Environmental Pollution 158 (2010) 1275–1280

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

EROD activity and stable isotopes in seabirds to disentangle marine food web contamination after the *Prestige* oil spill

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Two years after Prestige oil spill, seabirds were exposed to remnant oil related to their feeding habits with consequences on delayed lethality.

ARTICLE INFO

Article history:
Received 24 November 2009
Received in revised form
17 January 2010
Accepted 22 January 2010

Keywords:
Cytochrome p450
Seabird
Oil spill
Diet
Survival

ABSTRACT

In this study, we measured via surgical sampling hepatic EROD activity in yellow-legged gulls from oiled and unoiled colonies, 17 months after the *Prestige* oil spill. We also analyzed stable isotope composition in feathers of the biopsied gulls, in an attempt to monitor oil incorporation into marine food web. We found that yellow-legged gulls in oiled colonies were being exposed to remnant oil as shown by hepatic EROD activity levels. EROD activity was related to feeding habits of individual gulls with apparent consequences on delayed lethality. Capture–recapture analysis of biopsied gulls suggests that the surgery technique did not affect gull survival, giving support to this technique as a monitoring tool for oil exposure assessment. Our study highlights the combination of different veterinary, toxicological and ecological methodologies as a useful approach for the monitoring of exposure to remnant oil after a large oil spill.

1. Introduction

Large oil spills are one of the pollution events most likely to have dramatic effects on marine ecosystem components, including seabirds (Peterson et al., 2003). Compared to other marine organisms, seabirds appear to be at greater risk of suffering the negative impacts of oil spills. Large oil spills have indeed killed huge numbers of seabirds worldwide (e.g. Wiens et al., 1984; Wilhelm et al., 2007). Seabird casualties effectively reduce the number of reproductive individuals in populations (e.g. Velando et al., 2005a), though this effect is believed to be short-lasting (e.g. Dunnet, 1982; Votier et al., 2005). Nonetheless, marine organisms can also be affected by the chronic long-term exposure to the persistent and bioaccumulative components of oil via several indirect ecosystem processes (e.g. Broman et al., 1990; Peterson et al., 2003; Velando et al., 2005b; Hjermann et al., 2007). Direct lethal effects on seabirds immediately following an oil spill typically attract the greatest public and scientific concern. In contrast, sub-lethal effects due to chronic oil exposure have rarely been explored (some exceptions: Trust et al., 2000; Golet et al., 2002; Alonso-Alvarez et al., 2007a), though they have the potential to strongly impact the long-term dynamics of seabird populations (Peterson et al., 2003).

Sub-lethal effects as a result of the incorporation of oil into the marine food web are likely to be expected in seabirds because they are long-lived animals and upper trophic level consumers (Peterson et al., 2003), and because their populations tend to concentrate in habitats prone to high oil exposure (Clark, 1984). Sub-lethal effects impair seabird condition, which in turn, could have long-term consequences in survival and reproduction (Esler et al., 2000; Golet et al., 2002). Petroleum products are toxic to seabirds. Among other causes, toxic injury is due to oxidative stress and cellular damage associated with the metabolic response by which oil contaminants are biotransformed and eliminated from tissues as happens with the catalytic activity of cytochrome P450 in the liver (Gonzalez, 2005; Shimada, 2006; Ramos and García, 2007). Thus, hepatic P450 activity is currently recognized as a sensitive and fairly specific indicator of organic contaminants, such as the polycyclic aromatic hydrocarbons (PAH) found in petroleum products (Woodin et al., 1997; Kammann et al., 2005). P450 induction, measured by liver 7-ethoxyresorufin-O-deethylase (EROD) activity, has been observed in free-living seabirds environmentally exposed to residual oil several years after the *Exxon-Valdez* oil spill (Trust et al., 2000; Golet et al., 2002).

Here we used a combination of methodologies; including EROD activity measures in the liver tissue of live seabirds, stable isotope analysis and survival estimations, in an attempt to identify the likely paths of oil incorporation into marine food web after the

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doi:10.1016/j.envpol.2010.01.029

Prestige oil spill. The *Prestige* oil spill of 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 Brittany (France). The *Prestige* oil spill was the biggest catastrophe of its type in the Eastern North Atlantic and thousands of seabirds died in the following months. Since incorporation of oil from the *Prestige* is currently being detected in the marine food chain (e.g. Fernandez et al., 2006; Alonso-Alvarez et al., 2007b; Martínez-Gómez et al., 2009), chronic exposure of seabirds to oil would be expected, as they are long lived and upper trophic level consumers. The best evidence on the persistence of oil in the marine ecosystems of Galicia, for years after the *Prestige* spill come from breeding yellow-legged gulls (*Larus michahellis*). In this species, adults from colonies that were in the path of the oil spill consistently showed higher oil contamination levels compared to birds from unoiled colonies (Pérez et al., 2008). Moreover, the presence of PAHs in the blood of chicks from oiled colonies indicated that these pollutants were incorporated into the marine food chain as chicks were not directly exposed to crude-oil, but to contaminated organisms as part of their diets (Alonso-Alvarez et al., 2007b; Pérez et al., 2010).

In this study, we measured hepatic P450 response in yellow-legged gulls from oiled and unoiled colonies, 17 months after the *Prestige* oil spill, in order to assess potential continued exposure to residual oil in these high trophic level consumers. Typically, the measurement of EROD activity requires liver tissue samples preferably collected from freshly killed animals, although alternative techniques are possible (e.g. Degernes et al., 2002). In our study samples consisted on liver biopsies collected via surgical sampling. Moreover, besides checking the diet of breeding adults at the two colonies through pellet analysis we analyzed stable isotope composition in feathers of the biopsied gulls. Stable isotopes were employed in order to quantify the trophic status of individual gulls (reviewed in Fry, 2006) as a means to estimate the likely pathways of oil incorporation into the marine food web. Lastly, we estimated the apparent survival of the biopsied gulls, in order to evaluate the non-lethality of our sampling technique and the possible differences in adult survival between oiled and unoiled gull colonies.

2. Materials and methods

2.1. Study sites and animals

Yellow-legged gulls were sampled from oiled and unoiled coasts of Galicia, northwestern Spain (Fig. 1). For biopsies, we selected two breeding colonies, Coelleira island, located in an area that was not touched by the *Prestige* oil slick (unoiled colony) and Lobeiras islands, located in the pathway of the oil spilled from the *Prestige* (oiled colony). These colonies were selected because they were close enough to limit any geographic effects not related to the *Prestige* oil spill and because we had previously found strong differences in oil exposure in yellow-legged gulls likely related to the *Prestige* wreck (Pérez et al., 2008).

Ethical considerations were taken into account in the design of the study in order to avoid unnecessary harm to many animals while still eliciting a measurable response. In total, 20 adults (10 in each colony) were nest-trapped in 2004 while incubating (May 19–June 5), 17 months after the *Prestige* wreck. Gulls were weighed and several morphometries including wing and tarsus length (±1 mm) were determined to allow sexing birds by means of a discriminant function (Bosch, 1996). In addition, mantle feathers were collected and preserved in individual envelopes. We selected mantle feathers because they are typically moulted prior breeding (i.e. March–April; Harris, 1971). Adults were ringed with two rings, one on each leg: a numbered metallic ring provided by the Nature Protection Agency of Spain (Dirección General de Conservación de la Naturaleza, Ministerio de Medio Ambiente, Spain) and a plastic ring with an individual digit combination to facilitate identification from a distance.

2.2. Liver biopsy

Surgical liver biopsies were taken by a laparotomy wedge biopsy performed by an avian veterinarian in a field laboratory. Since atmospheric temperature was above 20 °C, the surgical site was set up in the open field close to the breeding area. Anesthesia was performed using a portable isoflurane anesthetic machine and an Ayre's T-piece breathing circuit. Birds were monitored with the aid of a stethoscope and a cloacal thermometer. Prior to surgery, all birds were premedicated with 0.4 mg/kg of butorphanol, 0.2 mg/kg of meloxicam and 100 mg/kg of oxytetracycline applied by intramuscular injection. Anesthesia was induced with 5% isoflurane delivered through a face mask and maintained at 1–2% isoflurane applied with an endotracheal tube. Laparotomy was performed through a midline ventral approach. A 2–3 cm incision, 0.5 cm caudal to the sternum, was made in the abdominal wall of each bird. Once the liver was identified and exposed, two 3/0 catgut overlapping 'guillotine' sutures were used to triangulate and isolate a wedge of tissue of the protruding margin of the right liver lobe. Then, the hepatic tissue distal to the ligatures was excised using a scalpel blade and mild pressure was applied to the site of incision until bleeding stopped. Afterwards, the abdominal wall was sutured in three different layers (abdominal musculature, subcutaneous fat and skin). All tissue layers were sutured with a simple interrupted pattern and 3/0 PDS suture material. Apart from some mild liver bleeding in a few birds, all surgeries were uneventful and all animals survived the procedure. After surgery, birds were allowed to recover from anesthetic inside individual cloth bags during 15–20 min. Once, fully recovered, they were released back to the wild.

2.3. EROD activity measurement

EROD activity was measured using a kinetic modification of the plate-based assay of Kennedy and Jones (1994). The liver tissue was first homogenized in 4 volumes in 0.15 M KCL in a Potter Teflon homogenizer. The homogenate was centrifuged at 9000 × g for 15 min. The supernatant was centrifuged in an ultracentrifuge at 100,000 × g for 60 min and the resulting microsomal pellet resuspended with resuspension buffer (50 mM Tris–HCl, 1 mM EDTA, 1 mM dithiothreitol and 20% v/v glycerol, pH 7.4) to give a protein concentration of approximately 20 mg/ml. EROD activity was determined in duplicate in fluorescence multiwell plate reader (Synergy HT-1) at 37 °C. Thus, 150 μl of sodium phosphate buffer (50 mM, pH 8) was added to each well, that then received microsomal suspension (10 μl) and ethoxyresorufin (50 μl of a methanol solution that was diluted 13-fold in sodium phosphate (50 mM, pH 8.0) immediately before addition to the wells; final concentration 1.0 mM). The plate was incubated at 37 °C for 5 min, and reactions were started with the addition of NADPH (25 μl of a 13.4 mM solution in sodium phosphate butter, pH 8.0; final concentration 1.0 mM) to each reaction well. Plates were placed into the fluorescence plate reader and scanned for resorufin with a 530 nm excitation filter and 590 nm emission filter for 10 min. Microsomal protein concentrations were quantified by a Lowry assay and EROD activities expressed as pmol resorufin min^{-1} mg $protein^{-1}$.

2.4. Stable isotope analysis and diet analyses

Mantle feathers collected from biopsied gulls were cut in 1-cm pieces, they were cleaned following the method used by avian isotope labs (L. I. Wassenaar personal communication) in a solution of chloroform:methanol (1:1) during 24 h. Afterwards, they were air dried in an air chamber during 24 h. To carry out stable isotope analyses, 0.5–1 mg of feather vein material was cut from the same location on each feather. Isotope analyses were performed in the Servicios Xerais de Apoio á Investigación (SXAIN, Universidade da Coruña). C and N contents and isotope analysis

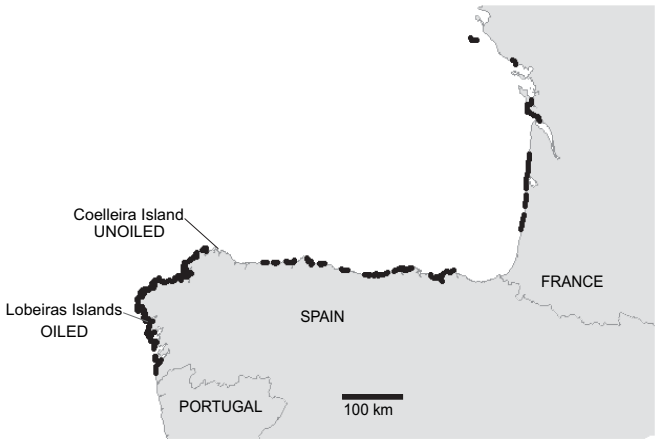

Fig. 1. Coastal areas affected by the *Prestige* oil spill (given in black) in northern Spain showing the location of the unoiled (Coelleira) and the oiled (Lobeiras) study colonies. (Source: Oficina Técnica de Vertidos Marinos, Ministerio de Educación y Ciencia. http://otvm.uvigo.es/accidentprestige/litoralafectado.html).

were determined using an elemental analyzer FlashEA1112 by ThermoFinnigan connected to an isotope ratio mass spectrophotometer DELTA plus by Finnigan MAT, using a ConFlo II interface.

Relative proportions of isotopes are estimated following:

$$\delta^{15}N‰ = [(^{15}N/^{14}N)\text{sample}/(^{15}N/^{14}N)\text{standard} - 1]1000$$

$$\delta^{13}C‰ = [(^{13}C/^{12}C)\text{sample}/(^{13}C/^{12}C)\text{standard} - 1]1000$$

Atmospheric N and VPDB (Pee Dee Belemnites) were used as standards for isotope analysis of N and C, respectively. In marine ecosystems a step-wise enrichment of ^{15}N typically occurs with each trophic level (Hobson et al., 1994). Additionally, $\delta^{13}C$ values can reveal sources of feeding, including inshore/benthic versus offshore/pelagic diet in marine habitats (Hobson et al., 1994).

Isotopic values in mantle feathers were compared with those in representative preys of yellow-legged gull in Coelleira and Lobeiras, inferred from adult pellet remains during adult sampling. The isotopic value of prey tissues was obtained from our reference material at Universidade da Coruña (see Carabel et al., 2009). Moreover, we carried out a taxonomical description of prey consumption based on 169 freshly regurgitated pellets collected throughout the breeding season (April–June), of which 122 were from Lobeiras and 47 from Coelleira. The results of food analyses are reported here as percent frequency of occurrence of a specific food type in a pellet sample (Table 1).

2.5. Estimation of survival probabilities

During the period 2004–2008, we gathered information on survival through intensive and extensive field surveys of banded gulls both at the colony and at the national scale. Focal colonies were intensively monitored during the breeding seasons of 2004–2007. Additionally, we consulted several resighting schemes in Galicia and Spain, with >2500 resightings between 2004 and 2008 of gulls ringed across Galicia, within the movement range of yellow-legged gulls (Munilla, 1997b). Modelling survival through capture–mark–recapture techniques requires large sample sizes, which are difficult to obtain through work such as ours. We thus relied on the analysis of apparent survival, assuming that mortality occurred in individuals not resighted during the 4-year period following biopsy; a reasonable assumption, given their population traits and the intensive monitoring effort described above.

2.6. Statistical analyses

Body condition was analyzed by a General Linear Model (GLM) with body mass as dependent variable, sex and colony (oiled vs unoiled) as factors and tarsus length as covariate (Velando and Alonso-Alvarez, 2003). The EROD activity was analyzed by a GLM, with colony and sex as factors and stable isotopes as covariates. Initially GLM with all explanatory variables and two-way interactions were fitted and then non-significant interactions and main terms were dropped sequentially to simplify the model. Data meet the assumptions of parametric analysis (homogeneity of variance: Levene's test: $P > 0.08$ and normality of residuals: Kolmogorov–Smirnov test: $P > 0.9$). Apparent survival (resighted during 4-year period after biopsies or not) was analyzed using a Generalized Linear Model (GENMOD) with binomial error and log link. We tested differences in survival between colonies and sex, and the relationship with EROD hepatic activity. Since EROD activity was highly related to colony (see results), we performed two separated analyses on survival for colony and EROD activity as independent variables to circumvent collinearity. Moreover, in order to avoid type II errors due to small sample size in variables with expected directional effect (oiled colony on EROD activity; oiled colony and EROD activity on survival) were 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 (Still, 1982). Data are expressed as mean ± SE.

Table 1
Frequencies of occurrence (%) of the main prey types found in pellets of breeding yellow-legged gulls at the two colonies studied, Coelleira (unoiled) and Lobeiras (oiled). Sample sizes are in parenthesis.

Prey type	Coelleira (47)	Lobeiras (122)	Total (169)
Earthworms	2.1	5.7	4.7
Refuse	17.0	7.4	10.1
Marine invertebrates			
Polybius henslowii	55.3	52.5	53.3
Pollicipes cornucopia	2.1	4.1	3.6
Mytilus galloprovincialis	0	3.3	2.4
Cephalopoda	4.3	0.8	1.8
Fishes			
Micromesistius poutassou	2.1	13.9	10.7
Sardina pilchardus	0	10.7	7.7
Trachurus trachurus	6.4	5.7	5.9
Trisopterus sp.	0	3.3	2.4
Other fishes	12.8	9.0	11.2

3. Results

3.1. EROD activity

Body mass of biopsied gulls did not differ between gulls from oiled and unoiled colonies (oiled: $F_{1,16} = 0.27$, $P = 0.61$; sex: $F_{1,16} = 6.71$, $P = 0.020$; tarsus: $F_{1,16} = 7.93$, $P = 0.012$). Hepatic EROD activity ranged from 5.42 to 288.65 pmol min^{-1} mg $protein^{-1}$ in biopsied gulls. In our study, 17 months after the spill, EROD activity in the liver of gulls from the oiled colony more than doubled (135%) the activity in gulls from the unoiled colony ($F_{1,15} = 12.24$, $P = 0.001$; Fig. 2). Moreover, we found that females showed higher EROD activity compared to males in both colonies (sex: $F_{1,15} = 7.58$, $P = 0.015$; sex*oiled: $F_{1,14} = 1.09$, $P = 0.31$, Fig. 2). Interestingly, EROD activity was negatively correlated with $\delta^{13}C$ (parameter estimate = −41.43, $F_{1,15} = 5.35$, $P = 0.035$), especially in gulls from the oiled colony, although the interaction was not significant ($\delta^{13}C$*oiled: $F_{1,14} = 0.81$, $P = 0.38$).

If the analysis was restricted to the gulls nesting in the oiled colony, we found that hepatic EROD activity was negatively and exponentially related to $\delta^{13}C$ ($R^2 = 0.41$, $P = 0.045$; Fig. 3a) and $\delta^{15}N$ ($R^2 = 0.46$, $P = 0.030$; Fig. 3b). These relationships suggest a higher exposure in those birds foraging on low trophic levels (low $\delta^{15}N$) and pelagic/offshore (low $\delta^{13}C$) preys, such as the pelagic crab *Polybius henslowii*, the most common prey in the diet samples (Fig. 3c; Table 1).

3.2. Survival

The sex of the bird did not influence subsequent apparent survival after biopsy ($\chi^2 = 0.97$, $P = 0.32$). In the unoiled colony 9 of 10 ringed gulls were alive for at least one year after the biopsy. In contrast, the survival of biopsied gulls in the oiled colony (0.5 ± 0.17) was reduced by close to a half compared with those at the unoiled colony (0.9 ± 0.10; $\chi^2 = 4.07$, $P = 0.022$). This reduction in survival was related to hepatic EROD activity (Fig. 4; $\chi^2 = 3.39$, $P = 0.032$).

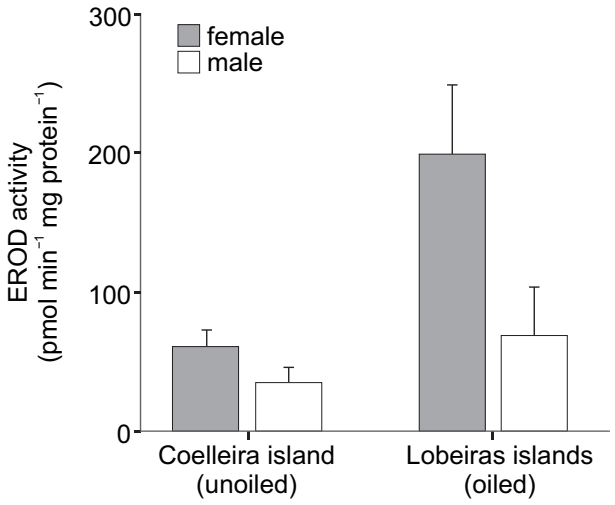

Fig. 2. Mean (±SE) hepatic EROD activity levels of yellow-legged gulls from an oiled (Lobeiras) and an unoiled (Coelleira) colony of Galicia (northwestern Spain).

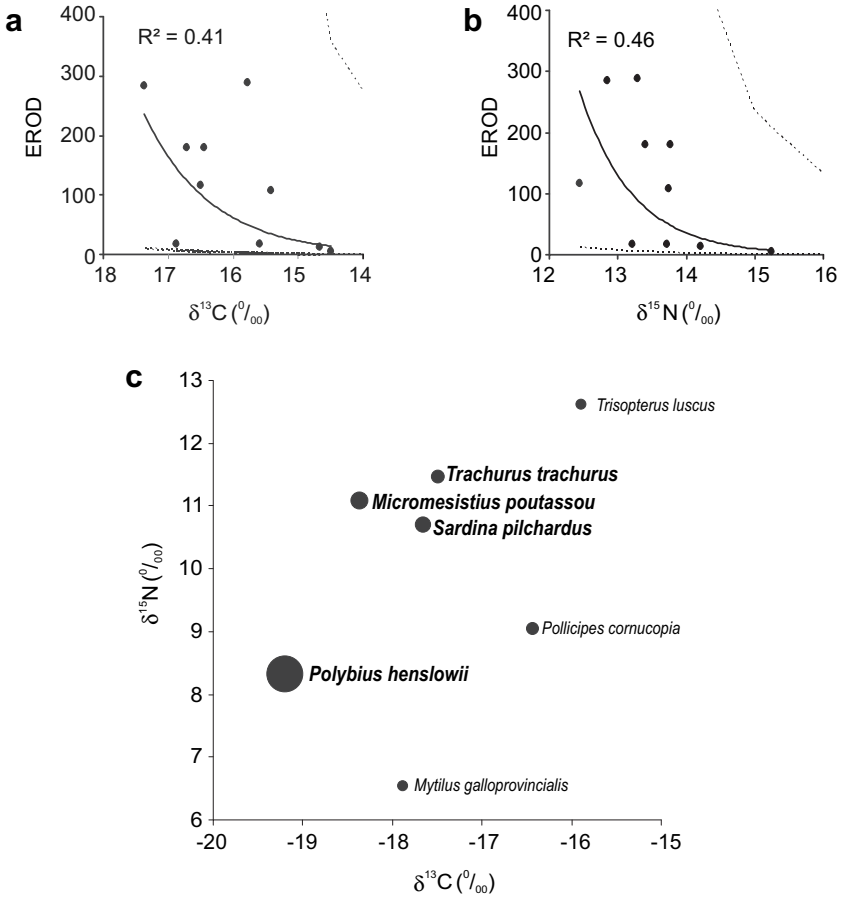

Fig. 3. Relationship between hepatic EROD activity and (a) stable-carbon relative abundance, $\delta^{13}C$ (b) stable-nitrogen relative abundance, $\delta^{15}N$, in mantle feathers of yellow-legged gulls from an oiled colony. Dashed lines represent 95% confidence limits. (c) Stable-carbon and nitrogen isotope abundance in the main prey items in the diet of the yellow-legged gull. Circle size is proportional to overall percent prey occurrence in the dietary sample.

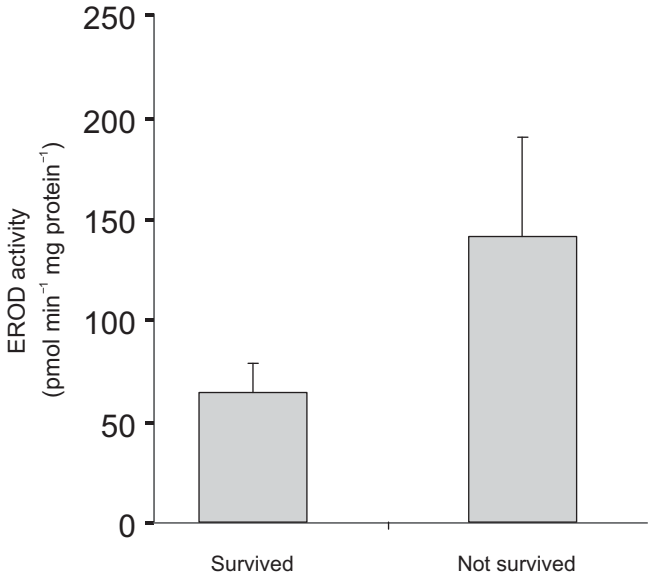

Fig. 4. Mean (±SE) hepatic EROD activity levels of yellow-legged gulls according to survival estimation. Survival was estimated assuming that mortality occurred in individuals that were not resighted during the 4-year period after biopsy.

4. Discussion

Our study suggests that gulls were being exposed to remnant oil 17 months after the *Prestige* catastrophe and validates the nondestructive use of seabirds as biomonitors of oil pollution in the marine environment. The results indicated that liver biopsies taken in the field have a great potential as a nonlethal invasive sample collection method in common species that allows for the monitoring of oil exposure in high trophic level marine organisms (e.g. seabirds). Thus, liver tissue samples taken from gulls biopsied in an oiled colony showed higher hepatic EROD activity levels than samples from gulls biopsied in an unoiled colony. Our results also highlight how information obtained from a combination of sources, such as biomarker activity measurements, stable isotope analysis and diet sampling, can be useful to investigate the presence and the pathways of residual oil in marine food webs.

Elevated EROD activity at the oiled site could have also been caused by exposure to contaminants that did not originate with *Prestige* oil spill including oil from other sources, PCB's and other chlorinated compounds (e.g. Borga et al., 2007). Nevertheless, pollution assessment studies conducted before the *Prestige* wreck failed to reveal differences in these and other contaminants between oiled and unoiled sampling areas (Alvarez-Piñeiro et al., 1995; Fernandes et al., 2008). In contrast, previous studies have documented the persistence of oil in the ecosystem and the chronic exposure of yellow-legged gull populations for years after the spill (Alonso-Alvarez et al., 2007a; Pérez et al., 2008). Indeed, our spatial study on seven seabird colonies found that 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 (Pérez et al., 2008). The cited study also found that Lobeiras was the colony most affected by remnant oil from the *Prestige*. Since blood cells are being continuously produced and have a lifespan of several weeks, the presence of PAHs in blood cells probably indicates a recent incorporation of the contaminants. In the present study, we found elevated hepatic cytochrome P450 levels in a colony affected by this remnant oil, consistent with a previous study that found that gulls sampled in oiled colonies were suffering damages on vital organs (i.e. liver and kidney; Alonso-Alvarez et al., 2007a). Overall, these studies suggested different sub-lethal effects on seabirds, associated to the long-term exposure to oil pollutants after the *Prestige* oil spill.

The incorporation of oil into the marine food web is further corroborated by the presence of PAHs in the blood of gull chicks from oiled colonies that were born almost two years after the spill, because nestlings would have been only exposed to contaminated organisms in the diet (Alonso-Alvarez et al., 2007a). Yellow-legged gulls are omnivorous top predators that feed mainly on marine animals (>85% in 2004 in our sampling colonies, including fishing discards, benthic and intertidal organisms) that they obtain around their breeding colonies, thus, former studies (Pérez et al., 2008; Alonso-Alvarez et al., 2007a), revealed that yellow-legged gulls were being chronically exposed to oil incorporated in the food web. An interesting result of this study, although based on small sample size, is that EROD activity correlated with the feeding habits of individual gulls. Stable isotope analysis of feather samples confirmed that birds occupying lower trophic positions (low $\delta^{15}N$) and feeding on pelagic/offshore (low $\delta^{13}C$) preys, probably marine invertebrates, showed high oil exposure, as indicated by increased hepatic EROD activity. In the marine environment bottom sediments and subsequently, benthic invertebrates are often the final destination of oil contaminants (Woodin et al., 1997). Hence, invertebrate feeders are more likely to ingest oil toxins than piscivorous feeders because marine invertebrates tend to accumulate toxins while fishes metabolize them rapidly (Varanasi, 1989). An important invertebrate species in the diet of the yellow-legged gull is the Henslow's swimming crab, *Polybius henslowii*, a bentho-pelagic invertebrate. This species is the most abundant decapod crab over the continental shelf of Galicia, and it is as a characteristic and even exclusive prey of yellow-legged gull populations in the Iberian Atlantic (Munilla, 1997a). Through pellet analysis we confirmed that *P. henslowii* was the most frequent prey at the time of sampling; although we do not have this kind of data for the period when feathers were formed (March–April). The sequestration of oil products by marine invertebrates can be responsible for the long-term exposure to oil contaminants in the seabirds that ingest them, which, in turn, is likely to have long-term population consequences (Peterson et al., 2003).

The high EROD activity levels observed in yellow-legged gulls after the *Prestige* oil spill agrees with previous findings on marine birds following the Exxon-Valdez oil spill. There, hepatic rates of EROD activity were higher in harlequin ducks (*Histrionicus histrionicus*), Barrow's goldeneyes (*Bucephala islandica*) and Pigeon guillemots (*Cepphus columba*) from oiled sites compared to birds from unoiled sites (Trust et al., 2000; Golet et al., 2002). These results suggest that in the aftermath of large oil spills, seabirds are susceptible to continued exposure to residual oil during several years. Thus, sub-lethal delayed effects due to continued oil contamination cannot be ignored if the impact of oil pollution on seabirds is to be assessed. Indeed, sub-lethal effects could eventually have a stronger impact on population dynamics than direct mortality (see Peterson et al., 2003). Interestingly, we also found that females showed higher EROD activity levels in liver, than males. This result agrees with previous findings on sex-specific harmful effects of oil pollution on gulls (Alonso-Alvarez et al., 2007a,b), and it may be due to sex-specific foraging habits (e.g. Pons, 1994) sex-related sensitivity to oil exposures due to physiological and nutritional stress associated to egg production (e.g. Morales et al., 2009; see also Alonso-Alvarez et al., 2007b, and references therein). Importantly, sex-specific effects of oil contamination on seabirds may have important demographic consequences, such as a reduction of reproductive pairs, constraining the recovery of seabird populations (Martínez-Abraín et al., 2006).

In total, 90% of the gulls that were subjected to biopsy in the unoiled colony were resighted in the four subsequent years, which is an apparent survival expected in large gulls (estimates are in the range of 0.800–0.935 per year; e.g. Lebreton et al., 1995; Pons and Migot, 1995; Wanless et al., 1996). This result suggests that the surgery technique did not affect survival of biopsied gulls. Note that surgical sampling could affect reproductive performance of biopsied gulls, but we had no data to evaluate this possibility. Sampled gulls did no differ in body condition between colonies. Nevertheless, we found that biopsied gulls in the oiled colony had reduced survival and that survival was correlated to former hepatic EROD activity. Although these data should be interpreted with caution due to small sample sizes and to any possible interacting effects of the biopsies, this result seems to suggest that continued exposure to residual oil impaired the gulls, thus promoting delayed lethal effects.

In conclusion, we found that yellow-legged gulls in oiled colonies have been exposed to remnant oil as shown by hepatic EROD activity levels, probably due to marine invertebrate diet. Moreover, this study emphasized that the combination of different veterinary, toxicological and ecological methodologies is a useful approach for the monitoring of exposure to remnant oil in the marine food web in the event of a large oil pollution pulses. In the future, monitoring programs based on such an integrate approach are therefore promising.

Acknowledgements

We want to express our gratitude to Dirección Xeral de Conservación da Natureza (Xunta de Galicia), Confraría de Pescadores de O Vicedo, Delegación da Consellería de Pesca en Celeiro and the "Punta Roncadoira" crew for logistic support. Marta Prieto Rodríguez for veterinary assistance, Carmen Díez Rivera for help during fieldwork and Jorge Mouriño and a great number of birdwatchers for sending us their sightings of the yellow-legged gulls ringed in this study. Xunta de Galicia gave working permissions and approved the study. I.M. was supported by a Xunta de Galicia "Parga Pondal" fellowship contract. The present study was financed by the program Plan Nacional I+D+I (VEM2003-20052, Ministerio de Educación y Ciencia, Spain).

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