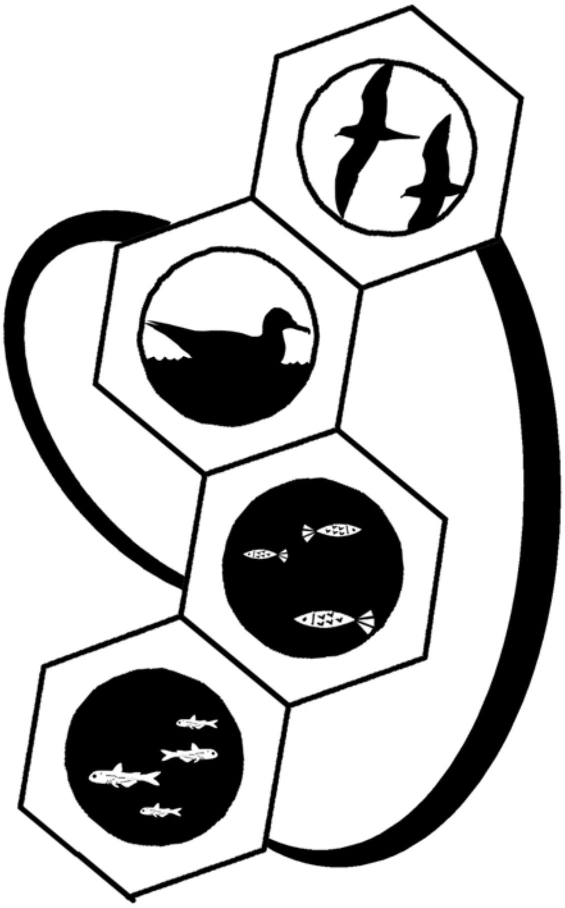

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Hidrocarburos aromáticos policílicos (PAHs) en aves pelágicas del Atlántico y del Mediterráneo: diferencias interespecíficas y espaciales.

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RESUMEN

Los estudios sobre la presencia de hidrocarburos aromáticos policíclicos (PAHs) en la fauna marina se han centrado principalmente en especies de invertebrados y en su mayor parte se han llevado a cabo en regiones costeras altamente contaminadas o en zonas afectadas por vertidos accidentales de crudo. Sin embargo, la capacidad de los PAHs para bioacumularse en los organismos que ocupan los primeros niveles de las cadenas tróficas, su larga vida media una vez incorporados en los sedimentos marinos y el transporte oceánico a gran distancia de estos compuestos pueden contribuir a la presencia de los mismos en regiones pelágicas aparentemente no expuestas a estos contaminantes. La presencia de PAHs en depredadores que ocupan los niveles superiores de las cadenas tróficas marinas se ha investigado en pocos estudios. No obstante, en algunos casos las aves marinas se han propuesto como candidatos ideales para monitorizar la presencia de contaminantes en nuestros océanos. De hecho, los resultados que han generado los escasos estudios centrados en aves marinas resultan contradictorios ya que mientras algún estudio sugiere determinadas aves marinas como buenos indicadores para estos contaminantes, otros indican lo contrario.

El principal objetivo consistió en evaluar el potencial de las aves marinas como posibles indicadoras de la contaminación basal por PAHs en el ambiente marino. Para ello se analizaron las diferencias interespecíficas en la presencia de PAHs en las aves seleccionadas, prestando especial atención a las posibles diferencias relacionadas con el área de cría, es decir, debidas a variaciones geográficas en los niveles basales de estos contaminantes. A tal fin, en el presente estudio se analizó la presencia de los 16 hidrocarburos aromáticos policíclicos propuestos por la EPA como compuestos prioritarios en hígado de 5 especies de aves pelágicas (Orden Procellariiformes). Específicamente, se seleccionaron 3 especies cuya zona de cría comprende el Atlántico noreste, petrel de Bulwer, paíño pechialbo y pardela cenicienta Atlántica, y dos especies que anidan en el Mediterráneo, la pardela Balear y la pardela cenicienta Mediterránea.

Los niveles de PAHs en hígados de aves pelágicas variaron significativamente entre especies, debido principalmente a los niveles más altos que mostraron las especies de petreles respecto a las de pardela. Aunque estudios previos han descrito niveles más altos de distintos contaminantes orgánicos, incluyendo los PAHs, en la cuenca Mediterránea comparada con la Atlántica, los niveles de hidrocarburos obtenidos en este estudio no mostraron diferencias significativas entre pardelas Atlánticas y Mediterráneas. No obstante, los perfiles de hidrocarburos obtenidos en las aves Mediterráneas indicaron un mayor aporte de fuentes antropogénicas para estos compuestos en la región Mediterránea respeto a la Atlántica. Sin embargo, estas diferencias entre los perfiles no fueron lo suficientemente relevantes como para que el análisis de componentes principales (PCA), realizado a partir de las contribuciones relativas de cada PAH analizado, separara las muestras en base a su lugar de procedencia. Esta ausencia de patrones geográficos significativos en la presencia de PAHs indica una menor influencia del área de cría como factor determinante de los niveles de estos contaminantes en aves pelágicas, Este hecho está posiblemente relacionado con la gran capacidad de estas aves para metabolizar y eliminar los PAHs.

Las diferencias en los niveles de hidrocarburos entre petreles (media 32.45 y 29.83 ng/g peso fresco para petrel de Bulwer y paíño pechialbo respectivamente) y pardelas (media 5.56 ng/g, 8.51 ng/g y 6.06 ng/g peso fresco para pardela cenicienta Mediterránea, Atlántica y pardela Balear respectivamente) posiblemente están asociados con las diferencias en la ecología trófica de estos dos grupos. Aunque todas las especies aquí incluidas están situadas en niveles altos de sus respectivas cadenas tróficas, por un lado, los petreles se alimentan principalmente de presas mesopelágicas (200-1000 m de profundidad) que migran verticalmente hasta la superficie, mientras que las pardelas explotan presas epipelágicas (0-200 m de profundidad). La distribución y los flujos verticales de PAHs en la columna de agua, que están marcadamente influenciados por las especies de zooplancton que realizan migraciones verticales, podrían explicar las diferencias en los niveles de hidrocarburos encontrados entre petreles y pardelas.

En conjunto este estudio pone de manifiesto a las aves pelágicas como especies poco apropiadas para la monitorización de hidrocarburos policíclicos aromáticos. No obstante nuestros resultados destacan la importancia de estudios basados en estrategias multiespecíficas como herramientas que ofrecen un mayor intervalo de niveles de contaminación que permitiría determinar mejor el riesgo asociado a las distintas especies. En resumen, las aproximaciones multiespecíficas representan una evaluación más integradora de la contaminación marina.

CHAPTER 3

Interspecies and spatial trends in polycyclic aromatic hydrocarbons (PAHs) in Atlantic and Mediterranean pelagic seabirds

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ABSTRACT: The presence of polycyclic aromatic hydrocarbons (PAHs) in marine wildlife has primarily been studied in invertebrate species from highly contaminated coastal regions or after accidental oil spills. However, the long-range transport of these pollutants could also contribute to significant background PAH levels in remote pelagic environments. In the present study, PAHs were analyzed in the livers of 5 species of pelagic seabirds: the Bulwer's petrel, the white-faced storm-petrel and the Cory's shearwater breeding in the northeast Atlantic, and the Scopoli's and the Balearic shearwater breeding in the west Mediterranean. The main objective was to assess the trophic and geographic trends of PAHs in seabirds to evaluate their suitability as bioindicators of chronic marine pollution by these compounds. Although higher levels of contamination, including PAHs, have been described in the Mediterranean Basin compared to other oceanic regions, we did not find significant spatial patterns and observed only minor effects of the geographic origin on seabird PAH burdens. Nevertheless, the PAH profiles suggested the importance of the anthropogenic sources of these pollutants within the Mediterranean, rather than the Atlantic Basin. Differences between species emerged as the main factors explaining the PAH levels and profiles in the seabirds studied. We found significantly higher PAH levels in the petrels (means 32.45 and 29.83 ng/g ww for Bulwer's petrel and white-faced storm-petrel, respectively) compared to shearwaters (mean 5.56 ng/g ww for Scopoli's, 8.51 ng/g for Cory's and 6.06 ng/g for Balearic shearwaters), which could be related to differences in their exploitation of mesopelagic and epipelagic resources, respectively, and the vertical dynamic of PAHs in the water column. Overall, in the present study, pelagic seabirds emerged as poor indicators of chronic PAH levels in pelagic environments. Nonetheless, this study enhances the need of multi-species approaches to obtain a broader interval of risk levels to represent a more comprehensive evaluation of marine pollution.

KEY WORDS: Organic pollutants; trophic ecology; marine environment; biomonitors; petrels; shearwaters.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants widely detected in both terrestrial (Edwards, 1983) and aquatic ecosystems (Hellou, 1996). PAHs have attracted a lot of scientific interest because they are considered a severe potential threat for wildlife due to their mutagenic, carcinogenic and toxic properties (World Health Organization, 1998; Laffon et. al., 2006) and their capability to bioaccumulate in invertebrate species (Meador et. al., 1995). Studies investigating PAHs have primarily focused on aquatic ecosystems. Once these compounds reach the aquatic medium, they become less susceptible to degradation and tend to be incorporated into sediments with long half-lives (MacRae and Hall, 1998). Therefore, PAHs can continually affect bottom-dwelling organisms and the whole ecosystem via the food web.

Hydrocarbons enter aquatic ecosystems because of human activities, mainly by incomplete combustion of organic matter with subsequent atmospheric deposition and industrial and urban runoff (Van Metre et. al., 2000; Nizzetto et. al., 2008). In marine environments, accidental or intentional discharges from oil tankers, ships and fuel extraction activities are other important sources of PAHs. All of these exposure pathways increase the risk for marine biota. In fact, most PAH studies have focused on marine ecosystems affected by oil spills or coastal areas near highly industrialized regions. However, the documented long-range transport of these pollutants could also contribute to significant background levels of PAHs in remote areas of the open sea (Nizzetto *et al.*, 2008). To our knowledge, few studies have monitored PAHs in less polluted areas, such as in pelagic ecosystems and especially in marine predators in high trophic levels (Kannan and Perrotta, 2008).

As top marine predators, seabirds have been proposed as suitable biomonitors for pollutants such as PCBs or heavy metals (Furness and Camphuysen, 1997). However, in the case of PAHs, the potential for seabirds to be biomonitors has been scarcely explored and their utility remains unclear. Recently, the yellow-legged gull (*Larus michahellis*) was proposed as a good indicator of PAH contamination after comparing levels from colonies polluted by the Prestige oil spill with non-affected areas (Pérez et al., 2008). However, whereas some seabird studies have found relationships between PAH levels and trophic or geographic variables, other studies have not. Studies of PAHs in seabird eggs have documented low concentrations for certain geographic areas and species, suggesting neither biogeographical trends nor interspecific patterns for PAH levels (Shore et. al., 1999; Franson et. al., 2004). Moreover, some authors have reported that PAHs are not commonly found in the tissues of birds from non-contaminated sites, and when PAHs are found, they tend to be present at low levels (Hall and Coon, 1988). In addition, studies reporting interspecific differences in PAH levels in birds found significantly lower levels in bird tissues than in their prey. These studies also found greater PAH burdens in birds feeding on invertebrates (low trophic positions) compared to those feeding on vertebrates, such as fish (higher trophic positions) (Broman et. al., 1990; Kayal and Connell, 1995; Lebedev et. al., 1998; Custer et. al., 2001). Overall, these results agree with studies on the trophodynamics of PAHs in marine food webs, which have revealed an inverse relationship between PAH burdens and trophic position and indicated that biomagnification in food webs is insignificant (Wan et. al., 2007; Perugini et. al., 2007; Nfon et. al., 2008). This general picture concerning PAHs is probably related to the low number of studies using seabirds to monitor PAHs.

In the present study, the PAHs proposed by the U.S. EPA as priority environmental contaminants were analyzed in the livers of pelagic seabirds (O. Procellariiformes). We used a multi-species approach, including petrel and shearwater species with different trophic niches and breeding, separately in the Atlantic and the Mediterranean (Brooke, 2004), to investigate whether PAHs in seabirds result from dietary differences or spatial differences in PAH exposure. The liver was selected as the target tissue to measure PAH exposure associated with the breeding areas of the seabirds. Birds rapidly metabolize and readily excrete PAHs (Broman et al., 1990; Troisi et. al., 2006). For example, 94% of PAHs injected into chicken eggs were metabolized within 14 days (Näf et. al., 1992). Therefore, the liver has been suggested to provide information regarding short-term exposure to PAHs in vertebrates due to its large and rapid detoxification capability (Hellou, 1996). Previous studies have also successfully related liver PAH burdens to the specific wintering grounds of birds (Custer et al., 2001). To evaluate the suitability of pelagic seabirds as indicators of marine PAH contamination, we investigated the relative contribution of the species' trophic ecology and geographic origin to explain their PAH burdens. We also evaluated the sex and age of the seabirds as possible sources of intraspecific variability in PAH levels.

MATERIAL AND METHODS

Species and sampling strategy: Five Procellariform species were selected: the Scopoli's shearwater (*Calonectris diomedea)*, the Cory's shearwater (*Calonectris borealis),* the Balearic shearwaters (*Puffinus mauretanicus*), the Bulwer's petrel (*Bulweria bulwerii*) and the white-faced storm-petrel (*Pelagodroma marina).* Whereas the Scopoli's and Balearic shearwaters breed in the Mediterranean, the others nest across the Macaronesian Archipelagos in the Northeast Atlantic.

Two sampling strategies were applied to obtain fresh whole carcasses from seabird specimens. In the Mediterranean Sea, 10 Balearic and 10 Mediterranean Cory's shearwaters were accidentally caught during their breeding periods between 2003 and 2007 by longliners in the Western Mediterranean. Atlantic specimens were obtained during the same period by the Wildlife Tafira Recuperation Center (Cabildo de Gran Canaria, Gran Canaria, Canary Islands), including 11 Atlantic Cory's shearwaters, 6

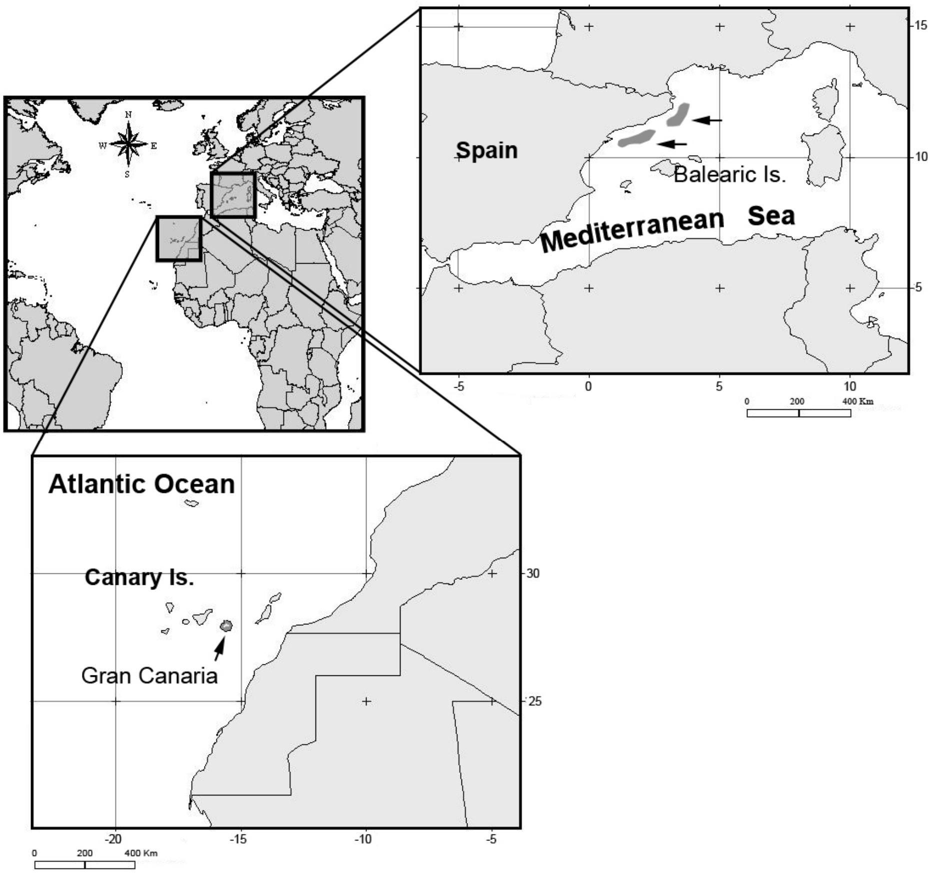

FIGURE 1. Seabird sampling locations. The dark grey areas and arrows indicate the Mediterranean and Atlantic zones where seabirds were collected.

Bulwer's petrels and 9 white-faced storm-petrels, and were all collected within their respective breeding periods. The sampling areas covered in this study are presented in Figure 1. To avoid potential selection biases from unhealthy birds, unrecoverable specimens with fractures euthanized upon arrival to the recuperation center were selected. All of the specimens were frozen and preserved at -24 ºC. Whole livers were extracted by dissection and immediately frozen until analyses.

During the dissection procedure, all birds were sexed and aged. Sex was determined by direct observation of the sexual organs during the dissections. Age was determined by the presence of the bursa of Fabricius (Glick, 1983; Broughton, 1994) and down feathers. Only specimens with no bursa traces were considered adults. Specimens with bursa and down feathers were considered fledglings from the breeding period when they were caught, and those with bursa and no down fathers were considered juveniles. Based on these criteria, all sampled specimens were classified as adults except three Atlantic Cory's shearwaters and two white-faced storm-petrels, which had bursa and down feathers and were therefore classified as fledglings.

PAH Analysis: Fifteen PAHs, among those selected by the U.S. EPA, were analyzed. PAHs ranged from di-aromatics to the hexa-aromatics: naphthalene (Naph), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Ant), fluoranthene

(Flt), pyrene (Pyr), chrysene and benz[a]anthracene (Chry_BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DahA), indeno[1,2,3,-c,d]pyrene (IcdP) and benzo[g,h,i]perylene (BghiP).

Depending on the species, 1 to 4 g of fresh liver was used for PAH analysis. PAHs in the liver samples were analyzed using the procedure described by Bordajandi et al. (2004) with some modifications. Briefly, the liver samples were homogenized with anhydrous sodium sulfate. Sample homogenates were Soxhlet extracted for 8 h with dichloromethane. Further cleanup was performed using an activated silica gel column. The first fraction, eluted with 25 mL of hexane, was discarded, and a second fraction containing the selected PAHs was eluted with 25 mL of hexane/dichloromethane (3:2, v/v). The extract was concentrated to a final volume of 1 mL in acetonitrile and filtered using a 0.45 μm syringe filter (Millex HV). Five liver samples and a blank sample were included in every batch.

PAHs were analyzed using an HPLC Jasco system consisting of a PU-1580 pump coupled to an HG-1580-31 mixer and an FP-920 fluorimetric detector with programmable excitation and emission wavelengths. Separation was achieved using a Spherisorb ODS2-C_{18} column (25cm x 4.6 mm and 5 μm particle size, Waters). A 20 μL loop was used to inject the samples. The initial mobile phase was acetonitrile/water (Mili-Q) (60:40, v/v), and separation was obtained using a gradient with acetonitrile increasing from 60 to 100% in 20 min at a flow rate of 1 mL/min. A calibration curve using the PAH MIX calibration mixture showed good linearity in 7 levels from 0.001 ng/μl to 0.2 ng/μl. The repeatability was determined at a concentration level of 0.02 ng/μL and presented relative standard deviations (RSDs) below 7% for all the PAHs, except pyrene (13%). The reproducibility was also satisfactory, with RSD values below 6%. The instrumental limit of detection (LOD), calculated as 3 times the signal-to-noise ratio, ranged between 0.7 and 8.4 pg/μL, except for dibenz[a,h]anthracene (13.0 pg/μL). Recovery studies showed acceptable percentages for most of the analytes, with values ranging from 70 to 110%.

Data analysis: SPSS 15.0 for Windows was used for statistical analysis. The contaminant concentrations were log transformed (using base 10 logarithms) to generate a normal distribution and homogeneity of variance. Concentrations below the detection limit were set to zero to calculate the mean and for statistical analysis. The distribution normality of the total PAH levels ($\sum_{15}$PAHs, the sum of the 15 individual PAHs analyzed) was validated by checking the Q-Q plots and the Shapiro-Wilk test (all $p>0.05$). To simultaneously assess the differences in the PAH exposures among species and sexes, we compared the ∑PAH levels of adult specimens using a two-way ANOVA. Then, we used post-hoc paired T-tests (with Bonferroni adjustment) to evaluate differences in PAH levels among species. Intraspecific comparisons between ∑PAH burdens in seabirds sampled in different years (only between years with more than 2 sampled individuals) were performed by means of Mann-Whitney U-test since non-normal distribution was found among years for each species. Species fingerprints were calculated as the average percent of each PAH with respect to the ∑PAHs. Comparisons among fingerprints were performed by grouping PAH congeners according to the number of aromatic rings: di- (Naph), tri- (Ace, Fl, Phen and Ant), tetra- (Flt, Pyr, Chry and BaA), penta- (BbF, BkF, BaP and DahA) and hexa-cyclic (IcdP and BghiP) PAHs. Finally, a principal component analysis (PCA) was applied to the contributions of all PAHs (except Ant, Chy, BaA and BkF which were under the limit of detection in all samples and thus cannot explain variability) to check whether seabird PAH profiles are associated with breeding locality or species. Concentrations are expressed in ng/g (wet weight basis) and as mean ± standard error, except where otherwise indicated.

RESULTS

PAHs were found in all of the 45 livers analyzed. We did not find any significant intraspecific relationships between sampling year and the ∑PAHs. Similarly, intraspecific comparisons (Mann-Whitney U-test) between seabirds sampled in different years did not result significant (all P>0.05), which suggests that the sampling year did not introduce a significant bias in our work. Intraspecific comparisons between age groups were not possible due to the low number of fledglings. Nevertheless, the ∑PAH concentrations (Table 1) and the number of PAHs detected (Table 2) in fledglings were markedly lower in fledglings than in adults. In fact, the ∑PAHs in Cory's shearwater and white-faced storm-petrel fledglings were below the range of the levels obtained in adults from each species.

TABLE 1. $\sum_{15}$PAH concentrations in the livers of pelagic seabirds breeding in the Mediterranean and the Atlantic

Species	Age	n	Arithmetic Mean	Standard Error	Geometric Mean	Range
Mediterranean						
Balearic shearwater	Adults	10	6.07	0.97	5.27	1.91 – 10.3
Scopoli's shearwater	Adults	10	5.56	0.96	4.64	1.59 – 8.93
Atlantic						
Cory's shearwater	Adults	8	8.52	1.57	7.47	3.32 – 17.1
	Fledglings	3	1.60	0.47	1.48	1.02 – 2.54
Bulwer's petrel	Adults	6	32.5	7.43	29.1	17.2 – 66.2
White-face storm-petrel	Adults	6	29.8	2.99	29.0	18.5 – 39.8
	Fledglings	2	11.1	3.85	10.4	7.27 – 14.9

Bulwer's petrels and white-faced storm-petrels showed the greatest concentrations for most of the PAHs among the adult specimens (Table 2). The two-way ANOVA tests showed that the ∑PAH concentrations (Table 1) differed significantly among species ($F_{40,4}$=20.47 and P<0.001), but neither sex nor the interaction species*sex showed a significant effect ($F_{40,1}$=3.40, P=0.08 and $F_{40,4}$=1.18 and P=0.34, respectively) (Figure 2). A post-hoc T-test (Bonferroni adjustment) showed that the petrels differed significantly from the shearwaters (P<0.05). No significant differences for the ∑PAHs were detected with the post-hoc T-test within petrel species or within shearwaters. Therefore, the differences between species were due to the higher levels found in the petrels compared to the shearwaters (Figure 2).

TABLE 2. Mean, standard error and range (in brackets) of PAHs (ng\g w.w.) analyzed in the livers of adult and fledgling Procellariiformes.

	Balearic shearwater	**Scopoli's shearwater**	**A. Cory's shearwater**		**Bulwer's petrel**	**White-faced storm-petrel**	
Age	Adult	Adult	Adult	Fledgling	Adult	Adult	Fledgling
n	10	10	8	3	6	6	2
Naph	1.68 ± 0.21 (0.87, 2.79)	0.84 ± 0.35 (–, 28)	3.54 ± 0.87 (0.92–8.18)	0.6 ± 0.38 (–, 1.29)	7.08 ± 1.35 (10.00-2.45)	9,79 ± 1,34 (4.46–12.91)	1.41 ± 0.71 (0.7-2.13)
Ace	0.31 ± 0.09 (–[a], 0.88)	0.09 ± 0.05 (–, 0.39)	0.04 ± 0.03 (–, 0.25)	–	1.7 ± 0.41 (–, 2.57)	1,14 ± 0,43 (–, 2.38)	–
Fl	1.05 ± 0.32 (–, 6.55)	0.07 ± 0.05 (–, 0.53)	0.24 ± 0.09 (–, 0.71)	–	13.30 ± 2.43 (8.48–22.82)	10,55 ± 3,4 (0.84-20.43)	–
Phen	0.49 ± 0.18 (–, 1.89)	0.62±0.17 (–, 1.71)	1.03 ± 0.26 (–, 2.26)	–	5.95 ± 2.7 (0.30-18.09)	2,23 ± 0,44 (0.65-3.74)	0.08 ± 0.08 (–, 0.16)
Ant	–	–	–	–	–	–	–
Flt	0.48 ± 0.08 (–, 0.62)	0.89 ± 0.31 (–, 3.24)	0.86 ± 0.28 (–, 2.39)	–	3.34 ± 2.05 (–, 12.21)	1,73 ± 0,50 (0.63-3.72)	–
Pyr	2.01 ± 0.38 (–, 3.25)	2.53 ± 0.54 (–, 5.63)	2.22 ± 0.56 (0.44–5.75)	1.00 ± 0.15 (0.72-1.25)	0.74 ± 0.39 (–, 2.52)	4,16 ± 2,57 (–, 16.70)	9.6 ± 3.07 (6.53-12.67)
Chry_BaA	–	–	–	–	–	–	–
BbF	–	0.02 ± 0.01 (–, 0.12)	0.03 ± 0.02 (–, 0.15)	–	0.32 ± 0.32 (–, 1.92)	–	–
BkF	–	–	–	–	–	–	–
BaP	–	0.07 ± 0.03 (–, 0.36)	0.11 ± 0.07 (–, 0.52)	–	–	–	–
DahA	–	0.27 ± 0.25 (–, 2.56)	0,34 ± 0.33 (–, 2.67)	–	0.12 ± 0.12 (–, 0.71)	0,14 ± 0,05 (–, 0.82)	–
IcdPyr	0.05± 0.01 (–, 0.46)	0.10 ± 0.05 (–, 0.47)	0,11 ± 0.05 (–, 0.42)	–	–	–	–
BghiPer	–	0.05 ± 0.04 (–, 0.35)	–	–	–	0,11 ± 0,11 (–, 0.66)	–

[a] – Below the detection limit or not detected.

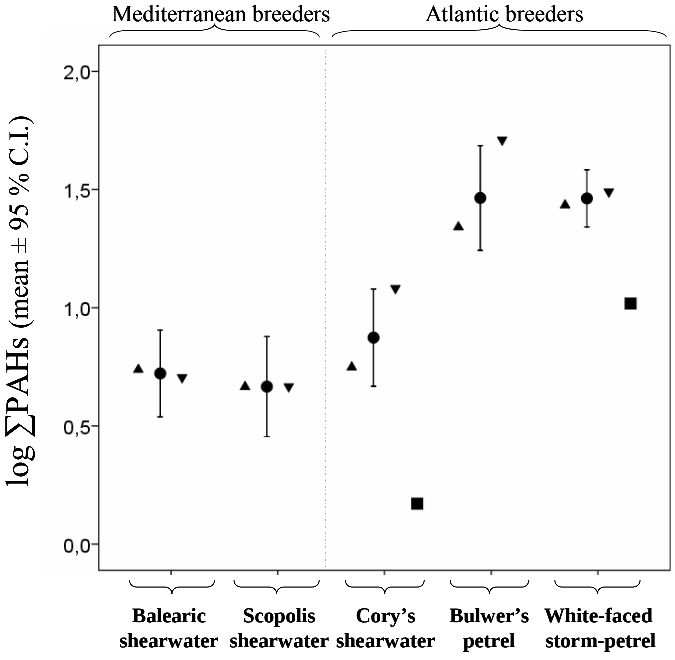

FIGURE 2. ∑PAH levels in the livers of Atlantic and Mediterranean adult seabirds (circles). Triangles and inverted triangles show the mean values obtained for males and females, respectively. Squares indicate the mean levels from Cory's shearwater and white-faced storm-petrel fledglings

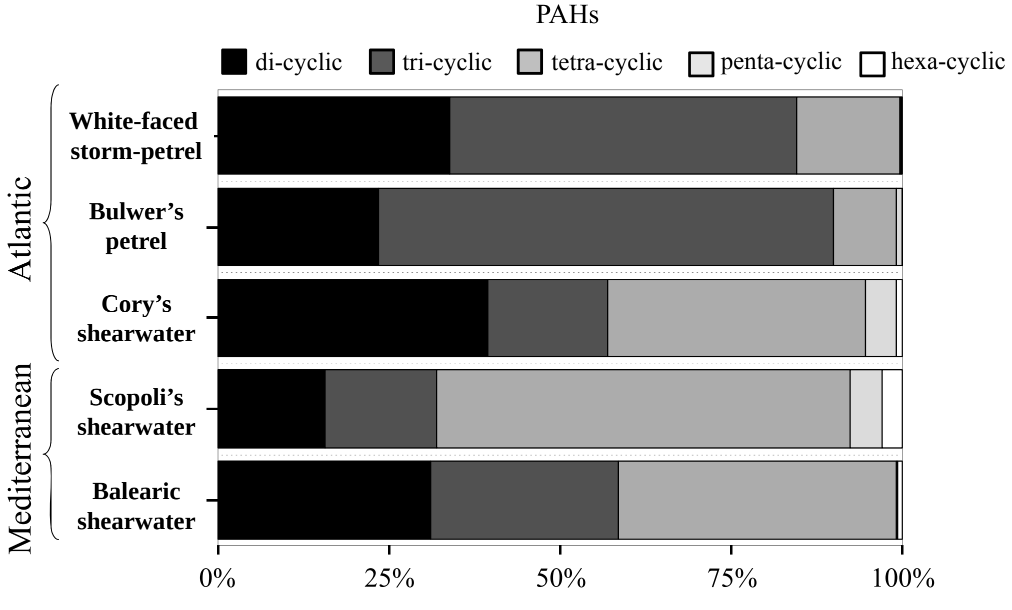

FIGURE 3. PAH profiles in adult seabird livers calculated as the percent of each PAH with respect to $\sum_{15}$PAH

Although PAH profiles in seabird livers differed among species (Figure 3), we did not find homogeneous patterns among species or among breeding regions (i.e. Altantic vs. Mediterranean Basins). That is, Cory's shearwaters and Balearic shearwaters breeding in the Atlantic and the Mediterranean, respectively, showed similar contribution of low (di- and tri-cyclic PAHs; 56.94% for Cory's shearwaters and 58.38% for Balearic shearwaters) and high (tetra-, penta- and hexa-cyclic) molecular weight PAHs in their profiles; pyrene (27.05% for Cory's shearwaters and 32.23% for Balearic shearwaters) and naphthalene (39.39% for Cory's shearwaters and 30.92% for Balearic shearwaters) were the most abundant congeners. However, whereas high molecular weight PAHs predominated in Scopoli's shearwaters (68.2%) breeding in the Mediterranean, low molecular weight PAHs were the most abundant compounds in livers from Bulwer's petrels (89.77%) and white-faced storm-petrels (84.43%) from the Atlantic. The abundance of tri-cyclic PAHs in livers from petrel species compared to the rest of species was mainly due to the greater contributions of fluorene (46.42% and 37.36% for Bulwer's petrels and white-faced storm-petrels, respectively), which was the most abundant PAH in their profiles. In the case of Scopoli's shearwaters, the dominant presence of tetra-cyclic PAHs was due to the abundance of pyrene (47.68%) compared to the remaining PAHs.

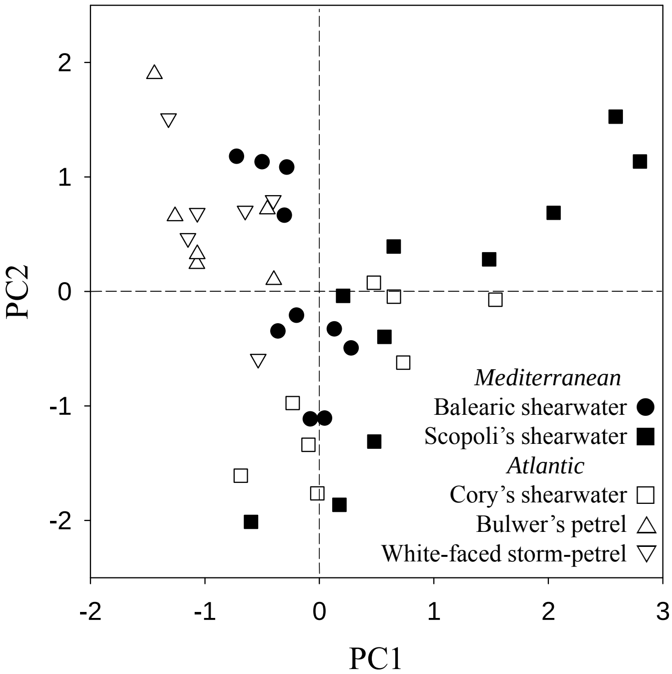

Figure 4. Principal components (PC1-PC2) extracted from the relative contributions of the analyzed PAHs

These results were in agreement with the principal component analyses (PCA) of the relative abundance of all the analyzed PAHs (Figure 4). We obtained two principal components, PC1 and PC2, accounting for 27.18 and 15.01 % of the variance, respectively. Their graphic representation did not allow the grouping of seabirds by species or by breeding locality alone. Although most petrels were clearly segregated from *Calonectris* shearwaters, Balearic shearwaters appeared within both groups (Figure 4). The component matrix indicated that PC1 was negatively associated with Fl, Ace and Naph (ordered from higher to lower coefficients) and positively with Pyr, BaP and Flt. PC2 was negatively associated with Phen abundance and positively with Ace and Fl. Therefore, PCA analysis segregated between *Calonectris* shearwaters and petrels mainly due to the major presence of di- and try-cyclic PAHs in petrels.

DISCUSSION

To our knowledge, this is the first study in which PAH levels have been evaluated in five pelagic seabirds breeding in two basins: the Northeast Atlantic Ocean and the Mediterranean Sea. Whereas the ∑PAH levels differed among species due to the significantly greater concentrations found in Bulwer's petrel and white-faced storm-petrels related to the shearwater species (Figure 2), sex was an insignificant factor in explaining seabird PAH burdens. Interspecies differences in PAH profiles also emerged mainly between petrels and shearwaters; PCA analysis mostly segregated petrel species from shearwaters (Figure 4). The lack of differences in PAHs among shearwaters breeding in the Atlantic and the Mediterranean reflected a minor effect of breeding locality on PAH levels in pelagic seabirds.

Temporal differences in PAH burdens have been documented in marine vertebrates mainly after local accidental discharges of petroleum into marine ecosystems (Marsili et. al., 2001; Soriano et. al., 2006; Pérez et. al., 2008). However, similar relevant oil spills have not been documented in our sampling areas from 2003 to 2007. As far as we know, few studies have reported differences related to the year of sampling in PAH burdens of vertebrates from areas not receiving direct pollution. In the few studies that did, temporal trends emerged in long-term temporal assessments, such as inter-decade comparisons (Kannan et al., 2008). In this study, we did not find any significant relationship between the ∑PAH levels and sampling years within the studied species. Therefore, although we cannot completely exclude some variability in PAHs due to different sampling years, it does not seem to shift our results.

Because interspecific comparisons of PAHs between regions can be confounded by trophic or detoxification efficiency differences among species, in this study, we focused on PAH differences among *Calonectris* shearwaters (Cory's and Scopoli's shearwaters) to evaluate possible spatial differences in PAHs. The Scopoli's and the Cory's shearwaters are closely related forms until recently considered subspecies of *C.diomedea* and show very similar morphology, ecology and physiology (Brooke, 2004; Gómez-Díaz and González-Solís, 2007; Gómez-Díaz et. al., 2009). Therefore, because no differences in the feeding ecology or metabolic capabilities of *Calonectris* shearwaters are expected, differences in PAHs between them should be related to geographic contamination patterns. Previous studies found marked geographic patterns in POPs, such as PCBs or DDTs, and heavy metals in *Calonectris* shearwater breeding in the Atlantic and the Mediterranean due to the greater levels of these pollutants in the Mediterranean Basin (Ramos et. al., 2009; Roscales et al., 2010). In fact, the Mediterranean Sea is a confined water mass with hydrogeographical conditions that have resulted in higher levels of contamination, including PAH contamination, compared with other regional seas (Martí et. al., 2001; Albaigés, 2005). However, we did not find significant differences among the PAH burdens of *Calonectris* shearwaters breeding in the Atlantic and

Mediterranean. This lack of differences is likely related to the rapid elimination of PAHs by birds, masking geographic patterns of baseline marine PAH levels. Nevertheless, some dissimilarities in Scopoli's and Cory's shearwater PAH profiles could be related to their breeding grounds.

The Scopoli's shearwater PAH profiles were clearly dominated by high-molecular-weight compounds (tri-, tetra-, penta- and hexa-cyclic; Figure 3). However, the Cory's shearwater profiles showed similar contributions of high and low-molecular-weight-PAHs. Low-molecular-weight-PAHs are the primary constituents of natural petroleum seeps, rather than urban sources of PAHs (Seruto et. al., 2005; Kannan *et al.*, 2008). Therefore, the presence of heavy PAHs in Scopoli's shearwaters could be related to the greater presence of anthropogenic sources of PAHs in the Mediterranean compared to the Atlantic. Pyrene (47.7%) was the most abundant compound in the Scopoli's shearwaters' PAH profiles, resulting in the dominance of tetra-cyclic compounds (60.48%) compared to the other PAHs. The greater abundance of pyrene is consistent with previous studies of PAHs in particulate matter in surface waters from the Western Mediterranean Sea. Particulate matter collected across the areas where the seabirds were collected and across their common feeding grounds on the Northeast Spanish coasts showed PAH profiles dominated by pyrene, likely caused by river runoffs (Dachs et. al., 1997). However, the PAH profiles were not different enough between Atlantic and Mediterranean species to be segregated by the PCA analysis, indicating only a minor importance of the breeding ground locations to PAH levels. In Balearic shearwaters, also breeding and feeding in the west Mediterranean, pyrene and tetra-cyclic PAHs were also the most abundant PAHs. Nevertheless, this species showed a greater proportion of low-molecular-weight-PAHs than Scopoli's shearwaters, which could be related to different feeding habits or metabolic capabilities between the species.

Differences in the PAH profiles and levels in this study emerged mainly when comparing petrels with the remaining species. The Bulwer's petrels and the white-faced storm-petrels showed significantly higher levels of the ∑PAHs than shearwaters. Moreover, the PCA segregated most petrels from the rest of the seabirds due to the higher presence of di- and try-cyclic PAHs in these species. Because the geographic origin did not explain the interspecific differences in PAH levels in seabirds, a different exposure to these contaminants through diet or different metabolic capability among species are the most probable hypotheses to explain the highest PAH burdens found in the petrels. Previous studies on Mixed-Function Oxidase (MFO) in seabirds found little enzymatic activity differences among pelagic species. A comparison between Leach's storm-petrels (*Oceanodroma leucorhoa*) and Manx shearwaters (*Puffinus puffinus*) showed similar activity between these species for most hepatic enzymes, with the exception of the aldrin epoxidase, 7-EROD and expoxide hydrolase, which showed higher activity in the case of the storm-petrel (Knight and Walker, 1982; Peakall et. al., 1987). These small differences in the MFO activity between storm-petrels and shearwaters could be related to the differences we found in the PAH profiles of Bulwer's petrels and white-faced storm-petrels. The greater abundance of low-molecular-weight-PAHs in petrels than in shearwaters may be explained by the greater capability of petrels to metabolize the larger PAH compounds, resulting in a low presence of high-molecular-weight PAHs in their profiles. Therefore, the greater PAH levels in petrels compared to *Calonectris* and Balearic shearwaters do not seem to be related to a major detoxification capability of shearwaters, but to a different exposure to these compounds through their diet.

The diet of Cory's shearwaters is mainly composed of epipelagic (0-200 m depth in the water column) fish, although cephalopods, crustaceans and plankton are also included in lower proportions (Granadeiro et. al., 1998). Bulwer's petrel is considered a mesopelagic (200-1000 m) feeder with a diet composed primarily of vertically migrating mesopelagic organisms (Monteiro et. al., 1996; Monteiro et. al., 1998). This petrel also feeds mainly on fish (Myctophidae), followed by cephalopods, crustaceans and plankton. To our knowledge, there are no studies investigating the diet of white-faced storm-petrels breeding in the Atlantic, but it is expected to be similar to the diet of Bulwer's petrel because this occurs with other petrels from the same archipelagos (Monteiro *et al.*, 1996; Bolton et. al., 2008). Although petrels and shearwaters feed on different prey types, both are essentially third-order consumers in their food chains, and previous studies suggested no differences in the trophic positions of these species (Monteiro et. al., 1995; Monteiro *et al.*, 1998). Therefore, our results suggest that mesopelagic feeders are exposed to higher PAH burdens than epipelagic consumers, which could be related to specific differences in the behavior and distribution of these pollutants across the water column. However, few works have focused on PAHs in mesopelagic food-chains or deep-sea organisms. Higher PAH burdens in mesopelagic, rather than epipelagic ecosystems have not been described, and previous studies showed a general surface-enrichment depth-depletion distribution for these pollutants in the water column (Dachs *et al.*, 1997; Schulz-Bull et. al., 1998; Martí *et al.*, 2001). Nevertheless, some authors suggested that the vertical distribution of PAHs in the Mediterranean and the Atlantic seems to be governed by planktonic bioaccumulations at open sea, with a submaxima concentration of PAHs in zooplanktonic spots at 300 m depth. This suggests that migratory zooplankton support the flux of hydrophobic pollutants in the water column (Hobson, 1993; Dachs *et al.*, 1997; Hodum and Hobson, 2000; Jaward et. al., 2004). Migratory Mycthopyds feed mainly on these spots of zooplankton, and they follow them in their daily vertical migration, which could contribute to a greater exposure to PAHs. Therefore, feeding specialization of seabirds on mesopelagic prey emerges as a possible explanation for our results and implicates mesopelagic food webs as the most susceptible to accumulate and transfer PAHs to marine predators. Further investigation of PAH levels in Myctophids and in the trophic and vertical flux of PAHs is needed to evaluate this hypothesis.

In this study, we also analyzed PAH levels in Atlantic Cory's shearwater and white faced storm-petrel fledglings. The PAH levels in fledglings were expected to reflect the local food web exposure because they are fed by their parents for several months until they fly. Differences between the white-faced storm-petrel and the Cory's shearwater fledglings were consistent with those found between adults. In addition, fledglings from both species showed lower ∑PAH levels than adults. The low PAH levels found in fledglings is probably related to the greater metabolic rate and the shorter exposure time of growing chicks. In addition, previous studies of seabirds have documented that some species tend to feed their chicks higher trophic levels preys (mainly fish) compared to the food the parents consume (Broman *et al.*, 1990). Because PAH levels were inversely related to trophic position, dietary differences could also attribute to the differences we found in this study between PAH levels in fledglings and adults.

Comparisons of PAH levels with other marine predators are problematic. PAH levels depend on the manner in which the concentrations are expressed (wet or dry weight) and vary broadly among tissues. Accordingly, we compared our results with those reported by Troisi et al. (2006) in liver of oiled common guillemots (*Uria aalge*) from the North Atlantic. They found a mean concentration of 250 ng/g ww for $\sum_{10}$PAHs (ranging from 40 to 970 ng/g). In general, our results showed lower total PAH concentrations than those found in common guillemots, which is expected for oiled seabirds. Nevertheless, petrels showed PAH burdens around 40 ng/g and one Bulwer's petrel specimen exceeded this value. Although the proportion of carcinogenic or high-molecular-weight PAHs found in our study is significantly lower than in oiled common guillemots, the ∑PAH found in some petrels from this study are similar to the concentrations obtained for oiled seabirds, which might be of concern.

CONCLUSIONS

Overall, our results indicate that PAH levels in seabirds depend mainly on the species. The lack of significant geographic patterns in seabird PAH levels, even comparing water masses with different baseline contaminant levels such as the Mediterranean and the Atlantic, suggest that pelagic seabirds are poor indicator species of background PAH levels in the marine environment. Nevertheless, species feeding on mesopelagic preys seem to be exposed to more PAHs than those feeding on surface resources. This suggests that mesopelagic food webs could be more susceptible to accumulate and transfer PAHs to marine predators. Although further studies are needed to verify this hypothesis, our results suggest that seabirds may be useful for biomonitoring of the vertical distribution of PAHs in the water column. Moreover, this study proposes a multi-species approach to achieve a broader interval of risk levels due to PAH exposition and to represent a more comprehensive evaluation of marine PAH contamination.

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CHAPTER 4

Geographic and trophic patterns of OCs in pelagic seabirds from the NE Atlantic and the Mediterranean archipelagos: a multi-species and multi-locality approach

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Capitulo 4.

Patrones geográficos e interespecificos en la presencia de OCs en aves pelágicas: una aproximación multi-especifica en diversas localidades del Atlántico NE y el Mediterráneo.

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RESUMEN

La ecología trófica y la distribución geográfica son dos factores clave que influyen fuertemente en los niveles de contaminantes organoclorados (OCs) en vertebrados marinos. Sin embargo la influencia relativa de estos dos factores es a menudo difícil de diferenciar, limitando la utilidad de las especies marinas como indicadores de la contaminación. Estudios previos han puesto de manifiesto la utilidad de los isótopos estables para examinar las diferencias tróficas entre poblaciones de una misma especie o entre especies distintas, permitiendo así evaluar la influencia de la ecología trófica sobre los niveles de OCs. Concretamente, los ratios de los isótopos estables de nitrógeno ($\delta^{15}N$) se han utilizado ampliamente para delinear la posición trófica de los consumidores marinos. De hecho, algunos trabajos han encontrado relaciones positivas y significativas entre los valores de $\delta^{15}N$ y los niveles de OCs en la fauna marina (a nivel inter- e intraespecífico) reflejando el proceso de biomagnificación de estos compuestos a lo largo de las cadenas tróficas.

Con el fin de evaluar la influencia relativa de la ecología trófica y la distribución geográfica sobre los niveles de OCs en aves marinas se determinaron los niveles de PCBs, DDTs y $\delta^{15}N$ en sangre de la mayor parte de especies de aves pelágicas cuya área de cría se extiende a lo largo del Atlántico NE y el Mediterráneo. Concretamente este estudio incluye Pardela cenicienta (del Mediterráneo y del Atlántico), Pardela chica, Pardela de Cabo Verde, Pardela chica de Cabo Verde, Pardela Balear, Pardela Mediterránea, Petrel de Bulwer, Paíño de Madeira y Paíño Europeo. Cada especie fue muestreada en diversas localidades con el objetivo de poder evaluar las variaciones geográficas en los niveles de OCs.

Los niveles de PCBs y DDTs mostraron variaciones significativas y consistentes tanto entre las especies como entre las distintas localidades muestreadas. Las

variaciones geográficas de los niveles de OCs fueron similares para todas las especies muestreadas. Igualmente las diferencias que se encontraron en los niveles de contaminación de las especies fueron similares en todas las localidades muestreadas. Sin embargo, estas variaciones interespecíficas en los niveles de OCs no se correspondieron con variaciones similares de δ^{15}N. De hecho, tan solo se encontró una relación significativa entre δ^{15}N y los niveles de OCs en el caso de las aves muestreadas en Azores, indicando que otros factores, además del nivel trófico están influenciando fuertemente la acumulación de estos contaminantes en las aves.

Los patrones geográficos encontrados en este estudio se caracterizaron por niveles de OCs claramente más altos en las especies que anidan en la cuenca Mediterránea. El carácter confinado del Mediterráneo así como el abundante vertido de estos contaminantes por parte de los países industrializados de Europa concuerdan con nuestros resultados. Además, dentro del Atlántico, los niveles de contaminantes aumentaron de sur (Cabo Verde y Canarias) a norte (Madeira y Azores) en todas las especies muestreadas y los perfiles de DDTs encontrados en Cabo Verde y Canarias sugieren un uso reciente de este pesticida en las zonas de alimentación de las aves de estos archipiélagos. Los niveles más altos encontrados en los archipiélagos de Azores y Madeira podrían estar relacionados con la influencia de la corriente del golfo sobre estas regiones, susceptible de transportar contaminantes desde las costas norte-americanas hasta el centro-norte del Atlántico. El uso reciente de DDT que sugieren los perfiles de las aves de Canarias y Cabo Verde está posiblemente asociado con el uso de este pesticida en los países subsaharianos.

Estudios previos así como el gran solapamiento que encontramos en los valores de δ^{15}N de las especies muestreadas indican que no existen diferencias marcadas en su posición trófica. Sin embargo, las especies de aves marinas que se alimentan de recursos mesopelágicos (200-1000 m), principalmente especies de peces (Mictófidos) que realizan diariamente migraciones verticales, mostraron niveles de OCs significativamente más altos que las que consumen recursos epipelágicos (0-200 m). Por tanto, nuestros resultados sugieren que las especies de aves que explotan niveles tróficos similares, pueden consumir también recursos de distintas profundidades, lo cual, resulta en niveles marcadamente diferentes de contaminantes.

En conjunto, este estudio corrobora a las aves marinas pelágicas no sólo como potenciales indicadores de las variaciones geográficas de los niveles de OCs en nuestros océanos, sino también como posibles indicadores de su distribución vertical en la columna de agua. Por tanto, nuestra aproximación multiespecífca supone una evaluación más exhaustiva del estado de contaminación por OCs y los posibles riesgos asociados a la misma en el caso de las aves marinas.

Geographic and trophic patterns of OCs in pelagic seabirds from the NE Atlantic and the Mediterranean: a multi-species/multi-locality approach

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ABSTRACT: Trophic ecology and geographic location are crucial factors explaining OC levels in marine vertebrates, but these factors are often difficult to disentangle. To examine their relative influence, we analyzed PCBs, DDTs and stable-nitrogen isotope signatures in the blood of several pelagic seabird species across multiple breeding localities from the northeast Atlantic and western Mediterranean. Large scale geographic patterns emerged due to the confined character and greater historical OC inputs in the Mediterranean compared to the Atlantic basin. Spatial patterns also emerged at the regional scale within the Atlantic basin, probably associated with long-range pollutant transport. Trophic ecology, however, also was a major factor explaining OC levels. We found clear and consistent OCs differences among species within each breeding locality. However, seabird $\delta^{15}N$ greatly overlapped and blood OC levels were not correlated with $\delta^{15}N$. This indicated that different seabird species may show similar trophic positions but, due to vertical migrations of some fish and squid species, may feed on prey from different levels of the water column, resulting in different OC burdens and obscuring the biomagnification processes. In sum, the present study suggests that multispecific approaches could be used to monitor the vertical dynamics of OCs in the marine environment, thereby providing a more comprehensive evaluation of marine pollution.

INTRODUCTION

Organochlorine contaminants (OCs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl ethane (DDT), are globally found in marine food chains and are known to have a wide array of adverse effects (*1*). These contaminants bioaccumulate and biomagnify throughout marine food webs due to their persistent and lipophilic properties (*2,3*). As a result, significant concentrations of OCs have been reported among marine organisms at high trophic positions, making them particularly susceptible to toxicological effects, but also providing opportunities for monitoring marine pollution (*4,5*). Among marine predators, seabirds have been proposed as useful bioindicators for OCs, mainly because many of them are placed at high trophic positions, breed at specific locations and show large-scale distributions (*6*). Despite the potential of seabirds to monitor contamination from different ocean habitats, most studies concerning OC levels in seabirds are restricted to coastal species, and few of them have dealt with species from pelagic ecosystems. This is unfortunate because pelagic seabirds can integrate contaminant levels of areas relatively unexploited by fisheries (*7*), and therefore, poorly explored and difficult to monitor for contaminants.

The limited data on OCs with respect to pelagic seabirds is probably related to the ethical and technical limitations of the sampling strategies (*8,9*), because common tissues for OC analysis (e.g., fat, liver or muscle) involve animal killing or unpractical sampling procedures, such as carcass collection. Nevertheless, thanks to the improvements in analytical skills, we can now survey a wide array of organic contaminants from small blood quantities obtained with negligible impact on the birds. Previous studies have validated the use of blood to evaluate OC levels in seabirds and have successfully used this tissue to evaluate recent exposition to marine contamination (*8,10-12*). In addition, blood can also be used to decipher seabird trophic ecology by means of stable isotope analysis. Specifically, the stable-nitrogen isotope ratio ($^{15}N/^{14}N$; $\delta^{15}N$) has been widely used to delineate the trophic position of seabirds (*13*). In fact, previous studies have found significant positive relationships between blood OC levels and $\delta^{15}N$, reflecting the biomagnification processes (*14,15*). However, because the accumulation of OCs is not determined by trophic level alone, partial differences between $\delta^{15}N$ and OC levels in seabirds have been reported, suggesting that geographic distribution, specific dietary habits or metabolic capabilities can also play a significant role in seabird pollution burdens (*9,16,17*). In this regard, multispecific and multilocality approaches can help to better understand the different contributions of trophic level compared to other factors on OC burdens.

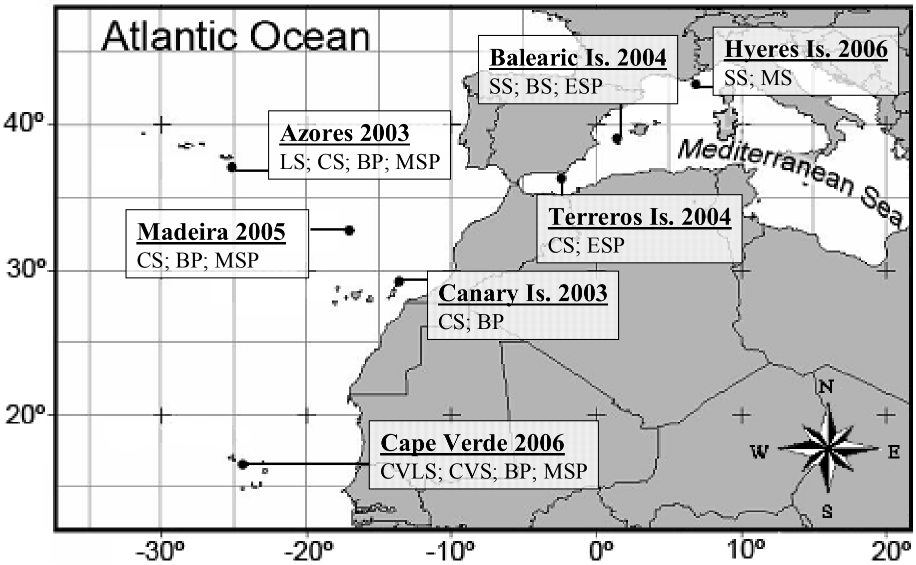
The present study focuses on OCs in pelagic seabirds breeding across the northeast Atlantic and the Mediterranean Sea. Some species are considered vulnerable or threatened, and previous studies have pointed out the need to improve our knowledge about the ecology and contaminant status of seabirds from these regions (*18,19*). However, few studies dealing with organochlorine contamination in pelagic vertebrate species have been conducted within these regions. In this study, organochlorine levels and stable isotope signatures of nitrogen were analyzed in blood from nine species of pelagic seabirds at seven breeding localities across the NE Atlantic and W Mediterranean archipelagos. We aimed (1) to evaluate the relative influence of geographic location and feeding ecology over OCs in pelagic seabirds; (2) to validate

OC geographic patterns and sources previously assessed trough a single-species approach (*15*); (3) to understand the biomagnification process of OCs by relating PCB and DDT levels from different species to their feeding ecology.

MATERIAL AND METHODS

Species, study area and sampling procedure: We sampled most petrels and shearwaters (9 species) breeding on the NE Atlantic Ocean and the west Mediterranean archipelagos (Figure 1). Some groups of species included in this study were parapatric species. That is, their range does not significantly overlap, but are immediately adjacent to each other and show similar morphology and ecology. This is the case with three superspecies of shearwaters, each including several paraspecies: the Cory's shearwater (*Calonectris diomedea*, *C. borealis* and *C. edwardsii)*, the little shearwater (*Puffinus baroli* and *P. boyidi*, among others) and the Manx shearwater *(Pyelkouan* and *P. mauretanicus,* among others) superspecies. These species are closely related forms, and until recently, long considered subspecies of the same species and in some cases their taxonomic status is still being debated (e.g., *Calonectris* shearwaters) (*20-22*). Therefore, regarding contamination, we will consider each superspecies as a single statistical unit and not a different species. The Scopoli's (*Calonectris diomedea*), the Cory's (*C. borealis*) and the Cape Verde (*C. edwardsii*) shearwaters breed across the Mediterranean Sea, the NE Atlantic (except Cape Verde) and the Cape Verde archipelago, respectively. The little shearwaters, Cape Verde (*Puffinus boydi*) and the Macaronesian (*P. baroli*), breed in Cape Verde and across the rest of the Macaronesian

FIGURE 1. Geographic distribution of the studied seabirds. Black points indicate the localities where seabirds were sampled, including the species and the sampling year.



CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer's petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory's shearwater; **SS**, Scopoli's shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

archipelagos, respectively. The Balearic (*P. mauretanicus*) and the Mediterranean (*Puffinus yelkouan*) shearwaters breed on the Balearic Islands and across the rest of the Mediterranean islands, respectively. Moreover, the present study also includes some wide-ranging breeding petrel species. The Bulwer's petrel (*Bulweria bulwerii*) and Madeiran storm-petrel (*Oceanodroma castro*) breed on most NE Atlantic archipelagos, while the European storm-petrel (*Hydrobates pelagicus*) breeds in both the Mediterranean and the Atlantic basins. The Madeiran storm-petrel has two distinct populations in most archipelagos; one breeding in summer and the other in winter. For this study, we selected populations breeding during summer (the so-called hot population) to make possible comparisons with the rest of summer-breeding seabird species (*23*).

Adult seabirds were sampled during their breeding season from 2003 to 2006 (Figure 1), particularly during incubation and chick rearing periods. Species from the same breeding locality were sampled within a single year. Previous analysis on *Calonectris* shearwaters suggested a negligible influence of the sampling year over shearwater OC levels within the considered period (2003-2006) (*15*). Depending on the size of the species, about 0.2-0.5 mL of blood was sampled from the brachial vein. Blood was transferred into vials with 1 mL of absolute ethanol and preserved at -24 ºC until analysis.

Chemical analysis: A sub-sample of the blood fixed with absolute ethanol was used for stable isotope analysis. Once the sub-samples were dried, about 0.36-0.40 mg of blood (weighed to the nearest µg) were placed into tin buckets for combustion. Isotopic analyses were carried out by elemental analysis-isotope ratio mass spectrometry (EA-IRMS), and stable isotope ratios were expressed in conventional notation as parts per thousand (‰).

From 0.2 to 0.02 g (depending on the species) of dried blood was used for OC determination. The following organochlorine compounds were analyzed: *ortho* PCB congeners #28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #183, #170, #189, #194 and DDTs, including *p,p'*-DDT and its two main metabolites, *p,p'*-DDD and *p,p'*-DDE. OC concentrations were determined following the procedure described in our previous work (*15*). PCB and DDT concentrations were determined by high-resolution gas chromatography with micro-electron capture detection. Further details of the sample treatment and the analytical procedure for the determination of stable isotope ratios and OC concentrations are described in the Supporting Information.

Data analysis: SPSS 15.0 for Windows was used for all of the statistical analysis. Distributions of PCB, DDT and $\delta^{15}N$ levels were inspected for normality. Values under the detection limit were set to zero. Contaminant concentrations were log transformed (using base 10 logarithms) in order to satisfy a normal distribution and the homogeneity of variance. The Shapiro-Wilk test and Q-Q plots revealed a normal distribution for pollutant concentrations (after log transformation) and $\delta^{15}N$ (all $P>0.05$). The correlation between total PCBs ($\sum_{20}$PCBs) and DDTs ($\sum_{3}$DDTs) was evaluated by means of linear regression analysis. Levels of both organochlorine families were strongly correlated ($F_{1,174}=1402.36$, $P<0.001$; $r^2=0.91$; slope=0.93) and thus, we evaluated seabird contaminant burden variability using the total amount of organochlorine contaminants ($\sum$OCs = $\sum_{20}$PCBs + $\sum_{3}$DDTs). Because the sampling design in this study was unbalanced (all sampled species do not breed in all localities), the analysis of variability of organochlorine contaminants was performed by applying a Generalized Linear Model analysis (SPSS v.15, GLM, Type IV SS for unbalanced designs) using $\sum$OCs as the response variable. We tested the main effects and interactions of the superspecies, breeding colony and $\delta^{15}N$. The final selected model was built following a forward stepwise procedure, which included only the significant effects retained. Because species and locality interacted significantly, we checked interspecies differences in OC levels within each breeding locality separately using one-way ANOVA tests. To evaluate the relationship of [OCs]-$\delta^{15}N$ among seabird species within each breeding locality, we applied ANCOVA analysis over the mean $\sum$OC and $\delta^{15}N$ levels of seabird species using breeding locality as a fixed factor and $\delta^{15}N$ as a covariable.

To assess the relative exposure to PCB and DDT congeners among seabirds, we examined their profiles at each breeding place (calculated as the average percent of each PCB or DDT related to $\sum_{20}$PCBs and $\sum_{3}$DDTs, respectively). Concerning PCBs, we grouped congeners depending on their chlorination degree (≤ tetra-, penta-, hexa- and ≥ hepta-chlorinated PCBs). Moreover, a principal component analysis (PCA) was applied to the normalized contributions of all PCBs analyzed to check whether PCB profiles segregated seabirds depending on their breeding locality or their taxonomic status. In the case of DDTs, we calculated DDE/DDT ratios to check seabird profile differences and to detect possible recent inputs of this pesticide (*24,25*).

RESULTS

Influence of species, δN^{15} and breeding locality on OC levels: Considering the levels of $\sum$OCs (Table 1), the GLM explained up to 91.5% of the initial variance and included five explanatory variables: breeding locality ($F_{6,174}=3.56$, $P=0.003$), superspecies ($F_{5,174}=111.39$, $P<0.001$), δN^{15} ($F_{1,174}=5.69$, $P=0.017$) and the interactions breeding locality*species ($F_{8,174}=4.92$, $P<0.001$) and breeding locality* δN^{15} ($F_{6,174}=4.11$,

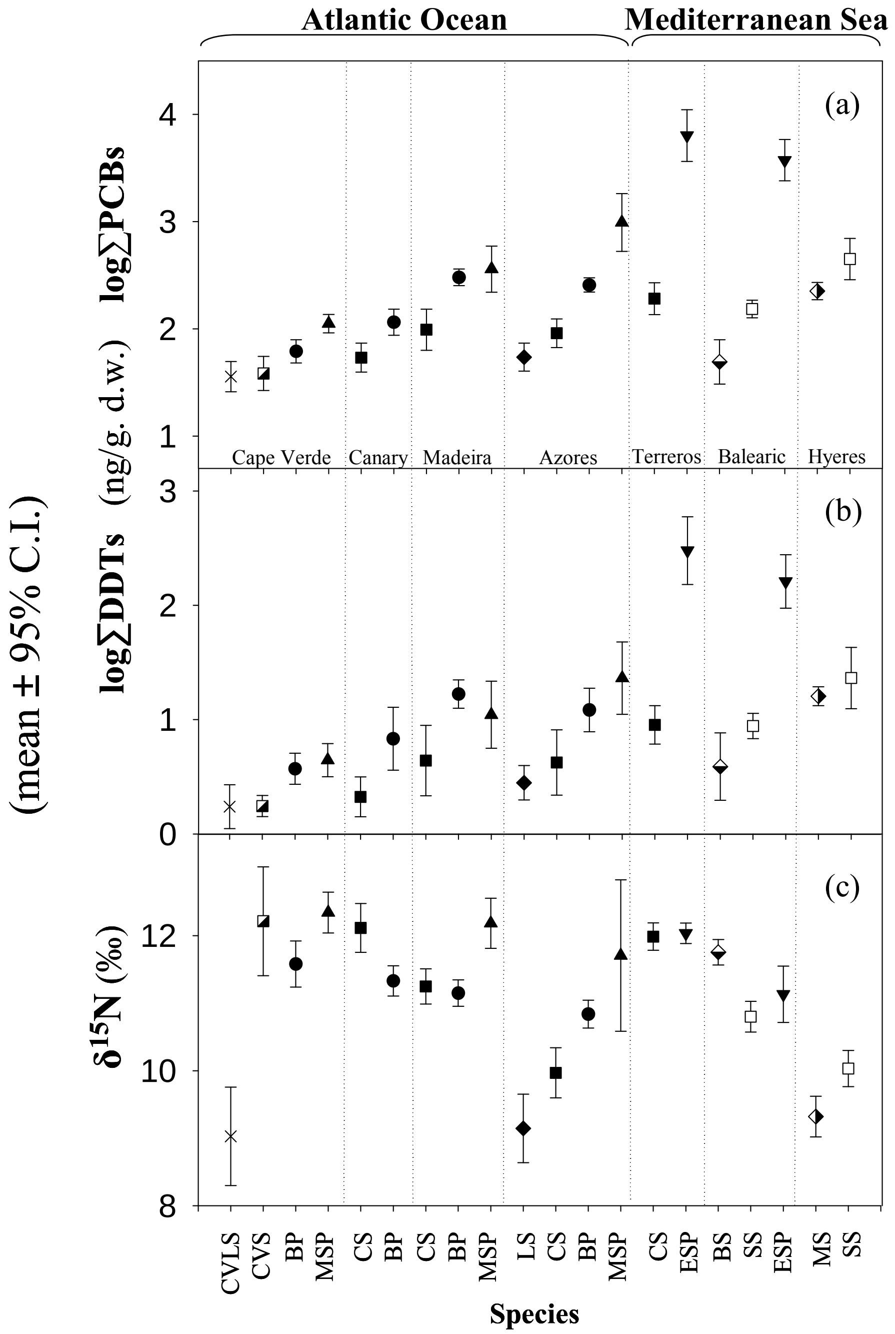
TABLE 1. Arithmetic and Geometric mean (AM and GM), standard error (SE) and range of $\sum_{20}$PCBs and $\sum_{3}$DDTs concentrations (ng g^{-1} d.w.) in blood from pelagic seabird species depending on their breeding locality.

Breeding colony				**∑PCBs**			**∑DDTs**	
Species	n	Year	AM ± SE	Range	GM	AM ± SE	Range	GM
ATLANTIC								
Cape Verde		2006						
CVLS	9		39.40 ± 7.06	21.12 - 92.68	35.94	7.201 ± 1.232	3.162 - 14.90	6.478
CVS	10		43.25 ± 7.31	17.74 - 97.35	38.42	7.691 ± 3.594	1.852 - 39.80	4.891
BP	10		65.22 ± 7.41	32.97 - 120.6	61.75	14.72 ± 1.71	5.866 - 23.80	13.74
MSP	9		115.1 ± 9.4	75.33 - 161.3	118.9	35.81 ± 7.43	16.08 - 82.46	30.80
Canary Is.		2003						
CS	10		59.22 ± 9.37	30.94 -114.8	53.93	13.13 ± 4.43	2.91 - 42.59	8.384
BP	9		122.3 ± 14.4	72.00 - 193.1	115.5	33.50 ± 6.48	15.40 - 68.46	29.28
Madeira		2005						
CS	10		125.3 ± 40.9	51.40 - 488.9	98.34	38.18 ± 19.28	11.03 - 208.7	22.06
BP	10		311.0 ± 24.5	200.2 - 442.2	302.4	89.43 ± 13.77	40.67 - 171.4	80.86
MSP	8		419.9 ± 84.0	186.5 - 784.2	361.4	172.1 ± 41.29	27.78 - 329.8	128.4
Azores Is.		2003						
LS	8		57.43 ± 6.49	26.66 - 79.80	54.52	12.52 ± 2.18	5.026 - 24.19	11.25
CS	10		99.33 ± 14.84	41.22 - 217.8	91.21	42.09 ± 21.48	12.49 - 234.3	25.13
BP	10		262.1 ± 17.5	(185.7 - 337.7	256.8	58.99 ± 11.73	23.13 - 146.8	50.77
MSP	5		1073 ± 206	441.7 - 1726.7	982.3	319.1 ± 85.07	88.69 - 546.9	264.1
MEDITERRANEAN								
Terreros Is.		2004						
CS	10		212.72 ± 34.54	91.68 - 463.6	191.5	100.1 ± 24.91	25.50 - 248.2	76.98
ESP	5		6843 ± 1213	3186 - 10495	6362	5816 ± 1500	2971 – 11561	5204
Balearic Is.		2004						
BS	6		53.99 ± 11.24	27.34 - 105.8	49.24	16.25 ± 8.80	4.724 - 59.93	10.08
SS	10		158.24 ± 13.38	102.1 - 227.7	153.3	69.57 ± 14.96	30.98 - 191.9	59.61
ESP	10		4504 ± 1022	1645 – 12298	3739	2781.5 ± 599.3	883.4 – 6266	2254
Hyeres Is.		2006						
SS	8		510.7 ± 107.8	216.2 – 1189	448.5	303.3 ± 96.81	96.26 - 911.7	233.58
MS	7		229.0 ± 16.1	155.95 - 283.12	225.3	81.03 ± 11.22	48.94 - 133.1	76.84

CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer’s petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory’s shearwater; **SS**, Scopoli’s shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

P=0.001). The significant effect of interactions indicated that interspecies differences of OC levels as well as the relationship [OCs]-δ^{15}N vary depending on the breeding locality.

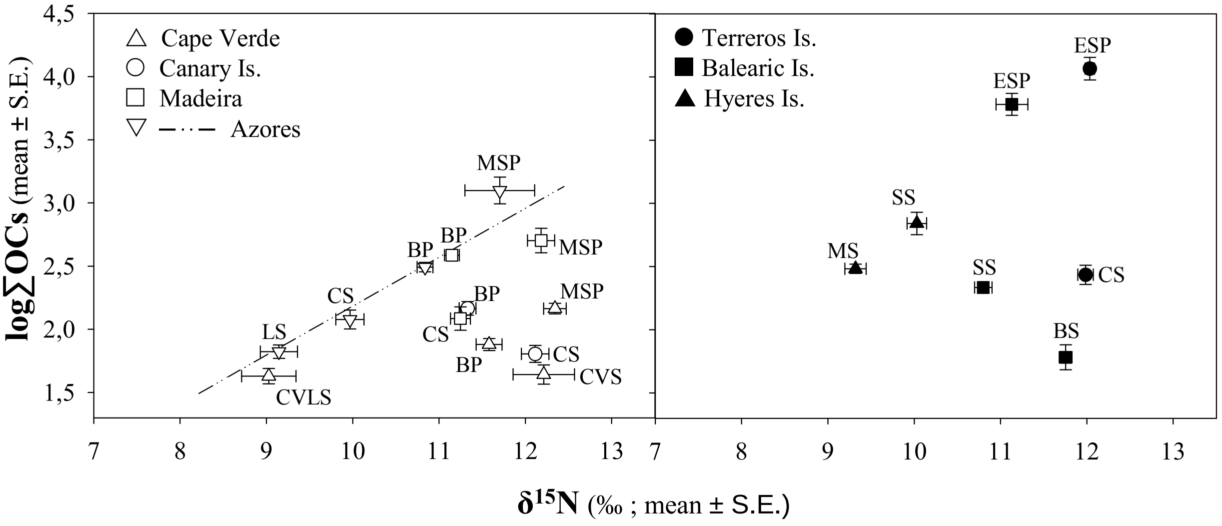
FIGURE 2. Mean and 95% intervals of confidence (IC) of PCBs (a), DDTs (b) and $\delta^{15}N$ (c) among breeding localities of pelagic seabirds from the NE Atlantic Ocean and the Mediterranean Sea.



×, **CVLS,** Cape Verde little shearwater; ◪, **CVS**, Cape Verde shearwater; ●, **BP**, Bulwer's petrel; ▲, **MSP**, Madeiran storm-petrel; ◆, **LS** Little shearwater; ■, **CS**, Cory's shearwater; □, **SS**, Scopolis shearwater; ⬙ **BS**, Balearic shearwater; ⬗, **MS**, Mediterranean shearwater

The GLM showed the significant effect of breeding locality on seabird OC levels. In fact, at intraspecific levels or within each superspecies group, seabirds showed consistent spatial patterns in their PCB and DDT burdens (Figure 2). That is, generally, storm-petrels and shearwaters from the Mediterranean showed respectively greater OC levels than those from the Atlantic. Moreover, within the Atlantic basin, PCB and DDT burdens increased from southern to northern breeding localities regardless of the species. Inter-species differences of ∑OCs at each breeding locality (Figure 2) were significant in all breeding localities (Cape Verde, $F_{3,38}$=17.71; Canary, $F_{1,19}$=16.85; Madeira, $F_{2,28}$=17.60; Azores, $F_{3,33}$=56.25; Terreros, $F_{1,15}$=171.01; Balearic, $F_{2,26}$=179.35; Hyeres, $F_{1,15}$=12.60; All P<0.001 except Hyeres with P=0.007). In general, PCB and DDT levels in seabird blood showed consistent inter-species patterns in all of the breeding localities (Figure 2). That is, within the Atlantic basin, little shearwaters showed the lowest mean values of both ∑PCBs and ∑DDTs, followed by *Calonectris* shearwaters, Bulwer's petrel and Madeiran storm-petrel. Post hoc tests revealed that differences in ∑OCs were significant for all pair comparisons with the exception of Bulwer's petrel vs. Madeiran storm-petrel and *Calonectris* vs. little shearwaters. Among Mediterranean breeders, the Balearic shearwater showed the lowest amount of PCBs and DDTs, followed by the Mediterranean and *Calonectris* shearwaters. The European storm-petrel showed the greatest ∑PCBs and ∑DDTs values in all of the breeding localities. All pair comparisons between species were significant. However, within both Atlantic and Mediterranean breeding localities, most seabirds showed $\delta^{15}N$ values that were strongly overlapped and did not follow the contamination level patterns (Figure 2).

FIGURE 3. $\delta^{15}N$ signatures and ∑OC concentrations in seabird blood. Significant relastionships between these variables within each breeding locality ar shown. Results are shown separately for Mediterranean and Atlantic seabirds for clarity purposes.



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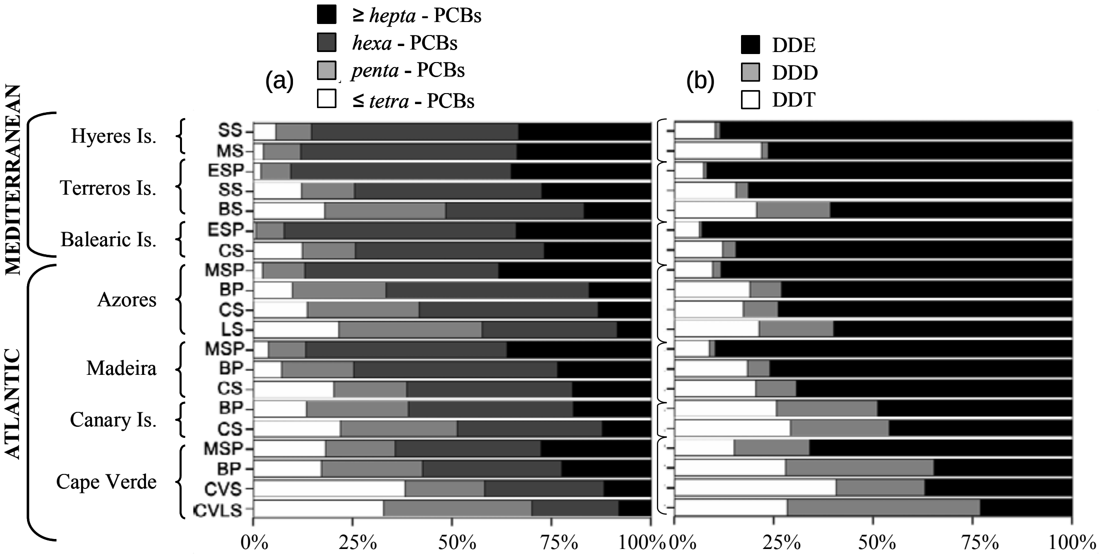
The differences between nitrogen and OCs were also evident when evaluating the relationship between ∑OCs and $\delta^{15}N$ (Figure 3) of seabird species within each breeding locality. Breeding locality, $\delta^{15}N$ and their interaction did not show a significant effect (ANCOVA test, all $P \geq 0.1$), indicating a lack of correlation between species OC levels and $\delta^{15}N$. In fact, the regression analysis between OC levels and $\delta^{15}N$ was only significant within seabirds from the Azores ($r^2=0.87$, $P=0.007$) due to the smaller overlap in Azorean seabird nitrogen signatures compared to the rest of breeding localities.

PCB and DDT profiles. Overall, among the analyzed PCB congeners, # 153, 138 and 180 were the most abundant in seabird blood samples. Although the relative contribution of ≤ tetra-, penta-, hexa- and ≥ heptachlorinated PCBs (Figure 4) varied among species and localities, in general, inter-species and spatial patterns of PCB congeners were consistent.

FIGURE 4. PCB and DDT profiles in the blood of pelagic seabirds depending on their breeding localities.



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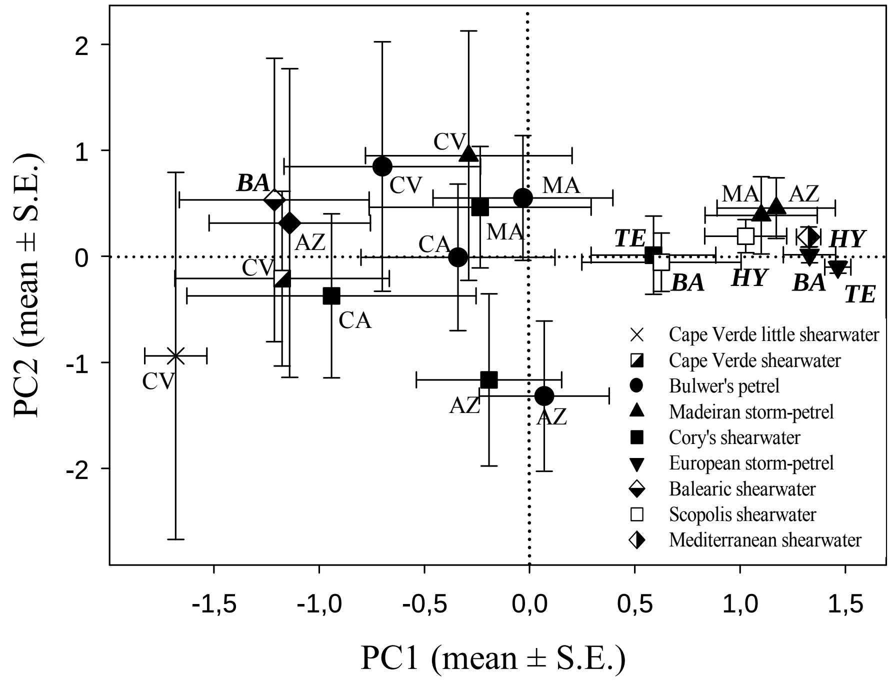
Hexachlorinated PCBs accounted for the greatest contributions (Figure 4) and concentrations (Table S1) in most seabird species. Only the Cape Verde and the little shearwaters showed PCB profiles dominated by low chlorinated PCBs (≤ tetra- or penta-PCBs). Generally, Mediterranean breeders showed a large proportion of higher chlorinated congeners (hexa- and ≥ heptachlorinated PCBs) with two exceptions: (1) Madeiran storm-petrels from the Azores and Madeira whose PCB profiles were clearly dominated by higher chlorinated PCBs, and (2) Balearic Shearwaters from the Balearic Islands, which showed a larger amount of lower chlorinated PCBs compared to the rest of the Mediterranean breeders. Furthermore, the principal component analysis (PCA) performed with the relative abundance of all analyzed congeners (Figure 5) segregated most Mediterranean breeders (all except the Balearic shearwater) and Madeiran storm-petrels from the rest of the seabirds due to the significant presence of higher chlorinated PCB congeners in these groups. Two principal components explained 50.59 % of the total variance. The component matrix indicated that PC1 was mostly positively associated with higher chlorinated PCBs and mainly negatively related to lower chlorinated congeners. PC2 did not show marked differences in its association with PCB congeners. Therefore, PC1 mostly represented a gradient of congener chlorination, and PC2, a general measure of PCB contamination.

FIGURE 5. Principal components (PC1-PC2) with the relative normalized contributions of all PCBs analyzed in pelagic seabird blood. Breeding localities from the Mediterranean Sea are indicated in bold type.



Atlantic breeding localities: CV, Cape Verde; CA, Canary Is.; MA, Madeira; AZ, Azores
Mediterranean breeding localities: ***TE***, Terreros Is.; ***BA***, Balearic Is.; ***HY***, Hyeres Islands.

Concerning DDTs, although *p,p'*-DDE, followed by *p,p'*-DDT and *p,p'*-DDD, showed the relative largest contributions in most seabirds (Table S2), DDT profiles varied among species and breeding localities (Figure 4). *p,p'*-DDE/*p,p'*-DDT ratios reflected these differences, showing mean values markedly lower in seabirds from Cape Verde and the Canary Islands (Table S2) compared to the rest of the breeding colonies. Specifically, *p,p'*-DDE/*p,p'*-DDT ratios in Cape Verde seabirds were lower than 1 in 44.7% of the cases and reached values between 1 and 2 in 21.1% of them. In the case of the Canary Islands, 31.6% and 15.9% of the seabirds also showed a DDE/DDT ratio lower than 1 and between 1 and 2, respectively. The rest of the seabirds from this study showed DDE/DDT ratios greater than two. These differences in the DDE/DDT ratios reflected a major abundance of *p,p'*-DDT in DDT blood profiles from seabirds breeding in the southern Macaronesian archipelagos compared to the rest of the sampled localities.

DISCUSSION

To our knowledge, this is the first large-scale study reporting PCB and DDT levels in several pelagic seabird species over multiple localities from the NE Atlantic and the Mediterranean Sea. Overall, our results show that both breeding locality and species have a marked influence on seabird organochlorine contamination. Further evidence of this combined influence was supported by PCA analysis, which did not allow group of the seabirds by species or breeding locality alone. Despite that, DDT and PCB levels in seabird blood showed some consistent spatial and inter-specific patterns.

Our results revealed a large homogeneity in the spatial patterns of OCs from pelagic seabird regardless of the species. On the whole, the geographic patterns of OCs reported in this study between and within the Atlantic and Mediterranean basins through a multispecific approach validate and give more consistence to those reported previously for *Calonectris* shearwaters, (*15,26*). That is, within the Atlantic seabirds, OC levels increased from the southern to the northern archipelagos. This latitudinal pattern is likely associated with the influence of North American coastal waters and pollution sources above the central-north Atlantic (including the Azorean region) (*27,28*). Moreover, most seabirds breeding in the southern Macaronesian archipelagos (Cape Verde and Canary Islands) showed DDE/DDT ratios that suggest recent inputs of DDT into their food webs. The nearby Sub-Saharan African countries, where DDT is still in use to control malaria outbreaks, represent the most probable source of these contaminants (*29*).

On a large scale, spatial patterns in seabird OCs emerged between the Mediterranean and the Atlantic. Mediterranean storm-petrels and shearwaters (except Balearic shearwaters) showed greater concentrations of OCs than their Atlantic counterparts. Moreover the principal component analyses segregated Mediterranean breeders (all except Balearic shearwater) and Madeiran storm-petrel from the rest of seabirds due to the greater degree of chlorination in their PCB profiles (Figure 5). In the case of Mediterranean seabirds, this global pattern is consistent with a confined sea with historically greater inputs of OCs, which ultimately results in greater levels of contamination and in a major a presence of the most persistent PCBs (*30*). However, the high chlorination degree in the PCB profiles of Madeiran storm-petrels is likely related to its dietary preferences or detoxification capabilities (see below).

OC levels differed significantly among seabird species within all the breeding localities. Overall, *Calonectris* shearwaters showed greater amounts of OCs related to the rest of shearwater species, but significantly lower levels than petrels (Figure 2). However, stable-nitrogen signatures of seabird species showed a significant overlap, which was not consistent with the variation pattern of their OC levels, pointing out that inter-species differences of OCs are not exclusively driven by seabird trophic level. In fact, $\delta^{15}N$ was a non-significant covariable explaining OC differences among seabird species and we only found a positive significant relationship between species ∑OCs and $\delta^{15}N$ in the case of the Azores. Among the studied seabirds, the Macaronesian little, the Cape Verde little and the Mediterranean showed the lowest OC burdens and the lowest stable-nitrogen signatures within their breeding localities. This result agrees with previous studies of heavy metals suggesting a relatively lower trophic position for little shearwaters (*31*). On the other hand, Bulwer's petrels, Madeiran storm-petrels and European storm-petrels showed higher OC concentrations than *Calonectris* shearwaters, regardless of the breeding locality. However, $\delta^{15}N$ values were only significantly greater in petrels than in *Calonetris* in the case of the Azores, reflecting a possible relatively lower trophic position for this shearwater species in relation to petrels within this breeding locality.

Overall, the small differences in blood stable-nitrogen signatures between petrel species and *Calonectris* shearwaters reported here suggest no differences in their trophic position. This agrees with previous studies about Azorean seabirds (*31,32*). However, similar isotopic values among species or individuals cannot necessarily be interpreted as evidence of similar diets (*33*). Therefore, the greater levels found in petrels could be related to different exposition trough their diet, but also to different metabolic capacity or energetic requirements among species. The energetic requirements, and hence the feeding rate, is greater in smaller species, which gives small species a greater exposition rate of contaminants. The petrel species included here are smaller than the *Calonectris* shearwater. However, the little shearwater is about a 73% lighter than *Calonectris* shearwaters, and only a 27% heavier than Bulwer's petrel, but showed significantly lower OC levels than *Calonectris* shearwaters. Moreover, all the analyzed species are phylogenetically close (O. Procellariformes) and biologically and ecologically similar compared to other seabird groups. Therefore, although we cannot completely exclude some differences in OC levels associated with the energetic requirements of the species, it seems to be a minor factor influencing seabird OC levels. Concerning the metabolic capabilities of seabirds, previous studies about Mixed-Function Oxidase (MFO) activity revealed a slightly greater activity for some enzymes in storm-petrels (*Oceanodroma leucorhoa*) compared to shearwaters (*Puffinus puffinus*) (*34,35*). These small differences in MFO activity between storm-petrels and shearwaters could be related to the lower presence of low chlorinated PCBs that we found in petrel species. Nonetheless, it could not explain the large difference in OC levels between these groups.

Although petrel and shearwater species included in this study feed mainly on pelagic fish (followed by lower proportions of cephalopods, crustaceans and plankton), they exploit different prey types. Madeiran storm-petrels and Bulwer's petrels are mainly nocturnal feeders specialized in exploiting daily vertical mesopelagic (from 200 to 1000 m of depth) migratory fishes, such as myctophids (*23,32,36*). *Calonectris* shearwaters are mainly epipelagic (0-200 m) diurnal feeders. The European storm-petrel feeds on a wide variety of marine resources (fish, crustacean and cephalopods), including miscellaneous fatty droplets, fisheries discards and scavenging on dead marine mammals (*37-39*). Dietary differences between petrels and shearwaters are probably associated with their OC levels and profiles. Previous studies in the Atlantic and Pacific oceans have reported higher burdens of OCs in mesopelagic and deep sea fishes compared to those from the upper water layers (*40-43*). Specifically, studies on Pacific mesopelagic fishes have revealed significantly greater burdens of organic pollutants in some daily vertical migrant and semi-migrant myctophid species in comparison to other mesopelagic fishes (*44*). Moreover, it was found that these myctophid species show OC profiles dominated by high molecular weight compounds, which include the higher chlorinated PCB congeners. Therefore, seabirds feeding on mesopelagic resources could be exposed to greater OC burdens than those of similar trophic position but feeding on epipelagic preys, which has also been reported previously in heavy metals studies (*45*).

In the case of European storm-petrels, fatty droplets could contribute to their elevated OC burdens but not their nitrogen signatures because OCs are lipophilic compounds but droplets lack protein content. In addition, previous studies have shown that seabird scavenging events also could contribute to upsetting the relationship between OC levels and $\delta^{15}N$ (*9,17*). In this study, we found a marked difference between $\delta^{15}N$ and OC concentrations in the case of one more species, the Balearic shearwater. Moreover, this species also showed markedly dissimilar PCB and DDT profiles in relation to the rest of the Mediterranean breeders. However, we did not find any satisfactory explanation for the unexpected levels and profiles in Balearic shearwaters in relation to its feeding ecology or metabolic capability.

In sum, inter-species differences in OCs reported in this study underline the importance of trophic level and specific dietary habits with respect to seabird OC burdens. Seabirds feeding on prey with similar trophic positions can also be feeding on prey from different levels of the water column, resulting in substantial differences in OC burdens that obscure the biomagnification process. Further studies on the trophic transfer of OCs and the relationship between predator and prey OC burdens are necessary to confirm this hypothesis as well as understand OC levels and profiles from some seabird species, such as the case of the Balearic shearwater. Nonetheless, this study illustrate that multispecies approaches can better help us to understand the relative contribution of geography and feeding ecology to the OC burdens than a single species-based strategy.. Our findings also suggest that these strategies in marine monitoring could be used to monitor not only regional differences of OCs, but also the vertical distribution of these contaminants. Moreover, this approach showed a broader interval of risk levels, which represents a more comprehensive evaluation of marine pollution.

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SUPPORTING INFORMATION AVAILABLE

Details of analytical methods and tables S1 and S2 (PCB congener and DDT metabolites concentrations) are available free of charge via the Internet at http://pubs.acs.org/.

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SUPPORTING INFORMATION

Geographic and trophic patterns of OCs in pelagic seabirds from the NE Atlantic and the Mediterranean: a multi-species/multi-locality approach

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INCLUDING MATERIALS

CARBON AND NITROGEN STABLE ISOTOPE ANALYSIS

In the laboratory, we sub-sampled 1-2 mg of blood previously homogenised by vortex stirred for 1 min. Sub-samples were dried at 60ºC for 24 h to remove ethanol. From 0.36 to 0.4 mg of homogenized blood were weighed to the nearest µg and placed into tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a ThermoFinnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer *via* a CONFLOIII interface. Stable isotope ratios were expressed in conventional notation as parts per thousand (‰), according to the following equation: $\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$ where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ and $^{13}C/^{12}C$. The standards for ^{15}N and ^{13}C were atmospheric nitrogen (AIR), and Peedee Belemnite (PDB), respectively. Precision and accuracy for $\delta^{13}C$ measurements was ≤ 0.1‰ and ≤ 0.3‰ for $\delta^{15}N$.

PCBS AND DDTS ANALYSIS

The following organochlorine compounds were analyzed: *ortho* PCB congeners #28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #183, #170, #189, #194 and DDTs, including *p,p'*DDT and its two main metabolites, *p,p'*DDD and *p,p'*DDE.

Elimination of ethanol from the blood samples (~0.5 mL) was carried out using a Speed-Vac (Jouan, RC.10.10) at 400 rev min^{-1} and 25° C coupled to a freeze-drier (Telstar, Cryodos-80) at 10 Pa and -80ºC for about 3h, until achieving constant weight of samples. Powdered residues were extracted with 3 mL of n-hexane and 2 mL of concentrated sulfuric acid. The tube was vortex stirred for 30 s and centrifuged at 4000 rpm for 10 minutes. The supernatant n-hexane phase was removed and the remaining sulphuric acid solution re-extracted twice with 2 mL of n-hexane. All hexane extracts were combined and the resulting 7 mL of n-hexane were purified by vortex stirring with 2 mL of sulfuric acid. Then the n-hexane phase was concentrated under a gentle stream of nitrogen.

PCB and DDT concentrations were determined by high resolution gas chromatography with micro-electron capture detection (HRGC-µECD, Agilent Technologies, model 6890N, Palo Alto, CA, USA). Before chromatographic analysis, 1,2,3,4-tetrachloro naphthalene (TCN) and PCB 209 were added as internal standards to correct for instrument variability. Samples were injected in hot splitless mode (1µl, 270ºC, splitless time 1.0 min). A DB-5 capillary column (J&W Scientific, USA; 60m, i.d. 0.25 mm, film thickness 0.25 µm) was used for chromatographic separation. The column temperature was programmed from 80ºC (2 min) to 185ºC (3 min) at a rate of 30ºC min^{-1}, then to 230ºC (10 min) at 1.5ºC min^{-1} and then to 270ºC (10 min). The detector temperature was set at 300ºC. Nitrogen was used as carrier gas (1.5 mL min^{-1}, constant flow) and as make-up gas (10 mL min^{-1}). Organochlorine compounds were identified on the basis of their relative retention times to the TCN and PCB 209 standards on the chromatographic column. Quantification was done within the linear range of the detector's seven-level calibration curve using HP ChemStation Plus program (Hewlett-Packard Co., Palo Alto, CA, USA). For every batch of 7 blood samples a blank sample was included. When results for blank samples were over the LODs they were subtract from the corresponding sample batch. Since sample preparation included an extra-step to remove ethanol from blood samples, five samples were previously spiked with PCB 209 to evaluate the method recovery. The recovery study showed high percentages with values ranging from 77 to 96%.

TABLE S1. Means and standard errors of PCB concentrations depending on their chlorination degree in the blood of pelagic seabirds breeding across the northeast Atlantic and the Mediterranean Sea.

Breeding colony Species	≤ *tetra*-PCBs	*penta*-PCBs	*hexa*-PCBs	≥ *hepta*-PCBs
ATLANTIC				
Cape Verde				
CVLS	11.92 ± 5.37	15.73 ± 11.69	8.85 ± 4.64	2.90 ±1.50
CVS	15.57 ± 8.80	8.23 ± 3.57	13.38 ± 10.28	6.07 ±7.17
BP	10.83 ± 3.57	16.29 ± 7.76	23.38 ± 12.03	14.73 ±6.28
MSP	21.13 ± 14.44	20.38 ± 8.81	41.86 ± 11.43	31.71 ±6.69
Canary Is.				
CS	11.87 ± 6.81	16.12 ± 6.20	23.06 ± 15.47	8.16 ±6.39
BP	16.54 ± 8.48	30.51 ± 11.63	51.80 ± 19.55	23.48 ±9.00
Madeira				
CS	19.10 ± 5.00	20.35 ± 14.50	58.56 ± 75.86	27.31 ±30.66
BP	11.53 ± 4.94	30.80 ± 15.16	137.07 ± 40.86	75.61 ±20.49
MSP	12.62 ± 7.99	38.23 ± 20.27	216.52 ± 128.27	152.55 ±79.16
Azores Is.				
LS	11.99 ± 3.99	20.91 ± 7.50	19.61 ± 5.92	4.92 ±2.14
CS	12.69 ± 5.48	28.09 ± 12.68	45.54 ± 25.25	13.01 ±6.49
BP	11.59 ± 5.95	28.52 ± 13.35	111.16 ± 27.20	54.82 ±11.10
MSP	18.33 ± 7.34	104.17 ± 32.71	541.04 ± 267.70	410.01 ±159.05
MEDITERRANEAN				
Terreros Is.				
CS	23.07 ± 8.73	26.76 ± 9.93	104.03 ± 57.44	58.86 ±32.05
ESP	38.59 ± 22.59	475.95 ± 163.86	3980.91 ± 1567.60	2348.52 ±888.00
Balearic Is.				
BS	8.22 ± 3.04	16.59 ± 8.27	19.57 ± 9.71	9.60 ±7.58
SS	18.60 ± 9.46	20.58 ± 5.38	75.38 ± 24.85	43.68 ±11.33
ESP	62.22 ± 45.60	313.68 ± 164.80	2507.55 ± 1792.33	1620.61 ±1149.09
Hyeres Is.				
SS	23.83 ± 12.58	43.61 ± 19.72	269.21 ± 169.87	174.00 ±102.71
MS	5.39 ± 1.37	21.44 ± 4.15	125.17 ± 26.40	77.02 ±12.47

CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer's petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory's shearwater; **SS**, Scopoli's shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

TABLE S2. Means and standard errors of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD concentrations (ng g^{-1} d.w.) and DDE/DDT in the blood of pelagic seabirds breeding across the northeast Atlantic and the Mediterranean Sea.

Breeding colony Species	DDT	DDE	DDD	DDE/DDT
ATLANTIC				
Cape Verde				
CVLS	2.03 ± 1.29	1.57 ± 2.45	3.60 ± 2.80	0.77 ± 0.88
CVS	1.83 ± 0.55	4.81 ± 10.98	1.05 ± 0.53	2.62 ± 4.07
BP	4.10 ± 2.19	5.26 ± 3.53	5.35 ± 2.86	1.39 ± 0.94
MSP	4.80 ± 2.06	25.60 ± 21.30	5.41 ± 5.21	5.01 ± 2.69
Canary Is.				
CS	2.54 ± 2.12	9.04 ± 12.04	1.54 ± 0.70	3.55 ± 2.83
BP	8.24 ± 3.31	20.01 ± 19.15	4.32 ± 3.61	2.42 ± 1.31
Madeira				
CS	8.48 ± 14.38	27.44 ± 45.08	2.26 ± 1.67	3.24 ± 1.18
BP	17.99 ± 7.35	63.61 ± 35.84	7.82 ± 4.39	3.55 ± 0.70
MSP	13.97 ± 8.70	156.09 ± 108.20	2.03 ± 1.52	11.17 ± 3.38
Azores Is.				
LS	2.47 ± 1.11	6.87 ± 2.41	3.17 ± 3.78	2.78 ± 1.32
CS	7.84 ± 13.99	31.55 ± 50.82	2.70 ± 3.23	4.03 ± 1.38
BP	13.12 ± 8.02	39.27 ± 29.58	6.60 ± 1.33	2.99 ± 4.31
MSP	26.77 ± 17.23	287.02 ± 176.71	5.34 ± 5.22	10.70 ± 6.13
MEDITERRANEAN				
Terreros Is.				
CS	10.16 ± 5.01	87.74 ± 73.75	2.16 ± 0.87	8.63 ± 3.05
ESP	340.80 ± 200.52	5441.57 ± 3168.87	34.17 ± 18.17	15.96 ± 10.11
Balearic Is.				
BS	3.26 ± 3.92	11.01 ± 15.75	1.97 ± 2.04	3.37 ± 1.11
SS	9.32 ± 3.38	58.51 ± 44.32	1.74 ± 0.65	6.27 ± 2.58
ESP	206.91 ± 149.40	2552.46 ± 1737.67	22.16 ± 19.02	12.33 ± 3.14
Hyeres Is.				
SS	30.26 ± 26.51	270.02 ± 252.61	2.99 ± 2.21	8.91 ± 2.63
MS	16.37 ± 3.74	63.43 ± 29.47	1.23 ± 0.11	4.00 ± 2.04

CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer's petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory's shearwater; **SS**, Scopoli's shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

Exposure to contaminants, such as Persistent Organic Pollutants (POPs) is currently regarded as a serious anthropogenic threat to marine predators and their food webs. Exposure to contaminants endured by marine wildlife is related to their trophic ecology, distribution and movements. However, the contribution of these biological features to contamination levels remains poorly understood in most marine predators, including seabirds. New methodologies, such as stable isotope analyses or a wide array of devices to track movement at different spatial and temporal scales can usefully provide new light into this issue. Although stable isotope signatures in marine wildlife provide valuable information about their trophic ecology, isotopic baseline levels also show geographical differences. Therefore, to understand isotopic differences among separate wildlife populations we first need to evaluate the influence of spatial variability in stable isotope signatures. Firstly, we evaluate in this thesis the relative influence of geographic origin and trophic ecology in shaping stable isotope signatures and contaminant burdens in pelagic seabirds. Then we present the spatial patterns in the isotopic signatures, OCs and PAHs that emerge among Mediterranean and northeast Atlantic seabirds. Secondly, we show that the trophic ecology of each seabird species is fairly constant throughout their breeding distributions, but clearly differ among species, which points out the marked relationship of seabird trophic ecology with their PCB, DDT and PAH burdens. Overall, this study underlines the usefulness of combining environmental chemistry methodologies with new approaches in the study of animal ecology, and thus shows the value of pelagic seabirds in marine contamination monitoring.

THE RELATIVE INFLUENCE OF GEOGRAPHIC ORIGIN AND TROPHIC ECOLOGY

Most seabird species included in this study show wide breeding distributions across the northeast Atlantic and the Mediterranean Sea. Moreover, these species exploit diverse resources, from epipelagic prey available throughout the day, to mesopelagic prey that migrate to the surface by night. Our multi-species approach offered an excellent opportunity to evaluate the relative influence of the geography (location of the breeding colony) and the trophic ecology (species) on the stable isotope signature of C ($\delta^{13}C$) and N ($\delta^{15}N$) and on the presence of POPs, in particular PCBs, DDTs and PAHs.

In the case of stable isotopes, we found marked differences in C and N signatures among most seabird species, whereas the spatial effect only emerged at large geographic scales. That is, most populations from the same species were grouped according to their isotopic values, regardless of their breeding archipelago (Figure 1).

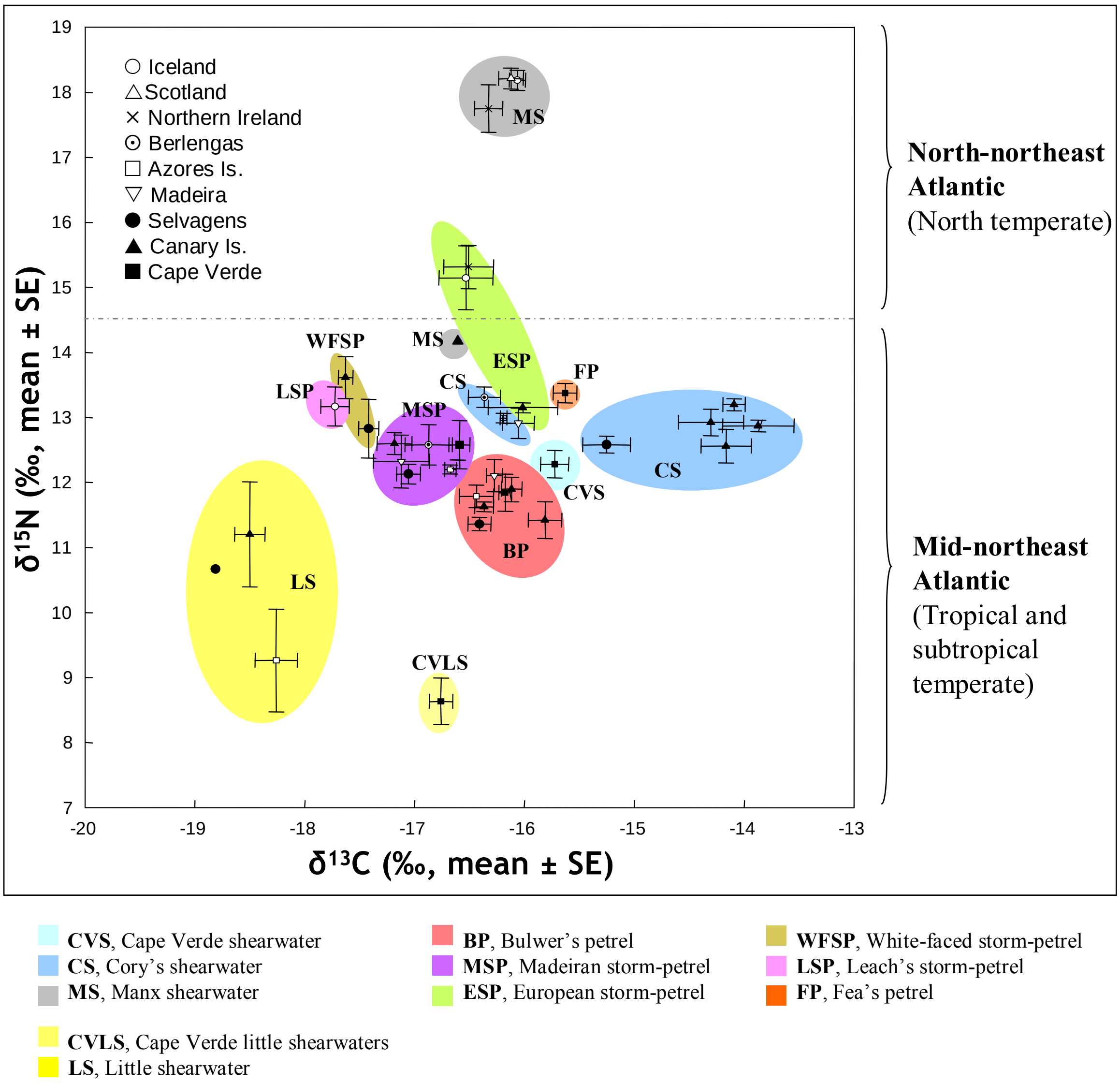


FIGURE 1. Carbon and nitrogen stable isotope signatures in feathers from Procellariiformes breeding across the northeast Atlantic Ocean. Sampled colonies with N=2 (Manx's shearwater from Canary Is. and little shearwater from Selvagens) are depicted without SE.

This result suggests that δ^{15}N and δ^{13}C signatures are primarily influenced by the feeding ecology of each species rather than by biogeographical trends in the isotopic baseline levels. Nonetheless, while stable isotope signatures in seabird feathers were geographically unstructured at regional scale (i.e. within the tropical and subtropical temperate regions), large scale comparisons (i.e. north related to tropical and subtropical temperate region) showed some differences, presumably attributable to shifts in the isotopic baseline levels among distant regions (see below).

Concerning PCB and DDT levels we found significant differences among breeding localities but also consistent interspecific differences (Appendix 1a and 1b). However, in the case of PAHs (Appendix 2), the geographic origin of seabirds showed a negligible effect over their PAH levels, even when the comparison was made between water masses with different baseline levels for these pollutants, like the Mediterranean and the Atlantic (Albaigés 2005; Martí *et al.* 2001; Dachs *et al.* 1997). Unlike OCs, differences in seabird PAH levels and profiles emerged mainly among species, regardless of their geographic origin.

Overall, our results emphasize the need to consider both, the geographic component and the feeding ecology, in the use of stable isotope methodology and in the study of seabird exposure to organic contaminants. Nevertheless, the seabird species explained a major proportion of variability in stable isotope signatures compared to breeding locality. In agreement, differences in POP levels among species were also markedly greater than those found among breeding localities at intraspecific level. Therefore, this study suggests species trophic niche as the major factor determining isotopic signatures and contaminant levels in marine predators across large geographic scales.

GEOGRAPHIC PATTERNS

Isotopic landscapes across the NE Atlantic: The use of Global Location Sensing (GLS) devices based on recording light levels successfully allowed us to assess the moulting areas of the first primary feather for most seabird species. In general, moulting areas of the tracked birds departed a few hundreds of kilometres from their colony sites (Figure 2), whereas populations included in this study are separated by several thousands of kilometres. Assuming that the proximity between breeding colony and moulting areas was true for all seabird species included in the study, we used breeding colony as the reference point to investigate isotopic spatial trends across the NE Atlantic. Since we could only track birds from a few populations and species, this generalization guarantees a homogeneous treatment for all sampled species, although we are aware that some moulting areas can move away several hundreds of kilometres from the breeding locality.

The study included breeding colonies from Cape Verde to Iceland, but spatial patterns in the isotopic signatures of seabird feathers were not apparent within large regions. That is, within the tropical and subtropical north temperate regions, distance among breeding colonies did not correlate with differences in stable isotope values for any of the petrel species. Moreover, neither latitudinal nor longitudinal location gradients within the species explained differences in $\delta^{13}C$ and $\delta^{15}N$ signatures among breeding colonies. This lack of geographical structure in seabird feather stable isotope signatures within large regions, such as the mid-northeast Atlantic, indicates a limited applicability of this methodology as an intrinsic marker to study the movements of marine predators at regional scales.

Spatial differences were only apparent in the case of $\delta^{15}N$ and only when considered between large regions. Seabirds from the north temperate region (Manx shearwater and European storm-petrels breeding in N. Ireland, Scotland and Iceland)

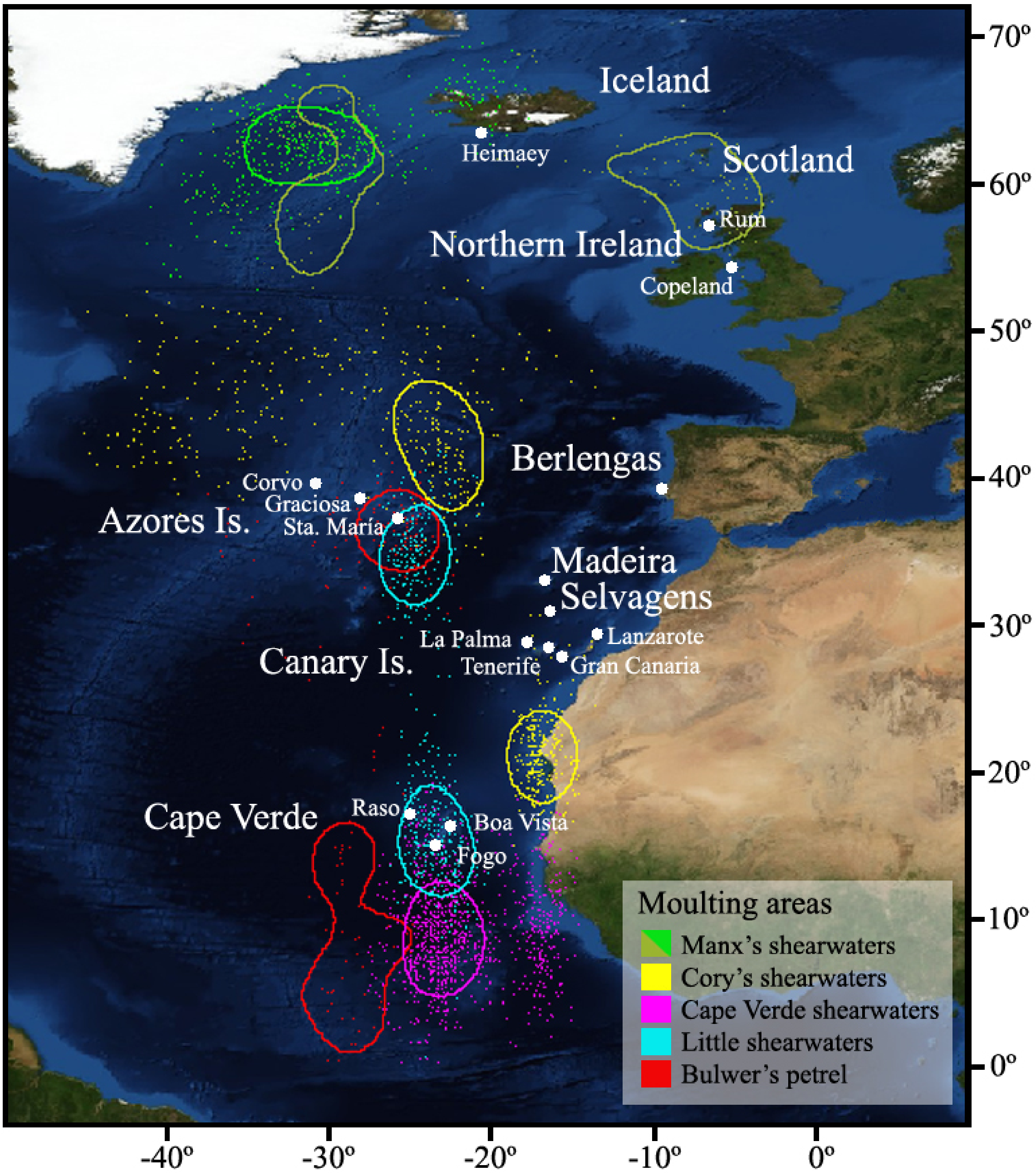


FIGURE 2. Sampled breeding colonies (white dots) and moulting areas (within drawn lines) of petrels and shearwaters from the northeast Atlantic Ocean. Moulting areas from north to south: Manx's shearwaters from Iceland in light green (N=10 in 2006) and from Scotland in dark green (N=4 in 2006 and N=2 in 2007); Cory's shearwaters in light yellow (N=9 in 2002), Bulwer's petrels in red (N=3 in 2007) and little shearwaters in blue (N=4 in 2007) from Azores; Cory's shearwaters from Canary Islands in light yellow (N=6 in 2002) and finally, Cape Verde shearwaters in magenta (N=24 in 2006), Cape Verde little shearwater in blue (N=7 in 2007) and Bulwer's petrel in red (N=5 in 2007) from Cape Verde.

showed significant greater $\delta^{15}N$ values at intra- and interspecific level than tropical and subtropical populations (Figure 1). These spatial differences resulted in a larger $\delta^{15}N$ variability (ranging up to 9‰) compared to previous seabird communities studies

(Hobson *et al.* 1994; Thompson *et al.* 1999). Trophic differences between seabird populations breeding in north and mid-northeast Atlantic regions could explain the δ^{15}N differences. However, we consider that this factor is unlikely to have a strong influence on the isotopic signatures, since the main prey (Clupeid fishes) of the seabird species included in the present analysis are abundant in both regions. Therefore, the enrichment in the nitrogen signatures of north Atlantic seabirds is probably due to changes in δ^{15}N baseline levels. This conclusion is consistent with previous studies, which found a general trend in seabirds and marine mammals to enrich nitrogen signatures with latitude, from north temperate to Arctic regions (Kelly 2000). Furthermore, marine chemistry studies based on δ^{15}N in zooplankton and marine nitrogen processes agree with the hypothesis of different baseline levels between north and mid-north Atlantic (Waser *et al.* 2000; Montoya *et al.* 2002; Mino *et al.* 2002; Graham *et al.* 2010). These spatial patterns enhance the potential of isotopic approaches to assess migratory movements among distant water masses, as recently shown in Cory's shearwaters (Ramos *et al.* 2009b). Therefore, although geographical differences in baseline isotopic signatures do not seem to be the most important source of isotopic variability within large regions, baseline isotopic changes need to be considered when comparing distant populations, not only in terrestrial but also in the marine environment.

Spatial patterns of OCs: Our study revealed marked spatial patterns in OC levels and profiles from seabirds. Inter-colony variations of blood PCBs and DDTs in pelagic seabirds were homogeneous regardless of the species, not only at large but also at regional scale (Figure 3). That is, all species showed similar inter-colony differences in their OC levels, emphasizing the influence of seabird distribution in OC contamination. Several factors could contribute to these patterns, but the two major ones may be attributed to diet differences among colonies and to geographic differences in pollutant baseline levels. However, it is unlikely that all the species would show dietary changes across their distribution which could explain similar spatial variations in their OC levels. Moreover, in the case of *Calonectris* shearwaters, a species which covers the whole geographic range of this study, blood δ^{15}N was significantly correlated with OC levels within breeding localities but not among them, reflecting a relationship between seabird contaminant burdens and trophic ecology at intra-locality level. Since OC levels and δ^{15}N were only correlated after controlling for breeding colony, inter-colony δ^{15}N variations in seabird blood probably indicate spatial variations in the isotopic baseline levels rather than seabird trophic variations. Therefore, inter-colony differences in pollutant levels seem to reflect geographic differences in OCs rather than differences in trophic ecology among localities.

Geographic differences in PCB and DDT levels mainly arose from the greater OC levels found in Mediterranean shearwaters and petrels compared to their Atlantic counterparts (Figure 3). Further evidences of global differences in organochlorine

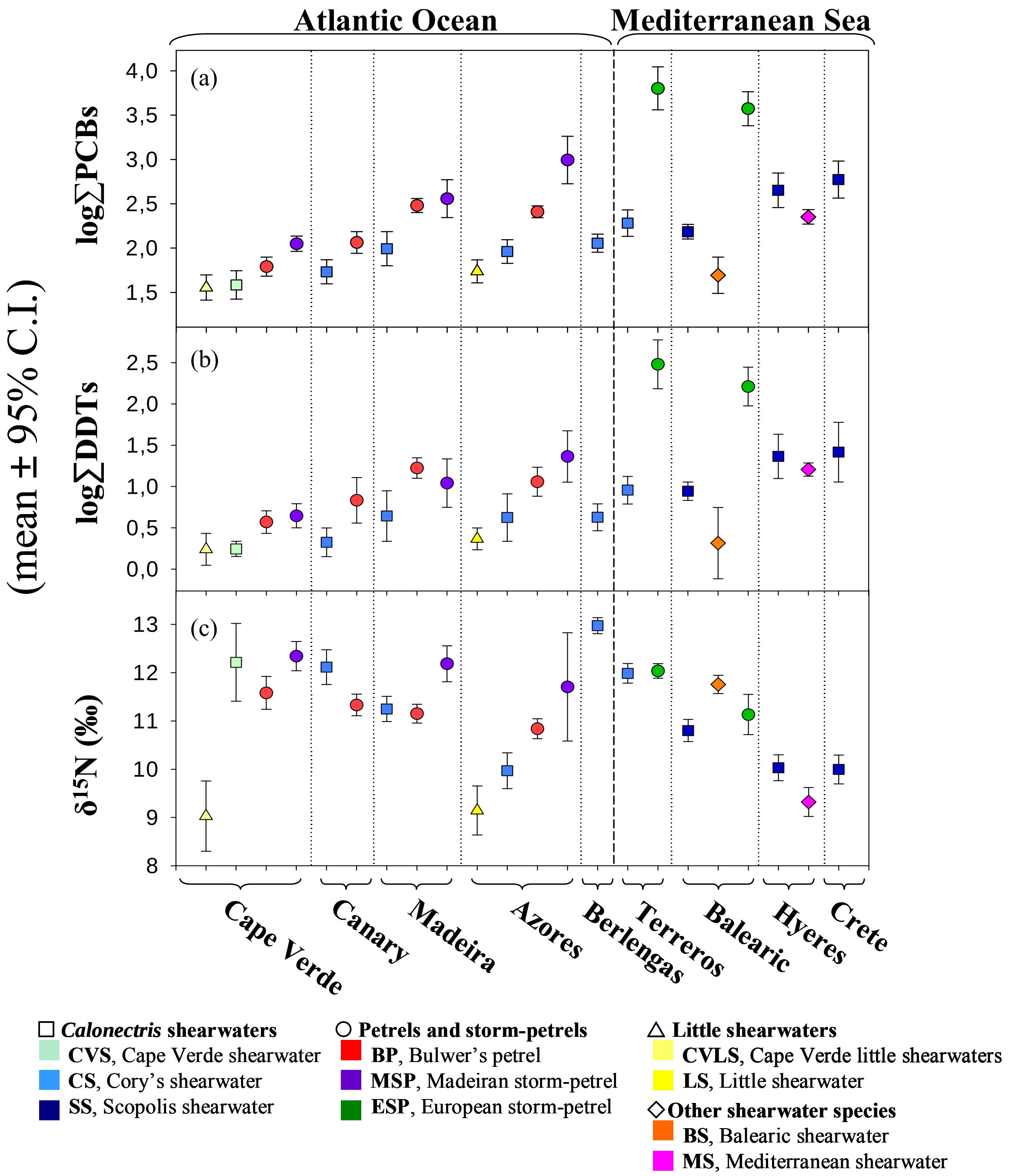


FIGURE 3. Mean and 95% intervals of confidence (IC) of total PCBs (a) and DDTs (b) and nitrogen stable isotope signatures (c) in blood of pelagic seabirds from the NE Atlantic Ocean and the Mediterranean Sea depending on their breeding localities.

contamination between these basins are supported by seabird PCB profiles (Figure 4). PCA analysis segregated Mediterranean breeders (except the Balearic shearwater) and Madeiran storm-petrel from the rest of seabirds, due to a greater degree of chlorination in their PCB profiles (Figure 5). The high chlorination degree in the PCB profiles of Madeiran storm-petrels is likely related to its dietary preferences and detoxification capabilities (see below). However, in the case of Mediterranean seabirds, this global pattern is consistent with the confined character of this sea and its great historical inputs

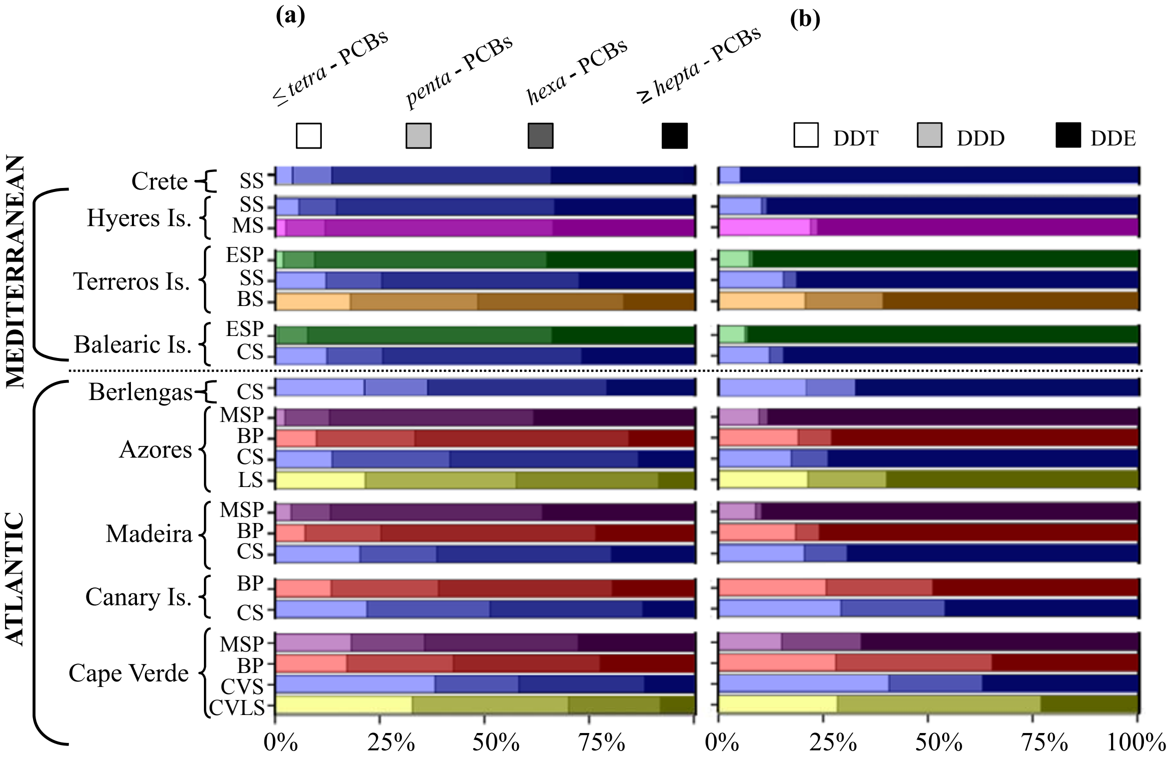


FIGURE 4. PCB and DDT profiles in blood of pelagic seabirds in relation to their breeding localities. Color and abbreviation legends are included in the Figure 3.

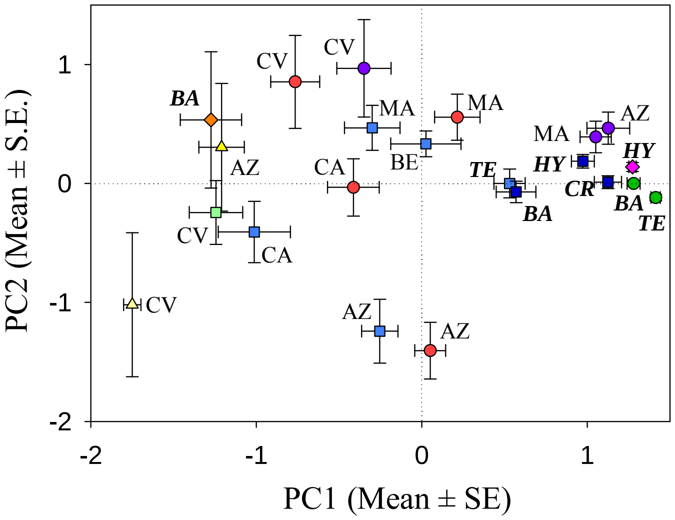


FIGURE 5. Principal components (PC1-PC2) with the relative normalized contributions of all PCBs analyzed in the blood of pelagic seabird species. Breeding localities from the Mediterranean Sea are indicated in bold and italic type. Color and symbol legends are included in the Figure 3.

Atlantic breeding localities: CV, Cape Verde; CA, Canary Is.; MA, Madeira; AZ, Azores; BE, Berlengas
Mediterranean breeding localities*: *TE*,** Terreros Is.**; *BA*,** Balearic Is.**; *HY*,** Hyeres Islands; ***CR, Crete.

of OCs, which ultimately, results in greater levels of contamination (Martí *et al.* 2001) compared to other regional seas or the Atlantic basin.

Within each basin we also found some consistent spatial patterns. OC levels increased from west to east in the case of Mediterranean *Calonectris* shearwaters, which were sampled along this water mass (Figure 3). Although most studies of OCs conducted in the Mediterranean have focused on the western region (probably due to the major development degree of the countries from this area) our results suggest greater levels of these pollutants in the eastern region. Further studies dealing with eastern Mediterranean contamination are needed to confirm this spatial pattern.

In the case of the Atlantic, PCB and DDT levels in the blood of seabirds increased from the south to the north regardless of the species (Figure 3). This latitudinal pattern could be related to long range pollutant transport from highly contaminated regions to remote open water. On one hand, the greater OC levels in northern (Azores and Madeira) compared to southern (Cape Verde and Canary Is.) seabird colonies could be related to the influence of the Gulf Stream and north Atlantic air currents over the central Atlantic region. Previous studies have suggested that these currents represent a flux of water, air and pollutants from North American coasts above the central north Atlantic, including the Azorean region (Santos *et al.* 1995; Stohl *et al.* 2002). On the other hand, the greater OC levels in shearwater blood from Berlengas Is. compared to the rest of Atlantic shearwaters may be related to the influence of Mediterranean waters along the southern Portuguese coast (Ambar *et al.* 2002). Although contamination levels were generally lower on seabirds from Canary and Cape Verde islands, their DDT profiles (Figure 4) suggest the nearby African countries as the most likely source for these contaminants. Seabirds breeding in these archipelagos tended to show a greater proportion of DDT compared to DDE. In fact, DDE/DDT ratios were lower than 1 in the 44.7% and 31.6% and lower than 2 in the 21.1% and 15.9% of seabirds from Cape Verde and Canary Is., respectively. The rest of seabirds from this study showed DDE/DDT ratios considerably greater than two. These results remark that seabirds from Cape Verde and Canary Is. have been recently exposed to DDT. Since the use of DDT was banned in Europe and North America during the 70s and the 80s, the source of this contamination is likely to be the nearby Sub-Saharan African countries, where DDT has been largely used to control malaria outbreaks (Walker *et al.* 2003).

The influence of geographic origin on seabird PAHs: Unlike OCs, our results showed a minor effect of breeding locality over PAH burdens in seabirds (Figure 6). This study includes measures of PAHs in liver from shearwater species breeding in the Atlantic and the Mediterranean and petrel species from the Atlantic basin. Since interspecific comparison of these pollutants between regions could be confounded due to trophic or detoxification efficiency differences between petrels and shearwaters, to evaluate spatial trends in PAHs we focused on PAH differences between *Calonectris* shearwaters. That is, *Calonectris* shearwaters include the Scopoli's and the Cory's shearwater, breeding in the Mediterranean and the Atlantic, respectively. They are closely related forms, which were considered subspecies of *C.diomedea* until recently and show very similar morphology, ecology and physiology (Brooke 2004; Gómez-Díaz & González-Solís 2007; Gómez-Díaz *et al.* 2009). Therefore, since no differences in the feeding ecology or metabolic capabilities of *Calonectris* shearwaters are expected, differences in PAHs between them should be related to geographic patterns in contamination (Roscales *et al.*

2010). However, we did not find significant differences in PAH burdens between them (Figure 6). Nonetheless, some dissimilarities in PAH profiles between Scopoli's and Cory's shearwater may be related to their different geographic origin.

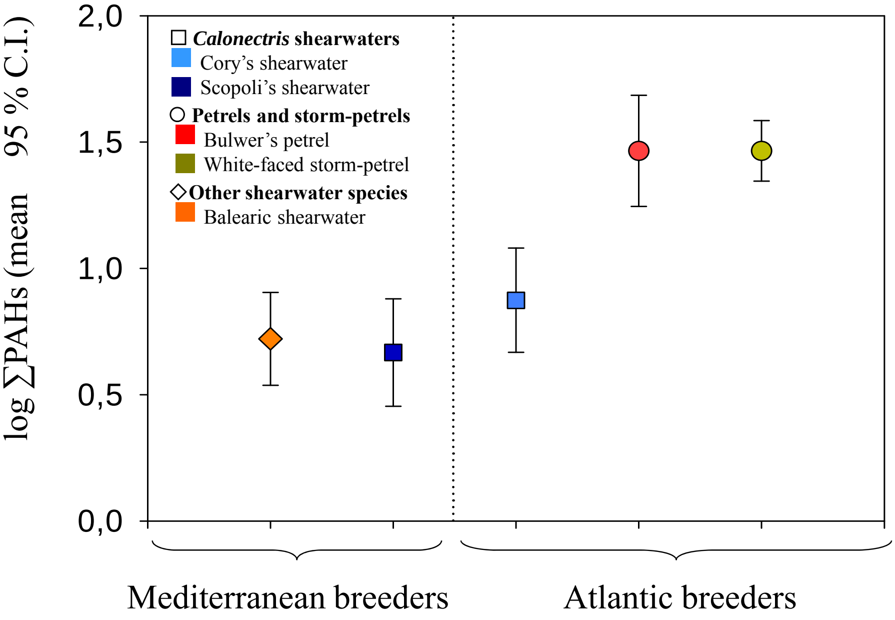


FIGURE 6. ∑PAH levels in livers of Atlantic and Mediterranean adult seabirds

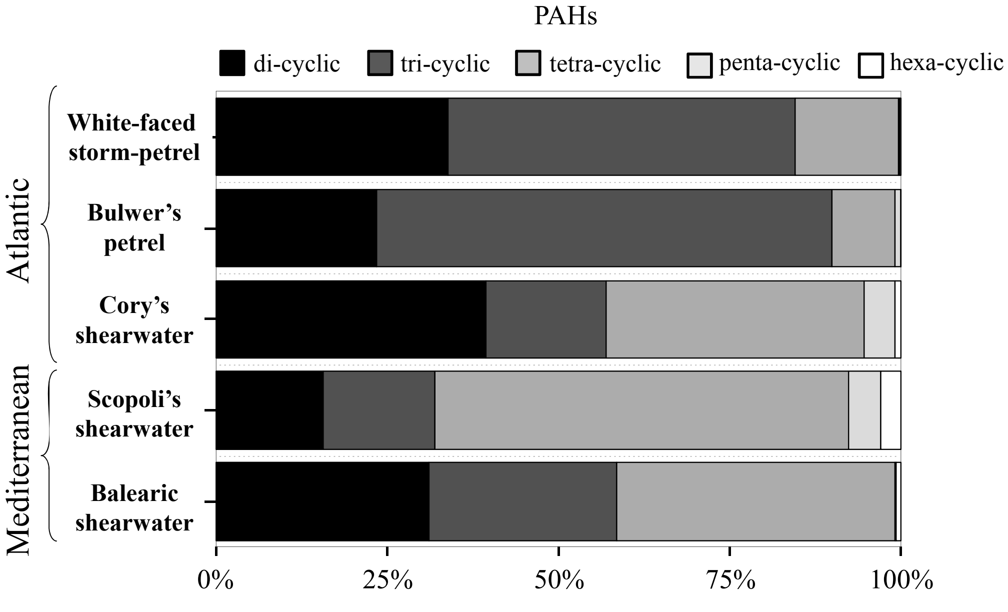


FIGURE 7. PAH profiles in adult seabird livers calculated as the percent of each PAH with respect to $\sum_{15}$PAH

The PAH profile of Scopoli's shearwaters was clearly dominated by high-molecular-weight compounds (tetra-, penta- and hexa-cyclic PAHs; Figure 7). However, Cory's shearwater profile showed similar contribution of high and low-molecular-weight-

PAHs. Low-molecular-weight-PAHs are the primary constituents of natural petroleum seeps, in contrast to urban sources of PAHs (Seruto *et al.* 2005; Kannan & Perrotta 2008). Therefore, the major presence of heavy PAHs in the case of Scopoli's shearwater could be related to a greater presence of anthropogenic sources for PAHs in the Mediterranean compared to the Atlantic. However, PAH profiles between Atlantic and Mediterranean species were not segregated by the PCA analysis (Figure 8), which indicates a limited importance of the species breeding ground over PAHs.

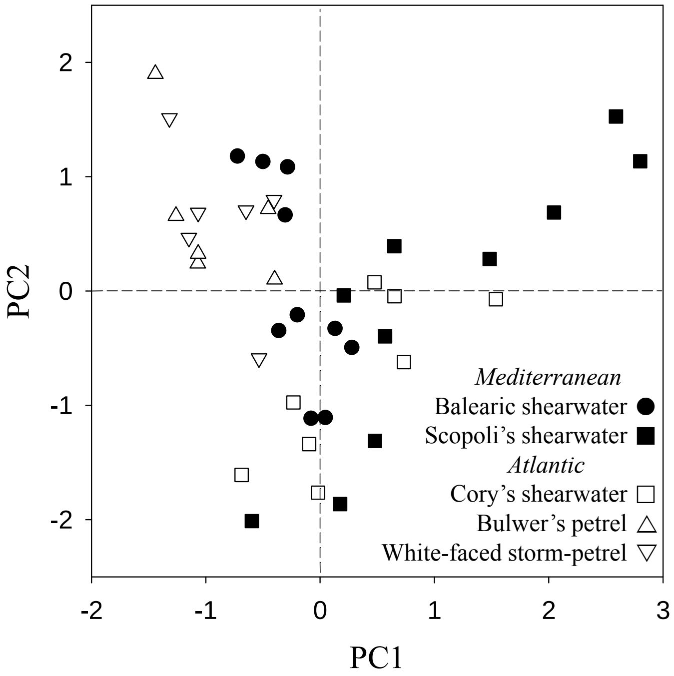


Figure 8. Principal components (PC1-PC2) extracted from the relative contributions of the analyzed PAHs in the liver of pelagic seabirds.

In contrast with OCs, the distribution of seabirds seems to have a minor effect over their PAH levels and profiles. That is the case even when comparing between the Atlantic and the Mediterranean, which have been proved to show different baseline levels for these contaminants (Martí *et al.* 2001; Albaigés 2005). Although OCs were analyzed in seabird blood and PAHs in liver, both tissues are assumed to indicate recent exposition to these pollutants (Hellou 1996; Bustnes *et al.* 2005b; Finkelstein *et al.* 2006; Yamashita *et al.* 2007; Kannan & Perrotta 2008). Therefore, the different influence of seabird distribution on their pollutant levels is probably related to the specific capability of the species to metabolize and eliminate OCs and PAHs. In the case of PAHs, it is known that birds rapidly metabolize and readily excrete PAHs (Broman *et al.* 1990; Troisi *et al.* 2006). For example, 94% of PAHs injected into chicken eggs are metabolized within 14 days (Näf *et al.* 1992) and previous studies have demonstrated insignificant PAH bioaccumulation and biomagnification in high trophic position predators (Wan *et al.* 2007). On the contrary, most OC compounds bioaccumulate in bird tissues since most congeners are hardly metabolized (D'Adamo *et al.* 1997; Drouillard *et al.* 2009). Consequently, our results suggest that the rapid elimination of PAHs by birds could be masking the likely existence of geographical patterns in baseline marine PAH levels.

ON THE ROLE OF TROPHIC ECOLOGY FROM AN ISOTOPIC PERSPECTIVE

Insights of stable isotope analyses on the trophic ecology of the NE Atlantic seabirds: As commented before, stable isotope signatures in feathers were grouped mainly by seabird species, but the major axis of interspecies variability was provided by $\delta^{13}C$ rather than $\delta^{15}N$ signatures. Indeed, seabird species from mid-north Atlantic showed a noticeable overlap in their $\delta^{15}N$. Most sampled colonies (66%) were grouped in a narrow range (about 3‰) of $\delta^{15}N$ signatures, which included all mid-north Atlantic breeders except the two little shearwater species (Figure 1).

Previous studies on seabird communities already found a strong overlap in seabird $\delta^{15}N$ (Forero *et al.* 2004) and their diet (Diamond 1983). Forero *et al.* (2004) offered a few dietary alternatives at each trophic level as the cause of overlap in stable nitrogen signatures of seabirds from Patagonia. This is also likely to occur in petrels breeding on mid-north Atlantic archipelagos, since this is an oligotrophic area, and thus, no rich pelagic communities are expected (Longhurst 1998). Considering that generally, $\delta^{15}N$ signatures enrich from 3‰ to 5‰ in each trophic level across marine food webs (Hobson & Welch 1992), the small difference in $\delta^{15}N$ found in feathers (Figure 1) and blood (Figure 3) of mid-north Atlantic shearwaters and petrels suggest that there are no differences in the trophic positions of most pelagic seabirds from this region. Nevertheless, isotopic signatures of little shearwaters reported here suggest that these species feed on a trophic level below the other mid-north Atlantic species. Unfortunately, we do not have details about the diet of little shearwaters. However, previous studies found lower Hg levels in little related to Cory's shearwaters, which is consistent with our results of a lower trophic level for little shearwaters (Monteiro & Furness 1995; Monteiro & Furness 1997; Monteiro *et al.* 1998).

In the case of $\delta^{13}C$, differences in size among species are probably associated to different flight and diving capabilities, which ultimately shape their trophic niche. Despite this, we could not recognize obvious general patterns correlating stable isotope signatures with the biological traits of the studied species. In some cases, however, differences in isotopic signatures among populations within a seabird species reflect the exploitation of different feeding locations. This is the case for the *Calonectris* shearwaters breeding in Canary, Selvagens and Cape Verde islands. These populations showed significantly greater $\delta^{13}C$ signatures than any other Cory's shearwater population breeding on the mid-north Atlantic colonies (Figure 1). As shown by the GLS devices (Figure 2), most Cory's shearwaters breeding on Canaries fed along the productive African shelf associated to the Canary current, resulting in a relatively enriched $\delta^{13}C$ values in their feathers, typical from inshore feeding (Kelly 2000). In contrast, Cory's shearwaters breeding in Azores fed on pelagic waters and showed depleted $\delta^{13}C$ values. Probably due to the larger distance to the African coast, Cory's shearwaters breeding in Selvagens and Cape Verde shearwaters make an intermediate use of the African shelf, resulting in intermediate $\delta^{13}C$ values. Carbon signatures of Cory's shearwaters breeding on the southernmost Macaronesian archipelagos were also substantially enriched compared to any other seabird species also breeding on the same archipelagos. This difference is probably reflecting the outstanding capability of Cory's shearwaters to reach remote areas such as the African continental shelf, compared to the more modest mobility of other small petrel species, such as the Bulwer's petrel.

In addition, our results also revealed some insights into the poorly known ecology of some seabird species from the northeast Atlantic, such as the Fea's petrel. Diet and movements of Fea's petrel are largely unknown, but its $\delta^{15}N$ signature suggests that this species feeds on higher trophic level preys than Cape Verde and Cory's shearwaters, and does it probably along the African shore, if we consider $\delta^{13}C$ signatures. To summarize, this study highlights the potential of combining isotopic and tracking approaches to reveal a meaningful view on the specific and interspecific trophic ecology of pelagic seabirds across a vast geographic range. Overall, our results showed a great homogeneity in the trophic ecology of most seabirds at intraspecific level but we also found clear differences among populations of some species. As the clearest example, Cory's shearwaters, which seems to specialize in the exploitation of specific resources, such as offshore or inshore preys, depending on the proximity of productive areas to their breeding colony. Therefore, not only interspecfic but also intraspecific sources of variation in the trophic ecology of seabirds could be influencing their contamination status.

The influence of individual trophic specialization on intraspecific OCs variability: To study the influence of trophic ecology over OCs in seabirds at intraspecific level, this research focused on *Calonectris* shearwaters. Concretely, we analyzed $\delta^{15}N$, $\delta^{13}C$, PCBs and DDTs in shearwater blood across the entire breeding range of Cape Verde, Cory's and Scopoli's shearwaters. We found a significant effect of $\delta^{15}N$ explaining PCB and DDT concentrations at intraspecific level after controlling for the breeding locality. In fact, all the colonies showed a consistently positive relationship between $\delta^{15}N$ and OC levels, except Terreros Is. and Cape Verde (Figure 9). Since $\delta^{15}N$ signatures increase with trophic levels, relationships between $\delta^{15}N$ signatures and OCs probably reflect the well known biomagnification properties of PCBs and DDTs. Exceptions, such as Terreros Is. and Cape Verde, are probably due to a differential use of feeding grounds with different baseline $\delta^{15}N$ levels, which obscures the biomagnification processes. For example, shearwaters from Terreros have been suggested to feed on both Mediterranean and Atlantic waters, with contrasting $\delta^{15}N$ baseline levels (Navarro *et al.* 2009a).

In the case of carbon, $\delta^{13}C$ did not show any significant effect explaining seabird OC levels because breeding locality and $\delta^{15}N$ accounted for most of the variability in shearwater OC levels. However, we found some evident patterns in shearwater $\delta^{13}C$ that were related to their OCs. That is, Cory's and Cape Verde shearwaters breeding in

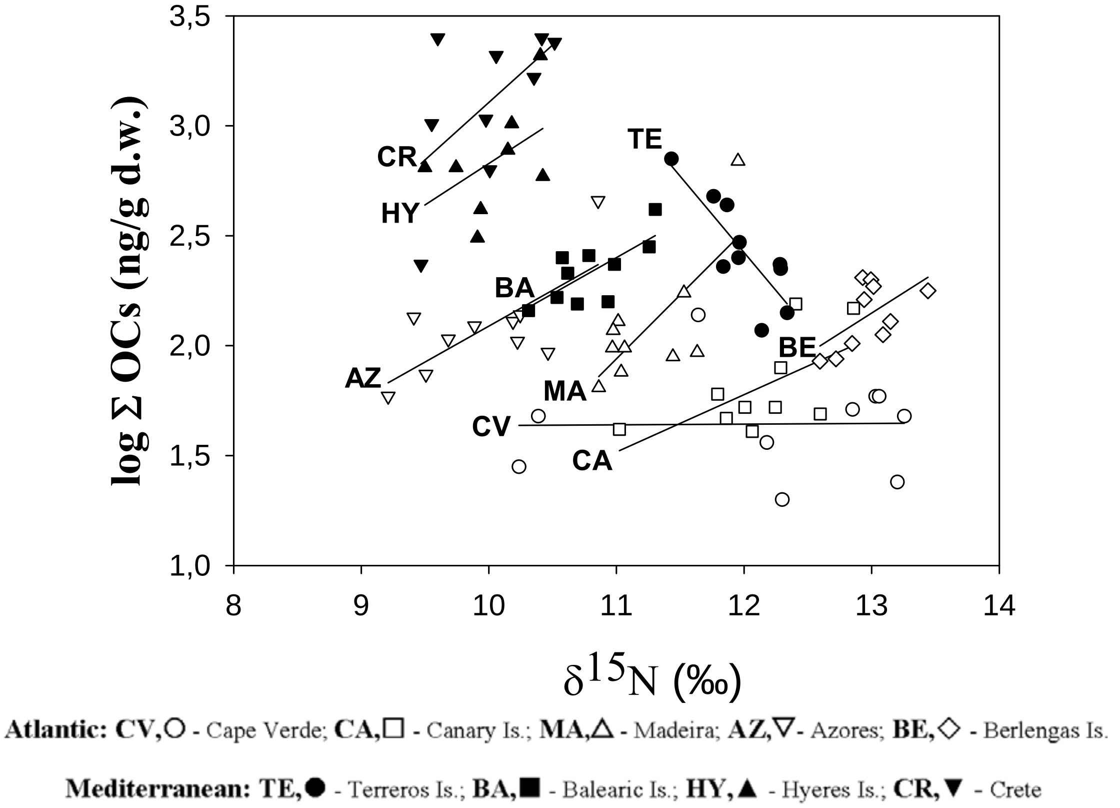


Figure 9: Relationships between $\delta^{15}N$ signatures and OC levels in *Calonectris* shearwaters. Linear regressions are shown for each breeding colony separately and identified by the abbreviation of the locality.

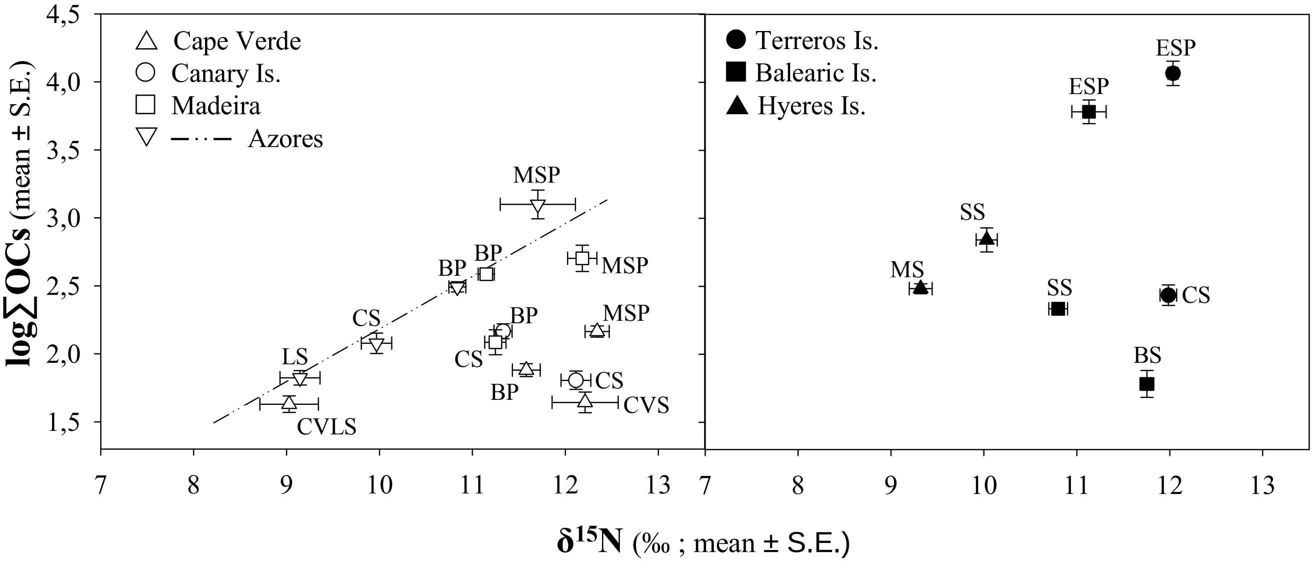
Canary and Cape Verde, respectively, showed significant greater blood $\delta^{13}C$ values compared to the remaining *Calonectris* shearwater populations (Appendix 1). In contrast, Cory's shearwaters breeding in Azores and Madeira showed the lowest mean $\delta^{13}C$ signatures. These dissimilarities in $\delta^{13}C$ reflect the differential exploitation of inshore and offshore resources among Atlantic *Calonectris* populations, which agree with our feather isotopic results. Specifically, as shown by GLS devices and previous studies, while shearwaters from Cape Verde and Canary Is. feed mainly over the Sub-Saharan African shelf (Navarro & González-Solís 2007; Ramos *et al.* 2009a), those from Azores and Madeira feed on pelagic waters that are far away from any coastal system (Ramos *et al.* 2009a). These differential feeding habits of shearwaters are related to the lower levels of PCBs and DDTs as well as to the specific OC profiles of shearwaters from Cape Verde and Canary islands, which suggests a recent exposition of these seabirds to DDT, as we stated previously.

Previous studies have pointed out sex as a major source of differences in pollutant levels due to the different trophic ecology between males and females (González-Solís *et al.* 2002; Martínez-Abraín *et al.* 2006; Wearmouth & Sims 2008) or to the different opportunities of males and females to excrete pollutants. The latter possibility usually refers to the additional route of females to excrete OCs by transferring them into the eggs (Drouillard & Norstrom 2000; Martinez-Abraín *et al.*

2006; Alonso-Alvarez *et al.* 2007). This study assessed if sex could explain variations of blood OC concentrations in *Calonectris* shearwaters which were sampled after egg laying. Sex was also considered to explain seabird PAH variability for all analyzed species. However, males and females did not show significant differences in OC or in PAH levels. The lack of sexual differences found in this study suggests similar ecology for both sexes, which agree with our isotopic results and previous studies (Navarro *et al.* 2009b). Moreover, these results also indicate that egg laying does not have a lasting effect on blood OC concentrations in these species. In the case of PAHs, since we sampled seabirds in different phases of their breeding period, we cannot confirm this hypothesis for liver PAH levels in the liver. Nonetheless, sex seems not to influence pollutant levels in the seabird species included in this study.

The relationship between nitrogen signatures and OCs found in this study indicates that biomagnification processes not only become apparent across species but can also be detected at the intra-specific level, which reflects the relevance of dietary preferences among seabird individuals. Moreover, $\delta^{13}C$ revealed the use of specific feeding grounds by seabird populations, which also result in different exposition to contaminants among individuals. Therefore, the present study confirms the utility of combining isotopic approaches with pollutant studies to provide new insights into the dynamics of contaminants in seabird populations. Interdisciplinary studies emerge as a powerful tool to properly understand the information that marine predators offer as biomonitors of marine pollution.

Interspecies patterns of seabird POPs: the relationship between trophic position and pollutant burdens. It is generally assumed that the biomagnification of PAHs in marine food webs is insignificant, so in the present research no relationship between seabird trophic position (inferred from $\delta^{15}N$) and their PAH burdens was expected. Conversely, previous studies have shown significant positive relationships between seabird $\delta^{15}N$ and their OC levels (Buckman *et al.* 2004; Elliott 2005; Ricca *et al.* 2008; Elliott *et al.* 2009). Authors attributed these findings to the biomagnification of these pollutants throughout marine food webs, highlighting trophic position as the main factor behind OC burdens in marine organisms. In agreement, we also found a marked influence of this factor upon OC burdens at intraspecific level. In the present study, organochlorine contaminant levels in seabird blood differed significantly among species within all the breeding localities. Overall, *Calonectris* shearwaters showed greater amounts of OCs compared to the rest of shearwater species but significant lower levels than petrels (Figure 3). This general pattern when comparing petrels and *Calonectris* shearwaters was also found in the case of their liver PAH levels. However, stable-nitrogen signatures of seabird species showed a great overlap which was not consistent with the variation pattern of their OC levels (Figure 3). In fact, $\delta^{15}N$ was a non-significant covariable that did not explain OC differences among seabird species and we only found a positive significant relationship between species ∑OCs and $\delta^{15}N$ in the case of Azorean seabirds (Figure 10).



CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer's petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory's shearwater; **SS**, Scopoli's shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

Figure 10: δ^{15} N signatures and ∑OC concentrations in seabird blood. Significant relationships between these variables within each breeding locality are shown. Relationships are shown separately for Mediterranean and Atlantic seabirds for clarity purposes.

Regarding organochlorine contamination among the studied seabirds the Macaronesian little, the Cape Verde little and the Mediterranean shearwaters showed the lowest OC burdens and also the lowest $\delta^{15}N$ signatures within their breeding localities. These results are consistent with previous studies of heavy metals suggesting a relative lower trophic position for little shearwaters (Monteiro *et al.* 1995) as well as with our isotopic results in feathers. On the other hand, Bulwer's petrels, Madeiran storm-petrels and European storm-petrels showed significant greater OC concentrations than *Calonectris* shearwaters, regardless of the breeding locality. However, $\delta^{15}N$ values in blood were only significantly greater in petrels than in *Calonectris* in the case of Azores, reflecting a possible relative lower trophic position for these shearwaters in relation to petrels within this breeding locality (Figure 10). As commented above, overall, the small differences in stable-nitrogen isotope signatures in blood and feathers of petrel species and *Calonectris* shearwaters reported here suggest no differences in their trophic position, which agrees with previous studies of contamination (Monteiro *et al.* 1995; Monteiro *et al.* 1998). Therefore, the upsets between $\delta^{15}N$ and OC levels as well as the interspecific differences in PAHs demonstrate the need to consider other factors apart from trophic position to understand the degree of bioaccumulation of these contaminants in seabirds. In this research we considered metabolic efficiencies and energetic requirements as well as dietary preferences of seabirds as possible factors obscuring the biomagnification of OCs and explaining the pollutant differences found in seabird species.

ALTERNATIVE FACTORS TO TROPHIC POSITION EXPLAINING POP LEVELS IN SEABIRD SPECIES

Metabolic efficiencies and energetic requirements: Differences in OCs and PAHs between shearwaters and petrels could be related to specific metabolic efficiencies or energetic requirements of the seabird species. The smaller the species, the greater their energetic requirements and therefore, the greater their feeding rates. This explains why small species are prone to accumulate greater contaminant levels. Petrel species included in this study are considerable smaller than *Calonectris* shearwaters (General introduction, Box 8), which may partly explain their greater pollutant levels. However, although the little shearwater is about 73% lighter than *Calonectris* shearwaters and only 26% heavier than Bulwer's petrel, little shearwaters showed significant lower OC levels than Cory's and Cape Verde shearwaters. Moreover, all the analyzed species are phylogenetically close (O. Procellariiformes) and biologically and ecologically similar, compared to other seabird groups (Heidrich *et al.* 1998; Austin 2004; Brooke 2004; Gómez-Díaz *et al.* 2009). Therefore, although we do not have extended details of the energetic requirements of the seabird species included here and thus we cannot completely exclude some differences in pollutant levels associated to this factor, it seems to have a minor influence.

Concerning metabolic capabilities of seabirds, previous studies on Mixed-Function Oxidase (MFO) in seabirds found little enzymatic activity differences among pelagic species after PCB and PAH exposure. Concretely, comparisons between Leach's storm-petrel (*Oceanodroma leucorhoa*) and Manx shearwater (*Puffinus puffinus*) showed similar activity in both species for most hepatic enzymes, with the exception of the aldrin epoxidase, 7-EROD and expoxide hydrolase, which showed higher activity in the case of the storm-petrel (Knight & Walker 1982; Peakall *et al.* 1987). These small differences in the MFO activity between storm-petrels and shearwaters could be related to the differences we found in OC and PAH profiles between petrel and shearwater species (Figures 4 and 7, respectively) . On one hand, the major presence of low-molecular-weight-PAHs in petrels than in shearwaters may be related to a major capability of petrels to metabolize larger PAH compounds. On the other hand, the lower presence in the petrel species of low chlorinated PCBs, which are generally more easily metabolized by vertebrates, is probably associated with a major detoxification activity in these species. Therefore, although metabolic activity differences among species are probably related to seabird pollutant profiles, this alone could not explain the greater pollutant levels of petrels compared to shearwaters.

The relevance of seabird dietary preferences on their pollutant levels: Overall, the study of $\delta^{15}N$ in blood and feathers of petrel species and *Calonectris* shearwaters suggested the absence of marked differences in their trophic position, which agree with previous studies about Azorean seabirds (Monteiro *et al.* 1995; Monteiro *et al.* 1998). However, similar isotopic values among species or individuals cannot necessarily be interpreted as an evidence for similar diets (Hobson *et al.* 1994). Although seabird species included in this study feed mainly on pelagic fish (followed by lower proportions of cephalopods, crustaceans and plankton), they exploit different prey types. Madeiran storm-petrels and Bulwer's petrels are mainly nocturnal feeders specialized in exploiting daily vertical mesopelagic (from 200 to 1000 m of depth) migratory fishes, such as myctophids (Monteiro *et al.* 1996; Monteiro *et al.* 1998; Bolton *et al.* 2008). *Calonectris* shearwaters are mainly epipelagic (0-200 m) diurnal feeders. The European storm-petrel feeds on a wide variety of marine resources (fish, crustacean and cephalopods) including miscellaneous fatty droplets, fisheries discards and scavenging events on dead marine mammals (D'Elbée & Hémery 1997; Valeiras 2003; Brooke 2004). These dietary differences between petrels and shearwaters probably influence their OC levels and profiles.

Previous studies in the Atlantic and Pacific oceans have reported greater burdens of OCs in mesopelagic and deep sea fishes compared to those from the upper water layers (Ballschmiter *et al.* 1997; Froescheis *et al.* 2000; De Brito *et al.* 2002; Mormede & Davies 2003). Specifically, studies on Pacific mesopelagic fishes have revealed significantly greater burdens of organic pollutants in some daily vertical migrant and semi-migrant myctophid species in comparison to other mesopelagic fishes (Takahashi *et al.* 2000). Therefore, seabirds feeding on mesopelagic resources could be exposed to greater OC burdens than those of similar trophic position but feeding on epipelagic preys, which has also been reported previously in mercury studies (Monteiro & Furness 1995). However, in the case of PAHs, only a few works have focused on these contaminants in mesopelagic food-chains or deep-sea organisms, and higher PAH burdens in mesopelagic than in epipelagic ecosystems have not been described. In fact, previous studies have shown a general surface-enrichment depth-depletion distribution for these pollutants in the water column (Dachs *et al.* 1997; Schulz-Bull *et al.* 1998; Martí *et al.* 2001). Nonetheless, some authors have suggested that the vertical distribution of PAHs in the Mediterranean and the Atlantic seems to be governed by planktonic bioaccumulation at open sea. They found a submaximum concentration of PAHs in zooplanktonic spots at 300 m depth, proposing that migratory zooplankton supports the flux of hydrophobic pollutants in the water column (Dachs *et al.* 1997; Jaward *et al.* 2004). Migratory mycthopyds feed mainly on these spots of zooplankton and they follow them in their daily vertical migrations, which could involve a greater exposure to PAHs. Therefore, feeding specialization of seabirds in mesopelagic preys emerge as a reasonable explanation for our results. This study suggests mesopelagic food webs as the most susceptible to accumulate and transfer persistent organic pollutants to marine predators. Nonetheless, further investigations in POP levels of myctophids as well as in the trophic and vertical flux of these compounds is needed in order to reinforce this hypothesis.

Dietary preferences of the European storm-petrel could also be contributing to the upset between OC burdens and $\delta^{15}N$ signatures of this species compared to *Calonectris* shearwaters. For instance, fatty droplets could contribute to elevate storm-petrel OC burdens but not their nitrogen signatures, since OCs are liphofilic compounds but droplets lack protein content. In addition, previous studies have shown that seabird scavenging events also could contribute to confound the relationship between OC levels and $\delta^{15}N$ (Fisk *et al.* 2001; Elliott 2005). In this study we found a marked upset between $\delta^{15}N$ and OC concentrations in the case of another species, the Balearic shearwater. This species also showed remarkable dissimilar PCB and DDT profiles compared to the rest of Mediterranean breeders. However, we did not find any satisfactory explanation for the unexpected levels and profiles in Balearic shearwaters in relation to its feeding ecology or metabolic capability. This finding constitutes a challenge for future research. Studies on the ecology and movements of this endangered species as well as on the OC levels of its potential preys are necessary to understand contaminant levels and profiles in the Balearic shearwater.

INTER-STUDIES COMPARISONS

Pollutant levels reported in previous studies of seabirds or marine predators can provide a useful context to evaluate the geographic trends in OCs and PAHs reported here and to assess possible risks due to marine pollution. Nonetheless, comparisons of the pollutant levels obtained in this study with those obtained from other marine predators are problematic. On one hand, POP levels depend on the manner in which concentrations are expressed (wet, dry or lipid weight) and vary broadly among tissues. On the other hand, since species sensitivity to pollutants varies widely and stress factors do not impact upon different species equally, inter-specific comparisons are difficult and some times scarcely representative (Thompson & Hamer 2000; Fisk *et al.* 2005). Therefore, although in this study we have focused mainly on comparisons between close species avoiding inter-tissues comparisons, they must be considered carefully. With the aim of evaluating possible risks for seabirds and draw possible temporal and spatial trends of marine pollution, we have compared our results with those from the studies included in the Tables 1 and 2.

Previous studies on seabirds have reported adverse ecological effects on the reproductive performance and behavior of gulls due to their OC burdens (Table 1) (Bustnes *et al.* 2001; Helberg *et al.* 2005; Bustnes *et al.* 2005a). The mean ∑PCB levels obtained in the present study were lower than those reported for gulls in all the species with the exception of European storm-petrels from the Mediterranean. However,

TABLE 1. Concentrations of PCBs in different tissues of seabirds

Location	Year	Species	Tissue	Concentration (ng/g dry wt)	Concentration (ng/g wet wt)	Remarks	Reference
Macaronesia **NE Atlantic**	2003-2005	Cory's shearwater *Calonectris borealis*	Blood		50.74[a] (5.53 – 87.41)	20 PCBs	This study
	2003-2006	Bulwer's petrel *Bulweria bulwerii*			34.07 (5.89 – 79.08)		
	2003-2006	Madeiran storm-petrel *Oceanodroma castro*			95.89 (13.45 – 308.73)		
Mediterranean	2004-2006	Scopoli's shearwater *Calonectris diomedea*	Blood		67.19 (16.38 – 212.59)	20 PCBs	This study
W Mediterranean	2004	Mediterranean shearwater *Puffinus yelkouan*	Blood		40.94 (27.88 – 50.62)	20 PCBs	This study
	2004	Balearic shearwater *Puffinus mauretanicus*			9.65 (4.828 – 18.91)		
	2004	European storm-petrel *Hydrobates pelagicus*			1014 (569.7 – 2198.9)		
Bear island **NE Atlantic**	1997-1998	Glaucous gull *Larus hyperboreus*	Blood		339.25 (52.40 – 1079)	8 PCBs	Bustnes et al. 2001
Bear island **NE Atlantic**	2000-2001	Glaucous gull *Larus hyperboreus*	Blood		351.21 (326 – 431)	6 PCBs	Bustnes et al. 2005
Loppa Island **NE Atlantic**	2001	Great Black-backed gull *Larus marinus*	Blood		101	8 PCBs	Helberg et al. 2005
Bear Island **NE Atlantic**	2005-2006	Fulmar *Fulmarus glacialis*	Plasma		24.58 (17.04 – 54.56)	23 PCBs	Knudsen et al 2007
Barents Sea **Arctic Ocean**	1992	Herring gull *Larus argentatus*	Egg	7686[b]		21 PCBs	Barrett et al. 1996
Ebro delta **W Mediterranean**	1992	Audouin's gull *Larus audouinii*	Egg	13166		16 PCBs	Pastor et al. 1995
Quiriquina Is. (Chile) **SE Pacific**	1995	Kelp gull *Larus dominicanus*	Egg	1011[b]		20 PCBs	Cifuentes et al. 2003
Macaronesia **NE Atlantic**	1982	Cory's shearwater *Calonectris borealis*	Egg	4190		24 PCBs	Renzoni et al. 1986
Balearic Is. **W Mediterranean**		Scopoli's shearwater *Calonectris diomedea*		95080			

a-Wet weight conversion is based on a moisture content of 82.12% (range 79.7 - 84.6%, n=23) in shearwater blood (This study)

b- Dry weight conversion is based on a moisture content of 76.16% in herring gull eggs (Norstrom et al. 2002)

Calonectris shearwaters breeding in the Mediterranean and Madeiran storm-petrels from the Atlantic showed a range of PCB levels that strongly overlap with those reported for gulls. In addition, some studies of albatross species from the Pacific have documented organochlorine levels believed to be of toxicological concern (Auman *et al.* 1997; Guruge *et al.* 2001; Finkelstein *et al.* 2006). In this case, although we found greater PCB concentrations than those reported for albatross (Table 1), our results are no directly comparable because we used whole blood and the authors used plasma to determine PCB concentrations. In this context, although we cannot assess the possible effects of OCs over pelagic seabird populations, the greater levels found in the Mediterranean compared to the Atlantic shearwaters as well as the great PCB levels found in storm-petrels, probably represent an extra stressor element for these populations. Therefore, monitoring OCs in pelagic seabirds from the northeast Atlantic and the Mediterranean seems appropriate in order to anticipate and prevent possible adverse impacts of OC pollution on their populations.

In the present thesis, greater OC levels were found in the Mediterranean compared to the Atlantic seabird populations. Previous studies on OC levels in gull eggs agree with this global spatial pattern (Table 1). In fact, OC levels from gull eggs suggest that the Mediterranean basin shows greater levels of these pollutants compared not only with the northeast Atlantic but also with other remote regions, such as the southeast Pacific or the Arctic Ocean. Renzoni et al. (1986) also reported the strong difference in PCB levels between the NE Atlantic and the Mediterranean by means of *Calonectris* shearwater eggs. PCB levels in shearwater eggs from northern Macaronesian archipelagos were 95.6% lower than those from Balearic Islands. In the present study, we found a smaller difference in shearwater PCB levels between these regions. PCB levels in shearwater blood from Canary Is. were 62.6% lower than those from Balearic Islands. The smaller difference that we found between Atlantic and Mediterranean shearwaters could be related to temporal variation in PCB levels. It is generally assumed that PCB contamination in marine ecosystems have decreased worldwide since their usage restriction in the 80s. However previous studies have pointed out a faster reduction of PCBs in enclosed marine regions such as the Mediterranean Sea compared to open oceans (Iwata *et al.* 1993; Loganathan & Kannan 1994; Tolosa *et al.* 1997; Bignert *et al.* 1998; Aguilar *et al.* 2002; Tanabe *et al.* 2003). A faster reduction of these pollutants in the Mediterranean basin compared to the open northeast Atlantic may be associated to the smaller difference that we found in PCB levels of Atlantic and Mediterranean shearwaters compared to that reported by Renzoni et al. 25 years ago.

Regarding PAHs, there is a general lack of studies dealing with these pollutants on seabird liver, which makes it difficult to establish useful comparisons with the PAH levels reported here. Previous studies on common eider ducks from the Baltic Sea showed greater PAH levels than those we found in Mediterranean shearwaters (Table 2). The greater levels of the eider are probably due to the major presence of invertebrate preys in their diet compared to shearwaters, which mainly feed on fish (Lebedev *et al.* 1998). Atlantic petrels and shearwaters showed PAH levels similar to those reported for eiders. Nevertheless, authors suggested that PAH burdens from eider livers were low and improbable to cause adverse toxicological effects. In the case of the Atlantic, Troisi et al. (Troisi *et al.* 2006) reported great levels of PAH in liver of oiled common guillemots (*Uria aalge*) from the North Atlantic (Table 1). In general, our results showed lower total PAH concentrations than those found in common guillemots, as we would expect from oiled seabirds. These differences agree with previous studies, which suggest seabirds as possible indicator species to compare PAH levels from oiled and clean marine regions (Pérez *et al.* 2008).

TABLE 2. Concentrations of PAHs in marine wildlife obtained in this and previous studies

Location	Year	Species	Tissue	Concentration (ng/g dry wt)	Concentration (ng/g wet wt)	Remarks	Reference
East Spain **W Mediterranean**	2003-2007	Balearic shearwater *Puffinus mauretanicus*	Liver	20.2[a] (6.3-34.4)	6,07 (1.91 – 10.3)	15 PAHs	This study
		Scopoli's shearwater *Calonectris diomedea*		18.5 (5.30-29.76)	5.56 (1.59 – 8.93)		
Canary Islands **NE Atlantic**	2003-2007	Cory's shearwater *Calonectris borealis*	Liver	28.4 (11.1-57.0)	8.52 (3.32 – 17.1)	15 PAHs	This study
		Bulwer's petrel *Bulweria bulwerii*		108.3 (57.3-220)	32.5 (17.2 – 66.2)		
		White-faced storm-petrel *Pelagodroma marina*		99.33 (61.7-132)	29.8 (18.5 – 39.8)		
E England **NE Atlantic**	2001-2002	Common guillemots *Uria aalge*	Liver		425 (43 - 972)	10 PAHs Oiled seabirds	Troisi *et al* (2006)
Baltic Sea	1986	Common eider duck *Somateria mollissima*	Liver	78		7 PAHs	Broman *et al.* (1989)
Ligurian Sea **W Mediterranean**	1993	Stripped dolphin *Stenella coeruleoalba*	Blubber		36205 (199.40-198369)	15 PAHs	Marsili *et al.* (2001)
Eastern China **NW Pacific**	2002-2004	Humpback dolphin *Sousa chinensis*	Blubber		6751	15 PAHs	Leung *et al.* (2005)
SE United States **NW Atlantic**	2003-2005	Bottlenose dolphin *Tursiops truncatus*	Blubber		757.1 (0 – 3199)	45 PAHs	Fair *et al.* (2010)

a - Dry weight conversion is based on a moisture content of 75% in Procellariiformes' liver (Elliott and Scheuhammer 1997)

Although in the present study PAH levels in liver of pelagic seabird did not show significant differences between Atlantic and Mediterranean basins, other studies have reported greater levels of these pollutants within the Mediterranean. For example, PAH levels in dolphin blubber obtained from the Mediterranean sea are markedly greater than those obtained in other regions, such as the northwest Atlantic and the Pacific (Table 2). The greater spatial differences found in PAH burdens of dolphins compared to those reported here in seabirds might be related to the use of different tissues for PAH analysis, fat in the case of dolphins and liver in the case of seabirds. However, previous studies have pointed out similar or lower content of PAHs in seabird adipose tissue than in liver (Broman *et al.* 1990), suggesting that the use of liver to evaluate seabird PAH levels do not represent a noticeable difference compared to adipose tissue. Although PAH levels in seabirds are not comparable with those reported in marine mammals, PAH levels in dolphin species suggest spatial differences in the exposition to these contaminants. Therefore, the lack of geographic differences in seabird PAH levels that we found in the present study point out the limited usefulness of these species to monitor background marine PAH contamination, at least, compared to other marine predators such as marine mammals.

SEABIRDS AS INDICATORS OF POPS IN THE MARINE ENVIRONMENT

The present study emphasizes the suitability of pelagic seabirds with wide breeding ranges as useful organisms to monitor marine contamination. We found that these seabird species can integrate contaminant levels from open sea areas, relatively unexploited by fisheries compared to seabirds (Karpouzi *et al.* 2007), and therefore, difficult to monitor for contaminants and poorly explored. Moreover, non-destructive sampling procedures such as seabird blood extraction emerge as suitable methodologies to evaluate marine pollution in these regions. Nonetheless, our results suggest that the potential of seabird species as indicators depends on the chemical contaminant family to be evaluated.

Pelagic seabirds showed marked spatial patterns in blood OCs regardless of the species, enhancing their viability to assess spatial trends in marine pollution at large and regional scales. On the contrary, seabirds showed a limited applicability to evaluate geographic patterns of PAHs, probably due to the efficient elimination of these compounds by vertebrates. In addition, we found that a multi-species approach in marine monitoring offers a more complete and integrated evaluation of marine pollution. Seabirds feeding on prey with similar trophic positions can also be feeding on prey from different levels of the water column, resulting in substantial differences in their pollutant burdens. Therefore, multispecific strategies in marine monitoring could be used to monitor not only regional differences of OCs and PAHs but also the vertical distribution of these contaminants. Moreover, this approach shows a broader interval of risk levels representing a more comprehensive evaluation of marine pollution.

The global picture of this study provides relevant guidelines for the implementation of effective pollution monitoring programs in the marine environment. The present study indicates that marine predators can accumulate contamination from distant regions, hundreds of kilometres away from their colony sites, and also from different depths in the water column. Moreover, marine predators can metabolize and eliminate some chemical contaminants and undergo different bioaccumulation rates. Therefore, the selection of indicator species in monitoring programs should be based on the specific objectives of the study. Factors like the specific target chemicals to be studied, the geographic area or the marine compartment of interest should be considered to select suitable species. Further integrative studies which explores the relationship between marine species ecology and their contamination, and well designed monitoring programs, including clear objectives with suitable species, could contribute to a better knowledge on the dynamic of POPs in the marine environment.

This research supports the knowledge of the global distribution of POPs and their high levels in marine wildlife feeding on remote marine regions. Spatial and temporal monitoring of POPs based on suitable sentinel species must be a priority and can contribute to the design of useful management strategies for marine ecosystems. In this context, seabirds with very extensive distributions such as *Calonectris* shearwaters could be used to assess large temporal trends of these pollutants across the North Atlantic and Mediterranean. These species offer the opportunity to explore the fate, sources and new inputs of marine pollutants. Moreover, although the possible effects of POPs in seabird populations were not assessed in this study, the great levels found in some species and populations could be considered as an element of concern. A leading question for research today concerns the environmental and human risks associated to pollution. Therefore, further studies focusing on the possible ecological and health impacts of POPs over marine ecosystems are necessary and suitable to develop effective conservation strategies for marine wildlife.

APPENDIX 1(a). Concentrations of $\sum_{20}$PCBs, $\sum_{3}$DDTs (ng g^{-1} d.w.), δ^{15}N and δ^{13}C (‰) in blood of pelagic seabird species from the NE Atlantic.

Breeding colony			$\sum$**PCBs**		$\sum$**DDTs**		δ^{15}**N**	δ^{13}**C**
Species	n	Year	AM^{a} ± SE (min – max)	GM^{b}	AM ± SE (min – max)	GM	AM ± SE (min – max)	AM ± SE (min – max)
ATLANTIC								
Cape Verde		2006						
CVLS	9		39.40 ± 7.06 (21.12 - 92.68)	35.94	7.201 ± 1.232 (3.162 - 14.90)	6.478	9.03 ± 0.75 (7.30 - 10.75)	-16.8 ± 0.2 (-17.9 - -16.2)
CVS	10		43.25 ± 7.31 (17.74 - 97.35)	38.42	7.691 ± 3.594 (1.852 - 39.80)	4.891	12.21 ± 0.32 (10.24 - 13.26)	
BP	10		65.22 ± 7.41 (32.97 - 120.6)	61.75	14.72 ± 1.71 (5.866 - 23.80)	13.74	11.58 ± 0.28 (10.92 - 12.57)	
MSP	9		115.1 ± 9.4 (75.33 - 161.3)	118.9	35.81 ± 7.43 (16.08 - 82.46)	30.80	12.34 ± 0.32 (11.78 - 12.88)	
Canary Is.		2003						
CS	10		59.22 ± 9.37 (30.94 -114.8)	53.93	13.13 ± 4.43 (2.91 - 42.59)	8.384	12.11 ± 0.18 (11.02 - 12.86)	-16.9 ± 0.2 (-17.9 - -16.0)
BP	9		122.3 ± 14.4 (72.00 - 193.1)	115.5	33.50 ± 6.48 (15.40 - 68.46)	29.28	11.33 ± 0.35 (10.81 - 11.87)	
Madeira		2005						
CS	10		125.3 ± 40.9 (51.40 - 488.9)	98.34	38.18 ± 19.28 (11.03 - 208.7)	22.06	11.25 ± 0.95 (10.86 - 11.95)	-18.8 ± 0.2 (-19.4 - -17.7)
BP	10		311.0 ± 24.5 (200.2 - 442.2)	302.4	89.43 ± 13.77 (40.67 - 171.4)	80.86	11.15 ± 0.52 (10.75 - 11.65)	
MSP	8		419.9 ± 84.0 (186.5 - 784.2)	361.4	172.1 ± 41.29 (27.78 - 329.8)	128.4	12.56 ± 0.50 (11.82 - 13.05)	
Azores Is.		2003						
LS	8		57.43 ± 6.49 (26.66 - 79.80)	54.52	12.52 ± 2.18 (5.026 - 24.19)	11.25	9.53 ± 0.36 (8.42 - 10.65)	
CS	10		99.33 ± 14.84 (41.22 - 217.8)	91.21	42.09 ± 21.48 (12.49 - 234.3)	25.13	9.97 ± 1.13 (9.21 - 10.86)	-18.9 ± 0.1 (-19.4 - -18.5)
BP	10		262.1 ± 17.5 (185.7 - 337.7)	256.8	58.99 ± 11.73 (23.13 - 146.8)	50.77	11.04 ± 0.50 (10.69 - 12.30)	
MSP	5		1073 ± 206 (441.7 - 1726.7)	982.3	319.1 ± 85.07 (88.69 - 546.9)	264.1	11.70 ± 0.29 (10.16 - 12.45)	
Berlengas								
CS	10		119.4 ± 11.9 (69.98 - 175.4)	113.9	25.29 ± 3.66 (10.17 - 49.33)	22.97	13.0 ± 0.1 (12.6 - 13.4)	-18.0 ± 0.1 (-18,2 - -17.2)

CVLS, Cape Verde little shearwater; **CVS**, Cape Verde shearwater; **BP**, Bulwer's petrel; **MSP**, Madeiran storm-petrel; **LS** Little shearwater; **CS**, Cory's shearwater

APPENDIX 1(b). Concentrations of $\sum_{20}$PCBs, $\sum_{3}$DDTs (ng g^{-1} d.w.), δ^{15}N and δ^{13}C (‰) in blood of pelagic seabird species from the Mediterranean.

Breeding colony			**∑PCBs**		**∑DDTs**		**δ^{15}N**	**δ^{13}C**
Species	n	Year	AM[a] ± SE (min – max)	GM[b]	AM ± SE (min – max)	GM	AM ± SE (min – max)	AM ± SE (min – max)
MEDITERRANEAN								
Terreros Is.		2004						
CS	10		212.72 ± 34.54 (91.68 - 463.6)	191.5	100.1 ± 24.91 (25.50 - 248.2)	76.98	11.99 ± 0.27 (11.43 - 12.34)	-18.2 ± 0.1 (-18.5 - -18.0)
ESP	5		6843 ± 1213 (3186 - 10495)	6362	5816 ± 1500 (2971 - 11561)	5204	12.03 ± 0.48 (11.89 - 12.20)	
Balearic Is.		2004						
BS	6		53.99 ± 11.24 (27.34 - 105.8)	49.24	16.25 ± 8.80 (4.724 - 59.93)	10.08	11.75 ± 0.12 (11.56 - 11.99)	
SS	10		158.24 ± 13.38 (102.1 - 227.7)	153.3	69.57 ± 14.96 (30.98 - 191.9)	59.61	10.80 ± 0.59 (10.31 - 11.31)	-18.5 ± 0.1 (-18.7 - -18.3)
ESP	10		4504 ± 1022 (1645 – 12298)	3739	2781.5 ± 599.3 (883.4 – 6266)	2254	11.13 ± 0.90 (10.44 - 12.03)	
Hyeres Is.		2006						
SS	8		510.7 ± 107.8 (216.2 – 1189)	448.5	303.3 ± 96.81 (96.26 - 911.7)	233.58	10.03 ± 0.45 (9.50 - 10.42)	-18.1 ± 0.1 (-18.3 - -17.8)
MS	7		229.0 ± 16.1 (155.95 - 283.12)	225.3	81.03 ± 11.22 (48.94 - 133.1)	76.84	9.25 ± 0.39 (8.63 - 9.81)	
Crete								
SS	9		678.9 ± 102.5 (145.1 - 1124)	593.0	895.3 ± 203.6 (89.45 - 1720)	641.4	9.99 ± 0.13 (9.47 - 10.5)	-17.7 ± 0.2 (-18.3 - -16.6)

SS, Scopoli’s shearwater; **BS**, Balearic shearwater; **MS**, Mediterranean shearwater

Appendix 2. $\sum_{15}$PAH (ng/g w.w.) concentrations in liver from pelagic seabird breeding in the Mediterranean and the Atlantic.

Species	Age	n	Arithmetic Mean	Standard Error	Geometric Mean	Range
Mediterranean						
Balearic shearwater	Adults	10	6.07	0.97	5.27	1.91 – 10.3
Scopoli's shearwater	Adults	10	5.56	0.96	4.64	1.59 – 8.93
Atlantic						
Cory's shearwater	Adults	8	8.52	1.57	7.47	3.32 – 17.1
	Fledglings	3	1.60	0.47	1.48	1.02 – 2.54
Bulwer's petrel	Adults	6	32.5	7.43	29.1	17.2 – 66.2
White-faced storm-petrel	Adults	6	29.8	2.99	29.0	18.5 – 39.8
	Fledglings	2	11.1	3.85	10.4	7.27 – 14.9

***TROPHIC ECOLOGY VS GEOGRAPHIC PATTERNS INFLUENCING ISOTOPIC SIGNATURES AND CONTAMINANT LEVELS OF SEABIRDS*:** Our results indicated that $\delta^{15}N$ and $\delta^{13}C$ signatures as well as OC and PAH levels in seabirds are primarily influenced by the feeding ecology of each species. Seabird trophic niche emerges as the most important factor determining isotopic signatures and contaminant levels in marine communities across large geographic ranges. Nevertheless, we also found some significant spatial patterns in the case of seabird stable isotope signatures and OCs. Therefore, the present study underlines the need to consider both, distribution and trophic ecology, when dealing with ecological isotopic approaches or contaminant studies of marine predators.

THE TROPHIC ECOLOGY OF SEABIRDS FROM AN ISOTOPIC POINT OF VIEW: The present study provides further evidences that stable isotope analysis on feather and blood combined with the use of tracking devices can reveal a meaningful view on the trophic ecology of pelagic seabirds. Overall, our results suggest that each species exploit specific trophic resources consistently throughout time and space and with little overlap among species. Nevertheless, this approach also revealed some variability in the trophic ecology of seabirds at intraspecific level. This study shows it is possible to associate intra- and interspecific seabird trophic ecology variations to their contaminants burdens. Therefore, stable isotope analyses can provide additional resources for the management authorities to increase the knowledge about trophic ecology of marine predators and to evaluate possible risks associated to marine pollution. The valuable information drawn from these approaches might be useful to design effective conservation strategies for marine wildlife.

THE INFLUENCE OF SEABIRD TROPHIC ECOLOGY OVER THEIR CONTAMINANT STATUS: Our results revealed that the biomagnification processes not only become apparent across species but can also be detected at the intra-specific level, reflecting the relevance of dietary preferences among seabird individuals. Moreover, inter-species differences in contaminant levels reported in this study not only underline the importance of trophic level but also of specific dietary habits. Marine predators with similar trophic positions can also be feeding on preys from different levels of the water column resulting in substantial differences in their contaminant burdens that obscure biomagnification processes. Therefore, this study points out the importance of trophic niche to understand the degree of accumulation of POPs, not only among species but also at intraspecific level.

***STABLE ISOTOPES AS OCEANIC TRACERS*:** Our results suggest that within large regions, such as mid-north Atlantic, isotopic variability is spatially unstructured. There were some geographic differences, but unrelated to distance among archipelagos, oceanographic traits, or geographic coordinates. Nevertheless, we found a clear enrichment in $\delta^{15}N$ signatures in the north temperate Atlantic compared to the mid-north Atlantic subtropical and tropical regions, likely due to baseline differences in isotopic signatures between regions. Therefore, this study points out the limited applicability of isoscapes in the north Atlantic to study migratory movements of marine organisms within large regions. On the contrary, stable isotope analyses can be a powerful tool to study long distance migrations between large geographic regions in the marine environment.

SEABIRDS AS MARINE POP INDICATORS: The present study reveals the suitability of pelagic seabirds with wide breeding ranges as useful organisms to biomonitor POP levels in pelagic ecosystems, which emphasizes their exceptional potential to be included in marine monitoring programs. Our results suggest multi-species sampling strategies could be used to monitor spatial differences in POPs among pelagic areas at regional or large scales and even among different heights of the water column. Nonetheless, the usefulness of pelagic seabirds largely depends on the contaminant family analyzed. This study suggests that the capability of seabirds to metabolize and eliminate some contaminants, such as PAHs, could be overriding geographic patterns from baseline marine levels.

MAPPING MARINE POLLUTION ACROSS THE NE ATLANTIC AND THE MEDITERRANEAN: Seabirds can provide a clear picture of the geographic variations in pollutant levels among large pelagic regions. Geographic differences in contaminant levels determine the exposure of seabirds to OCs, enhancing the importance of predator distribution in the study of marine contamination. At global scale, our results confirm the Mediterranean basin as a more polluted region compared to the NE Atlantic and other remote marine regions. Moreover, at regional scale, we found that OC levels increased from west to east within the Mediterranean and from south to north in the case of mid-north Atlantic. The greatest OC levels found in some seabird populations range within those previously reported to cause adverse ecological effects, which could represent an element of concern for the conservation of these populations.

MULTIDISCIPLINARY STUDIES AS INNOVATIVE TOOLS IN MARINE POLLUTION MONITORING: Overall, this study shows the utility of combining new methodologies in the study of animal ecology, i.e. stable isotope signatures and tracking devices, with the analysis of pollutant levels to understand the dynamic of contaminants in marine food webs across vast geographic regions. While tracking devices can reveal the feeding grounds of wide-ranging species, $\delta^{15}N$ and $\delta^{13}C$ signatures help linking feeding ecology to contaminant burdens. This integrative approach underlines the relative contribution of trophic ecology and distribution over the contaminant levels and profiles of marine wildlife. Therefore, multidisciplinary studies offer a comprehensive view on the role of marine predators integrating pollutant levels over vast geographic areas and their potential as biomonitors of marine pollution.

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ENTRE LA ECOLOGÍA Y LA QUÍMICA AMBIENTAL: LAS AVES PELÁGICAS COMO INDICADORES DE LA CONTAMINACIÓN MARINA

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INTRODUCCIÓN

La contaminación de nuestros océanos constituye hoy en día una de las amenazas más patentes para los ecosistemas marinos. Además de los efectos tóxicos agudos que causa la contaminación sobre la fauna marina, también son de crucial importancia los efectos crónicos de estas sustancias a largo plazo así como las capacidades sinérgicas que existen entre las distintas amenazas que se ciernen sobre el medio marino. Por ejemplo, estudios recientes en el Ártico han puesto de manifiesto que los efectos derivados del cambio climático agravarán los efectos nocivos derivados de la contaminación marina, especialmente en el caso de las especies que ocupan los niveles superiores de la cadena trófica. Por tanto, a pesar de que la monitorización de los niveles de contaminación en el medio marino así como el estudio de la dinámica de los contaminantes y los factores que determinan la exposición a los mismos han constituido una importante línea de investigación durante los últimos 20 años, aun hoy constituyen un reto de futuro y una prioridad para la conservación de nuestros océanos.

Buena parte de los estudios sobre la contaminación marina se han centrado en la presencia de los contaminantes orgánicos persistentes (POPs, abreviación comúnmente utilizada proveniente del nombre de estos compuestos en inglés). El gran interés científico y social que despiertan los POPs se debe principalmente a que todos estos compuestos químicos se caracterizan por su elevada toxicidad, su persistencia en el medio y su capacidad de bioacumulación, por lo que representan un riesgo evidente para la salud de los ecosistemas a escala global. De hecho, con el objetivo de eliminar y restringir el uso y producción de buena parte de estos contaminantes, más de 160 países aunaron esfuerzos para frenar la grave amenaza que suponen estos compuestos mediante la firma del Convenio de Estocolmo (2001). Entre las distintas familias de contaminantes que incluyen los POPs, los hidrocarburos aromáticos policíclicos (PAHs) y los compuestos organoclorados, como es el caso de los bifenilos policlorados (PCBs) y el dicloro-difenil-tricloroetano (DDT), destacan por sus propiedades carcinogénicas y mutagénicas, siendo bien conocido el gran abanico de efectos fisiológicos, bioquímicos y ecológicos nocivos que tienen sobre los ecosistemas marinos. Además, una vez introducidos en el medio, los PAHs y los OCs son transportados lejos de sus fuentes de origen mediante las corrientes de aire y agua afectando de este modo también a los ecosistemas y la fauna de regiones remotas como son el Ártico o amplias zonas pelágicas, de mar abierto, muy alejadas de las costas continentales. Por todo esto, la presencia de estos contaminantes, su distribución y sus fuentes en el medio marino han sido intensamente estudiados durante los últimos años.

La reciente Estrategia Marítima Europea (EMS) ha puesto de manifiesto que uno de los mayores retos para la investigación consiste en el desarrollo de redes, sistemas y metodologías adecuadas para la monitorización de la contaminación marina a gran escala. Esta estrategia global, incluye la utilización de diversas herramientas entre las que destaca el uso de indicadores ecológicos que permitan evaluar los impactos de las actividades humanas sobre los ecosistemas marinos, así como la respuesta de los mismos a estas perturbaciones. Un indicador ecológico ha sido definido como una característica cuantificable relacionada con la estructura, composición o funcionamiento de los sistemas ecológicos. Muchas de las aplicaciones de los indicadores ecológicos, especialmente en el caso del estudio de la contaminación, se han centrado en el nivel de especie, dando lugar al concepto de especie indicadora o bioindicador. Principalmente, estas especies se han usado para evaluar el estado de salud de los ecosistemas así como para explorar la causa de los cambios, estrés y disfunciones de los ecosistemas. El uso de los bioindicadores está firmemente arraigado en la monitorización de la contaminación así como en el estudio de la dinámica de los contaminantes, la ecología, la ecotoxicología y la conservación de los ecosistemas marinos.

Generalmente, los estudios centrados en la contaminación marina se han basado en especies de su macrofauna, especialmente en macroinvertebrados acuáticos, peces y aves. La taxonomía de la especies seleccionadas suele estar relacionada con la familia de contaminantes a estudiar (por ejemplo, los PAHs han sido comúnmente monitorizados usando invertebrados, siendo escasos los estudios con vertebrados) así como con los objetivos específicos del estudio (ej. moluscos para monitorizar la contaminación en puertos o bahías y aves marinas como gaviotas para evaluar el estado general de zonas costeras). Como resultado, un gran número de especies, incluyendo crustáceos, moluscos, peces, aves y mamíferos marinos han sido propuestos como indicadores para el estudio de la contaminación en el medio marino. Entre todas ellas ha sido especialmente destacado el gran potencial de las aves marinas. Por ejemplo, su utilización como bioindicadores para el estudio de metales pesados o vertidos de crudo está ampliamente aceptada. Sin embargo, su utilidad como bioindicadores de POPs ha sido poco explorada, especialmente en el caso de las aves pelágicas ya que los estudios se han centrado principalmente en las regiones y las especies costeras.

A pesar del gran número de especies utilizadas para monitorizar la contaminación, diversos estudios han puesto de manifiesto la gran dificultad que existe para interpretar la información que ofrecen dichas especies sobre el estado y situación real de los ecosistemas marinos así como del resto de especies que los componen. Los factores que limitan la aplicabilidad del estudio de especies marinas como indicadores están ligados al gran desconocimiento que existe sobre la ecología, fisiología y la dinámica espacio-temporal de muchas de ellas. Por ejemplo, gran parte de los estudios llevados a cabo hasta la fecha asumen que la dieta y posición trófica de la especies empleadas para monitorizar son las mismas que las descritas con anterioridad en especies próximas o en la misma especie pero estudiada en una región diferente. Sin embargo, la dieta y la posición trófica de los depredadores marinos varía a lo largo de su distribución geográfica e incluso dentro de una misma población debido a la explotación de recursos tróficos específicos. Otros factores como el sexo o la edad también implican dietas o zonas de forrajeo específicas así como diferentes oportunidades para la excreción de los contaminantes (ej. las hembras de las aves excretan parte de su carga de contaminantes en los huevos) que pueden resultar en niveles diferenciados de contaminación entre machos y hembras, o por ejemplo, entre adultos e inmaduros. Por tanto, y teniendo en cuenta que la dieta es la principal ruta de entrada de los contaminantes en la mayor parte de los depredadores marinos, es relativamente obvio el relevante papel que juega la ecología trófica influyendo sobre las cargas de contaminantes que presentan los consumidores marinos.

Por otro lado, los movimientos migratorios de gran parte de la fauna marina no han sido estudiados en profundidad. Igualmente, las áreas de alimentación, especialmente en el caso de las especies que poseen una gran movilidad y que pueden alimentarse a cientos de kilómetros de sus áreas de cría o de la zona donde han sido muestreadas, son un misterio en el caso de muchos depredadores marinos. Puesto que la presencia de los contaminantes y sus fuentes no están uniformemente distribuidas en el medio marino, el conocimiento de las zonas de alimentación así como de los cuarteles de invernada son aspectos claves para entender la información espacial que ofrece la especie objeto de estudio. Por tanto, la distribución y movimientos así como la ecología trófica son dos factores clave que influyen sobre los niveles de contaminación de los depredadores marinos. Sin embrago, su influencia relativa se ha estudiado escasamente. Por ejemplo, cuando encontramos diferencias en las cargas de contaminantes entre poblaciones distintas de una misma especie o de especies distintas, si las diferencias observadas se deben a diferencias geográficas en los niveles basales de contaminantes o a diferencias específicas en la dieta o la posición trófica de las poblaciones estudiadas es incierto. En base a todo esto, más allá de la simple monitorización de contaminantes en la fauna marina, el uso de especies indicadoras requiere a su vez el estudio de su ecología trófica, de su homogeneidad a lo largo de la distribución geográfica de la especie así como de sus movimientos, no sólo migratorios, sino también para la búsqueda de alimento.

Afortunadamente, durante los últimos años, el desarrollo y empleo de nuevas metodologías para el estudio de la ecología y los movimientos de la fauna marina han aumentado enormemente nuestro conocimiento sobre los depredadores marinos. Concretamente, el análisis de isótopos estables y el uso de sistemas de seguimiento remoto como los geolocalizadores se han utilizado ampliamente para estudiar diversos aspectos de los ecosistemas y las especies que los componen, desde la estructuración trófica de comunidades marinas hasta la delineación de las rutas migratorias transecuatoriales de depredadores marinos como la pardela cenicienta. Los geolocalizadores permiten conocer la posición exacta de un animal marcado con gran exactitud, por lo que son singularmente útiles para estudiar la dinámica espacio-temporal de la fauna marina. En el caso del análisis de isótopos estables, el supuesto básico sobre el que se sustenta su aplicación es que la señal isotópica (la proporción entre los dos isótopos estables mayoritarios de un determinado elemento) en los tejidos de los consumidores deriva directamente de aquellas que presentan sus presas. Concretamente, buena parte de los estudios que emplean el análisis de isótopos estables se han centrado específicamente en el análisis de las firmas isotópicas de Carbono ($^{13}C/^{12}C$; $\delta^{13}C$) y Nitrógeno ($^{15}N/^{14}N$; $\delta^{15}N$). Mientras que las firmas isotópicas de Nitrógeno se han utilizado principalmente para estudiar y determinar la posición trófica de los consumidores marinos, las de carbono permiten diferenciar entre la explotación de recursos costeros (ecosistemas neríticos) y la de presas procedentes de mar abierto (pelágicas). Además, ambas firmas isotópicas sufren variaciones geográficas en sus niveles basales que también se reflejan en los tejidos de los consumidores marinos. Por ejemplo, mediante la utilización de las firmas isotópicas de N se puede distinguir si una pluma ha crecido en el Atlántico o en el Mediterráneo. Los patrones geográficos en las variaciones de los niveles basales $\delta^{15}N$ y $\delta^{13}C$ se han utilizado con éxito para delinear las zonas de alimentación de distintas especies o el estudio de sus movimientos migratorios, identificando mediante los isótopos estables los principales cuarteles de invernada.

En el estudio de la ecología trófica mediante el análisis de isótopos estables es muy importante la selección del tejido apropiado ya que la composición isotópica de cada tejido cambia a lo largo del tiempo de acuerdo con su tasa de renovación, por lo que cada uno de ellos integra la señal isotópica de la dieta ingerida en un plazo de tiempo distinto. Por ello, tejidos metabólicamente activos, con una elevada tasa de renovación, proporcionan información sobre los alimentos asimilados recientemente (ej. días en el caso de plasma e hígado), mientras que los tejidos con una tasa de renovación menor proporcionan información sobre la dieta de un período de tiempo mayor (ej. semanas en el caso del músculo y sangre, meses o años en el caso del hueso), algo especialmente útil para determinar los hábitos alimenticios a corto y a largo plazo. Los tejidos metabólicamente inactivos (ej. los tejidos de queratina como uñas, pelo o plumas) proporcionarán información sobre los alimentos asimilados en el momento de su formación, ya que una vez que se vuelven inertes sus señales isotópicas permanecen inalteradas. Por tanto, en estos tejidos, a diferencia de los tejidos metabólicamente activos, el momento del muestreo es independiente de la información isotópica integrada en ellos.

La combinación del análisis de isótopos estables en el tejido adecuado, el uso de geolocalizadores y el estudio de la contaminación en la fauna ofrece un amplio abanico de posibilidades para explorar el potencial de los organismos marinos como indicadores de la contaminación. Por ejemplo, estudios recientes han encontrado relaciones significativas entre $\delta^{15}N$ y los niveles de contaminantes organoclorados en distintos organismos marinos. Dichas relaciones reflejan un aumento de la carga de OCs cuanto mayor es la posición trófica lo cual posiblemente se debe a la capacidad de biomagnificación de estos contaminantes. Igualmente, estudios que han combinado el uso de geolocalizadores y el análisis de isótopos estables y PCBs en sangre de distintas especies de albatros que crían simpátricamente en el Pacífico han puesto de manifiesto que las diferentes cargas de estos contaminantes que presentan las especies de albatros se deben a la explotación de diferentes zonas del Pacifico y no a diferencias en su dieta.

OBJETIVOS

El principal objetivo de esta tesis es contribuir a la comprensión de la influencia relativa que ejercen la ecología trófica y la distribución de la fauna marina sobre sus firmas isotópicas y los niveles de contaminación que presentan. Esta combinación integradora entre el estudio de la ecología y la contaminación constituye además una sólida vía para la evaluación del potencial de los organismos marinos como indicadores de la contaminación por POPs.

A tal fin, el diseño experimental de este estudio se basa en una estrategia multi-especifica y multi-localidades centrada en el estudio de la mayor parte de las especies de aves pelágicas (Orden Procellariiformes) que crían en el Atlántico NE, en el Mediterráneo o en ambos. En conjunto, dentro del Atlántico este estudio incluye la pardela cenicienta (*Calonectris borealis),* pardela pichoneta (*Puffinus pusffinu*), pardela chica (*Puffinus assimilis*), pardela de Cabo Verde (*Calonectris edwardsii*), pardela chica de Cabo Verde (*Puffinus boydi*), petrel de Bulwer (*Bulweria bulewerii*), paíño de Madeira (*Oceanodroma castro*), paíño Europeo (*Hydrobates pelagicus*), paíño pechialbo (*Pelagodroma marina*) y petrel de Fea (*Pterodroma feae*). En el caso del Mediterráneo se muestrearon pardela cenicienta (*Calonectris diomedea*), pardela Balear (*Puffinus mauretanicus*), pardela Mediterránea (*Puffinus yelkouan*) y paíño Europeo. Por otro lado, cada especie se muestreo en varias localidades a lo largo de su área de cría. Por ello, el estudio cuenta con muestras obtenidas en Cabo Verde, Canarias, Madeira, Selvagens, Azores, Berlengas (Portugal), Irlanda, Escocia e Islandia dentro del Atlántico, y de Almería, Baleares, Hyeres (Francia) y Creta en el caso del Mediterráneo.

Esta tesis se estructura en distintos capítulos que, dependiendo de los objetivos específicos de cada uno, incluyen distintas metodologías y tejidos diana. En el primer capítulo, se ha combinado el análisis de los ratios isotópicos en plumas con la aplicación de técnicas de seguimiento mediante geolocalización. Específicamente, se muestrearon y determinaron las firmas isotópicas de Carbono y Nitrógeno en la primera pluma primaria (n=609) de la mayor parte de las especies (11 especies) de aves pelágicas que crían a lo largo del Atlántico noreste (16 localidades). Además, se han empleado geolocalizadores en 6 especies para delimitar las áreas donde mudan la primera pluma primaria, en este caso también en varias colonias de cría para cada especie. De esta forma, se consigue relacionar los valores isotópicos encontrados en las plumas con regiones geográficas concretas. Los objetivos específicos de este estudio son (***1***) entender la influencia relativa de la ecología trófica y la distribución geográfica sobre las firmas isotópicas en aves pelágicas, (***2***) explorar los patrones geográficos en los niveles basales de las firmas isotópicas de Carbono y Nitrógeno y (***3***) investigar la estructuración trófica de las comunidades de aves pelágicas del Atlántico NE y evaluar la consistencia del nicho trófico de las mismas a lo largo de su distribución geográfica. En los capítulos 2, 3 y 4 se determinaron las concentraciones de OCs y PAHs en sangre e hígado respectivamente. A lo largo de estas secciones se han explorado las variaciones en los niveles de estos contaminantes en las aves pelágicas en base a su distribución geográfica y su ecología trófica, estudiada a través del análisis de isótopos estables. Además, otras fuentes de variabilidad en los niveles de contaminación, como son el sexo o la edad, fueron inspeccionadas. Con esta aproximación se ha evaluado el potencial de las especies seleccionadas como posibles indicadores de la contaminación marina por POPs así como la dinámica de estos contaminantes en el medio marino. Estos tres capítulos cubren objetivos específicos comunes que son: (***4***) aportar nueva información sobre los niveles de POPs en aves pelágicas derivados de la contaminación crónica en el Atlántico y en el Mediterráneo, (***5***) entender la influencia relativa de la ecología trófica y la distribución geográfica sobre las concentraciones de POPs en aves pelágicas, (***6***) describir los patrones geográficos de OCs y PAHs así como sus posibles fuentes en el Atlántico NE y el Mediterráneo y (***7***) relacionar las variaciones intra- e interespecíficas en los niveles de POPs con la ecología trófica de las aves estudiadas mediante el uso de los isótopos estables, prestando especial atención al proceso de biomagnificación de estos compuestos.

RESULTADOS Y DISCUSIÓN

A lo largo de este estudio, en primer lugar se evaluó la influencia relativa de la ecología trófica y la distribución geográfica sobre las firmas isotópicas y las cargas de contaminantes en aves pelágicas. A continuación, se describieron los patrones geográficos en los niveles basales de $\delta^{13}C$, $\delta^{15}N$, PCBs, DDTs y PAHs que surgen a lo largo de Atlántico NE y del Mediterráneo. Por otro lado, este estudio muestra la gran consistencia de la ecología trófica de las distintas especies de aves pelágicas seleccionadas en toda su distribución geográfica así como las fuertes diferencias que existen entre las especies, poniendo de manifiesto la marcada relación que existe entre los niveles de PCBs, DDTs y PAHs en aves pelágicas y su ecología y dinámica espacio-temporal. En conjunto, este trabajo destaca la utilidad de combinar el estudio de la contaminación con el de la ecología en los depredadores marinos, poniendo de manifiesto el gran potencial de las aves pelágicas como indicadores de la contaminación marina.

Las firmas isotópicas de Carbono y Nitrógeno en la primera pluma primaria (crecida en el área de cría) variaron significativamente entre las especies y las localidades de cría. Sin embargo, las variaciones interespecíficas fueron mucho más acusadas que las debidas a la localización geográfica. De hecho, los patrones de variación que se encontraron para los valores de $\delta^{15}N$ y $\delta^{13}C$ no mostraron una estructuración geográfica dentro de la zona central del Atlántico noreste, que comprende los archipiélagos Macaronésicos y se corresponde con las regiones tropical y subtropical templada del Atlántico norte. En concreto, al combinar las firmas de ambos isótopos, las muestras se agruparon principalmente en base a la especie, independientemente del origen geográfico de la misa, con la excepción de las muestras de aves obtenidas en las localidades más norteñas (Irlanda, Islandia y Escocia). En el caso de los contaminantes, lo niveles de PCBs y DDTs en las aves mostraron variaciones geográficas e interespecíficas significativas. No obstante, las diferencias en los niveles de OCs fueron mucho mas acusadas entre especies que entre colonias, indicando una mayor influencia del primer factor. Por el contrario, las concentraciones de PAHs mostraron variaciones significativas entre las especies, pero no entre las distintas áreas de cría, incluso comparando entre el Atlántico y el Mediterráneo, dos cuencas con marcadas diferencias en sus niveles de contaminación. En conjunto, estos resultados apuntan al nicho trófico como el principal factor que influye sobre las firmas isotópicas y los niveles de PCBs, DDTs y PAHs en depredadores marinos, aves pelágicas en este caso, a lo largo de extensas regiones geográficas.

El principal patrón geográfico encontrado en las firmas isotópicas en las plumas de las aves del Atlántico NE se basa en los valores significativamente mas altos de $\delta^{15}N$ (de un 3 a un 6‰) que reflejaron las poblaciones de aves pelágicas del Atlántico Norte (Islandia, Escocia e Irlanda del Norte), ya sea a nivel intra- o interespecífico. Estas diferencias tan marcadas sugieren un cambio en los niveles basales de $\delta^{15}N$ entre las zonas tropical y subtropical templadas respecto a la región templada del norte, lo cual concuerda con estudios isotópicos previos en depredadores marinos y en zooplancton. Mientras que la falta de estructuración geográfica que encontramos en las firmas isotópicas de las aves de la zona Tropical y Subtropical del Atlántico noreste indica que los isótopos estables constituyen una metodología muy limitada para el estudio de los movimientos migratorios a escala regional, las marcadas diferencias entre esas zonas y la región templada del Norte puede resultar altamente útil para el estudio de los movimientos a mayor escala geográfica en el medio marino.

Las variaciones geográficas de los niveles de OCs fueron similares para todas las especies de aves estudiadas. Los patrones geográficos encontrados en este estudio se caracterizaron por niveles de OCs significativamente más altos y una mayor presencia de los compuestos con mayor grado de cloración en las especies que anidan en la cuenca Mediterránea. El carácter confinado del Mediterráneo así como el abundante vertido de estos contaminantes por parte de los países industrializados de Europa concuerdan con nuestros resultados. Resulta de gran interés destacar que los niveles de OCs encontrados en las algunas especies de aves pelágicas del Mediterráneo se encuentran dentro del intervalo de niveles de contaminación para los cuales estudios previos con gaviotas han demostrado efectos ecológicos adversos.

Dentro del Atlántico, los niveles de contaminantes aumentaron de sur (Cabo Verde y Canarias) a norte (Madeira y Azores) en todas las especies estudiadas y los perfiles de DDTs encontrados en Cabo Verde y Canarias sugieren un uso reciente de este pesticida en las zonas de alimentación de las aves de estos archipiélagos. Los niveles más altos encontrados en los archipiélagos de Azores y Madeira podrían estar relacionados con la influencia de la corriente del golfo sobre estas regiones, susceptible de transportar contaminantes desde las costas norte-americanas hasta el centro-norte del Atlántico. El uso reciente de DDT que sugieren los perfiles de las aves de Canarias y Cabo Verde está posiblemente asociado al uso de este pesticida para el control de la malaria en los países subsaharianos. En el caso de los PAHs no se han encontrado diferencias espaciales significativas ni en los niveles ni en los perfiles de estos contaminantes en las aves. Esta ausencia de patrones geográficos significativos en la presencia de PAHs indica una menor influencia del área de cría como factor determinante de los niveles de estos contaminantes en las aves marinas, pudiendo ser debido a la gran capacidad de las mismas para metabolizar y eliminar los PAHs.

Como se ha comentado previamente, al combinar las firmas isotópicas de C y N en las plumas de las aves pelágicas que crían en Atlántico NE, las muestras se agruparon principalmente en base a la especie, independientemente del origen geográfico de las mismas. Además, los ratios isotópicos obtenidos mostraron muy poco solapamiento entre las distintas especies, especialmente en el caso del Carbono, lo que sugiere que cada especie explota recursos tróficos específicos y de forma consistente a lo largo de toda su distribución. No obstante, algunas especies mostraron diferencias isotópicas claras en el caso de algunas de sus poblaciones. Este es el caso de la pardela cenicienta. Las pardelas muestreadas en diferentes colonias de las Islas Canarias mostraron los valores de $\delta^{13}C$ mas altos de este estudio, ya sea comparando con el resto de colonias de esta especie o con el resto de especies. Los geolocalizadores mostraron que las pardelas de las colonias Canarias se alimentan principalmente en la plataforma continental Africana, lo cual explicaría sus elevados valores de $\delta^{13}C$. Por el contrario, las pardelas cenicientas de Azores y Madeira mostraron firmas isotópicas de C marcadamente más bajas que el resto de colonias de esta especie. Esto posiblemente se debe a la gran distancia que existe entre las área de cría de estas poblaciones y los sistemas costeros altamente productivos, forzando a estas pardelas a alimentarse exclusivamente de presas pelágicas en mar abierto, tal y como mostraron los geolocalizadores. El resto de poblaciones de pardela cenicienta, es decir, las muestreadas en Selvagens y Cabo Verde, mostraron valores intermedios entre los de las de Canarias y las de Azores o Madeira. Estos valores intermedios posiblemente se deban a que estas pardelas explotan también las zonas neríticas próximas a la costa Africana, si bien en este caso, la mayor distancia entre las colonias y el continente comparada con el caso Canario, resulta en un uso intermedio de presas pelágicas y neríticas.

En el caso del $\delta^{15}N$, todas las especies que crían en la Macaronesia mostraron un gran solapamiento en sus firmas isotópicas en la primera pluma primaria, agrupándose en un rango de apenas un 3‰ con la excepción de las pardelas chicas. Teniendo en cuenta que de forma general se asume un enriquecimiento de un 3‰ a un 5‰ por cada nivel trófico a lo largo de las cadenas tróficas marinas, las pequeñas diferencias encontradas en $\delta^{15}N$ en las plumas sugiere un posición trófica similar para la mayor parte de las especies de aves pelágicas del Atlántico NE, lo que también concuerda con nuestros resultados isotópicos en sangre. No obstante, las firmas isotópicas más bajas (tanto en pluma como en sangre) de las Pardelas chicas sugiere una posición trófica inferior para estas especies, lo cual concuerda con estudios previos sobre metales pesados en las aves pelágicas de Azores.

Las diferencias descritas en la ecología trófica de las especies, así como las diferencias entre las poblaciones de una misma especie, mostraron relaciones muy claras con sus niveles de contaminantes. Para explorar las variaciones en los niveles de contaminantes debidos a variaciones intraespecíficas de la ecología trófica este estudio se centró en el caso del las pardelas cenicientas del Atlántico, del Mediterráneo y de Cabo Verde (*Calonectris* sp.) ya que se han considerado una única especies hasta hace muy poco tiempo. Su gran similitud ecológica, morfológica y fisiológica nos permite considerarlas como una única especie a efectos estadísticos en el caso de la contaminación. En este caso, cabe destacar especialmente la relación positiva y significativa que se encontró entre $\delta^{15}N$ y los niveles de OCs en la sangre de las aves dentro de la mayoría de las colonias de pardelas muestreadas, es decir, a mayor posición trófica del individuo dentro de una misma colonia, mayor carga de OCs. Sin embargo, esta relación no se encontró al combinar todos los datos independientemente de la colonia, indicando que las variaciones de $\delta^{15}N$ entre colonias posiblemente se deban a variaciones de los niveles basales de las firmas isotópicas y no a variaciones en la posición trófica de las pardelas. La relación que se ha encontrado entre la posición trófica de los individuos ($\delta^{15}N$) y sus niveles de OCs en este estudio probablemente refleja la capacidad de biomagnificación de estos contaminantes. Esta relación refleja la especialización de los individuos hacia el consumo de presas de determinados niveles tróficos como un factor clave para explicar los niveles de contaminación en aves pelágicas. Igualmente, los resultados obtenidos sugieren que dicha especialización supone que el proceso de biomagnificación no sólo pueda detectarse a lo largo de las cadenas tróficas sino también a nivel intraespecífico.

A nivel interespecífico las concentraciones de OCs y PAHs mostraron variaciones significativas y consistentes, es decir, las diferencias que se encontraron en los niveles de contaminación de las especies fueron similares en todas las localidades muestreadas. Sin embargo, estas variaciones interespecíficas en los niveles de OCs no se correspondieron con variaciones similares de $\delta^{15}N$ en el caso de varias de las especies estudiadas. De hecho, tan solo se encontró una relación significativa entre $\delta^{15}N$ y los niveles de OCs a nivel interespecifico en el caso de las aves muestreadas en Azores, indicando que otros factores, además del nivel trófico *per se,* están influenciando fuertemente la acumulación de estos contaminantes en las aves.

La explicaron más coherente para explicar la falta de relación entre el nivel trófico de algunas especies y su carga de contaminantes concierne a las diferencias que existen en la dieta de las aves pelágicas. Estudios previos así como el gran solapamiento que encontramos en los valores de $\delta^{15}N$ de las especies muestreadas indican que no existen diferencias marcadas en su posición trófica. Sin embargo, una posición trófica similar no implica una dieta similar. Concretamente, especies de aves marinas como el petrel de Bulwer y el paíño de Madeira, que se alimentan de recursos mesopelágicos (200-1000 m), principalmente de especies de peces (Mictófidos) que realizan diariamente migraciones verticales, mostraron niveles de OCs y PAHs significativamente más altos que las que consumen recursos epipelágicos (0-200 m). Esto concuerda con estudios previos que han destacado los altos niveles de contaminantes orgánicos que se encuentran en especies de peces que habitan zonas profundas (>200m de profundidad) de los océanos. Por tanto, nuestros resultados sugieren que las especies de aves que explotan niveles tróficos similares, pueden consumir también recursos de distintas profundidades, resultando en niveles de contaminación marcadamente diferentes entre ellas. Por tanto este estudio sugiere que las aves marinas pelágicas podrían utilizarse no sólo como indicadores potenciales de las variaciones geográficas de los niveles de contaminantes en nuestros océanos, sino también como posibles indicadores de su distribución vertical en la columna de agua.

De manera global, este estudio revela el gran potencial de las aves pelágicas como bioindicadores de la contaminación marina. Además, queda patente que el diseño de estudios multidisciplinares, combinando la ecología y la contaminación, ofrecen un marco integrador que permite una evaluación mas precisa de la dinámica de los contaminantes en los ecosistemas marinos así como de los factores que determinan sus niveles en la fauna de los mismos. Por tanto, la inclusión de aves pelágicas de amplia distribución, como es el caso de la pardela cenicienta, en los programas de monitorización de la contaminación marina así como el desarrollo de nuevos estudios que integren los niveles de contaminación de la fauna marina con el estudio de los factores que los explican, constituyen líneas sólidas para avanzar en la investigación de la contaminación marina.

CONCLUSIONES

- **Las firmas isotópicas de N y C así como los niveles de OCs y PAHs están principalmente influenciados por el nicho trófico de las especies**. No obstante, también se han encontrado algunos patrones geográficos claros en el caso de los isótopos estables y los OCs. Por tanto, ambos factores, la distribución geográfica y ecología trófica deben considerarse en la aplicación de los isótopos estables al estudio de la fauna marina así como en el estudio de sus niveles de la contaminación.

- Este estudio destaca la utilidad de **combinar sistemas de seguimiento remoto como los geolocalizadores con el análisis de los isótopos estables** para el estudio de la ecología de los depredadores marinos. En conjunto esta aproximación sugiere que **las aves pelágicas poseen nichos tróficos singulares que no se solapan entre especies y que son consistentes a lo largo de su distribución geográfica**.

- Queda patente en este trabajo que **la posición trófica y la dieta de las aves pelágicas tienen una influencia decisiva sobre sus niveles de contaminación tanto a nivel interespecífico como a nivel intraespecífico**:
    - Por un lado, se ha comprobado que la **biomagnificación** de los contaminantes no sólo es un proceso que ocurre a lo largo de las cadenas tróficas entre las distintas especies que las componen sino que también se puede detectar **a nivel intraespecífico** debido a la especialización de los individuos en presas de niveles tróficos específicos.
    - Además, a nivel ineterespecífico este estudio revela que las especies de aves que explotan niveles tróficos similares, pueden **consumir también recursos de distintas profundidades, resultando en niveles de contaminantes marcadamente diferentes**.

- Los resultados obtenidos ponen de manifiesto que **los isótopos estables constituyen una metodología muy limitada para el estudio de los movimientos migratorios a escala regional** (Zonas tropical y subtropical templada) pero altamente útil para el estudio de los movimientos a mayor escala geográfica (entre las regiones Norte y Tropical y Subtropical templada) en el medio marino.

- Los niveles de **OCs encontrados en las aves pelágicas revelan niveles marcadamente más altos de estos compuestos en la región Mediterránea comparada con el Atlántico NE**. Además los niveles de OC aumentan de Oeste a Este dentro del Mediterráneo, y de Norte a Sur en el Atlántico NE. Los altos niveles de contaminación detectados en las aves Mediterráneas son susceptibles de causar efectos ecológicos nocivos en estas poblaciones, pudiendo representar **un riesgo a tener en cuenta para la conservación** de las mismas.

- Podemos concluir que **las aves marinas pelágicas son indicadores útiles de la contaminación marina que permiten detectar variaciones geográficas** en los niveles basales de POPs así como **identificar las fuentes** potenciales de los mismos. Además, nuestros resultados indican que los estudios multi-específicos combinando la utilización de especies cuya dieta se compone de presas de diferentes profundidades pueden ofrecer también **la oportunidad de evaluar la distribución vertical de los contaminantes** en la columna de agua. No obstante, la utilidad de las aves marinas como indicadoras es limitada en el caso de aquellos contaminantes que son fácilmente metabolizados, como es el caso de los PAHs.

- Este estudio revela que **las aproximaciones multidisciplinares representan potentes herramientas en la evaluación de la contaminación marina**. Nuestra aproximación multi-específica combinando el estudio de la ecología y la contaminación resulta especialmente útil para comprender la influencia de distintos factores clave como la distribución, movimientos y ecología trófica sobre los niveles de contaminación que se encuentran en los organismos marinos. Todo ello ofrece **una visión integradora de la información que ofrecen las especies marinas como indicadores de la contaminación**.