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Sublethal effects on seabirds after the *Prestige* oil-spill are mirrored in sexual signals

Cristobal Pérez[1,*], Ignacio Munilla[2], Marta López-Alonso[3] and Alberto Velando[1]

[1]*Departamento de Ecoloxía e Bioloxía Animal, Facultade de Bioloxía, Universidade de Vigo, Lagoas-Marconsende, Vigo 36310, Spain*
[2]*Departamento de Botánica, Facultade de Bioloxía, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain*
[3]*Departamento de Patoloxia Animal, Facultade de Veterinaria, Universidade de Santiago de Compostela, Lugo 27002, Spain*
**Author for correspondence (cristobal@uvigo.es).*

It has been suggested that sexual signals may be a useful measure of environmental quality as they represent the sum of environmental pressures on the animal. Accordingly, it has been proposed that carotenoid-based coloration may be especially valuable in monitoring and detecting the sublethal effects of toxic pollutants in the environment. Here, we evaluate whether the carotenoid-based coloration in the bill of adult yellow-legged gulls reflects oil-induced sublethal effects in breeding colonies affected by the *Prestige* oil spill. In 2004, we took blood samples from 27 adult birds at four insular breeding colonies located in the pathway of the *Prestige* oil spill. We measured the size of the red bill spot area and analysed plasma biochemical parameters indicative of sublethal effects of oil contamination in gulls, including glucose, total protein, creatinine, inorganic phosphorus, aspartate aminotransferase (AST) and gamma-glutamyl transferase. We showed that the size of their red bill spot area was positively related to body condition, while negatively related with AST levels, an enzyme that is commonly used as an indication of hepatic damage in birds. Hence, the present study provides support for the idea that carotenoid-based colour integuments may be a useful measure of environmental quality.

Keywords: oil pollution; sublethal effects; coloration; seabird

1. INTRODUCTION

The expression of ornamental traits often signals reliable information about the physical condition of the bearer (Andersson 1994). Moreover, owing to their high phenotypic plasticity, the expression of sexual signals is particularly sensitive to the cascade of physiological mechanisms produced by stressful events (Buchanan 2000). Accordingly, it has been suggested that sexual signals may be a useful measure of environmental quality as they represent the sum of environmental pressures on the animal (Hill 1995).

Carotenoid-based colorations exhibited by fishes and birds can be considered as good examples of reliable condition-dependent signals (Olson & Owens 1998). Thus, it has been proposed that carotenoid-based coloration may be especially valuable in detecting and monitoring the sublethal effects of toxic pollutants in the environment, because mechanisms underlying both coloration and pollutant damage are interconnected (Dauwe & Eens 2008). Indeed, we have demonstrated experimentally that oil exposure promotes carotenoid mobilization with negative consequences for carotenoid pigmentation (red bill spot) in the yellow-legged gull (*Larus michahellis*) (Pérez *et al.* submitted), a sexual selected trait in both sexes (Morales *et al.* 2009). These results suggest that coloration may be useful for monitoring sublethal effects on seabirds following catastrophic oil pollution pulses at sea. Here, this hypothesis is evaluated, to our knowledge, for the first time in the aftermath of a real oil pollution event; the *Prestige* oil spill occurred in November 2002.

The *Prestige* oil spill was the biggest catastrophe of its type in Europe and thousands of seabirds died in the following months (e.g. Velando *et al.* 2005; Martínez-Abraín *et al.* 2006). Although acute mortality resulting in large numbers of seabird casualties draws much public attention, long-term sublethal exposures to petroleum products have commonly been ignored. One of the few exceptions to date is the yellow-legged gull in northwestern Spain, which has been studied following the *Prestige* oil spill. In this species, adults from colonies that were in the path of the oil spill showed consistently higher oil contamination levels compared with birds from unoiled colonies (Pérez *et al.* 2008*b*). Moreover, recent research has shown that the *Prestige* oil spill was responsible for a delayed impact of a sublethal nature, inducing damages to vital organs, such as the liver and kidney (as shown by higher levels of two aminotransferases: aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT); Alonso-Alvarez *et al.* 2007*a*,*b*). Here, we further evaluate whether variation in sexual-selected coloration reflected these oil-induced sublethal effects in gulls affected by the *Prestige* wreck.

2. MATERIAL AND METHODS

This study was carried out at four insular yellow-legged gull-breeding colonies of northwestern Spain (Cíes, Ons, Vionta and Lobeiras), located in the pathway of the *Prestige* oil spill (Pérez *et al.* 2008*b*). In total, 27 adult birds were nest-trapped in 2004 while incubating (May 23–June 5), 17 months after the *Prestige* spill. For each bird, the head, bill, tarsus length (to the nearest 1 mm) and body mass (to the nearest 10 g) were measured. Tarsus length allowed determination of sex by means of a discriminant function (Bosch 1996). A body condition index was derived using the residuals of the regression of body mass against wing length and sex. The bill was photographed with a digital camera and the size of the red spot area was measured with the aid of image analysis software (for more details see Pérez *et al.* 2008*a*). We focused on the size of the red carotenoid-based spot of the lower mandible because of previous experimental evidence which showed that exposure to the *Prestige* oil did not affect the 'redness' of the bill spot but it resulted in a reduction in the size of the red bill spot area (Pérez *et al.* submitted).

A blood sample was taken from the ulnar vein and centrifuged at the end of the day. Blood cells were analysed to determine and quantify haematological levels of polycyclic aromatic hydrocarbons (PAHs) that were present in the oil spilled by the *Prestige* (see Pérez *et al.* 2008*b*). Likewise, plasma chemicals indicative of sublethal effects triggered by the *Prestige* oil spill were analysed: glucose, total protein, creatinine, inorganic phosphorus, AST and GGT (see Alonso-Alvarez *et al.* 2007*a*,*b*).

We investigated the effect of the blood levels of PAHs and plasma biochemicals on the size of the red bill spot area using a general linear

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model. In the model, colony and sex were included as factors while date of capture, body condition, AST, GGT, creatinine, inorganic phosphorus, glucose, total protein and PAHs were included as covariates. Non-significant terms were backward dropped using a stepwise elimination procedure. Data are expressed as means ± s.e.

3. RESULTS

In the sampled yellow-legged gulls, the size of the red bill spot area was not related to date of capture, colony or sex ($p > 0.15$ in all cases). The final model for the size of this red ornament included only two variables, body condition ($F_{1,25} = 7.50$, $p = 0.011$) and plasma levels of AST ($F_{1,25} = 6.54$, $p = 0.017$). Thus, the size of the red bill spot area was positively correlated with individual condition (figure 1, $r = 0.48$) and negatively with plasma levels of AST (figure 2, $r = -0.46$). Blood levels of PAHs and the rest of the biochemical parameters did not correlate with the size of the red bill spot area ($p > 0.3$, in all cases).

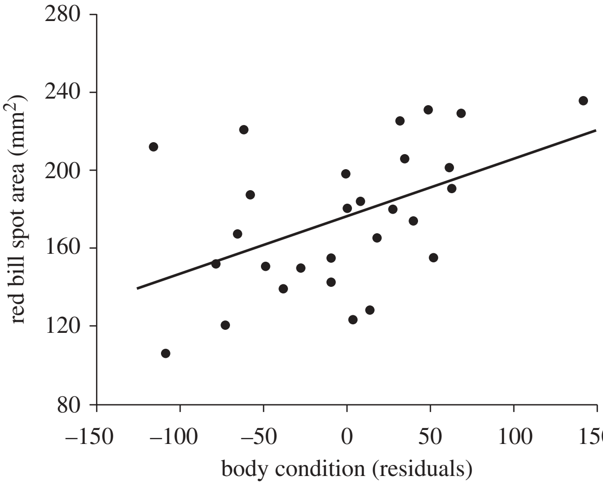

Figure 1. Relationship between the size of the red bill spot area and body condition (estimated as the residuals between body weight, body size and sex) in breeding yellow-legged gulls from oiled colonies.

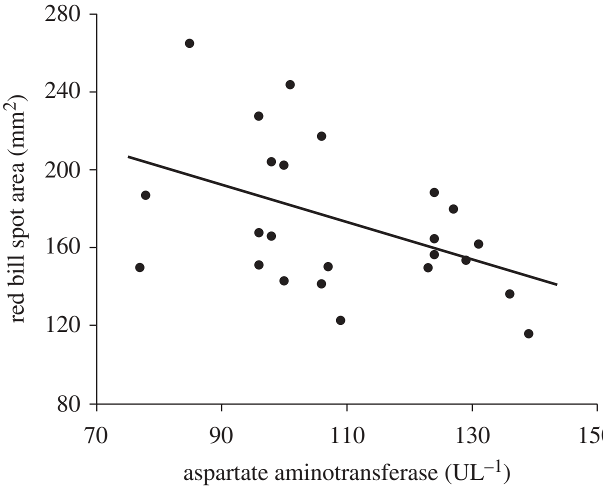

Figure 2. The relationship between the size of red bill spot area and plasma levels of AST.

4. DISCUSSION

In this study, we found that individuals with high plasma levels of AST showed reduced red bill spots. The AST enzyme is commonly used as an index of hepatocellular disease in birds (Harr 2002). Previous studies indicate that high plasma AST levels were induced by exposure to *Prestige* oil (Alonso-Alvarez *et al.* 2007*a*,*b*). We sampled gulls in colonies exposed to residual *Prestige* oil (Pérez *et al.* 2008*b*). The AST plasma levels increase after oil pollution owing to the activation of the hepatic cytochrome P450 (see Golet *et al.* 2002). After oil exposure, carotenoids are mobilized to overcome the harmful effects of PAH ingestion (Pérez *et al.* submitted), thus combating the pro-oxidant substances generated by the degradation of oil hydrocarbons (Yilmaz *et al.* 2007) and counteracting the immunodepressive effects of oil exposure (White *et al.* 1994). Hence, birds with high levels of hepatic damage, as shown by high levels of AST, should be diverting carotenoids away from sexual signals to use them in the oil detoxification process (Pérez *et al.* submitted).

In addition to hepatic damage, we have also found that the size of the red bill spot area was a good indicator of body condition in yellow-legged gulls, in accordance with a recent study on wild black-backed gulls (*Larus marinus*), a closely related species (Kristiansen *et al.* 2006). Processes involving carotenoid use may explain the reduced red spot signal shown by gulls in poor condition (e.g. Velando *et al.* 2006). The absorption and transport of carotenoids can be sensitive to lipids and lipoproteins (Solomon & Bulux 1993) that are reduced during poor nutritional conditions (Alonso-Alvarez & Ferrer 2001). In the *Prestige* oil spill, there was no evidence that oil exposure affected the nutritional condition of yellow-legged gulls (Alonso-Alvarez *et al.* 2007*a*,*b*). Thus, our results suggest that, in gulls, the size of the red bill spot area may be sensitive to several independent environmental pressures, such as nutritional conditions and damage from exposure to toxic chemicals, as evidenced by AST plasma levels.

Interestingly, in the sampled gulls, the size of the red bill spot area was not related to blood levels of total PAHs. Because vertebrates have great capacity to metabolize PAHs and their erythrocytes have a finite lifespan (30 days in birds), blood levels of PAHs are indicative of recent ingestion (Pérez *et al.* 2008*b*), rather than the long-term exposure typically involved in sublethal effects. In fact, the size of the red bill spot area was negatively affected by experimental heavy fuel oil exposure, although it did not correlate with blood levels of PAHs (Pérez *et al.* submitted).

In conclusion, our study supports the hypothesis proposed by Hill (1995) that carotenoid-based colour integuments might be useful measures of environmental quality. Because carotenoid-based traits have evolved for social reasons, their disruption as a result of exposure to oil pollution may have significant consequences in decision-making by gulls during the breeding period, thus affecting reproductive output. Thus, there is a risk of underestimating the impact of oil pollution on seabirds by overlooking the behavioural and population consequences of long-term sublethal effects, such as those derived from impaired sexual signals. Therefore, an evaluation of sexual signals in the animals affected will add further knowledge to the assessment of the real impact of oil pollution on wildlife.

Xunta de Galicia gave working permissions and approved the study.

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Alonso-Alvarez, C. & Ferrer, M. 2001 A biochemical study of fasting, subfeeding, and recovery processes in yellow-legged gulls. *Physiol. Biochem. Zool.* **74**, 703–713. (doi:10.1086/322932)

Alonso-Alvarez, C., Munilla, I., López-Alonso, M. & Velando, A. 2007*a* Sublethal toxicity of the *Prestige* oil spill on yellow-legged gulls. *Environ. Int.* **54**, 7733–7781.

Alonso-Alvarez, C., Pérez, C. & Velando, A. 2007*b* Effects of acute exposure to heavy fuel oil from the *Prestige* spill on a seabird. *Aquat. Toxicol.* **84**, 1033–1110.

Andersson, M. 1994 *Sexual selection*. New York, NY: Princeton University Press.

Bosch, M. 1996 Sexual size dimorphism and determination of sex in yellow legged gulls. *J. Field Ornithol.* **67**, 5343–5541.

Buchanan, K. L. 2000 Stress and the evolution of condition-dependent signals. *Trends Ecol. Evol.* **15**, 1563–1160. (doi:10.1016/S0169-5347(99)01812-1)

Dauwe, T. & Eens, M. 2008 Melanin- and carotenoid-dependent signals of great tits (*Parus major*) relate differently to metal pollution. *Naturwissenschaften* **95**, 9693–9973. (doi:10.1007/s00114-008-0400-1)

Golet, G. H. *et al.* 2002 Long-term direct and indirect effects of the 'Exxon Valdez' oil spill on pigeon guillemots in Prince William Sound, Alaska. *Mar. Ecol. Prog. Ser.* **241**, 2873–2304. (doi:10.3354/meps241287)

Harr, K. E. 2002 Clinical chemistry of companion avian species: a review. *Vet. Clin. Pathol.* **31**, 1403–1151. (doi:10.1111/j.1939-165X.2002.tb00295.x)

Hill, G. E. 1995 Ornamental traits as indicators of environmental health. *BioScience* **45**, 253–231. (doi:10.2307/1312532)

Kristiansen, K. O., Bustnes, J. O., Folstad, I. & Helberg, M. 2006 Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. *J. Avian Biol.* **37**, 63–12.

Martínez-Abraín, A., Velando, A., Oro, D., Genovart, M., Gerique, C., Bartolomé, M. A., Villuendas, E. & Sarzo, B. 2006 Sex-specific mortality of European shags after the *Prestige* oil spill: demographic implications for the recovery of colonies. *Mar. Ecol. Prog. Ser.* **318**, 2713–2276.

Morales, J., Alonso-Álvarez, C., Pérez, C., Torres, R., Serafino, E. & Velando, A. 2009 Families on the spot: sexual signals influence parent-offspring negotiations. *Proc. R. Soc. B* **276**, 24 773–22 483. (doi:10.1098/rspb.2008.1942)

Olson, V. A. & Owens, I. P. F. 1998 Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 5103–5514. (http://dx.doi.org/10.1016/S0169-5347(98)01484-0)

Pérez, C., Lores, M. & Velando, V. 2008*a* Availability of non-pigmentary antioxidants affects red coloration in gulls. *Behav. Ecol.* **19**, 9673–9973.

Pérez, C., Velando, A., Munilla, I., Lopez-Alonso, M. & Oro, D. 2008*b* Monitoring PAH pollution in the marine environment after the *Prestige* oil-spill by means of seabird blood analysis. *Environ. Sci. Technol.* **42**, 7073–7713.

Pérez, C., Lores, M. & Velando, V. Submitted. Carotenoids are mobilized after exposure to heavy fuel oil, with negative consequences on bird coloration.

Solomon, N. W. & Bulux, J. 1993 Effects of nutritional status on carotene uptake and bioconversion. *Ann. NY Acad. Sci.* **691**, 963–109.

Velando, A., Munilla, I. & Leyenda, P. M. 2005 Short-term indirect effects of the *Prestige* oil spill on a marine top predator: changes in prey availability for European shags. *Mar. Ecol. Prog. Ser.* **302**, 2633–2274. (http://dx.doi.org/10.3354/meps302263)

Velando, A., Beamonte, R. & Torres, R. 2006 Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia* **149**, 5433–5552. (doi:10.1007/s00442-006-0457-5)

White Jr, K. L., Kawabata, T. T. & Ladics, G. S. 1994 Mechanisms of polycyclic aromatic hydrocarbon immunotoxicity. In *Immunotoxicology and immunopharmacology*, 2nd edn (eds J. H. Dean, M. I. Luster, A. E. Munson & I. Kimber), pp. 123–142. New York, NY: Raven Press.

Yilmaz, B., Ssempebwa, J., Mackerer, C. R., Arcaro, K. F. & Carpenter, D. O. 2007 Effects of polycyclic aromatic hydrocarbon-containing oil mixtures on generation of reactive oxygen species and cell viability in MCF-7 breast cancer cells. *J. Toxicol. Environ. Health A* **70**, 11 083–11 115. (doi:10.1080/15287390701208545)