Monitoring Polycyclic Aromatic Hydrocarbon Pollution in the Marine Environment after the Prestige Oil Spill by Means of Seabird Blood Analysis

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In this study we tested the use of seabird blood as a bioindicator of polycyclic aromatic hydrocarbon (PAH) pollution in the marine environment. Blood cells of breeding yellow-legged gulls (Larus michahellis) were able to track spatial and temporal changes consistent with the massive oil pollution pulse that resulted from the Prestige oil spill. Thus, in 2004, blood samples from yellow-legged gulls breeding in colonies that were in the trajectory of the spill doubled in their total PAH concentrations when compared to samples from unoiled colonies. Furthermore, PAH levels in gulls from an oiled colony decreased by nearly a third in two consecutive breeding seasons (2004 and 2005). Experimental evidence was gathered by means of an oil-ingestion field experiment. The total concentration of PAHs in the blood of gulls given oil supplements was 30% higher compared to controls. This strongly suggested that measures of PAHs in the blood of gulls are sensitive to the ingestion of small quantities of oil. Our study provides evidence that seabirds were exposed to residual Prestige oil 17 months after the spill commenced and gives support to the nondestructive use of seabirds as biomonitors of oil pollution in marine environments.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are globally distributed environmental contaminants which attract considerable concern because of their known toxic and bioaccumulative effects in animals (1, 2). In humans, health risks associated to PAH exposure include cancer (3) and DNA damage (4). The major sources affecting the presence and distribution of PAHs in the environment are anthropogenic (5). In the marine environment, these include large oil spills from tankers, oil discharges by all kinds of ships, and activities associated with offshore oil and gas exploration and production (6).

Immediate negative impacts are expected from oil pollution in coastal and offshore environments through acute mortality of marine organisms directly exposed to oil (7, 8). For example, lethal short-term effects of large oil spills often involve substantial seabird losses (9). Nonetheless, marine organisms can also become affected to the long-term exposure of the persistent and bioaccumulative components of oil via several indirect processes mediated through the ecosystem (10, 2). Direct effects immediately following an oil spill typically attract the greatest public and scientific concern (11, 7). In contrast, sublethal effects due to chronic oil exposure have rarely been explored (some exceptions: see refs 12 and 13). Such research is more costly to conduct because it involves longer time frames and requires evaluation of multiple mechanisms of potential impact to biological systems (14).

Petroleum products are toxic to seabirds (15). Life history characteristics of seabirds make them particularly vulnerable to oil pollution (14) because they spend much of their lives on the ocean’s surface and because their populations concentrate in habitats prone to high oil exposure (16). Moreover, because seabirds are placed in high trophic positions, they are likely to be good candidates to monitor the marine ecosystem (16). In fact, seabirds have also been used to follow polluting agents such as heavy metals and organochlorines (17, 18). Nevertheless, very few studies have monitored PAH concentrations in bird tissues and these studies were mainly based upon the examination of birds either found dead or sacrificed, (10, 19–21) though eggs have also been used to follow the Sea Empress oil spill (22). Scarcity of data about PAHs in seabird tissues probably reflects the view that vertebrates are not good models to assess oil contamination because of their great ability to metabolize PAHs (23, 24). In common with all vertebrates, birds have well-developed mixed function oxygenase (MFO) systems that can rapidly metabolize parent PAHs into hydrophilic products that are more easily excreted, thereby making it difficult to determine the chemical structure of the original compound. For example, PAHs were metabolized by chicken embryos within two weeks after injection into eggs (25). Consequently, only minor concentrations of parent compounds are usually detectable in vertebrate tissues, (26, 27) and it has been postulated that directly measuring oil constituents in bird tissues does not accurately reflect exposure to xenobiotic parent compounds (28). Alternative techniques such as PAH metabolite bile burden or the induction of cytochrome P450 (12, 21, 28) have been developed. However, these measures normally require freshly killed animals.

Here, we present the analysis of PAHs in seabird blood as a convenient and relatively rapid method with little disturbance to birds for monitoring PAH contamination in the marine environment. Since blood cells are continuously being produced and have a lifespan of several weeks (29), the presence of PAHs in blood cells probably indicates a recent incorporation during erythropoiesis. As far as we know, no previous studies have investigated the presence of PAHs in the blood of birds exposed to oil (but see ref 30 for an example in mammals). We evaluated the adequacy of yellow-legged gulls (Larus michahellis formerly Larus cachinnans) as indicators of PAH pollution derived from the Prestige oil spill by measuring the concentration of 15 Prestige oil PAHs in their blood.
The Prestige wreck, off Galicia (NW Spain) in November 2002, was one of the most recent examples of a large marine oil spill. It resulted in the release to the marine environment of approximately 60,000 tonnes of oil products in the eight months following the wreck, spreading pollution from Northern Portugal to France (Figure S1 in the Supporting Information (SI)). The Prestige oil spill is considered the biggest large-scale catastrophe of its type in Europe. Since incorporation of oil from the Prestige is currently being detected in the marine food chain (31, 32, 4), chronic exposure of seabirds would be expected, as they are long-lived and upper trophic level consumers.

In the present study, two complementary approaches were used: first, we compared PAH levels in the blood of adult yellow-legged gulls captured in unoiled and oiled breeding colonies 17 months after the event. Second, we performed an oil-ingestion experiment by supplementing a sample of gulls with oil (33). This experiment allowed us to evaluate whether seabird blood reflected direct exposure to PAHs and to study the dynamics of PAH incorporation in blood (34).

In addition, since it is expected that oil incorporation in the food web from the spill will lessen with time, we compared PAH values from gulls sampled at the oiled colony of Illas Cíes in two consecutive years.

Materials and Methods
Spatial Study. Bird sampling was performed in seven insular yellow-legged gull breeding colonies distributed along the coast of Northwestern Spain (Figure S1). Since yellow-legged gulls feed mainly on marine organisms (35; >80% in 2004) at an average distance of less than 40 km away from the breeding colony (36), PAHs in blood probably indicate contamination at the local scale. Three of the colonies were located in an area that was free from the impact of the Prestige oil spill (unoiled colonies: Coelleira, Ansarón, and Pantorgas), whereas the other four were in the pathway of the spill (oiled colonies: Cies, Ons, Vionta, and Lobeiras). In total, 61 adults (32 females and 29 males) were nest-trapped in 2004 while incubating (May 19 to June 5), 17 months after the Prestige wreck.

Oil-Ingestion Experiment. In order to evaluate the effect of oil ingestion on the presence of PAHs in the blood of gulls, we performed a field experiment at the Illas Cíes breeding colony (Figure S1). At the end of April 2005, during the courtship period of gulls, we randomly allocated 36 breeding pairs to the experiment of which 16 were fed oil (oil-supplemented group) and 20 were treated as controls (control group). Between 1 and 30 days after egg laying was complete (i.e., the third egg was laid), 18 control (10 females and 8 males) and 12 (8 females and 4 males) oil-supplemented gulls were trapped at the nest (one gull per pair), and a blood sample was taken (see further details in Supporting Information). The comparison between the concentration of PAHs in control adults with respect to that in the adults sampled in 2004 were used to estimate temporal changes in the PAH contamination after the Prestige oil spill.

Blood Sampling and PAH Analysis. Blood cells were analyzed to determine and quantify hematological levels of PAHs. A blood sample (1–2 mL, depending on body mass) was taken from the ulnar vein with a heparinized 25G needle. Blood was immediately transferred to plastic tubes that were kept cool in ice boxes (4 °C), and the samples were centrifuged at the end of the day. Blood cells were transferred into cryovials which were kept frozen at −80 °C until analysis. The PAHs that were selected for analysis were the 15 PAHs (Table 1) constituents of the oil spilled by the Prestige according to PAH priority pollutants listed by the United States Environmental Protection Agency (U.S. EPA) (38). PAH levels were determined by high-performance liquid chromatography (HPLC) coupled to a wavelength programmable fluorescence detector (see further details in Supporting Information).

Statistical Analysis. Spatial comparisons of PAH values were tested by means of a generalized mixed model (PROC MIXED in SAS software; SAS Institute, 2001) including the area (oiled vs unoiled) as a fixed factor and the identity of each colony as a random factor. In order to avoid type II errors due to small sample size (see ethical considerations in Supporting Information), the effect of oil ingestion was analyzed using one-tailed tests with significance levels set at 0.05, as recommended in studies which involve manipulations that are potentially detrimental (39). For each PAH, regression curves were fitted to data from the oil-supplemented group as a means to examine significant nonlinear relationships between the blood levels at the time of capture and time since ingestion. Furthermore, data were subject to a principal component analysis (PCA), in order to analyze the underlying effect of the Prestige oil spill on the individual concentrations of the PAHs found in the blood of gulls. This analysis included the adults sampled in the temporal study and the experimental birds as well. Data are expressed as mean ± SE.

Results
Spatial and Temporal Distribution of PAH Pollution. In 2004, 17 months after the Prestige disaster, the concentration of ΣPAHs in the blood cells of gulls from unoiled colonies was, on average, 120% higher than concentrations found in gulls from unoiled colonies (F₁,38 = 5.44, p = 0.011; Figure 1A). Gulls from Lobeiras, the colony most heavily affected by the spill, showed the highest ΣPAHs values (Figure 1A). Differences between unoiled and oiled colonies were significant for four compounds (naphthalene, fluorene, anthracene, and pyrene; Table 1), and in the unoiled colonies, PAH profiles in gull blood were clearly dominated by naphthalene (Table 1). The temporal comparison between gulls sampled in 2004 and 2005 (control group in the experimental study) at Illas Cíes showed an overall decrease in ΣPAHs levels with time (Table 1, the ΣPAHs in blood decreased by 170%). Accordingly, the majority of oil compounds showed reduced concentrations in blood in 2005 (Table 1).

Oil Ingestion Experiment. The oil-supplemented group showed higher ΣPAHs concentrations in blood than control gulls (Figure 1B; F₀,18 = 1.87, p = 0.036). Overall, specific PAH concentrations in oil-supplemented gulls were significantly higher for five compounds (anthracene, fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene; Figure S2). The relative abundances of individual hydrocarbons in the blood samples of oil-supplemented gulls was not in accordance with their proportions in the oil supplements (r = −0.14, p = 0.61). Moreover, their relative abundances in blood correlated inversely with molecular weight (r = −0.71, p = 0.003) and the number of rings (r = −0.749, p = 0.001). When the effect of time after ingestion was analyzed, a specific pattern for each compound was found. Thus, six compounds showed significant nonlinear responses (Figure 2). Of these, fluorene, fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene showed similar response patterns: oil-supplemented gulls trapped at the end of the experiment consistently showed higher blood concentrations than birds trapped in the few days after ingestion (Figure 2). In contrast, the concentration of indeno(1,2,3-cd) pyrene decreased according with the time of capture and benzo(b+j)-fluoranthene concentration started to decrease in birds captured 15 days after the oil ingestion. The other compounds did not show a significant relationship with the time from oil ingestion (p > 0.05).

Principal Component Analysis. The factorial analysis revealed the presence of three main factors accounting for 61% of the total variance observed. The first component (PC1),...
<table>
<thead>
<tr>
<th>PAHs (ng/g)</th>
<th>unoi</th>
<th>2004</th>
<th>oiled</th>
<th>2005</th>
<th>p</th>
<th>CI (n = 18)</th>
<th>p</th>
</tr>
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<tr>
<td></td>
<td>PA (n = 6)</td>
<td>AN (n = 7)</td>
<td>CO (n = 12)</td>
<td>LO (n = 15)</td>
<td>VI (n = 7)</td>
<td>ON (n = 7)</td>
<td>CI (n = 7)</td>
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<td>naphthalene</td>
<td>4.67 ± 0.67</td>
<td>8.14 ± 0.85</td>
<td>8.98 ± 2.72</td>
<td>50.32 ± 30.20</td>
<td>49.02 ± 14.78</td>
<td>37.14 ± 11.67</td>
<td>58.85 ± 48.54</td>
</tr>
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<td>acenaphthene</td>
<td>1.19 ± 0.28</td>
<td>1.55 ± 0.53</td>
<td>3.33 ± 1.08</td>
<td>5.93 ± 2.42</td>
<td>0.69 ± 0.45</td>
<td>3.50 ± 2.97</td>
<td>0.57 ± 0.37</td>
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<td>fluorene</td>
<td>3.14 ± 0.60</td>
<td>3.82 ± 0.78</td>
<td>5.38 ± 0.75</td>
<td>29.52 ± 18.94</td>
<td>10.46 ± 2.20</td>
<td>20.37 ± 13.62</td>
<td>11.00 ± 5.73</td>
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<tr>
<td>phenanthrene</td>
<td>4.10 ± 0.47</td>
<td>5.17 ± 0.97</td>
<td>16.45 ± 2.99</td>
<td>11.94 ± 1.49</td>
<td>30.51 ± 9.45</td>
<td>15.11 ± 3.53</td>
<td>30.07 ± 20.55</td>
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<tr>
<td>anthracene</td>
<td>8.95 ± 2.72</td>
<td>9.73 ± 2.93</td>
<td>6.90 ± 3.13</td>
<td>17.78 ± 7.62</td>
<td>13.64 ± 3.28</td>
<td>15.46 ± 2.94</td>
<td>15.00 ± 4.89</td>
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<tr>
<td>fluoranthene</td>
<td>1.18 ± 0.43</td>
<td>1.54 ± 0.61</td>
<td>6.33 ± 1.31</td>
<td>7.83 ± 2.06</td>
<td>2.24 ± 1.08</td>
<td>3.99 ± 2.29</td>
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<td>pyrene</td>
<td>6.91 ± 2.13</td>
<td>9.18 ± 2.31</td>
<td>8.52 ± 2.81</td>
<td>13.87 ± 4.31</td>
<td>9.57 ± 2.37</td>
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<td>3.14 ± 1.51</td>
<td>29.03 ± 15.84</td>
<td>11.71 ± 3.61</td>
<td>12.47 ± 4.48</td>
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<td>1.05 ± 0.49</td>
<td>2.01 ± 1.04</td>
<td>2.29 ± 0.82</td>
<td>22.23 ± 17.96</td>
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<td>1.81 ± 0.61</td>
<td>1.58 ± 0.37</td>
<td>4.93 ± 2.83</td>
<td>9.86 ± 7.79</td>
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<td>2.32 ± 0.66</td>
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<td>0.07 ± 0.02</td>
<td>0.25 ± 0.09</td>
<td>1.58 ± 0.99</td>
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<td>0.58 ± 0.34</td>
<td>0.36 ± 0.16</td>
<td>1.58 ± 0.55</td>
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<td>dibenz[a,h]anthracene</td>
<td>0.31 ± 0.24</td>
<td>0.22 ± 0.15</td>
<td>0.34 ± 0.10</td>
<td>0.52 ± 0.28</td>
<td>0.05 ± 0.00</td>
<td>0.06 ± 0.01</td>
<td>0.21 ± 0.11</td>
</tr>
<tr>
<td>benzo[g,h,i]perylene</td>
<td>1.05 ± 0.49</td>
<td>2.01 ± 1.04</td>
<td>2.29 ± 0.82</td>
<td>22.23 ± 17.96</td>
<td>1.88 ± 0.60</td>
<td>2.69 ± 1.14</td>
<td>1.69 ± 0.36</td>
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<tr>
<td>indeno[1,2,3-cd]pyrene</td>
<td>0.05 ± 0.00</td>
<td>1.51 ± 0.88</td>
<td>0.21 ± 0.08</td>
<td>1.81 ± 1.00</td>
<td>0.66 ± 0.55</td>
<td>1.41 ± 0.98</td>
<td>1.42 ± 0.60</td>
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<td>ΣPAH</td>
<td>75.15</td>
<td>101.17</td>
<td>71.71</td>
<td>228.33</td>
<td>136.20</td>
<td>132.34</td>
<td>154.53</td>
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</tbody>
</table>

* Colony abbreviations are as follows: PA = Pantorgas, AN = Ansarón, CO = Coelleira, LO = Lobeiras, VI = Vionta, ON = Ons, CI = Cíes; n = sample size.
explaining 28.3% of the total variance, probably represents total oil pollution, thus it is highly correlated with PAHs

\( r = 0.92, \ p = 0.003 \). The second and third components explained 18.4 and 13.9% of the variance, respectively. These two components clearly separated oiled from unoiled colonies (Figure 3): oiled colonies showed positive values in PC2 and PC3, whereas unoiled colonies showed negative values in PC2. Thus, PC2 ordered the colonies according to their degree of exposure to the Prestige oil. In the experimental birds, the supplementation of Prestige oil increased the PC2 but not the PC3 values, further validating the PC2 component as indicator of Prestige pollution. Accordingly, the PC3 component (highly correlated with benz(a)anthracene and pyrene) probably indicates oil pollution from other sources. Interestingly, the gulls sampled at Illas Cies in 2005 (CIO5; Figure 3) displayed lower values in the PC2 and PC3 components when compared to the 2004 samples (CI; Figure 3), suggesting a reduced exposure to oil contamination for gulls in 2005.

**Discussion**

To our knowledge, this is the first field study in which levels of PAHs were measured nondestructively in a vertebrate with the purpose to monitor oil pollution in the marine environment after a large oil spill. Overall, our study provides reliable support to the potential use of seabird blood as a monitoring tool for oil exposure. This view is based upon observational and experimental evidence. First, the technique was able to track spatial and temporal changes consistent with the massive oil pollution pulse that resulted from the Prestige wreck in 2002 (40). Thus, yellow-legged gulls sampled in oiled colonies doubled total PAH concentrations when compared to gulls from unoiled colonies. Furthermore, PAH levels in gulls from a colony in the trajectory of the spill (Illas Cies) decreased by nearly a third in one year. On the other hand, our field experiment strongly suggested that the profile of PAHs in the blood of gulls is likely to be influenced by the composition of recently ingested oil and that measures of PAHs in the blood of gulls are sensitive to the ingestion of small quantities of oil.

Polycyclic aromatic hydrocarbons are constituents of oil that, upon ingestion, are rapidly metabolized, thereby making it difficult to determine the chemical structure of the original compound. For this reason, it has been postulated that low concentrations of parent PAHs should be expected in vertebrate tissues (25–27). Nonetheless, we found higher concentrations of parent PAHs in the blood of yellow-legged gulls that were exposed to the Prestige oil (either experimentally or at the moment of the spill) with respect to unexposed gulls. The mean concentration of parent PAH compounds (\( n = 15 \)) analyzed in blood cells of yellow-legged gulls, were 139.53 ± 21.42 ng/g dry weight (range 6.40–860.78 ng/g; equivalent to 86.12 ± 13.22 ng/g wet weight (ww)) in the range of values reported for other seabird tissues. Thus, for example, in muscle tissues of silver gulls (Sarus novaehollandiae) and Australian pelicans (Pelecanus conspicillatus) the mean concentration values were 85 and 75 ng/g ww, respectively (\( \Sigma_{10} \)PAHs; 19); in herring gull (Larus argentatus) muscle the mean value was 37.8 ± 12.5 ng/g ww (\( \Sigma_{12} \)PAHs; 41), whereas in the liver of oil-exposed guillemots (Uria aalge) the mean value was 250 ± 90 ng/g (range 40–970 ng/g; \( \Sigma_{18} \)PAHs; 21). Interspecific comparisons of PAHs levels should be treated with caution due to high intraspecific variability as shown by our results and because PAHs concentrations probably differ broadly among tissues. Thus, for example, in eider ducks (Somateria mollissima), the mean value was 7.8 ng/g dw in liver, 46 ng/g in gallbladder, and 9.7 ng/g in adipose tissue (\( \Sigma_{12} \)PAHs; 10), suggesting important intraindividual variability.

The spatial comparison of PAH levels in the blood of yellow-legged gulls breeding in oiled versus unoiled colonies strongly suggests that yellow-legged gulls were exposed to residual Prestige oil 17 months after the spill commenced. Acute toxicity is expected when seabirds exposed to the spill ingest oil by preening (42). However, contaminated prey are also a potential source of contamination, and continued incorporation of oil products through trophic processes has been documented for seabird species after a large oil spill (12). The life history characteristics of yellow-legged gulls make them susceptible to continued exposure to remnant oil (13) because they frequently occur and feed in coastal and nearshore environments, which are the same areas that received much of the oil spilled from the Prestige. Adult yellow-legged gulls in Northwestern Spain are sedentary and feed extensively on benthic and intertidal marine organisms (35). Sublethal effects derived from continued oil exposure have been recently documented for yellow-legged gulls in Northwestern Spain (13).

In the oiled colonies, most of the PAH profiles in gull blood were dominated by naphthalene (22–38%), indicating a petrogenic (i.e., derived from petroleum) source (43). Although after the wreck the composition of the Prestige oil was probably altered by weathering (44), naphthalene was also the dominant parent compound found in subsurface waters (45) and intertidal sediments (46) from oiled areas immediately after spill. In contrast, gulls from unoiled colonies showed low naphthalene percentages (6–12%), and profiles were dominated by PAHs with a large number of benzene rings (≥4 rings), especially in Pantogras and Ansarón colonies, indicative of a rather pyrogenic source of contamination. In other studies, naphthalene and tricyclic PAHs also dominated samples from seabird species, including gulls, affected by petrogenic contamination (19, 21). The differences on PAH profiles between the gull blood and the Prestige crude oil can be due to oil alterations by weathering, changes in PAH composition in the prey tissues, or specific metabolization of PHA compounds by gulls (see below).

There is no information about PAH levels in the blood of yellow-legged gulls before the Prestige wreck to complete the classic before-after-control-impact (BACI) approach (47).
Nevertheless, the comparison of gulls sampled at Illas Cíes in 2004 and 2005 is consistent with the expected reduction in PAH levels with time after acute oil incorporation during the spill. Thus, ΣPAHs concentrations in the blood of gulls decreased 3-fold in just one year, down to the 2004 values from unoiled colonies. Interestingly, the reduction in PAH levels with time also suggest that PAH concentrations right after the wreck may even have been higher than those found in 2004 samples (17 months later). Except for five compounds, the majority of hydrocarbons decreased their concentrations abruptly. This reduction was not related to molecular weight nor the number of aromatic rings, suggesting an overall reduction in oil exposure by yellow-legged gulls in coastal Northwestern Spain in 2005. Although the reduction in PAH levels should be treated with caution because it was estimated in a single colony, our results are in agreement with studies on other marine organisms (mussels, *Mytilus galloprovincialis*) that found that ΣPAHs also decreased substantially with time after the *Prestige* event (48).

In our experiment, gulls fed with oil increased their blood concentration of PAHs by 30% with respect to controls, hence revealing that PAH levels in the blood of yellow-legged gulls were in some extent directly related with oil ingestion. A rough extrapolation from the experiment indicates that the ingestion of 3.25 µg of ΣPAHs resulted in an increase of 1 ng/g of PAHs in blood. However, the relative abundances of PAHs in blood were not in accordance with the composition of the oil ingested. Interestingly, heavier compounds showed lower concentrations in blood, suggesting that gulls mobilized and metabolized PAH compounds differentially depending on their number of rings or molecular weight. Note that vertebrate erythrocytes have a finite programmed lifespan in blood circulation (30 days in birds; 29), thus PAHs found in blood cells were mobilized recently. However, the incorporation of ingested PAHs into the blood cells during erythropoiesis is complex and specific of each compound, while differences in metabolization should also be expected (49, 25). Differences in the mobilization and metabolization of PAHs by gulls were also evident in the study of the temporal pattern of PAHs in blood due to oil ingestion. Although our experiment was not designed to entirely cover the metabolism of these compounds in seabird blood, six of the PAHs analyzed presented significant short-term patterns of change. In four compounds, the highest concentrations in blood were measured toward the end of the experiment. In vertebrates, ingested PAHs are transported into the liver and some fraction is transformed in excretable compounds, but some PAHs remain in the enterohepatic circulation thus extending the residence time of PAHs in the body (50). The increase of some PAHs in oil-fed gulls at the end of the experiment may be due to the incorporation of enterohepatic circulating PAHs during erythropoiesis. Interestingly, different temporal patterns of PAH compounds in experimental gulls probably
indicates different rates of metabolization and residence in the liver. The experimental study suggests that using gull blood as a monitoring tool may underestimate the exposure to heavier PAHs and that acute exposure to some PAH may not be adequately reflected if samples are taken too shortly after an oil pollution event.

Lastly, the factorial analysis revealed that the variance in the blood concentration of PAHs could be grouped in three main factors. While the first factor (PC1) represented total oil pollution in blood, the other two components (PC2 and PC3) clearly segregated oiled and unoiled colonies. In addition, PC2 probably indicated exposure to the Prestige oil. Two main lines of evidence further support the use of this component as proxy of Prestige pollution. First, the PC2 was highly correlated with the amount of ΣPAHs in the sediments close to the colonies shortly after the Prestige spill ($r = 0.96, p = 0.01$; data from Gonzalez 2006). Moreover, experimental gulls fed with Prestige oil increased their PC2 but not their PC3 scores. The PC3 scores probably indicated oil contamination from other sources (i.e., chronic). Interestingly, the PC3 score of Illas Cíes was lower in 2005 than in 2004, suggesting that lower levels of (chronic) oil pollution were operating. Enforcement of controls of illegal oil discharges from passing ships after such a large and visible spill could explain this pattern (6).

In summary, our study not only provides evidence on the temporal and spatial patterns of oil contamination in the marine ecosystems of Northwestern Spain after the Prestige oil spill but also gives support to the use of seabirds as biomonitors of oil pollution in a nondestructive manner. Monitoring programs based upon the analysis of PAHs in seabird blood are therefore promising, providing that harm and disturbance to seabird individuals and populations are kept to a minimum.

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Supporting Information Available
Map of coastal areas affected by the Prestige oil spill, details of the oil-ingestion experiment and the PAH analyses, the mean PAH levels in the blood cells of yellow-legged gulls subject to an oil ingestion experiment, and the PAH profiles in the oil used in the ingestion experiment are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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