Monitoring PAH pollution in the marine environment after the *Prestige* oil-spill by means of seabird blood analysis

Cristóbal Pérez[1], Alberto Velando[1*], Ignacio Munilla[1], Marta López-Alonso[2], Daniel Oro[3]

[1]Departamento de Ecoloxía e Bioloxía Animal. Facultade de Bioloxía. Universidade de Vigo. Campus Lagoas-Marconsende. 36310 Vigo, Spain

[2]Departamento de Patoloxía Animal. Facultade de Veterinária. Universidade de Santiago de Compostela. 27002 Lugo, Spain

[3]IMEDEA (CSIC-UIB). C/Miquel Marqués 21. 07190 Esporles, Majorca, Spain

*Author for correspondence:
Alberto Velando
Departamento de Ecoloxía e Bioloxía Animal
Campus Lagoas-Marcosende
Universidade de Vigo. 36310 Vigo, Spain
e-mail avelando@uvigo.es
Tel +34 986812590
Fax: +34 986812556

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 provide evidence that seabirds were exposed to residual *Prestige* oil 17 months after the spill commenced and gives support to the non destructive 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: 12-13). 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 also been used to follow polluting agents as heavy metals and organochlorines (17, 18). Nevertheless, very few studies have monitored PAH concentrations in bird tissues; in these studies the approaches mainly used are 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 high ability in metabolizing PAHs (23, 24). In common with all vertebrates, birds have well-developed mixed function oxygenase (MFO) systems that can rapidly metabolise 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 embryo 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 as PAH metabolite bile burden have been developed or the induction of cytochrome P450 (12, 21, 28). 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 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 released to the marine environment of approximately 60,000 tonnes of oil products in the eights 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; firstly, we compared PAHs levels in the blood of adult yellow-legged gulls captured in unoiled and oiled breeding colonies, seventeen months after the event. Secondly, we performed an oil-

77 ingestion experiment by supplementing a sample of gulls with oil (33). This experiment
78 allowed us to evaluate whether seabird blood reflected direct exposure to PAHs and to
79 study the dynamics of PAHs incorporation in blood (34). In addition, since it is expected
80 that oil incorporation in the food web from the spill will lessen with time, we compared
81 PAH values from gulls sampled at the oiled colony of Illas Cíes in two consecutive years.
82

83 ## Materials and Methods

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

93 **Oil-ingestion experiment.** In order to evaluate the effect of oil ingestion on the
94 presence of PAHs in the blood of gulls, we performed a field experiment at the Illas Cíes
95 breeding colony (Figure S1). At the end of April 2005, during the courtship period of gulls,
96 we randomly allocated 36 breeding pairs to the experiment of which 16 were fed oil (oil-
97 supplemented group) and 20 were treated as controls (control group). Between one and
98 thirty days after egg laying was complete (i.e.: the third egg was laid) 18 control (10 females
99 and 8 males) and 12 (8 females and 4 males) oil-supplemented gulls were trapped at the
100 nest (one gull per pair) and a blood sample was taken (see further details in supporting
101 information). The comparison between the concentration of PAHs in control adults with

respects to 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 haematological 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 (4ºC), and centrifuged at the end of the day. Blood cells were transferred into cryovials which were kept frozen at -80ºC until analysis. The PAHs that were selected for analysis were the 15 PAHs (Table 1) constituents of the oil spilled by the *Prestige* (37) according to PAH priority pollutants listed by the United States Environmental Protection Agency (US 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 fixed factor and the identity of each colony as a random factor. Temporal comparisons between birds sampled at Cíes in 2004 and 2005 as well as the effect of oil ingestion on the concentration of PAHs in the blood of experimental gulls were type II errors due to small sample size (see ethical considerations above), the effect of oil ingestion was analyzed using one-tailed tests and significance levels set at 0.05, as recommended in studies which involve manipulations that are potentially detrimental to animals (39). For each PAH, regression curves were fitted to data from the oil-supplemented group as a means to examine significant non-linear 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.analyzed using *t*-tests. Sex of the bird did not show significant effects on PAH concentration ($p > 0.09$ in all cases) and was not included in the analyses. In order to avoid

Results

Spatial and temporal distribution of PHA pollution. In 2004, 17 months after the *Prestige* disaster, the concentration of ΣPAHs in the blood cells of gulls from oiled colonies was, on average, 120% higher than concentrations found in gulls from unoiled colonies ($F_{1,59} = 5.44$, $p = 0.011$; Figure 1A). Gulls from Lobeiras, the colony most heavily affected by the spill, showed the highest ΣPAHs values (Figure 1A). Differences between oiled and unoiled colonies were significant for four compounds (naphthalene, fluorene, anthracene and pyrene; Table 1) and in the oiled 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; $t_{28} = 1.87$, $p = 0.036$). 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 non-linear responses (Figure 2). Of these, fluorene, fluorantene, 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) 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 others sources. Interestingly, the gulls sampled at Illas Cíes in 2005 (CI05; 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 non-destructively 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 evidences. 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 Cíes) 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 cells of yellow-legged gulls that were exposed to the *Prestige* oil (either experimentally or at the moment of the spill) 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 6.48 − 860.78 ng/g; equivalent to 86.12 ± 13.22 ng/g wet weight) in the range of values reported for other seabird tissues. Thus, for example, in muscle tissues of silver gulls (*Larus novaehollandiae*) and australian pelicans (*Pelecanus conspicillatus*) the mean concentration values were 85 and 75 ng/g ww respectively (Σ_{12}PAHs; 19); in herring gull (*Larus argentatus*) muscle the mean values were 37.8 ± 12.5 ng/g ww (Σ_{18}PAHs; 41), whereas in the liver of

206 oil exposed guillemots (*Uria aalge*) the mean values were 250 ± 90 ng/g (range 40 - 970
207 ng/g, ww; Σ_{10}PAHs; 21). Inter-specific comparisons of PAHs levels should be treated with
208 caution due to high intra-specific variability as shown by our results and because PAHs
209 concentrations probably differ broadly among tissues. Thus, for example, in eider ducks
210 (*Somateria mollissima*), the mean value was 7.8 ng/g dw in liver, 46 ng/g in gallbladder and
211 9.7 ng/g in adipose tissue (Σ_7 PAHs; 10), suggesting important within organism variability.
212 The spatial comparison of PAH levels in the blood of yellow-legged gulls breeding
213 in oiled versus unoiled colonies, strongly suggests that yellow-legged gulls were exposed to
214 residual *Prestige* oil 17 months after the spill commenced. Acute toxicity is expected when
215 seabirds exposed to the spill ingest oil by preening (42). However, contaminated prey are
216 also a potential source of ingestion and continued incorporation of oil products through
217 trophic processes has been documented for seabird species after a large oil spill (12). The
218 life history characteristics of yellow-legged gulls make them susceptible to continued
219 exposure to remnant oil (13) because they frequently occur and feed in coastal and
220 nearshore environments, which are the same areas that received much of the oil spilled
221 from the *Prestige*. Adult yellow-legged gulls in North-western Spain are sedentary and feed
222 extensively on benthic and intertidal marine organisms (35). Sublethal effects derived from
223 continued oil exposure have been recently documented for yellow-legged gulls in North-
224 western Spain (13).
225 In the oiled colonies, most of the PAH profiles in gull blood were dominated by
226 naphthalene (22 - 38%), indicating a petrogenic (i.e.: derived from petroleum) source (43).
227 Although after the wreck, the composition of the *Prestige* oil was probably altered by
228 weathering (44), naphthalene was also the dominant parent compound found in subsurface
229 waters (45) and intertidal sediments (46) from oiled areas immediately after spill. In
230 contrast, gulls from unoiled colonies showed low naphthalene percentages (6 - 12%), and
231 profiles were dominated by PAHs with a large number of benzene rings (≥4 rings),

232 especially in Pantorgas and Ansarón colonies, indicative of a rather pyrogenic source of
233 contamination. In other studies, naphatalene and tricyclic PAHs also dominated samples
234 from seabird species, including gulls, affected by petrogenic contamination (19, 21). The
235 differences on PHA profiles between the gull blood and the *Prestige* crude oil can be due to
236 oil alterations by weathering, changes in PAH composition in the prey tissues, or specific
237 metabolization of PHA compounds by gulls (see below).

238 There is no information about PAH levels in the blood of yellow-legged gulls
239 before the *Prestige* wreck to complete the classic before-after-control-impact (BACI)
240 approach (47). Nevertheless, the comparison of gulls sampled at Illas Cíes in 2004 and
241 2005 is consistent with the expected reduction in PAH levels with time after acute oil
242 incorporation during the spill. Thus, ΣPAHs concentrations in the blood of gulls decreased
243 threefold in just one year, down to the 2004 values from unoiled colonies. Interestingly, the
244 reduction in PAH levels with time also suggest that PAH concentrations right after the
245 wreck may even have been higher than those found in 2004 samples (17 moths later).
246 Except for five compounds, the majority of hydrocarbons decreased their concentrations
247 abruptly. This reduction was not related to molecular weight or the number of aromatic
248 rings, suggesting an overall reduction in oil exposure by yellow-legged gulls in coastal
249 North-western Spain in 2005. Although the reduction in PAH levels should be treated with
250 caution because it was estimated in a single colony, our results are in agreement with
251 studies on other marine organisms (mussels, *Mytilus galloprovincialis*), that found that ΣPAHs
252 also decreased substantially with time after the *Prestige* event (48).

253 In our experiment, gulls fed with oil increased their blood concentration of PAHs
254 by 30% with respect to controls, hence revealing that PAHs levels in the blood of yellow-
255 legged gulls were in some extent directly related with oil ingestion. A rough extrapolation
256 from the experiment indicates that the ingestion of 3.25 μg of ΣPAHs resulted in an
257 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 since 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 towards the end of the experiment. In vertebrates, ingested PAHs are transported to the liver and some fraction is transformed in excretable compounds, but some PAHs remain in the enterohepatic circulation 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 during the erythropoiesis of enterohepatic circulating PAHs. 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 oiling incident as the *Prestige* 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 North-western Spain after the Prestige oil spill but also gives support to the use of seabirds as biomonitors of oil pollution in a non destructive 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 is kept to a minimum.

Acknowledgments

We want to express our gratitude to Parque Nacional de las Islas Atlánticas de Galicia, Naviera Mar de Ons, Confraría de Celeiro and the Punta Roncadoira crew (Delegación da Consellería de Pesca en Celeiro) for logistic support and the IEO for kindly providing a sample of Prestige oil. Carmen Díez, Julio Eiroa, David Álvarez and Manolo Pajuelo assisted in field work. A.V. was supported by a Ramon y Cajal Fellowship (Ministerio de Educación y Ciencia, Spain). The present study was founded by the program Plan Nacional I+D+I 2004-2007 (Ministerio de Educación y Ciencia, Spain).

Supporting Information Available

Map of coastal areas affected by the *Prestige* oil spill. Details on oil-ingestion experiment and PAH analyses are given. Mean of PAHs in the blood cells of yellow-legged gulls subject to an oil ingestion experiment. PAH profiles in the oil used in the ingestion experiment.

Literature Cited

(1) Moore, M. N.; Livingstone, D. R.; Widdows, J. Hydrocarbons in marine mollusks: Biological effects and ecological consequences. In: Varanasi, U. (Ed.), *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*. CRC Press Inc., **1989**, 291–328.

(2) Meador, J. P.; Stein, J. E.; Reichert, W. L.; Varanasi, U. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev. Environ. Contam. Toxicol.* **1995**, 143, 79-165.

(3) Baars, B. J. The wreackage of the oil tanker "Erika" – human health risk assessment of beach cleaning, sunbathing and swimming. *Toxicol. Lett.* **2002**, 128, 55–68.

(4) Laffon, B.; Fraga-Iriso, R.; Pérez-Cadahía, B.; Méndez, J. Genotoxicity associated to exposure to Prestige oil during autopsies and cleaning of oil-contaminated birds. *Food Chem. Toxicol.* **2006**, 44, 1714–1723.

(5) van Metre, P. C.; Mahler, B. J.; Furlong, E. T. Urban sprawl leaves its PAH signature. *Environ. Sci. Technol.* **2000**, 34, 4064-4070.

(6) Wells, P. G. Oil and seabirds: The imperative for preventing and reducing the continued illegal oiling of the sea by ships. *Mar. Pollut. Bull.* **2001**, 42, 251-252.

(7) Paine, R T.; Ruesink, J. L.; Sun, A.; Soulanille, E. L.; Wonham, M. J.; Christopher D. G.; Harley, C. D. G.; Brumbaugh, D. R.; Secord D. L. Trouble on oiled waters: Lessons from the *Exxon Valdez* Oil Spill. *Annu. Rev. Ecol. Syst.* **1996**, 27, 197–235.

(8) daSilva, E. M.; PesoAguiar, M. C.; Navarro, M. D. T.; Chastinet, C. D. E. A. Impact of petroleum pollution on aquatic coastal ecosystems in Brazil. *Environ. Toxicol. Chem.* **1997**, 16, 112-118.

(9) Votier, S. C.; Hatchwell, B. J.; Beckerman, A.; McCleery, R. H.; Hunter, F. M.; Pellatt, J.; Trinder, M.; Birkhead, T. R. Oil pollution and climate have wide-scale impacts on seabird demographics. *Ecol. Lett.* **2005,** 8, 1157–1164.

(10) Broman, D.; Näf, C.; Lundbergh, I., Zebühr, Y. An in situ study on the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (PAHs) in an aquatic food chain (Seston-*Mytilus edulis* L.-*Somateria mollissima* L.) from the Baltic: an ecotoxicological perspective. *Environ. Toxicol. Chem.* **1990**, 9, 429-442.

(11) Salomone, M. Ecological riches threatened as oil-spill history repeats itself. *Nature* **2002**, 420, 347.

(12) Esler, D.; Bowman, T. D.; Trust, K. A.; Ballachey, B. E.; Dean, T.A.; Jewett, S. C.; O'Clair, C. E. Harlequin duck population recovery following the "Exxon Valdez" oil spill: progress, process and constraints. *Mar. Ecol. Prog. Ser.* **2002**, 241, 271-286.

(13) Alonso-Alvarez, C.; Munilla, I.; López-Alonso, M.; Velando, A. Sublethal toxicity of the *Prestige* oil spill on yellow-legged gulls. *Environ. Int.* **2007**, 33, 773-781.

(14) Peterson, C. H.; Rice, S. D.; Short, J. W.; Esler, D.; Bodkin, J. L.; Ballachey, B. E.; Irons, D. B. Long-term ecosystem response to the Exxon Valdez oil spill. *Science* **2003**, 302, 2082-2086.

(15) Leighton, F. A. The toxicity of petroleum oils to birds:an overview. In *The effects of oil in wildlife: research, rehabilitation and general concerns*. Ed. by White, J. and Frink, L. Sheridan Press, Hanover, PA **1991**

(16) Clark, R. B. Impact of oil pollution on seabirds. *Environ. Pollut.* **1984**, 33, 1-22.

(17) Arcos, J. M.; Ruíz, X.; Furness, R. W. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. *Mar. Ecol. Prog. Ser.* **2002**, 232, 281-290.

(18) Braune, B. M. Temporal trends of organochlorines and mercury in seabird eggs from the Canadian Arctic, 1975-2003. *Environ. Pollut.* **2007**, 148, 599-613.

(19) Kayall, S.; Connell, D. W. Polycyclic aromatic hydrocarbons in biota from the Brisbane River Estuary, Australia. *Estuar. Coast. Shelf Sci.* **1995**, 40, 475-493.

(20) Custer, T. W.; Custer, C. M.; Hines, R. K.; Sparks, D. W. Trace elements organochlorines, polycyclic aromatic hydrocarbons, dioxins and furans in lesser scaup wintering on the Indiana Harbor Canal. *Environ. Pollut.* **2000**, 110, 469-482.

(21) Troisi, G. M.; Bexton, S.; Robinson, I. Polyaromatic hydrocarbon and PAH metabolite burdens in oiled common guillemots (*Uria aalge*) stranded on the east coast of England (2001-2002). *Environ. Sci. Technol.* **2006**, 40, 7938-7943.

(22) Shore, R. F.; Wright, J.; Horne, J. A.; Sparks, T. H. Polycyclic aromatic hydrocarbon (PAH) residues in the eggs of coastal-nesting birds from Britain. *Mar. Pollut. Bull.* **1999**, 38, 509-513.

(23) Hall, R. J.; Coon, N. C. Interpreting residues of petroleum hydrocarbons in wildlife tissues. *Biological Report* **1988**, 88 (15). US Fish and Wildlife Service, Washington, DC, USA

(24) Varanasi, U.; Stein, J. E.; Nishimoto, M. *Metabolism of polycyclic aromatic hydrocarbon in the aquatic environment*. Varanasi, U. Ed., CRC Uniscience Series, CRC Press: Boca Raton, FL. **1989**, 93-150.

(25) Naf, C.; Broman, D.; Brunstrom, B. Distribution and metabolism of polycyclic aromatic hydrocarbons (PAHs) injected into eggs of chicken (*Gallus domesticus*) and common eider duck (*Somateria mollissima*). *Environ. Toxicol. Chem.* **1992**, 11, 1653-1660.

(26) Ariese, F.; Kok, S. J.; Verkaik, M.; Gooijer, C.; Velthorst, N. H.; Hofstraat, J. W. Synchronous fluorescence spectrometry of fish bile: A rapid screening method for the biomonitoring of PAH exposure. *Aquat. Toxicol.* **1993**, 26, 273-286.

(27) Di Giulio, R. T.; Benson, W. H.; Sanders, B. M.; van Veld, P.A. Biochemical mechanisms: metabolism, adaptation and toxicity. In: Rand G. M., editor. *Fundamentals of Aquatic Toxicology*. Washington: Taylor and Francis, **1995**, 523-561.

(28) Trust, K. A.; Esler, D.; Wooding, B. R.; Stegeman, J. J. Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Mar. Pollut. Bull.* **2000**, 40, 397-403.

(29) Clark M. R. Senescence of red blood cells: progress and problems. *Physiol. Rev.* **1988**, 68, 503-554.

(30) Laurent, C.; Feidt, C.; Lichtfouse, E.; Grova, N.; Laurent, F.; Rychen, G. Milk-blood transfer of ^{14}C-tagged polycyclic aromatic hydrocarbons (PAHs) in pigs. *J. Agric. Food Chem.* **2001**, 49, 2493-2496.

(31) Fernandez, N.; Cesar, A..; Gonzalez, M.; DelValls, T. A. Level of contamination in sediments affected by the Prestige oil spill and impact on the embryo development of the sea urchin. *Cienc. Mar.* **2006**, 32, 421-427.

(32) Morales-Caselles, C.; Jimenez-Tenorio, N.; de Canales, M. L. G.; Sarasquete, C.; DelValls, A. T. Ecotoxicity of sediments contaminated by the oil spill associated with the tanker "Prestige" using juveniles of the fish *Sparus aurata*. *Arch. Environ. Contam. Toxicol.* **2006**, 51, 652-660.

(33) Butler, R. G.; Lukasiewicz, P. A field study of the effect of crude oil on Herring gull (*Larus argentatus*) chick growth. *Auk* **1979**, 96, 809-812

(34) Leighton, F. A.; Lee,Y. Z.; Rahimtula, A. D.; O'Brien, P. J.; Peakall, D. B. Biochemical and functional disturbances in red blood cells of herring gulls ingesting Prudhoe Bay crude oil. *Toxicol. Appl. Pharmacol.* **1985**, 81, 25-31.

(35) Munilla, I. Henslow's swimming crab (*Polybius henslowii*) as an important food for yellow-legged gulls (*Larus cachinnans*) in NW Spain. *ICES J. Mar. Sci.* **1997**, 54, 631-634.

(36) Oro, D.; Bosch, M.; Ruiz, X. Effects of a trawling moratorium on the breeding success of the yellow-legged gull *Larus cachinnans*. *Ibis* **1995**, 137, 547-549.

(37) CSIC (Consejo Superior de Investigaciones Científicas). *CSIC technical reports* **2003**. No. 01 and 02 (in Spanish). http://csicprestige.iim.csic.es/Informes.htm

(38) Keith, L. H.; Telliard, W. A. Priority pollutants. I. A perspective view. *Environ. Sci. Technol.* **1979**, 13, 416-423.

(39) Still, A. W. On the numbers of subjects used in animal behaviour experiments. *Anim. Behav.* **1982**, 30, 873-880.

(40) Bosch, X. Prestige wreck - For Spain, oil spill disaster is in the bag. *Science* **2003**, 302, 1485.

(41) Wan, Y.; Xiaohui, J.; Hu, J.; Jin, F. Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environ. Sci. Technol.* **2007,** 41, 3109-3114.

(42) Briggs, K. T.; Yoshida, S. H.; Gershwin, M. E. The influence of petrochemicals and stress on the immune system of seabirds. *Regul. Toxicol. Pharmacol.* **1996**, 23, 145–155.

(43) Page, D. S.; Boehm, P. D.; Douglas, G. S.; Bence, A. E.; Burns, W.A.; Mankiewicz, P.J. Pyrogenic polycyclic aromatic hydrocarbons in sediments record past human activity: a case study in Prince William Sound, Alaska. *Mar. Pollut. Bull.* **1999**, 38, 247-260.

(44) Fernández-Varela, R.; Gómez-Carracedo, M. P.; Fresco-Rivera, P.; Andrade, J. M.; Muniategui, S.; Prada, D. Monitoring photooxidation of the Prestige's oil spill by attenuated total reflectance infrared spectroscopy. *Talanta* **2006**, 69, 409-417

(45) Gonzalez, J. J.; Vinas, L.; Franco, M. A.; Fumega, J.; Soriano, J. A.; Grueiro, G.; Muniategui, S.; Lopez-Mahia, P.; Prada, D.; Bayona, J. M.; Alzaga, R.; Albaiges, J. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. *Mar. Pollut. Bull.* **2006**, 53, 250-259.

(46) Rodríguez, J. G.; Incera, M.; de la Huz, R.; López, J.; Lastra, M. Polycyclic aromatic hydrocarbons (PAHs), organic matter quality and meiofauna in Galician sandy beaches, 6 months after the *Prestige* oil-spill. *Mar. Pollut. Bull.* **2007**, 54, 1046-1052.

(47) Osenberg, C. W.; Schmitt, R.J.; Holbrook, S. J.; Abusaba, K. E.; Flegal, A. R. Detection of environmental impacts: natural variability, effect of size, and power analysis. *Ecol. Appl.* **1994**, 4, 16-30.

(48) Soriano, S. J. A.; Franco, H. A.; Vinas, D. L.; Cambeiro, C. B.; Gonzalez, F. J. J. Preliminary data on polycyclic aromatic hydrocarbons (PAHs) in wild mussels from the Cantabrian coast (N Spain) following the Prestige oil spill. *Cienc. Mar.* **2006**, 32, 457-463.

(49) Lee, Y. Z.; Leighton, F. A.; Peakall, D. B.; Norstrom, R. J.; O'Brien, P. J.; Payne, J. F.; Rahimtul, A. D. Effects of ingestion of Hibernia and Prudhoe Bay crude oils on hepatic and renal mixed-function oxidase in nestling herring gulls (*Larus argentatus*). *Environ. Res.* **1985**, 36, 248-255.

(50) Ramesh, A.; Walker, S.; Hood, D. B.; Guillén, M. D.; Schneider, K.; Weyand, E. H. Bioavailability and risk assessment of orally ingested Polycyclic Aromatic Hydrocarbons. *Int. J. Toxicol.* **2004**, 23, 301-333.

TABLE 1. Mean (±SE) PAH concentrations (ng/g) in the blood cells of yellow-legged gulls sampled in oiled and unoiled colonies in April-May 2004 and sampled at the colony of Illas Cíes in 2005. (Colony abbreviations are: PA=Pantorgas, AN=Ansarón, CO=Coelleira, LO=Lobeiras, VI=Vionta, ON=Ons, CI=Cíes; *n* =sample size).

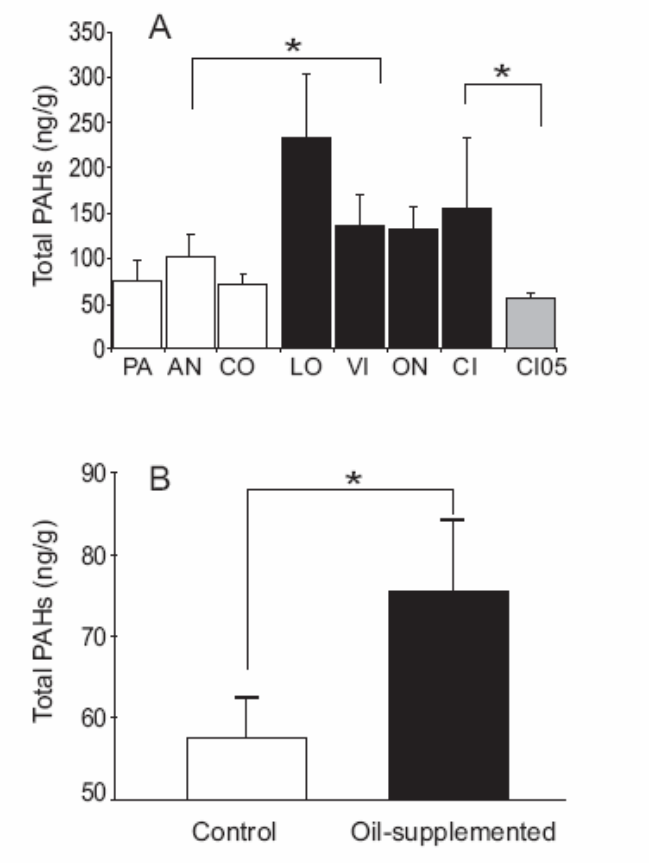
	2004								2005	
	Unoiled			Oiled						
PAHs (ng/g)	PA (*n*=6)	AN (*n*=7)	CO (*n*=12)	LO (*n*=15)	VI (*n*=7)	ON (*n*=7)	CI (*n*=7)	*p*	CI (*n*=18)	*p*
Naphthalene	4.67 ±0.67	8.14 ±0.85	8.98 ±2.72	50.32 ±30.20	49.02 ±14.78	37.14 ±11.67	58.85 ±48.54	0.015	14.71 ±2.33	0.07
Acenaphthene	1.19 ±0.28	1.55 ±0.53	3.33 ±1.08	5.93 ±2.42	0.69 ±0.45	3.50 ±2.97	0.57 ±0.37	0.32	4.33 ±0.51	<0.001
Fluorene	3.14 ±0.60	3.82 ±0.78	5.38 ±0.75	29.52 ±18.94	10.46 ±2.20	20.37 ±13.62	11.00 ±5.73	0.050	1.72 ±0.60	0.008
Phenanthrene	4.10 ±0.47	5.17 ±0.97	16.45 ±2.99	11.94 ±1.49	30.51 ±9.45	15.11 ±3.53	30.07 ±20.55	0.085	5.35 ±1.00	0.030
Anthracene	8.95 ±2.72	9.73 ±2.93	6.90 ±3.13	17.78 ±7.62	13.64 ±3.28	15.46 ±2.94	15.00 ±4.69	0.036	8.82 ±0.84	0.015
Fluoranthene	1.18 ±0.43	1.54 ±0.61	6.33 ±1.31	7.83 ±2.06	2.24 ±1.08	3.99 ±2.29	4.57 ±2.56	0.25	0.65 ±0.15	0.010
Pyrene	6.91 ±2.13	9.18 ±2.31	8.52 ±2.81	13.87 ±4.31	9.57 ±2.37	13.92 ±3.63	15.59 ±4.06	0.039	8.68 ±0.94	0.012
Benz[a]anthracene	38.96 ±15.01	53.76 ±14.54	3.14 ±1.51	29.03 ±15.84	11.71 ±3.61	12.47 ±4.48	9.54 ±2.94	0.20	6.77 ±0.60	0.09
Chrysene	1.71 ±1.14	0.70 ±0.15	1.04 ±0.19	4.73 ±3.24	1.25 ±0.59	0.86 ±0.24	2.03 ±0.48	0.16	0.50 ±0.06	<0.001
Benzo[b+j]fluoranthene	1.05 ±0.49	2.01 ±1.04	2.29 ±0.82	22.23 ±17.96	1.88 ±0.60	2.69 ±1.14	1.69 ±0.36	0.17	2.01 ±0.29	0.26
Benzo[k]fluoranthene	1.81 ±0.61	1.58 ±0.37	4.93 ±2.83	9.86 ±7.79	1.88 ±0.38	2.32 ±0.66	1.68 ±0.41	0.31	0.57 ±0.13	0.001
Benzo[a]pyrene	0.07 ±0.02	0.25 ±0.09	1.58 ±0.99	0.73 ±0.21	0.58 ±0.34	0.35 ±0.16	1.58 ±0.55	0.45	1.82 ±0.37	0.36
Dibenz[a,h] anthracene	0.31 ±0.24	0.22 ±0.15	0.34 ±0.10	0.52 ±0.28	0.05 ±0.00	0.06 ±0.01	0.21 ±0.11	0.44	1.23 ±0.26	0.012
Benzo[g,h,i]perylene	1.05 ±0.49	2.01 ±1.04	2.29 ±0.82	22.23 ±17.96	1.88 ±0.60	2.69 ±1.14	1.69 ±0.36	0.20	2.01 ±0.29	0.01
Indeno[1,2,3-cd]pyrene	0.05 ±0.00	1.51 ±0.88	0.21 ±0.08	1.81 ±1.00	0.66 ±0.55	1.41 ±0.98	1.42 ±0.60	0.07	0.35 ±0.14	0.01
Σ PAH	75.15	101.17	71.71	228.33	136.20	132.34	154.53	0.02	57.65	0.03

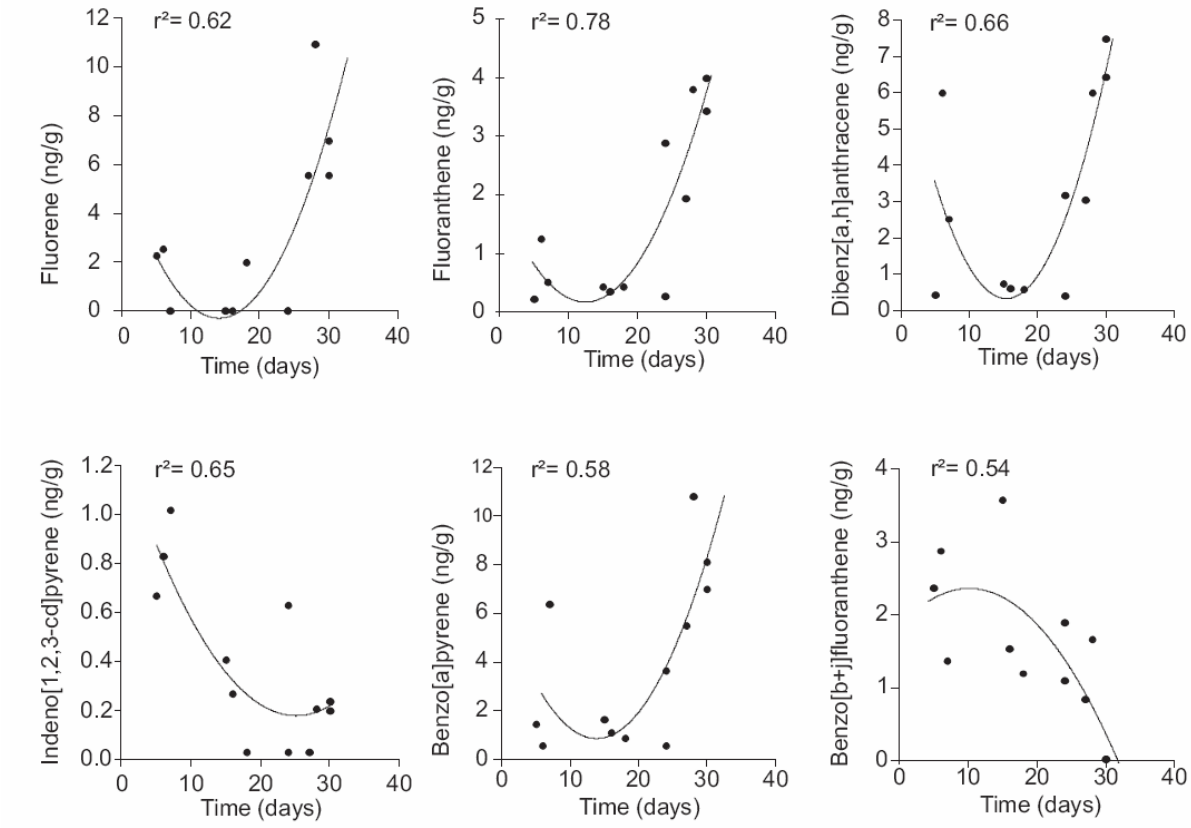
FIGURE LEGENDS

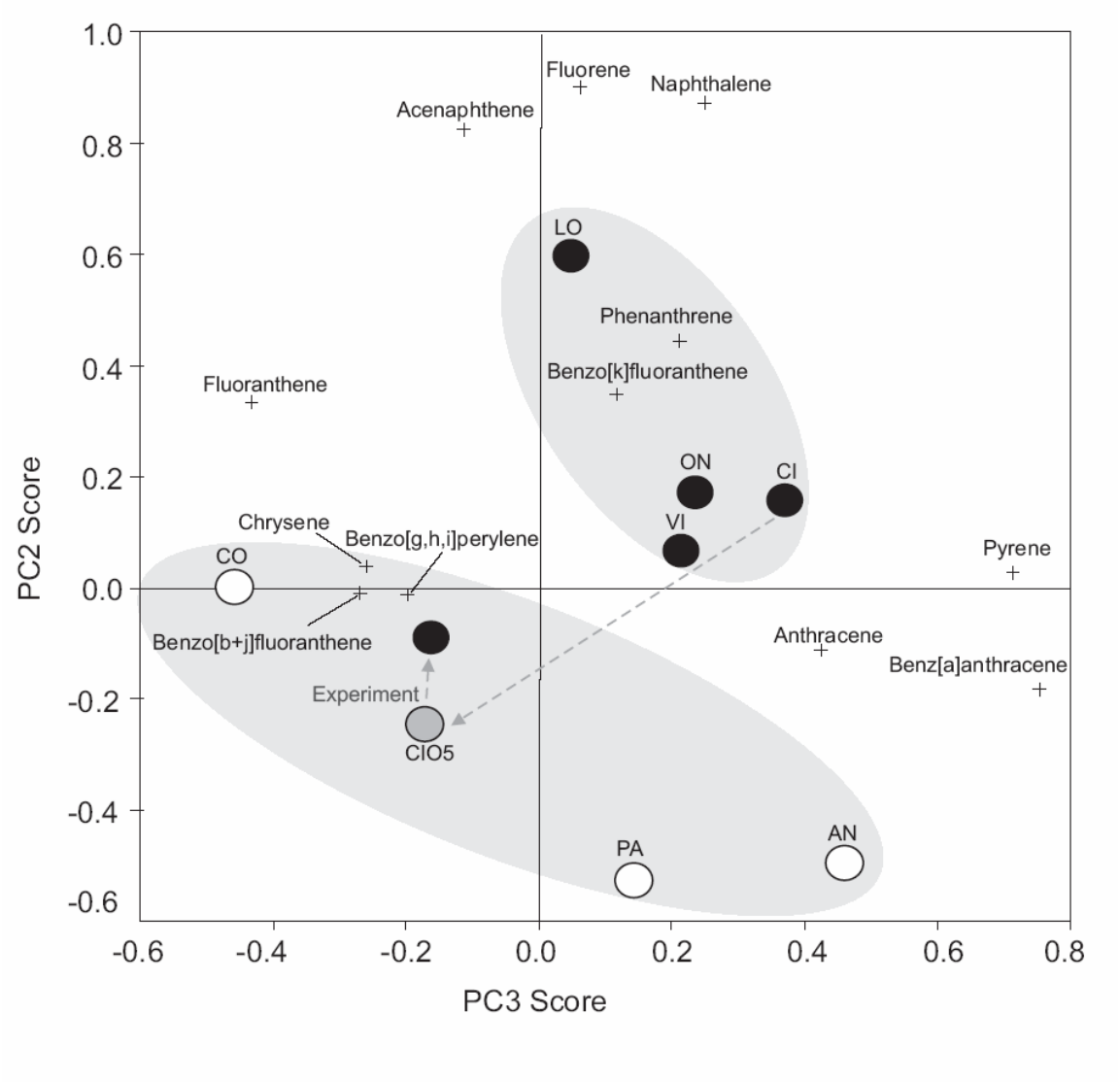
FIGURE 1. Mean (±SE) PAH levels in the blood cells of yellow-legged gulls from A) unoiled and oiled colonies (open and black bars, respectively) and illas Cíes in 2005, and B) from gulls fed vegetable oil (control group, open bar) and vegetable oil plus *Prestige* oil (oil-supplemented group, black bar), (Colony abbreviations are: PA=Pantorgas, AN=Ansarón, CO=Coelleira, LO=Lobeiras, VI=Vionta, ON=Ons, CI=Cíes 2004 and CI05=Cíes 2005). * $p < 0.05$

FIGURE 2. Significant relationship of PAHs of blood cells levels from gulls fed with *Prestige* heavy fuel oil and elapsed time between the end of oil feeding and the capture of gulls.

FIGURE 3. Principal Component Analysis (PCA) diagram of 15 *Prestige* oil PAHs, PAH levels in oiled colonies (closed circles) and unoiled colonies (open circles) and PAH levels in gulls subject to the oil ingestion experiment. The long broken line shows the comparison between gulls sampled at Cíes in the 2004 and 2005 (CI05, control group in the experiment) breeding periods. The closed circle at the end of the shorter arrow shows PAH levels from oil-supplemented gulls in the experiment (Colony abbreviations are: PA=Pantorgas, AN=Ansarón, CO=Coelleira, LO=Lobeiras, VI=Vionta, ON=Ons, CI=Cíes 2004 and CI05=Cíes 2005).


A
Total PAHs (ng/g)
350
300
250
200
150
100
50
0
*
*
PA
AN
CO
LO
VI
ON
CI
CI05
B
Total PAHs (ng/g)
90
80
70
60
50
*
Control
Oil-supplemented


r²= 0.62
Fluorene (ng/g)
Time (days)
r²= 0.78
Fluoranthene (ng/g)
Time (days)
r²= 0.66
Dibenz[a,h]anthracene (ng/g)
Time (days)
r²= 0.65
Indeno[1,2,3-cd]pyrene (ng/g)
Time (days)
r²= 0.58
Benzo[a]pyrene (ng/g)
Time (days)
r²= 0.54
Benzo[b+j]fluoranthene (ng/g)
Time (days)


1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
PC2 Score
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
PC3 Score
Fluorene
Naphthalene
Acenaphthene
LO
Phenanthrene
Benzo[k]fluoranthene
Fluoranthene
ON
CI
VI
Chrysene
Benzo[g,h,i]perylene
CO
Pyrene
Benzo[b+j]fluoranthene
Anthracene
Benz[a]anthracene
Experiment
ClO5
PA
AN

Brief

This study shows that seabirds were exposed to *Prestige* oil 17 months after the spill, supporting the use of seabird blood to monitor oil pollution.

SUPPORTING INFORMATION

MANUSCRIPT TITLE: Monitoring PAH pollution in the marine environment after the Prestige oil-spill by means of seabird blood analysis

AUTHORS: Cristóbal Pérez, Alberto Velando, Ignacio Munilla, Marta López-Alonso, Daniel Oro

This supporting information (7 pages including this cover page) contains details of analytical methods, 2 figures and 2 Tables:

OIL-INGESTION EXPERIMENT

Ethical considerations were taken into account in the design to avoid unnecessary harm to animals while still eliciting a measurable response. Thus, the number of experimental subjects was kept as low as possible (1) and we opted for an amount of oil that was well below the dosage used in previous experiments (2, 3). The oil-supplemented group was restricted to 16 pairs that were fed daily with 0.04 ml of Prestige oil (kindly provided by Instituto Español de Oceanografía under the control of the Spanish Technical Bureau of Marine Spills; otvm.uvigo.es) during seven consecutive days (0.3 ml in total per pair; individual daily PAHs dose: 59.15 μg; Table S2). Oil was dissolved in 6 ml of vegetable oil and spread over a slice of white bread. To minimize the risk of theft by non-target birds, the oiled bread was placed in the territory hidden in vegetation as close to the nest as possible (4). Pairs from the control group were fed in a similar manner with bread and vegetable oil.

ANALYSIS OF PAHS

After microwave extraction with a 1:1 mixture of acetone and hexane, the extract was cleaned-up using a deactivated alumina column with hexane as eluant. PAH levels were determined by high performance liquid chromatography (HPLC) coupled to a wavelength programmable fluorescence detector (5). Samples (100 μl) were injected into a HPLC system fitted with a Waters PAH analytical column (250 mm x 4.6 mm x 5μm). The mobile phase was acetonitrile:water in gradient elution and at a flow rate of 1.2 ml/min. The column oven temperature was maintained at 27 °C. For every group of 10 blood samples, a blank sample was included and processed through extraction and cleanup procedures to check for any external sources of contamination. From the analysis of serial dilution of standards (SRM 2977), the limit of detection was calculated (Table S1). Recovery of PAHs was analyzed by adding a mixture of PAHs (200 ng/g of each compound) to a pool of blood cells and compared with the original values (Table S1).

The accuracy and precision of the analytical procedure were tested using two different methods. Since no certified quality controls on blood samples are available, we used a mussel tissue, a rich lipid matrix. First, we compared PAH levels analyzed by HLPC and GC-MS in the same mussel samples. The PAH concentrations determined by HPLC did not differ than those concentrations by GC-MS ($p > 0.1$), except for fluorene concentration that was 8% lower in HPLC compared with GC-MS. In addition, certified

quality control from the National Institute of Standard and Technology (Gaithersburg, USA; NIST SRM 2977) was used to compare with our analytical procedure. All PAH compounds were within the certified range except for benz[a]anthracene, benzo[g,h,i]perylene levels that were 6 and 11%, respectively, lower and fluorine, benzo[b+j]fluoranthene that were 6 and 5%, respectively, higher than certified values. Moreover, our analytical procedure was involved in the "Prestige 2004 Intercalibration Trial" held by the Instituto Español de Oceanografía (unpublished data). From the basis of IUPAC classification (6) the method proficiency was judged as satisfactory ($|z|<2$).

Literature Cited

1. Still, A.W. On the numbers of subjects used in animal behaviour experiments. *Anim. Behav.* **1982**, 30, 873-880.
2. Butler, R.G.; Lukasiewicz, P. A field study of the effect of crude oil on Herring gull (Larus argentatus) chick growth. *Auk* **1979**, 96, 809-812
3. Leighton, F.A. The toxicity of petroleum oils to birds:an overview. In *The effects of oil in wildlife: research, rehabilitation and general concerns*. Ed. by White, J. and Frink, L. Sheridan Press, Hanover, PA **1991**
4. Pérez, C.; Velando, A.; Dominguez, J. Parental food conditions affect sex-specific embryo mortality in the yellow-legged gull (Larus michahellis). *J. Ornithol.* **2006**, 147, 513-519.
5. Viñas-Diéguez, L. Evaluación de Hidrocarburos Aromáticos Policíclicos (HAPs) por Cromatografía Líquida de Alta Eficacia (CLAE) en el Entorno Marino Gallego. PhD dissertation. Universidade de Vigo. Spain. **2002**.
6. Thompson, M.; Wood, R. The International harmonized protocol for the proficiency testing of (chemical) analytical laboratories. *Pure Appl. Chem.* **1993**, 65, 2123-2144.

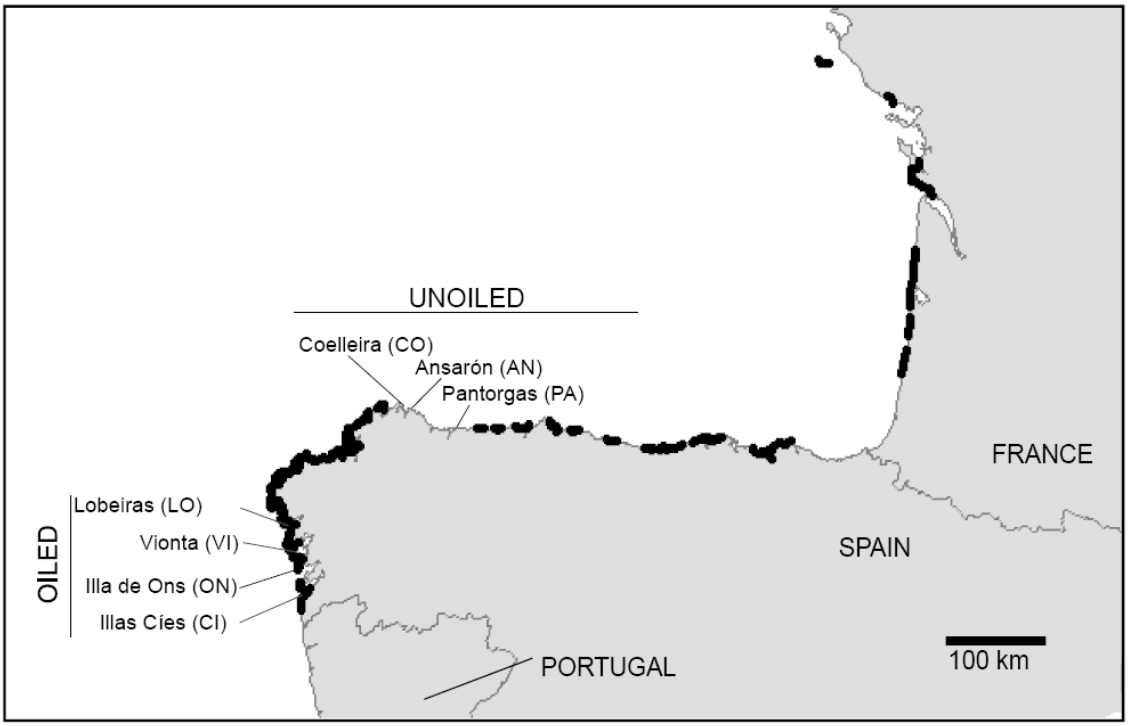


FIGURE S1. Coastal areas affected by the *Prestige* oil spill. Unoiled and oiled colonies are shown (Source: Oficina Técnica de Vertidos Marinos, Ministerio de Educación y Ciencia. http://otvm.uvigo.es/accidentprestige/litoralafectado.html). Colony codes in parentheses as given in text.

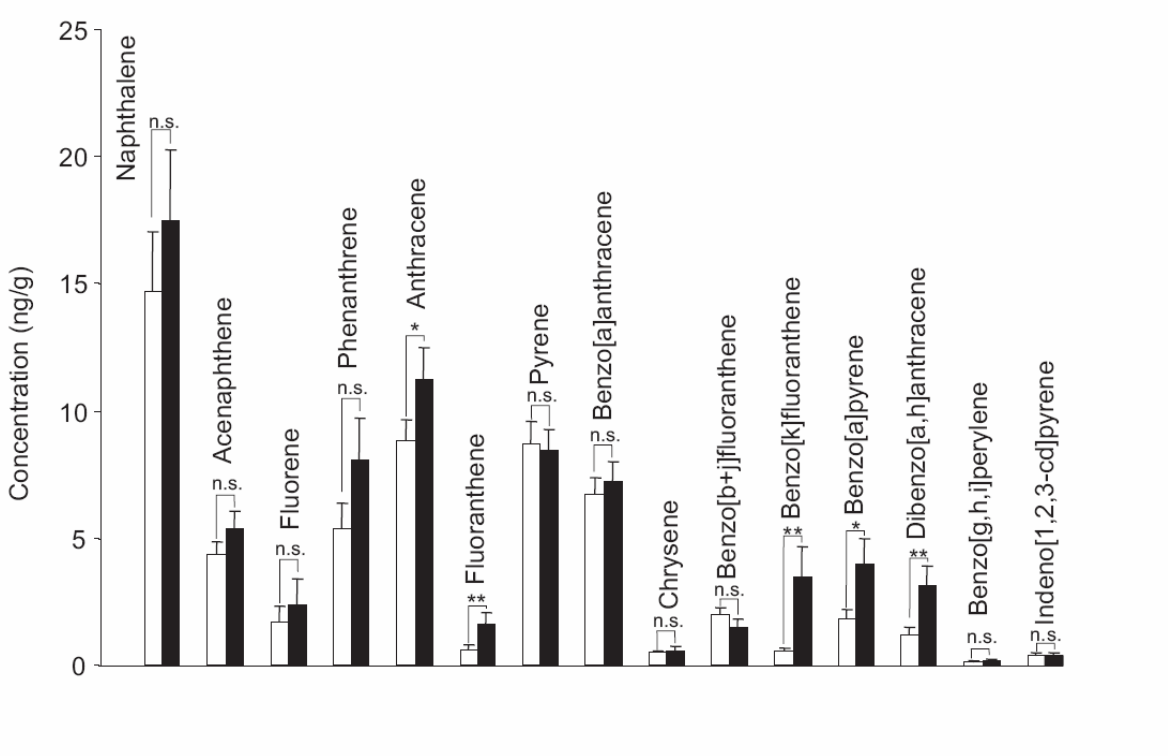


FIGURE S2. Levels of 15 PAHs in the blood cells of yellow-legged gulls subject to an oil ingestion experiment: control group (open bars) and oil-supplemented group (black bars). n.s. $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

TABLE S1. Limits of detection and percentage recoveries (±SE) of 15 PAHs analyzed.

Polycyclic Aromatic Hydrocarbons	Detection limit (ng/g)	Recovery (% ± SE)
Naphthalene	0.02	77.44 ± 1.53
Acenaphthene	0.01	84.50 ± 1.78
Fluorene	0.01	102.11 ± 2.04
Phenanthrene	0.01	94.68 ± 2.70
Anthracene	0.02	90.92 ± 1.71
Fluoranthene	0.01	94.44 ± 1.36
Pyrene	0.01	97.12 ± 1.13
Benz[a]anthracene	0.04	81.71 ± 1.79
Chrysene	0.03	95.11 ± 0.78
Benzo[b+j]fluoranthene	0.05	93.13 ± 0.94
Benzo[k]fluoranthene	0.05	93.55 ± 1.09
Benzo[a]pyrene	0.05	96.47 ± 4.01
Dibenz[a,h] anthracene	0.02	97.53 ± 1.66
Benzo[g,h,i]perylene	0.01	94.58 ± 0.34
Indeno[1,2,3-cd]pyrene	0.05	93.75 ± 0.29

TABLE S2. Relative composition (%) of 15 PAHs in the oil used in the ingestion experiment and the individual daily dose ingested by experimental yellow-legged gulls.

Polycyclic Aromatic Hydrocarbons	%	Total[1] (μg)	Dose[2] (ng/g)
Naphthalene	14.54	8.60	9.82
Acenaphthene	5.61	3.32	3.78
Fluorene	1.01	0.60	0.68
Phenanthrene	3.79	2.24	2.55
Anthracene	26.32	15.57	17.76
Fluoranthene	5.78	3.42	3.90
Pyrene	4.55	2.69	3.06
Benz[a]anthracene	17.50	10.35	11.80
Chrysene	6.48	3.83	4.37
Benzo[b+j]fluoranthene	9.04	5.35	6.10
Benzo[k]fluoranthene	1.35	0.79	0.91
Benzo[a]pyrene	2.16	1.28	1.46
Dibenz[a,h] anthracene	0.27	153	0.18
Benzo[g,h,i]perylene	0.12	0.07	0.08
Indeno[1,2,3-cd]pyrene	1.13	0.67	0.77
Σ PAH	100	59.15	67.47

[1]Total amount of PAHs present in on the crude oil daily ingested by individual gulls

[2]PAH dose in relation to adult body mass (876.7±41.4 g)