Ardeola 67(2), 2020, 325-339 DOI: 10.13157/arla.67.2.2020.ra6

CARBON δ^{13}C ISOTOPIC MARKER VALUES CORRELATE WITH CAROTENOID-BASED BILL COLOURATION IN ADULT YELLOW-LEGGED GULLS *LARUS MICHAHELLIS*

UN MARCADOR ISOTÓPICO DEL CARBONO δ^{13}C SE CORRELACIONA CON LA COLORACIÓN GENERADA POR CAROTENOIDES EN LA GAVIOTA PATIAMARILLA *LARUS MICHAHELLIS*

Nere Zorrozua[1] *, Carlos Alonso-Álvarez[2], Beñat Díaz[1],
Carola Sanpera[3, 4], Lluís Jover[4] † and Juan Arizaga[1]

Summary.—The carotenoid-based colouration that many birds present may honestly reflect the quality of the bearer, as carotenoids have to be obtained through diet. These pigments are important for many physiological functions and individuals could find a trade-off between the allocation of carotenoids for these uses and their accumulation for secondary-sexual and social traits. The Yellow-legged Gull *Larus michahellis* is an opportunistic species that feeds on a wide spectrum of prey. We studied whether carotenoid-based colouration (bill, red spot, eye-ring) is related to the trophic ecology inferred by the isotopic composition of adults' feathers at three colonies of this species. The isotopic marker δ^{13}C negatively correlated with carotenoid-based colouration (red spot area). Contrarily to *a priori* prediction, the marker indicated that higher consumption of landfill scraps and terrestrial prey might lead to a larger red bill spot in adult Yellow-legged Gulls independently of sex. No correlation was detected between isotopic signatures and egg volume in females. The finding that higher consumption of marine prey may imply a smaller red spot supports some results from other avian studies that report that feeding on human-related terrestrial food sources has an apparently positive effect on the adult phenotype. The results may suggest that higher consumption of marine prey may not be as advantageous as supported

---

[1] Department of Ornithology, Aranzadi Sciences Society, Zorroagagaina 11,
E-20014 Donostia-San Sebastián, Spain.

[2] Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (MNCN),
Consejo Superior de Investigaciones Científicas (CSIC), José Gutiérrez Abascal 2,
28006 Madrid, Spain.

[3] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia,
Universitat de Barcelona, Avda. Diagonal 643, E-08028 Barcelona, Spain.

[4] Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Avda. Diagonal 643,
E-08028 Barcelona, Spain. † Deceased in October 2018.

* Corresponding author: nzorrozua@aranzadi.eus

by previous studies. Nonetheless, the effects of potentially toxic compounds that may come from some feeding sources should be further studied, including any delayed sub-lethal effects on the physiology of adults and, probably, chicks. —Zorrozua, N., Alonso-Álvarez, C., Díaz, B., Sanpera, C., Jover, Ll. & Arizaga, J. (2020). Carbon δ^{13}C isotopic marker values correlate with carotenoid-based bill colouration in adult Yellow-legged Gulls *Larus michahellis*. *Ardeola*, 67: 325-339.

Key words: carotenoid-based colouration, individual quality, seabirds, stable isotopes, trophic ecology.

RESUMEN.—La coloración basada en carotenoides que presentan muchas aves puede reflejar honestamente la calidad del individuo, ya que deben ser obtenidos mediante la dieta. Estos pigmentos son importantes para varias funciones fisiológicas y los individuos pueden buscar compensar entre la distribución de los carotenoides para estos usos y su acumulación para atributos sexuales secundarios y sociales. La gaviota patiamarilla *Larus michahellis* es una especie oportunista que se alimenta de una gran variedad de presas. En este trabajo hemos estudiado si la coloración basada en carotenoides (pico, mancha roja, anillo orbital) está relacionada con la ecología trófica inferida a partir de la composición isotópica de plumas de aves adultas en tres colonias de esta especie. El marcador isotópico del δ^{13}C correlacionó negativamente con la coloración basada en carotenoides (área de la mancha roja). Contrariamente a la predicción hecha a priori, el marcador indica que un mayor consumo de presas terrestres y recursos de vertedero podría ayudar a generar una mancha roja del pico mayor en gaviotas de cualquier sexo. En hembras, no se detectó ninguna correlación entre las signaturas isotópicas y el volumen de los huevos. El resultado de que un consumo mayor de presas marinas implica una mancha roja menor apoya otros descritos en estudios anteriores que muestran efectos aparentemente positivos de comer en ambientes terrestres humanos sobre el fenotipo de aves adultas. Los resultados pueden sugerir que un mayor consumo de presas marinas puede no ser tan positivo como el presentado en estudios previos. Sin embargo, el efecto de los componentes tóxicos potenciales que se pueden obtener de algunas fuentes de alimentación debería ser estudiado más a fondo. Deberíamos de considerar también los probables efectos subletales retardados asociados a estos hábitats y que se ejercerían sobre los adultos y, probablemente también, sobre los pollos. —Zorrozua, N., Alonso-Álvarez, C., Díaz, B., Sanpera, C., Jover, Ll. y Arizaga, J. (2020). Un marcador isotópico del carbono δ^{13}C se correlaciona con la coloración generada por carotenoides en la gaviota patiamarilla *Larus michahellis*. *Ardeola*, 67: 325-339.

Palabras clave: aves marinas, calidad individual, coloración basada en carotenoides, ecología trófica, isótopos estables.

INTRODUCTION

Human activities generate different selective pressures that can have an impact on birds in very different ways. Food subsidies are among those factors that can shape the diet and, accordingly, all the life-history aspects that can depend on trophic ecology (Ramos *et al*., 2009; Gilbert *et al*., 2016). However, not all food sources provide similar amounts of macro- (energy/structural) or micro- (e.g. vitamins) nutrients (Eldridge & Krapu, 1988; Pierotti & Annett, 1990; Annett & Pierotti, 1999). Consequently, the type of food consumed by an individual or a population can affect different fitness-related traits, whether directly or through carry-over effects. Such traits include individual body condition, (Acquarone *et al.,* 2002; Laursen *et al.,* 2009), survival (Oro & Furness, 2002; Le Galliard *et al.,* 2005) or reproductive success (Pierotti & Bellrose, 1986; Eldridge & Krapu, 1988; Clifford & Anderson, 2001; Reynolds *et al.,* 2003). Given this, in a scenario of global change where many species feed on resources of anthropogenic origin

(Oro *et al.,* 2013), it is essential for the implementation of conservation and management strategies to determine how these influence individual phenotypes and, as a result, reproductive output.

In addition to the energy reward, some micronutrients in the diet, such as carotenoids, may strongly influence the phenotype. In particular, carotenoids may affect the expression of the secondary sexual traits that serve as signals during the breeding season. Many such traits are coloured ornaments among which those produced by carotenoid pigments leading to yellow-orange-red colours are probably the most studied (e.g. Hartley & Kennedy, 2004; McGraw, 2006). In many birds and other vertebrates, carotenoid-based colouration seems to have evolved to act as a sexual signal reliably showing the quality of the bearer during the courtship and breeding season or during intra-sexual contests to acquire a mate (e.g. Grether *et al.,* 1999; Hill, 2006). Carotenoid pigments cannot be directly synthesised by the animal and have to be obtained through diet (Goodwin, 1984; Brush, 1990). It has often been assumed that carotenoids are scarce in natural food sources and so costly to obtain (Endler, 1980; Grether *et al.,* 1999; Kodric-Brown, 1985; Hill, 1990). The production costs of signals may thus constitute a handicap that only high-quality individuals would be able to overcome, ensuring the reliability of the signal (according to the 'handicap' theory; Zahavi, 1975; Grafen, 1990). Moreover, it is generally accepted that carotenoids are essential to homeostasis, fulfilling important functions as immune boosters and antioxidants (Lozano, 1994; von Schantz *et al.,* 1999; Alonso-Álvarez *et al.,* 2004; Pérez-Rodríguez, 2009; but see also Hartley & Kennedy, 2004; Costantini & Møller, 2008). Therefore, animals could face a trade-off between the allocation of carotenoids for homeostasis versus their accumulation in some conspicuous features for purposes of sexual and social signalling (von Schantz *et al.,* 1999; Møller *et al.,* 2000; Alonso-Álvarez *et al.*, 2008). Recently, it has also been suggested that carotenoid-based signalling may also serve to highlight the efficiency of cell (mitochondrial) respiration (Hill & Johnson, 2012; Koch & Hill, 2018); this would only apply to red (keto) carotenoid-based colouration (García-de Blas *et al.,* 2016; Lopes *et al.*, 2016). Hence, it is suggested that signals based on red ketocarotenoids would be the most reliable indicators of individual quality among avian species as they should be the costliest to acquire (Weaver *et al.,* 2018). In summary, only the fittest individuals would be able to feed on prey rich in carotenoids and so to produce carotenoid-based colouration of the highest intensity.

Marine prey has a rich concentration of carotenoids (Goodwin, 1984: Maoka, 2011) owing to its dependence on primary producers that are also rich in such pigments (Olson & Owens, 1998; Maoka, 2011; de Carvalho & Caramujo, 2017). Therefore, it may be expected that birds whose diet comprises a high proportion of marine prey should exhibit more intense carotenoid-based colouration than those with a higher dependence on terrestrial or landfill food, which should, in turn, show paler traits.

Gulls (Laridae) offer a good model to answer these questions since they are broadly distributed and there are many populations that depend to an extent on food subsidies of human origin. Moreover, foraging on marine prey has been associated with the production of higher quality offspring in this avian taxon. Thus, those adult gulls specialised in foraging on marine food enjoy a higher fledging rate and offspring winter survival than those relying on other sources such as landfills (Pierotti & Annett, 1987; Annett & Pierotti, 1999). Better body condition and growth rates have also been found in chicks of other seabirds that feed mostly fish (Paiva *et al.,* 2006; Forero *et al.*, 2014). These results can be attributed to the fact that, compared to other prey types, fish have a higher concentration of micronutrients (e.g. calcium that should favour skeletal development) and also a higher energy content (i.e. lipids; Clarke & Prince, 1980; Anthony *et al*., 2000; Forero *et al*., 2014). Therefore, it can be predicted *a priori* that marine prey (mostly fish) should constitute a better nutritional resource than terrestrial (earthworms) or landfill-based food. Accordingly, adults that forage more on marine prey should have better body condition and could invest more in reproductive traits such as colours.

The Yellow-legged Gull *Larus michahellis* is the commonest gull of the south-western Palearctic (Olsen & Larson, 2004). It is an opportunist that feeds on a very wide variety of prey, such as fish and other marine prey, terrestrial prey like earthworms or organic scraps from landfills (Duhem *et al.,* 2005; Ramos *et al.,* 2009; Arizaga *et al.,* 2013). The Yellow-legged Gull displays carotenoid-based colouration in the eye-ring, legs and bill (Cramp & Simmons, 1983). The expression of this colouration is intensified during the mating and breeding period (Pérez *et al.,* 2008; Morales *et al.,* 2009). In particular, the red bill spot has been thought to play an important role in adjusting reproductive investments, as it is grasped by begging chicks, stimulating food release by parents (Tinbergen, 1948). It could have also evolved to act during courtship feeding, thus ultimately being involved in sexual selection (Velando *et al.,* 2013). Furthermore, we know that gulls with more intense carotenoid-based colouration show better body condition and breeding output: greater egg volumes and clutch sizes (Kristiansen *et al.,* 2006; Blévin *et al*., 2014), and are also more successful during mate choice (Hammouda *et al*., 2016). The yellow-orange-red bill colouration of Yellow-legged Gulls involves a number of carotenoids, including the red (keto) carotenoids (astaxanthin, canthaxanthin) and the yellow xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) (Pérez *et al*., 2008). Unfortunately, the relative contribution (concentration) of each in the distinct ornament areas (yellow-orange background and red spot) was not determined (Pérez *et al*., 2008). Nonetheless, on the basis of avian literature (e.g. McGraw, 2006), we may assume that the red bill spot should mainly comprise red ketocarotenoids and the yellow part of the bill and feet, could be mostly the result of yellow carotenoid accumulation.

We addressed the relationship between the expression of carotenoid-based colouration in adult Yellow-legged Gulls captured at the breeding colony and isotopic markers of diet composition during the preceding winter. We measured the isotopic signatures of stable isotopes in feathers, which provided us with information on the diet during the moult period in which the tissue was formed (Hobson *et al*., 1994). The δ^{13}C and δ^{15}N isotopes were used to obtain insights into the foraging habitat and trophic position, respectively (Schoeninger & DeNiro, 1984; Hobson *et al*., 1994; Cherel & Hobson, 2007). In addition, we tested for the relationship between the isotopic markers and individual body condition (size-corrected body mass) and egg volume (in females only), since they would contribute to understanding the link between individual fitness and dietary composition. Assuming that a diet containing a higher proportion of marine prey should be positive in terms of fitness, we predicted that the gulls that foraged more on this food type would also be those in better body condition, expressing more intense or larger carotenoid-based colouration; such females would also be able to lay larger eggs. As far as we know, only one previous study has analysed the link between isotopic indices of diet composition and carotenoid-based colour intensity in seabirds (in Brown Boobies *Sula leucogaster brewsteri* by using δ^{13}C only: Michael *et al.,* 2018). The cited work used $\delta^{13}C$ to detect differences in colouration between individuals feeding to different extents on pelagic or benthic prey, but it also showed that carotenoid-based colouration might indicate the individual capacity to obtain carotenoid-rich food (Michael *et al.,* 2018). Here, we analyse whether isotopic markers reveal a link between the consumption of marine food and the expression of these coloured secondary sexual traits.

MATERIALS AND METHODS

Study area and data collection

The study was carried out at three Yellow-legged Gull colonies in Gipuzkoa, northern Iberia, from west to east: Getaria (43°18'N 2°12'W), Santa Clara (43°19'N 1°59'W), Ulia (43°20'N 1°57'W), hosting about 92, 85 and 520 adult breeding pairs, respectively (Arizaga *et al.,* 2009).

During the 2016 breeding season (May), 33 adults were captured using spring traps when incubating (12 in Getaria, 11 in Santa Clara and 10 in Ulia). Once captured, each bird was ringed, and the sixth secondary, S6 numbered ascendingly, was collected for analysis of stable isotopes. S6 grows during autumn-winter (Olsen & Larson, 2004) and, hence, reflects the diet just before the current breeding season; i.e. an S6 of a bird captured in 2016 reflects what that bird ate during winter 2015-16. We also measured body mass (with a PESOLA balance, 20g accuracy) and tarsus length (with a digital calliper; 0.1mm accuracy). We took three photos of the head of each individual using a Sony DSC-HX90V camera with flash placed at the top of a box *c.* 24cm high, with a lateral hole to insert the head into the box base. The whole box was covered with opaque fabric to exclude external light. A homogeneous grey panel and a measurement scale were placed at the base of the box for standardisation purposes. Also, a cork support *c.* 1cm high was placed below the bill to ensure the same position for all individuals in the photographs. All measurements and photographs were taken by the same person to minimise potential bias. All birds were sexed at the University of Navarra using a subsample (calamus) of the innermost primary feather for DNA analyses (Griffiths *et al.*, 1998).

In addition, for nests where adults were captured, we also recorded the clutch size and the length and width of each egg, measured with a digital calliper (mm). The egg volume was then obtained as indicated by Harris (1964): Volume = 0.476 × length × width^2.

Data preparation: colouration analysis and spot ratio

Colouration analyses were conducted using the head photos. In particular, we selected a number of reference points (Figure 1) both on the bill and the eye-ring. We used Adobe Photoshop CS software to measure colour values on the RGB scale at each reference point. The same zoom was used for all measurements; Magic Wand tool (tolerance: 40) allowed us to select an area around each reference point, which was then averaged (using Filter > Blur > Average) before taking the RGB colour values. In addition, an area of 350 × 350 pixels of the panel was selected from the photograph, to obtain mean RGB values. All these values were later transformed into Luminosity (lum) and Hue values (Saravanan *et al.*, 2016). Hue values are inverse to colour intensity. For the different colouration values obtained, repeatability was estimated using the three photos taken for each individual bird (rptR package): Bill hue: $R = 0.86$, $p < 0.001$; Bill lum: $R = 0.61$, $p < 0.001$; Eye-ring hue: $R = 0.97$, $p < 0.001$; Eye-ring lum: $R = 0.42$, $p < 0.001$; Spot hue: $R = 0.95$, $p < 0.001$; Spot lum: $R = 0.59$,

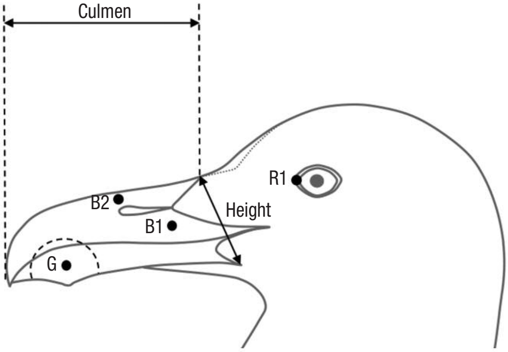

FIG. 1.—Reference points used to record colouration: B1-B2, bill colouration (yellow); R1, orbital-ring colouration (red); G, gonydeal spot colouration (red dot). We also measured the gonydeal spot area in relation to the bill area, culmen and height.

[Puntos usados para medir coloración: B1-B2, coloración del pico (amarillo); R1, coloración del anillo orbital (rojo); G, coloración de la mancha del gonys (punto rojo). Además, también se midió el área de la mancha del gonys con relación al área del pico, culmen y altura.]

$p < 0.001$; Grey panel hue: R = 0.73, $p < 0.001$; Grey panel lum: R = 0.35, $p < 0.004$.

We also measured a number of variables to determine the relative proportions of the red spot surfaces associated with the gonydeal and the yellow area (Figure 1), using the scale in the photograph to standardise length. In particular, and in order to avoid complications when measuring bill area (parts may be covered with feathers or a gull's expression may affect the bill edge position), culmen and height were measured to estimate a fixed area for the bill and the red spot (Figure 1).

Data preparation: egg volume

Only females were considered in models on egg volume, since this variable is mostly determined by the female's diet (Ramírez *et al.,* 2011; Poisbleau *et al.,* 2017). We considered the mean volume of each clutch for the analysis. A t-test was run to see whether the mean volume differed between clutches of 2 or 3 eggs. As no differences were detected (t = –0.32, df = 7.98, $P = 0.757$), the (remote) possibility that a 2-egg clutch was incomplete when sampled would not influence our results and so both 2- and 3-egg clutches were considered.

Stable isotope analyses

The signatures provided by the δ^{13}C and δ^{15}N stable isotopes retained in S6 were used to assess the Yellow-legged Gull winter diet (Arizaga *et al.,* 2013; Zorrozua *et al.,* 2020). Feathers were washed in 1M sodium hydroxide solution and oven-dried at 60ºC before being placed in an impactor mill (Freezer/mill 6750-Spex Certiprep) that operates with liquid Nitrogen (ca. –200ºC) to be homogenised into a fine powder. Weighed subsamples (*c*. 0.3mg for δ^{13}C and δ^{15}N) of this powder were introduced in capsules for combustion and isotopic analysis in elemental analysis-isotope ratio mass spectrometry (EA-IRMS), using a ThermoFinnigan Flash 1112 analyser coupled to a Delta isotope ratio mass spectrometer via a ConFlo III interface (Serveis Científico-Tècnics, University of Barcelona).

The values obtained for δ^{13}C and δ^{15}N stable isotopes analysed in the feathers would suggest a higher or lower proportion of a certain feeding resource (Arizaga *et al*., 2013).

Statistical modelling

Statistical models were run to determine to what extent the isotopic marker values were related to adult body condition, carotenoid colouration and egg volume. For this, we used individual body mass and five dependent (object) variables described above (bill, eye-ring and gonydeal spot colouration-Hue; red spot area; egg volume).

Variance inflation factors (VIF) were calculated initially to test for the existence of a $\delta^{13}C$-$\delta^{15}N$ correlation. As no association was detected (VIF = 1.25), both isotopic signatures were included simultaneously in the same model.

For the different dependent variables considered, we conducted General Linear Models (GLM) with a linear-link function and normal distribution errors, and the following control fixed factors or covariates: C-winter ($\delta^{13}C$ values for the S6), N-winter ($\delta^{15}N$ values for the S6), and sex (this last excluded from models to test for the effect on egg volume; only females were considered). For the models testing body condition (size-corrected body mass) and red spot area, we included respectively tarsus length and a variable with the area calculated as culmen × height (Cul × Hei) as covariates. The colony identity was included as a fixed factor. For models testing for the bill, eye-ring and gonydeal spot colouration intensity (inverse Hue), component one of a Principal Component Analysis (PCA) conducted for Hue and Luminosity of the grey panel used to take the photographs was introduced as a covariate. PCA resulted in two components, the first (PC1) explaining 56% of the variance (eigenvalue = 1.13) and high values indicating high values of Hue and low values of lum (factor loadings for PC1: Hue = 0.71, lum = –0.71). Mean values of the three photos of each individual were considered for each of the colour values.

Results have been provided for simplified and full models. In the model testing body mass variability, the tarsus length covariate was always maintained to test size-independent body mass (condition). The same was done with the first component obtained for the grey panel to avoid subtle effects of variation in ambient light on colour measurements. For the red spot area model, Cul × Hei was also always maintained. Normality of the model residuals was confirmed during the model selection procedure.

Statistical analyses used R 3.3.2 (R Development Core Team 2011).

RESULTS

The mean $\delta^{13}C$ and $\delta^{15}N$ values (± SD) were −18.3 ± 1.3 and 13.1 ± 1.5. Neither of the two isotopes (N and C) analysed had any association with body mass ($\delta^{15}N$: $F_{1,25} = 1.50$, $P = 0.232$, $\delta^{13}C$: $F_{1,26} = 1.71$, $P = 0.203$). We detected a significant effect of sex and colony on body mass (Table 1, see Supplementary Material, Appendix 1, Table A1, for the full models). The tarsus length did not show a significant association with body mass and males were found to be heavier than females (female: 804.44 ± 52.94g; male: 925.71 ± 62.48g). Individuals from Ulia were heavier than individuals from the other two colonies.

Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ showed no association with any of the analysed colour-associated traits, except the red spot area, which was found to be negatively associated with carbon values (Table 1, Supplementary Material, Appendix 1, Table A1). In this case, gulls foraging less on marine prey showed a larger gonydeal spot in their bills (Figure 2).

Other effects related to colour-associated traits revealed that (1) individuals from Ulia showed paler eye-rings than individuals from the other two colonies (Table 1); and (2) the intensity of the red spot tended to be lower in males (female: –6.75 ± 2.88; male: –9.52 ± 4.89; Table 1).

Regarding reproductive output, egg volume did not covary with $\delta^{13}C$ and $\delta^{15}N$ signatures, but in relation to colony identity, with Getaria having the larger eggs and Ulia the smallest ones (Getaria: 77.93 ± 2.83mm^3; Santa Clara: 72.79 ± 5.76mm^3; Ulia: 70.15 ± 4.46mm^3; Table 1).

TABLE 1

Beta-parameter estimates (± SE; below: *P*-values) of the best Generalized Linear Models on different dependent variables. Reference beta-parameter values (beta = 0): Sex = Female; Colony = Getaria. Only females were considered for egg volume models. Egg volume was the mean volume of the clutch.
[Beta-parámetros estimados (± SE; abajo: P-valores) de los mejores Modelos Lineales Generalizados para las diferentes variables dependientes. Valores de referencia de los beta-parámetros (beta = 0): Sexo = Hembra; Colonia = Getaria. Para los modelos del volumen de los huevos sólo se consideraron hembras. El volumen de los huevos fue el volumen promedio de la puesta.]

Covariates/ Factors	Body mass	Bill yellow intensity	Eye ring redness	Spot redness	Spot area	Egg volume
Intercept	596.11 ± 304.63 $P = 0.061$	−42.94 ± 0.41 $P < 0.001$	−8.53 ± 1.10 $P < 0.001$	−6.28 ± 1.34 $P < 0.001$	0.03 ± 0.81 $P = 0.969$	77.93 ± 2.06 $P < 0.001$
PC1 Grey		1.29 ± 0.39 $P = 0.003$	−0.43 ± 0.65 $P = 0.509$	−0.35 ± 0.66 $P = 0.594$		
Tarsus	2.92 ± 5.04 $P = 0.567$					
Culmen-Height					−0.00 ± 0.00 $P = 0.338$	
Sex: Male	116.09 ± 27.19 $P < 0.001$			−2.83 ± 1.34 $P = 0.044$		
Colony: Santa Clara	25.39 ± 23.70 $P = 0.294$		−0.97 ± 1.60 $P = 0.550$	1.18 ± 1.65 $P = 0.481$		−5.14 ± 2.79 $P = 0.087$
Colony: Ulia	58.97 ± 24.37 $P = 0.023$		−6.16 ± 1.59 $P < 0.001$	−2.75 ± 1.63 $P = 0.103$		−7.77 ± 2.79 $P = 0.015$
C-winter					−0.10 ± 0.04 $P = 0.026$	

DISCUSSION

We detected a statistically significant relationship between feather δ^{13}C values and the expression of a bill colour trait of adult gulls, suggesting that their feeding habitat may influence carotenoid-based colouration signalling. By contrast, we did not find evidence supporting that the isotopic signatures had any significant correlation with other bill-associated colour traits considered here and size-corrected body mass (body condition) or egg volume. Nonetheless, accepting the null hypotheses needs caution since our sample sizes were relatively small.

The size of the red bill spot was associated negatively with δ^{13}C values. Studies have shown that although ketocarotenoids could be derived from the diet (e.g. marine prey consumption), the red carotenoid-based colouration is a more reliable signal when compared with yellow xanthophyll-based traits (Johnson & Hill, 2013; Cantarero & Alonso-Álvarez, 2017; Weaver *et al.*, 2018). Hence, only the highest quality individuals would be able to synthesise the red pigment

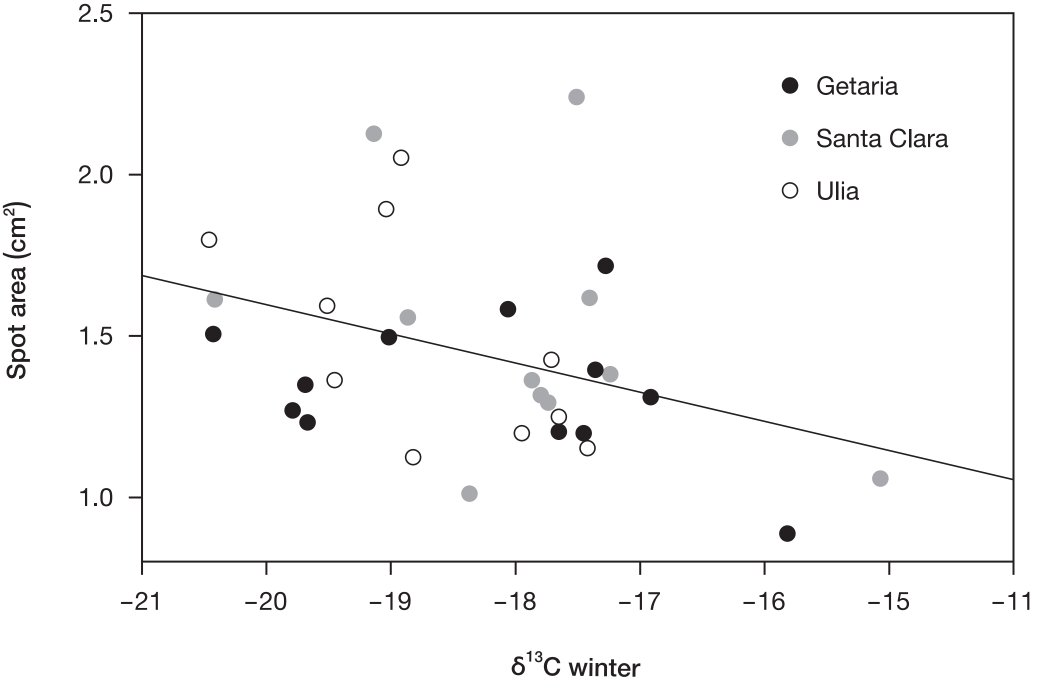

FIG. 2.—Red spot area in relation to carbon isotopic values obtained from a winter-grown feather. *[Relación del área de la mancha roja con los valores isotópicos del carbono obtenidos a partir de una pluma crecida en invierno.]*

from yellow substrates and allocate these ketocarotenoids to the coloured traits. Also, and similarly to our results, red spot size, but not colour intensity, was found to be affected when the gulls' diet was supplemented with the antioxidant vitamin E (Pérez *et al*., 2008), suggesting that spot colouration is sensitive to dietary composition. It is worth noting that Pérez *et al*. (2008) worked with males only; we did not find any differences between sexes.

The size of the red bill spot has previously been related to parental chick-feeding behaviour as mothers with larger spots were found to trigger more intense chick begging (Velando *et al.,* 2013). Also, mates of individuals of either sex with artificially enlarged red spots were found to increase food provision to chicks (Morales *et al.,* 2009). Here we show that red spot size is associated with an isotopic marker of the feeding habitat. Therefore, we can argue that the gulls' foraging behaviour can ultimately influence significant life-history traits that, in turn, depend on the expression of carotenoid-based coloured ornaments.

Previous studies on trophic ecology carried out at the Gipuzkoa colonies showed that higher δ^{13}C values indicated higher prey consumption within marine habitats (Arizaga *et al*., 2013, Zorrozua *et al*., 2020). Our results suggest, therefore, that greater exploitation of terrestrial prey, including scraps from landfills, promotes larger red bill spots, as compared to marine habitats. This conclusion is intriguing as we initially predicted that higher consumption of marine prey (mostly fish) should result in a larger intake of carotenoids, which hence would facilitate a larger red spot when compared with birds feeding on other food items. However, we should also consider that marine food may also include coastal prey taken from intertidal areas in winter (Moreno *et al*., 2010; we did not detect this kind of prey in summer, e.g., Arizaga *et al*., 2013). Preliminary data from GPS-tracked gulls have shown that use of intertidal flats, even in winter, is scarce (Arizaga *et al*., 2017; unpublished data). In any event, the Basque coast, where our study population lives, comprises relatively steep cliffs with no relevant intertidal flats where gulls might forage, as occurs elsewhere (Irons, 1998).

Regarding landfills, we must consider that rubbish could contain lower carotenoid levels than fish and also more pollutants, which would increase oxidative stress and, in turn, lead to utilisation of carotenoids as antioxidants or their direct bleaching (e.g. Hartley & Kennedy, 2004). The literature suggests that toxic compounds found in landfill sites or other human habitats induce a loss of carotenoid-based colouration (Giraudeau *et al*., 2015; Dauwe & Eens, 2008; Vallverdú-Coll *et al*., 2016). Nonetheless, some exceptions to this negative effect exist. Thus, adult Red-legged Partridges *Alectoris rufa* exposed to high levels of herbicide or lead showed increased red colouration (García-de Blas *et al*., 2016; Vallverdú-Coll *et al*., 2015). These latter effects could be due to an increased redox state consequent upon higher oxidative stress linked to pollutants, which, in turn, could favour the oxidation of yellow-substrate carotenoid pigments to generate red pigments (García-de Blas *et al.,* 2016). Alternatively, mild exposure to toxins could trigger some compensatory (hormetic) mechanism (Costantini *et al.,* 2010; Losdat *et al.,* 2018), e.g. improving the capacity to absorb carotenoids from food. However, not all pollutants originate from terrestrial anthropogenic habitats. Marine prey can also be a source of high levels of mercury or fuel oil residues after an oil spill (Wiener *et al.,* 2002; Pérez *et al.,* 2010). Further studies are necessary to analyse the presence of toxins and their influence on oxidative stress and bare-part colouration in Yellow-legged Gulls.

We must also note that carotenoid-based bare-part colouration (skin, bill) can change very rapidly, over a few days or weeks (Faivre *et al.,* 2003; Alonso-Álvarez *et al.,* 2004; Velando *et al.,* 2006). Hence, we may ask why and how the winter diet could have an impact on the red spot area in the spring. A reliable scenario is that our gulls forage on similar trophic resources both in winter and spring, so that winter carbon values may be correlated with those existing in spring. However, long-term effects are also plausible. For example, nestling captive Zebra Finches *Taeniopygia guttata* exposed to an antioxidant synthesis blocker for just one week had redder ketocarotenoid-based bills in adulthood (Romero-Haro & Alonso-Álvarez, 2015).

We must also consider that the relationship between red spot area and our carbon marker could mostly be driven by the fact that some birds fed on terrestrial food. The consumption of terrestrial food (chiefly earthworms) in winter, however, was rather low in our population (Zorrozua *et al.,* 2020). We may hypothesise that only higher quality birds are able to feed on this probably limited resource in winter and/or that the birds foraging on terrestrial prey would also perform longer flights. The latter possibility was previously considered to be a factor favouring the production of red carotenoid-based colourations in birds. In this regard, studies of Common Crossbills *Loxia curvirostra* suggested that the generation of red pigments could be linked to the individual capacity to transform yellow carotenoids to red, which may increase with individual flying capacity (Völker, 1957; Weber, 1961). Under this scenario, the larger red spots in our gulls may be a consequence of diverse individual behaviours implying metabolic differences rather than being directly associated with particular food resources.

In conclusion, we obtained evidence supporting that the diet, in a broad sense, had a significant effect on Yellow-legged Gull bare-part colouration. In particular, individuals foraging more on marine prey in winter had smaller red spots in their bills during the breeding season. This suggests that higher levels of marine prey consumption may not be as favourable as supported by previous studies, at least regarding the expression of carotenoid-based signals. Birds feeding on terrestrial or landfill food had larger red spots, ultimately favouring higher breeding success. Nonetheless, we should also consider the possibility of long-term sub-lethal effects derived from exposure to pollutants in anthropogenic habitats, which could also exert an impact on seabird demography (e.g. Bustnes *et al*., 2008; Goutte *et al*., 2014).

ACKNOWLEDGEMENTS.—We are grateful to those who helped with the fieldwork, especially members of the Txingudi Ringing Station. The research was partially funded by the Basque Government, the Deputation of Gipuzkoa and Donostia City Council. N. Zorrozua was supported by a pre-doctoral fellowship from the Basque Government. Gipuzkoa Administration provided permissions for this study. All applicable international, national and institutional guidelines for the care and use of animals were followed. The associated editor (Alberto Velando) and two anonymous reviewers provided very valuable comments that helped us to improve an earlier version of this work.

AUTHOR CONTRIBUTIONS.—NZ and JA conceived the ideas and designed the study; NZ and BD carried out the sampling; NZ, CA, CS, LJ and JA analysed the data; NZ, CA and JA took the lead in writing the manuscript. All authors provided critical feedback to the drafts and gave final approval for publication.

REFERENCES

Acquarone, C., Cucco, M., Cauli, S.L. & Malacarone, G. (2002). Effects of food abundance and predictability on body condition and health parameters: experimental tests with the Hooded Crow. *Ibis*, 144: 155-163.

Alonso-Álvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B. & Sorci, G. (2004). An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am. Nat*., 164: 651-659.

Alonso-Álvarez, C., Pérez-Rodríguez, L., Mateo, R., Chastel, O. & Viñuela, J. (2008). The oxidation handicap hypothesis and the carotenoid allocation trade-off. *J. Evol. Biol.,* 21: 1789-1797.

Annett, C.A. & Pierotti, R. (1999). Long-Term Reproductive Output in Western Gulls: Consequences of Alternate Tactics in Diet Choice. *Ecology*, 80: 288-297.

Anthony, J.A., Roby, D.D. & Turco, K.R. (2000). Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *J. Exp. Mar. Biol. Ecol*., 248: 53-78.

Arizaga, J., Galarza, A., Herrero, A., Hidalgo, J. & Aldalur, A. (2009). Distribución y tamaño de la población de la gaviota patiamarilla *Larus michahellis lusitanius* en el País Vasco: tres décadas de estudio. *Revista Catalana d'Ornitologia*, 25: 32-42.

Arizaga, J., Jover, L., Aldalur, A., Cuadrado, J.F., Herrero, A. & Sanpera, C. (2013). Trophic ecology of a resident Yellow-legged Gull (*Larus michahellis*) population in the Bay of Biscay. *Mar. Environ. Res*., 87-88: 19-25.

Arizaga, J., Laso, M., Zorrozua, N., Delgado, S., Aldalur, A. & Herrero, A. (2017). Uso del espacio por adultos de gaviota patiamarilla *Larus michahellis* Naumann, 1840 durante el periodo reproductor: resultados preliminares en relación al uso de vertederos. *Munibe*, 65: 67-80.

Blévin, P., Tartu, S., Angelier, F., Leclaire, S., Bustnes, J.O., Moe, B., Herzke, D., Gabrielsen, G.W. & Chastel, O. (2014). Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (*Rissa tridactyla*). *Sci. Total Environ*., 470-471: 248-254.

Brush, A.H. (1990). Metabolism of carotenoid pigments in birds. FASEB J., 4: 2969-2977.

Bustnes, J.O., Erikstad, K.E., Lorentsen, S.H. & Herzke, D. (2008). Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. *Environ. Pollut.,* 156: 417-424.

Cantarero, A. & Alonso-Álvarez, C. (2017). Mitochondria-targeted molecules determine the redness of the zebra finch bill. *Biol. Lett.*, 13: 20170405.

Cherel, Y. & Hobson, K.A. (2007). Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar. Ecol. Prog. Ser.*, 329: 281-287.

Clarke, A. & Prince, P.A. (1980). Chemical composition and calorific value of food fed to Mollymauk chicks Diomedea melanophris and D. Chrysostoma at Bird island, South Georgia. *Ibis*, 122: 488-494.

Clifford, L.D. & Anderson, D.J. (2001). Food limitation explains most clutch size variation in the Nazca booby. *J. Animal Ecol.,* 70: 539-545.

Costantini, D. & Møller, A.P. (2008). Carotenoids are minor antioxidants for birds. *Funct. Ecol.*, 22: 367-370.

Costantini, D., Metcalfe, N.B. & Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecol. Lett.,* 13: 1435-1447.

Cramp, S. & Simmons, K.E.L. (1983). *The birds of the Western Palearctic*, *vol 3*. Oxford University Press, Oxford.

Dauwe, T. & Eens, M. (2008). Melanin- and carotenoid-dependent signals of great tits (*Parus major*) relate differently to metal pollution. *Naturwissenschaften,* 95: 969-973.

de Carvalho, C.C.C.R. & Caramujo, M.J. (2017). Carotenoids in Aquatic Ecosystems and Aquaculture: A Colorful Business with Implications for Human Health. *Front. Mar. Sci.,* 4: 93.

Duhem, C., Vidal, E., Roche, P. & Legrand, J. (2005). How Is the Diet of Yellow-legged Gull Chicks Influenced by Parents' Accessibility to Landfills? *Waterbirds*, 28: 46-52.

Eldridge, J.L. & Krapu, G.L. (1988). The influence of diet quality on clutch size and laying pattern in Mallards. *Auk*, 105: 102-110.

Endler, J.A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, 34: 76-91.

Faivre, B., Grégoire, A., Préault, M., Cézilly, F. & Sorci, G. (2003). Immune activation rapidly mirrored in a secondary sexual trait. *Science,* 300: 103.

Forero, M.G., Hobson, K.A., Bortolotti, G.R., Donázar, J.A., Bertellotti, M. & Blanco, G. (2014). Food resource utilisation by the Magellanic penguin evaluated through stable-isotope analysis: segregation by sex and age and influence on offspring quality. *Mar. Ecol. Prog. Ser.*, 234: 289-299.

García-de Blas, E., Mateo, R. & Alonso-Álvarez, C. (2016). Specific carotenoid pigments in the diet and a bit of oxidative stress in the recipe for producing red carotenoid-based signals. PeerJ, 4: e2237.

Gilbert, N.I., Correia, R.A., Silva, J.P., Pacheco, C., Catry, I., Atkinson, P.W., Gill, J.A. & Franco, A.M.A. (2016). Are white storks addicted to junk food? Impacts of landfill use on the movement and behaviour of resident white storks (*Ciconia ciconia*) from a partially migratory population. *Mov. Ecol.*, 4: 1-13.

Giraudeau, M., Mateos-González, F., Cotín, J., Pagani-Nuñez, E., Torné-Noguera, A. & Senar, J.C. (2015). Metal exposure influences the melanin and carotenoid-based colorations in great tits. *Sci. Total Environ.*, 532: 512-516.

Goodwin, T.W. (1984). *The Biochemistry of the Carotenoids: Animal, Vol. II*. London: Chapman & Hall.

Goutte, A., Barbraud, C., Meillère, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Delord, K., Cherel, Y., Weimerskirch, H. & Chastel, O. (2014). Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. *Proc. R. Soc. B*, 281: 20133313.

Grafen, A. (1990). Biological signals as handicaps. *J. Theor. Biol.,* 144: 517-546.

Grether, G.F., Hudon, J. & Millie, D.F. (1999). Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc. R. Soc. Lond. B*, 266: 1317-1322.

Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G. (1998). A DNA test to sex most birds. *Mol. Ecol.*, 7: 1071-1075.

Hammouda, A., Hamza, F., Ayadi, T., Pearce-Duvet, J. & Selmi, S. (2016). Assortative mating for carotenoid colouration but not size in the Yellow-legged Gull *Larus michahellis*. *Bird Study*, 63: 289-292.

Harris, M.P. (1964). Aspects of the breeding biology of the gulls *Larus argentatus, L. fuscus* and *L. marinus*. *Ibis*, 106: 432-456.

Hartley, R.C. & Kennedy, M.W. (2004). Are carotenoids a red herring in sexual display? *Trends Ecol. Evol.*, 19: 353-354.

Hill, G.E. (2006). Female mate choice for ornamental coloration. In, G.E. Hill & K.J. McGraw (eds.): *Bird coloration. Function and evolution*, pp. 137-200. Harvard University Press, Cambridge.

Hill, G.E. (1990). Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Animal Behaviour*, 40: 563-572.

Hill, G.E. & Johnson, J.D. (2012). The Vitamin A-Redox Hypothesis: A Biochemical Basis for Honest Signaling via Carotenoid Pigmentation. *Am. Nat.,* 180: E127-E150.

Hobson, K.A., Piatt, J.F. & Pitocchelli, J. (1994). Using stable isotopes to determine seabird trophic relationships. *J. Anim. Ecol.,* 63: 786-798.

Irons, D.B. (1998). Foraging area fidelity of individual seabirds in relation to tidal cycles and flock feeding. *Ecology*, 79: 647-655.

Johnson, J.D. & Hill, G.E. (2013). Is carotenoid ornamentation linked to the inner mitochondria membrane potential? A hypothesis for the maintenance of signal honesty. *Biochimie*, 95: 436-444.

Koch, R.E. & Hill, G.E. (2018). Do carotenoid-based ornaments entail resource trade-offs? An evaluation of theory and data. *Funct. Ecol.*, 32: 1908-1920.

Kodric-Brown, A. (1985). Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.*, 17: 199-205.

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: 6-12.

Laursen, K., Asferg, K.S., Frikke, J. & Sunde, P. (2009). Mussel fishery affects diet and reduces body condition of Eiders Somateria mollissima in the Wadden Sea. *J. Sea Res.*, 62: 22-30.

Le Galliard, J.-F., Ferrière, R. & Clobert, J. (2005). Juvenile growth and survival under dietary restriction: are males and females equal? *Oikos*, 111: 368-376.

Lopes, R.J., Johnson, J.D., Toomey, M.B., Ferreira, M.S., Araujo, P.M., Melo-Ferreira, J., Andersson, L., Hill, G.E., Corbo, J.C. & Carneiro, M. (2016). Genetic Basis for Red Coloration in Birds. *Curr. Biol.*, 26: 1427-1434.

Losdat, S., Blount, J.D., Marri, V., Maronde, L., Richner, H. & Helfenstein, F. (2018). Effects of an early-life paraquat exposure on adult resistance to oxidative stress, plumage colour and sperm performance in a wild bird. *J. Anim. Ecol.,* 87: 1137-1148.

Lozano, G.A. (1994). Carotenoids, parasites, and sexual selection. *Oikos*, 70: 309-311.

Maoka, T. (2011). Carotenoids in Marine Animals. *Mar. Drugs*, 9: 278-293.

McGraw, K.J. (2006). Mechanics of carotenoid-based coloration. In, G.E. Hill & K.J. McGraw (eds.): *Bird coloration: mechanisms and measurements*. Cambridge, M.A.: Harvard University Press.

Michael, N.P., Torres, R., Welch, A.J., Adams, J., Bonillas-Monge, M.E., Felis, J., López-Márquez, L., Martínez-Flores, A. & Wiley, A.E. (2018). Carotenoid-based skin ornaments reflect foraging propensity in a seabird, *Sula leucogaster*. *Biol. Lett.*, 14: 20180398.

Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N. & Surai, P.F. (2000). Carotenoid-dependent signals: Indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult. Biol. Rev.*, 11: 137-159.

Morales, J., Alonso-Álvarez, C., Pérez, C., Torres, R., Serafino, E. & Velando, A. (2009). Families on the spot: sexual signals influence parent-offspring interactions. *Proc. R. Soc. Lond. B*, 276: 2477-2483.

Moreno, R., Jover, L., Munilla, I., Velando, A. & Sanpera, C. (2010). A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Mar. Biol.*, 157: 545-553.

Olsen, K.M. & Larson, H. (2004). *Gulls of Europe, Asia and North America*. Christopher Helm. London.

Olson, V.A. & Owens, I.P.F. (1998). Costly sexual signals: Are carotenoids rare, risky or required? *Trends Ecol. Evol.*, 13: 510-514.

Oro, D. & Furness, R.W. (2002). Influence of food availability and predation on survival of kittiwakes. *Ecology*, 83: 2516-2528.

Oro, D., Genovart, M., Tavecchia, G., Fowler, M.S. & Martínez-Abraín, A. (2013). Ecological and evolutionary implications of food subsidies from humans. *Ecol. Lett.*, 16: 1501-1514.

Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N. & Nielsen, S. (2006). Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. *Ardea*, 94: 241-255.

Pérez, C., Lores, M. & Velando, A. (2008). Availability of nonpigmentary antioxidant affects red coloration in gulls. *Behav. Ecol.*, 19: 967-973.

Pérez, C., Lores, M. & Velando, A. (2010). Oil pollution increases plasma antioxidants but reduces coloration in a seabird. *Oecologia*, 163: 875-884.

Pérez-Rodríguez, L. (2009). Carotenoids in evolutionary ecology: re-evaluating the antioxidant role. *BioEssays*, 31: 1116-1126.

Pierotti, R. & Bellrose, C.A. (1986). Proximate and ultimate causation of egg size and the "third-chick disadvantage" in the western gull. *Auk*, 103: 401-407.

Pierotti, R. & Annett, C.A. (1987). Reproductive consequences of specialization and switching in an ecological generalist. In, A.C. Kamil, J.R. Krebs & H.R. Pulliam (eds.): *Foraging behaviour*, pp. 417-442. Plenum Press, New York,

Pierotti, R. & Annett, C.A. (1990). Diet and Reproductive Output in Seabirds. *BioScience*, 40: 568-574.

Poisbleau, M., Beaulieu, M., Dehnhard, N., Demongin, L., Lepoint, G., Sturaro, N. & Eens, M. (2017). Extreme intra-clutch egg size dimorphism is not coupled with corresponding differences in antioxidant capacity and stable isotopes between eggs. *Comp. Biochem. Physiol. A*, 205: 77-85.

Ramírez, F., Ramos, R., Carrasco, J.L., Sanpera, C., Jover, L. & Ruiz, X. (2011). Intra-clutch pattern of albumen $\delta^{13}C$ and $\delta^{15}N$ in yellow-legged gulls *Larus michahellis*: Female dietary shift or resource allocation strategy? *J. Avian Biol.*, 42: 239-246.

Ramos, R., Ramírez, F., Sanpera, C., Jover, L. & Ruiz, X. (2009). Diet of Yellow-legged Gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. *J. Ornithol.*, 150: 265-272.

Reynolds, S.J., Schoech, S.J. & Browman, R. (2003). Nutritional quality of prebreeding diet influences breeding performance of the Florida scrub-jay. *Oecologia*, 134: 308-316.

Romero-Haro, A.A. & Alonso-Álvarez, C. (2015). The level of an intracellular antioxidant during development determines the adult phenotype in a bird species: a potential organizer role for glutathione. *Am. Nat.*, 185: 390-405.

Saravanan, G., Yamuna, G. & Nandhini, S. (2016). *Real time implementation of RGB to HSV/HSI/HSL and its reverse color space models*. 2016 International Conference on Communication and Signal Processing (ICCSP). DOI: 10.1109/ICCSP.2016.7754179.

Schoeninger, M.J. & DeNiro, M.J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim. Cosmochin. Acta*, 48: 625-639.

Tinbergen, N. (1948). Social Releasers and the Experimental Method Required for Their Study. *The Wilson Bulletin*, 60: 6-51.

Vallverdú-Coll, N., Mougeot, F., Ortiz-Santaliestra, M.E., Rodriguez-Estival, J., López-Antia, A. & Mateo, R. (2016). Lead exposure reduces carotenoid-based coloration and constitutive immunity in wild mallards. *Environ. Toxicol. Chem.*, 35: 1516-1525.

Vallverdú-Coll, N., Ortiz-Santaliestra, M.E., Mougeot, F., Vidal, D. & Mateo, R. (2015). Sublethal Pb exposure produces season-dependent effects on immune response, oxidative balance and investment in carotenoid-based coloration in red-legged partridges. *Environ. Sci. Technol.*, 49: 3839-3850.

Velando, A., Kim, S.Y. & Noguera, J.C. (2013). Begging response of gull chicks to the red spot on the parental bill. *Anim. Behav.*, 85: 1359-1366.

Velando, A., Beamonte-Barreiros, 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: 543-552.

Völker, O. (1957). Die experimentelle Rotfärbung des Gefieders beim Fichtenkreuzschnabel (*Loxia curvirostra*). *J. Ornithol*., 98: 210-214.

von Schantz, T.V., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. (1999). Good genes oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B,* 266: 1-12.

Weaver, R.J., Santos, E.S.A., Tucker, A.M., Wilson, A.E. & Hill, G.E. (2018). Carotenoid metabolism strengthens the link between feather coloration and individual quality. *Nat. Commun*., 9: 73.

Weber, H. (1961). Uber die Ursache des Verlustes der roten Federfarbe bei gekäfigten Birkenzeisigen. *J. Ornithol*., 102: 158-163.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H. & Scheuhammer, A.M. (2002). Ecotoxicology of mercury. In, Hoffman, D.J., Rattner, B.A., Burton, G.A. & Cairns, J. (eds.): *Handbook of Ecotoxicology*. CRC Press.

Zahavi, A. (1975). Mate selection-a selection for a handicap. *J. Theor. Biol*., 53: 205-214.

Zorrozua, N., Aldalur, A., Herrero, A., Diaz, B., Delgado, S., Sanpera, C., Jover, L. & Arizaga, J. (2020). Breeding Yellow-legged Gulls increase consumption of terrestrial prey after landfill closure. *Ibis*, 162: 50-62.

SUPPLEMENTARY ELECTRONIC MATERIAL

Additional supporting information may be found in the on-line version of this article. See the volume 67(2) on www.ardeola.org

Appendix 1. Table A1. Beta-parameter estimates (± SE; below: P-values) of the full models predicting different dependent variables.

Submitted: July 23, 2020
Major revisión: September 17, 2019
Second version arrived: December 20, 2019
Minor revisión: January 23, 2020
Third version arrived: February 04, 2020
Accepted: February 11, 2020

Editor: Alberto Velando

© Juan Varela