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Breeding Yellow-legged Gulls increase consumption of terrestrial prey after landfill closure

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The ecology of opportunistic foragers can be highly dependent on anthropogenic food sources, such as landfills, resulting in changes in several ecological and demographic aspects. The total closure of several landfill sites and the use of deterrence systems to prevent access to the remaining open landfill sites in a region in the northern Iberian Peninsula provided an excellent opportunity to evaluate the consequences of landfills on the trophic ecology of an opportunistic forager, the Yellow-legged Gull *Larus michahellis*, using these resources. Based on stable isotope analyses, we used mixing models to estimate changes in diet before and after the closure of the majority of landfills in the breeding and the non-breeding season. We found a decrease in the consumption of food from landfills after their closure, which was accompanied by an increase in feeding on terrestrial prey (mostly earthworms), but only in the breeding season. Interestingly, we observed no increase in marine prey consumption after the landfill closures. In winter there was a decrease in terrestrial prey consumption, whereas the consumption of marine and, despite their reduce availability, landfill resources increased. Thus, and unlike when all the landfills were open, we detected significant trophic differences between breeding and non-breeding seasons. Additionally, we found significant trophic differences among colonies that were quite close to each other, but not between breeding adults and chicks. In conclusion, landfill closure or the use of deterrence systems had a direct impact on the trophic ecology of Yellow-legged Gulls; loss of refuse was mainly compensated for by prey of terrestrial origin when breeding, but not in winter. Thus, this species may experience foraging constraints in winter with potential effects on other life-history aspects including their dispersal, breeding and survival that needs further evaluation.

Keywords: change in food availability, diet, fish prey, generalist forager, refuse tips, seabird, stable isotope mixing model.

The exploitation of anthropogenic feeding sources is one of the main factors currently driving the ecology and population dynamics of several opportunistic species worldwide (Garrott *et al.* 1993, Oro *et al.* 1995, 2013, Rock 2005, Neves *et al.* 2006, Duhem *et al.* 2008, Bicknell *et al.* 2013, Real *et al.* 2017, Arizaga *et al.* 2018). Behavioural changes to take advantage of such resources tend to be rapid and the effects are seen in a very short time at both the individual and the population level, including important trophic changes, reduced dispersal distances and improvements in survival and breeding parameters (Belant *et al.* 1993, Bosch *et al.* 1994, Oro *et al.* 1995, Sol *et al.* 1995, Bukacínska *et al.* 1996, Oro *et al.* 2004, but see Pierotti & Annett 1991 and Annett & Pierotti

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1999). This is particularly true when the foraging sites are stable and predictable over time (Oro *et al.* 2013, Real *et al.* 2017). Increased protection of birds since the 20th century (Coulson 1963, Coulson & Coulson 2009) and increased availability of anthropogenic food resources, such as landfills, has supported the population growth of several avian species, including gulls, herons and storks (Auman *et al.* 2008, Arizaga *et al.* 2009, Coulson & Coulson 2009, but see Coulson 2015). European laws, and Directive 1999/31/UE in particular, oblige all Member States to close all open-air landfills by 2020, thus exposing opportunistic species using these sites for foraging to a scenario of restricted access to food in the near future.

Feeding at landfills by gulls is well known, although its frequency may vary between gull species, as well as between colonies and age classes. For instance, estimates of the use of landfills by foraging Yellow-legged Gulls *Larus michahellis* range from 0% of foraging at landfills (e.g. on offshore islets situated very far from any landfill, Ramos *et al.* 2009a) to > 80% (e.g. in south-eastern France, Duhem *et al.* 2005). Closure of landfills could be even more critical to gulls if policies aimed at banning fishing discards, another important source of food (Oro *et al.* 1995, 1996, Ramos *et al.* 2009a, Arizaga *et al.* 2010a, 2013a), are implemented (Calado *et al.* 2017). Understanding how populations deal with curtailments of foraging opportunities, and what alternative food sources could be used (if available), is of crucial importance in ascertaining how flexibly these species can respond to environmental change, and to what extent they are able to overcome rapid and dramatic changes in current food availability.

The most common gull of the southwestern Palaearctic is the Yellow-legged Gull. Their numbers increased rapidly in the study region from the 1980s to 2000 (Olsen & Larson 2004) and the overall population continues to increase in number (BirdLife International 2017). The diet of the species has been reported to depend heavily on landfills across practically all of its distribution (Duhem *et al.* 2005, Neves *et al.* 2006, Moreno *et al.* 2010, Arizaga *et al.* 2013a, but see Ramos *et al.* 2009a), especially in those colonies close to large urban areas. The Yellow-legged Gull population along the Basque coast was *c.* 4500 breeding pairs in 2007 and the population grew *c.* 150% between 1980 and 2000 (Arizaga *et al.* 2009). In 2007–2009, approximately 40% of its diet (by biomass) came from landfills (Arizaga *et al.* 2013a). However, since 2008, local administrations have started closing landfills, or taking measures to deter gulls from feeding on them. This change in landfill availability, and considering that the local year-round resident gull population is highly dependent on local foraging sites (Arizaga *et al.* 2010b, 2013b, Egunez *et al.* 2018), provides an opportunity to examine the effect of landfill closures on the species' trophic ecology throughout the whole year.

It has been reported that adults consume more refuse themselves than they feed to their chicks, which are often fed with a higher proportion of marine prey, mainly fish discards (Moreno *et al.* 2010). This is attributed to the fact that fish is of higher value in terms of both energy and nutrients during the growth period of the chicks (Annett & Pierotti 1999). Previous studies carried out in the region have revealed a trophic overlap between age-classes (Arizaga *et al.* 2013a), and we would not expect deviations from this pattern unless chicks began to be fed with a different proportion of marine/terrestrial prey as compared with adults once landfills were unavailable. Trophic differences between the sexes have not yet been analysed in our study population and only rarely in other studies on gulls (e.g. Pons 1994, Washburn *et al.* 2013). The diet of gulls and their use of landfill resources may also vary between seasons (Ramos *et al.* 2011, Ceia *et al.* 2014). In some Mediterranean colonies, consumption of marine prey is known to increase during the winter, which is associated with a decrease in landfill discharges linked to the reduced number of tourists during these months (Ramos *et al.* 2011). When landfill sites were still open, no seasonal variation in the diet was found in our study population (Arizaga *et al.* 2013a) but this might have changed after landfill closure, depending on the availability of alternative feeding sources in the different seasons. Recent analyses of movement patterns of Yellow-legged Gull within the Basque region have revealed that the use of landfills and marine prey is strongly dependent on the distance from the colony to landfill sites (Egunez *et al.* 2018) and fishing harbours (Arizaga *et al.* 2013a), respectively. Thus, trophic variation between colonies at a very local scale would also be expected, depending on the closeness of the colony to major food sources, such as harbours or landfills.

The diet of gulls can be assessed by stable isotopes of inert body tissues that can reconstruct trophic categories individual birds consumed during the entire period over which the tissues were growing (Hobson *et al.* 1994). Measured in feathers, stable isotopes allow diet to be estimated over the time it has taken the feather to develop. δ^{15}N can be used to estimate trophic position, whereas δ^{13}C can provide information on the foraging habitat (Schoeninger & DeNiro 1984, Hobson *et al.* 1994, Forero & Hobson 2003, Cherel & Hobson 2007). Here, using stable isotopes (δ^{13}C and δ^{15}N) of feathers, the main aims of this work were: (1) to examine potential differences in the trophic ecology of a resident Yellow-legged Gull population (three colonies situated within *c.* 20 km that host a total population of *c.* 500 breeding pairs; Arizaga *et al.* 2009) between a period of high availability of food from landfills (2007–2009), and one of low availability (2014–2016); and (2) to identify alternative food resources consumed by gulls within the region, and any potential new conflicts with human activities. We expected a decrease in the consumption of food originating from landfills during 2014–2016, which should be compensated for by an increase in other alternative food resources (of either terrestrial or marine origin). A specific aim was to explore variation in diet in relation to age, sex, season and colony.

METHODS

Study area and data collection

The study was carried out in three Yellow-legged Gull colonies in Gipuzkoa (northern Iberian Peninsula): Getaria (43°18′N, 02°12′W), Santa Clara (43°19′N, 01°59′W) and Ulia (43°20′N, 01°57′W; Fig. 1). All three colonies are situated on cliffs with similar characteristics; in 2007 these colony sites hosted 92, 85 and 526 breeding pairs in Getaria, Santa Clara and Ulia, respectively (Arizaga *et al.* 2009). The population trends at Getaria, which is situated near one of the most active fishing harbours in Gipuzkoa (Fig. 1), and Santa Clara are not known; the third colony appears to be decreasing (trends obtained for 2000–2013; Juez *et al.* 2015).

Within a 75-km radius of our study colonies, as most local Yellow-legged Gulls do not move beyond this distance during their whole life (Arizaga *et al.* 2013b, J. Arizaga unpubl. data), are five landfill sites (San Marcos, Urteta, Zaluaga, Sasieta and Jata, see Fig. 1) that dumped *c.* 286 292 tonnes of refuse per year (from 2007 until now/closure).

Throughout the breeding season of 2007–2009 and 2014–2016, both chicks and adults were captured in the colonies for the collection of feathers from the body (chicks) or wing (adults) for stable isotope analyses (δ^{13}C and δ^{15}N). Chicks were caught by hand at the age of *c.* 20 days. Four to six dorsal (mantle) feathers were taken and the chick body mass and tarsus length were also measured. To avoid pseudo-replication, we only considered one chick per nest. Adults were captured using spring traps while incubating. They were chosen at random in those sites of the colony where trapping was possible. We aimed to sample the inner, first primary feather (P1) (which grows just after breeding) and the secondary S6 (grown in autumn–winter; Olsen & Larson 2004), their isotopic values reflecting the diet during the preceding reproductive period and during the previous non-breeding period, respectively. In cases where the gulls had already moulted one of these feathers (usually P1), the next non-moulted feather was taken (*c.* 80% of the cases). The primary feather was collected close in time to when moulted naturally, and the gap produced by removing the secondary feather in the inner part of the feather is small, so that the impact on the individual's flight ability was likely to be minimal. Chicks were sampled in all the breeding seasons for the entire period (years 2007–2009 and 2014–2016), but adults were only sampled in 2008–2010 in Ulia and 2016 in all three colonies (Table S1). Adults were sexed using small remains of dried blood in the calamus of collected primary feathers by analysing that DNA (Griffiths *et al.* 1998) at the University of Navarra.

Stable isotope analysis and mixing models

Stable isotopes allow the occurrence of groups of prey at the main trophic levels to be estimated (prey of marine origin, terrestrial, etc.), and their level of accuracy is sufficient for most research (Forero & Hobson 2003, Sanpera *et al.* 2007, Moreno *et al.* 2010, Ceia *et al.* 2014). Mixing models and models of niche width have been very useful in studies of trophic ecology (Bearhop *et al.* 2004,

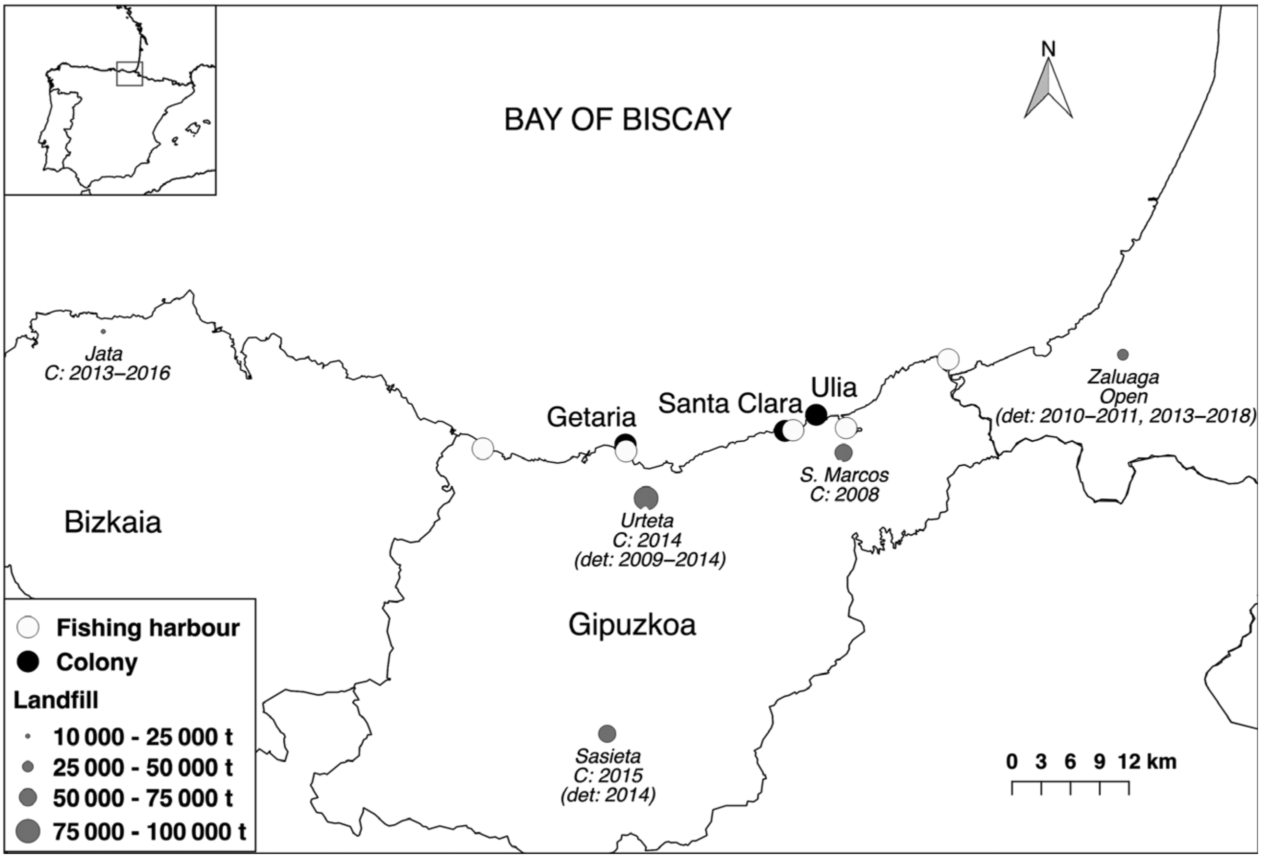

Figure 1. Location of the Yellow-legged Gull breeding colonies in Gipuzkoa, northern Iberian Peninsula, where the study was carried out. Fishing harbours are also indicated, along with the landfill sites in the area and, where relevant, their year of closure (C), the years when deterrence measures (det) were used, which included falconry, pyrotechnics and distress calls, and mean discharge (shown by size of circle, see key).

Navarro *et al.* 2009, Ramos *et al.* 2009b, Arizaga *et al.* 2013a), as long as reference prey are adequately identified.

Based on previous δ^{13}C, δ^{15}N and δ^{34}S values for the period 2007–2009 for the three colonies (Arizaga *et al.* 2013a), we looked at the reliability of using only two isotopes (δ^{13}C and δ^{15}N) to estimate prey consumption. These isotopes can distinguish between three different resources: landfills, terrestrial and marine (Table 1). We found that the 95% credible intervals of results using two isotopes (δ^{13}C and δ^{15}N) considerably overlapped with the results using all three isotopes (Fig. S1). Consequently, to minimize costs we ran the analyses using only δ^{13}C and δ^{15}N. The methodology for the stable isotope analyses used in the present work was the same as that in Arizaga *et al.* (2013a) in order to obtain comparable results. Feathers were washed in a solution of 1 M NaOH, dried (60 °C) and then homogenized into fine powder using an impactor mill (Freezer/mill 6750-Spex, Certiprep) operating at liquid nitrogen temperature. Weighed sub-samples of the powdered feathers (*c.* 0.3 mg for δ^{13}C and δ^{15}N) were placed in tin capsules and isotopic analysis was carried out by elemental analysis-isotope ratio mass spectrometry using a ThermoFinnigan Flash 1112 coupled to a Delta isotope ratio mass spectrometer via ConFlo III interface. Analyses were carried out by the Centres Científics i Tècnics (CCiT) at the University of Barcelona. Stable isotope signatures were reported in conventional δ notation (‰) relative to Peedee Belemnite for δ^{13}C and atmospheric nitrogen for δ^{15}N. The δ^{13}C standard was Vienna PeeDee Belemnite (VPDB) calcium carbonate, and the δ^{15}N standard was atmospheric nitrogen (N_2). International standards (ammonium sulphate, potassium nitrate and glutamic acid for δ^{15}N and polyethylene, sucrose and glutamic acid for $\delta^{13}C$) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy was $\leq 0.1‰$ for $\delta^{13}C$ and $\leq 0.3‰$ for $\delta^{15}N$.

Statistical analyses

To test for possible differences between the two periods (2007–2009 vs. 2014–2016) we focused on chicks, as these were sampled in all colonies and years. To this end, we used general linear mixed models (GLMMs) on $\delta^{13}C$ and $\delta^{15}N$, with colony and period as fixed factors; year was considered as a random factor in order to control for annual variations in diet (Arizaga *et al.* 2013a, Ceia *et al.* 2014).

To compare the diet of adults and chicks across periods we focused on data from Ulia. In this case we only used P1 feathers, which grow in summer, coinciding with the chick-rearing period, meaning that the data from adult primary feathers are comparable with those from chick feathers collected the previous year. Here we also used GLMMs with $\delta^{13}C$ or $\delta^{15}N$ as response variables, period and age as fixed factors, and year as a random factor. For this analysis, potential differences in the assimilation of diet in chicks and adults were not taken into account.

To test for possible seasonal variations in the trophic ecology we focused on adults (P1 and S6 feathers). We ran GLMMs on $\delta^{13}C$ or $\delta^{15}N$ as response variables, including period (2007–2009 and 2014–2016) and feather type (P1 or S6) as fixed factors, and year and individual as random factors (as P1 and S6 were taken from the same individuals). Finally, we also tested for differences in $\delta^{13}C$ and $\delta^{15}N$ between the sexes, using adult samples taken in 2016 in a GLMM with the feather type (P1 and S6), colony and sex as fixed factors, and individual as a random factor. All statistical analyses were carried out in R 3.3.2 (R Development Core Team 2011) and normality of distribution was checked for isotopic values.

To assess the relative contribution of each resource category to the assimilated diet, we used a Bayesian multi-source stable isotope mixing model (package 'siar' in R, Parnell *et al.* 2010) with default values (chain length = 200 000; burning = 50 000; thinning = 15, dirichlet distribution prior = 1/3). To obtain comparable results, we used the isotopic values reported by Arizaga *et al.* (2013a) for the same food resource categories (landfill, marine, terrestrial; Table 1) collected in the same area. Using the isotopic values of $\delta^{15}N$ and $\delta^{13}C$, in addition to obtaining the relative importance of each prey category for 2014–2016, a diet reconstruction for 2007–2009 was made, as the work by Arizaga *et al.* (2013a) also included the isotopic values of $\delta^{34}S$. The results are presented as 95, 75 and 50% credible intervals for the three types of food sources considered.

To compare trophic niche widths between periods (2007–2009 vs. 2014–2016), between seasons (summer vs. winter) and between age classes (chicks vs. adults), SIBER analyses were performed on the isotopic values (Jackson *et al.* 2011). We calculated the standard ellipse area with correction for small sample sizes (SEAc) to represent the trophic niche width for each colony (the larger the area of the ellipse, the wider the trophic niche) and their overlap between periods (for chick feathers), seasons (for 2016 adult feathers) and age classes (for adult P1 and chick mantle feathers from Ulia). With the SIBER package (Jackson *et al.* 2011) the relative overlap was estimated between two ellipses. Lack of overlap between the ellipses suggests that trophic niche differs, but the occurrence of overlap may not necessarily indicate that feeding sources are shared (Gallagher *et al.* 2017).

Table 1. Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ (‰) (mean ± 95% confidence interval) of different prey categories obtained from regurgitates of chicks (Arizaga *et al.* 2013a). Isotopic discrimination values for $\delta^{15}N$ and $\delta^{13}C$ were, respectively: landfill + 5.0 and + 2.2‰; terrestrial + 4.0 and + 2.7‰; marine + 3.0 and + 0.9‰ (Peterson *et al.* 1985, Hobson & Clark 1992, Bearhop *et al.* 2002, Hobson & Bairlein 2003, Ramos *et al.* 2009a), with sd = 0.1.

Prey category	*n*	$\delta^{13}C$	$\delta^{15}N$
Terrestrial[a]	5	−23.84 ± 2.90	8.86 ± 2.82
Landfill[b]	3	−21.12 ± 1.17	4.35 ± 1.88
Marine fish[c]	8	−18.04 ± 0.65	11.14 ± 1.87

[a] Includes annelids (earthworms) and molluscs (family Arionidae). [b] Pork, beef or chicken. [c] Includes fish prey from the families Carangidae, Clupeidae (pelagic), Sparidae, Trachinidae and Gadidae (benthic).

RESULTS

The trophic ecology of chicks showed significant changes between the periods 2007–2009 and

2014–2016 (Figs 2 & 3). Models considering period-by-colony interactions revealed that this interaction was not significant (δ^{13}C: colony-by-period, $F_{2,258} = 1.34$, $P = 0.265$; δ^{15}N: colony-by-period, $F_{2,258} = 1.38$, $P = 0.254$). The diets of chicks during the earlier years, when adults could access landfills, were enriched in δ^{13}C, and both isotopic signatures also differed between colonies (δ^{13}C: colony, $F_{2,260} = 10.31$, $P < 0.001$; period, $F_{1,4} = 11.53$, $P = 0.027$; δ^{15}N: colony, $F_{2,260} = 7.03$, $P = 0.001$; period, $F_{1,4} = 0.24$, $P = 0.653$; Fig. 2). *Post hoc* Tukey tests indicated that there was a significant difference between Santa Clara and the other two colonies for both δ^{13}C (Santa Clara – Getaria, $P < 0.001$; Santa Clara – Ulia, $P < 0.001$) and δ^{15}N (Santa Clara – Getaria, $P = 0.002$; Santa Clara – Ulia, $P = 0.007$). In Fig. 3, from 'siar' results it can be seen that consumption of landfill resources dropped from *c.* 40% to 20–30% from before to after landfill closures. In contrast, the consumption of terrestrial prey increased substantially when the landfill site was closed in all three colonies, from a mean of < 20% to *c.* 40% (Fig. 3).

When comparing δ^{15}N and δ^{13}C values from feathers of chicks and adults (P1) from Ulia, we observed no significant differences between age classes (δ^{13}C: period, $F_{1,4} = 12.93$, $P = 0.023$; age, $F_{1,171} = 0.07$, $P = 0.790$; δ^{15}N: period, $F_{1,4} = 0.03$, $P = 0.872$; age, $F_{1,171} = 0.07$, $P = 0.797$; Fig. 4), indicating that the two age classes had a similar trophic ecology, at least during the breeding season. Interactions between age and period were not significant (δ^{13}C: period-by-age, $F_{1,170} = 0.35$, $P = 0.553$; δ^{15}N: period-by-age, $F_{1,170} = 0.02$, $P = 0.894$).

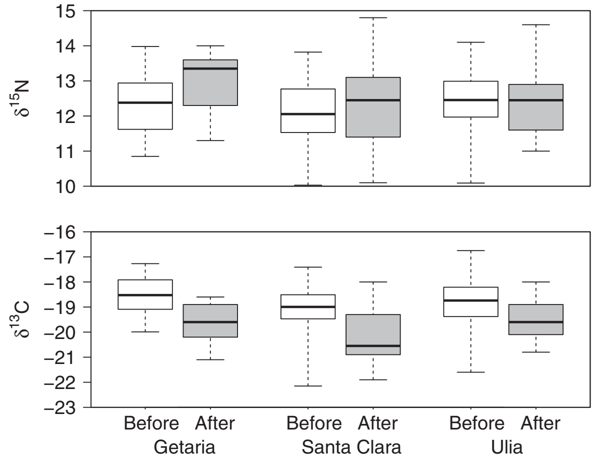

Figure 2. Isotopic signatures of δ^{13}C and δ^{15}N (‰) of chick mantle feathers in the colonies of Getaria, Santa Clara and Ulia, before (unfilled boxes) and after (grey-shaded boxes) the closure of the neighbouring landfills. Median, first and third quantile are represented and the whiskers extend to the most extreme data point.

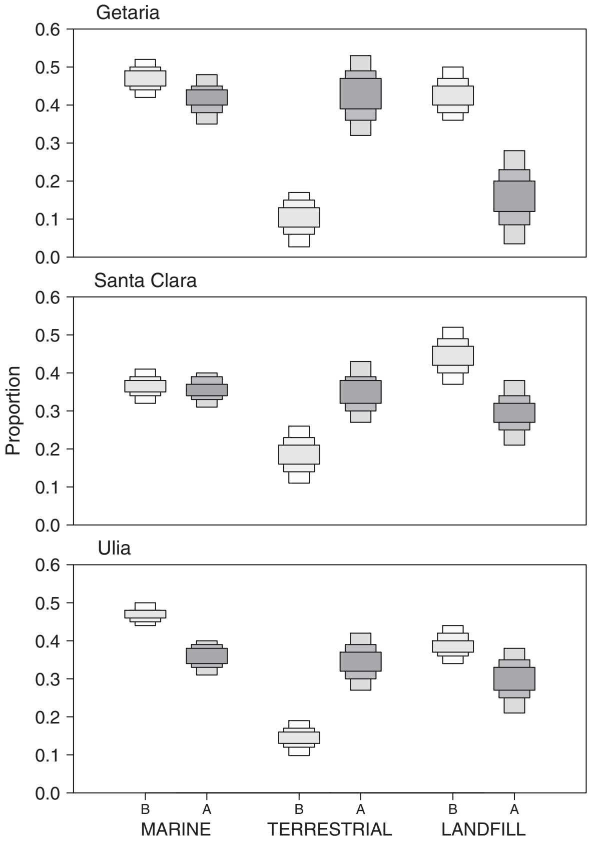

Figure 3. The 'siar' results (± 95, 75 and 50% credible intervals) showing source contribution to chick diet before (B, 2007–2009) and after (A, 2014–2016) landfill closure in Gipuzkoa. Results for each colony are considered separately.

Comparing feathers from adults (P1 and S6) from Ulia revealed a significant period-by-season interaction for δ^{13}C (period, $F_{1,2} = 2.11$, $P = 0.281$; season, $F_{1,49} = 7.03$, $P = 0.011$, period-by-season, $F_{1,49} = 6.38$, $P = 0.015$), but not for δ^{15}N (period, $F_{1,53} = 2.24$, $P = 0.140$; season, $F_{1,47} = 0.09$, $P = 0.770$, period-by-season, $F_{1,47} = 1.91$, $P = 0.173$; Fig. 4). Separate models for seasons revealed a significant effect of period on δ^{13}C only during the breeding season (breeding time: period, $F_{1,44} = 8.89$, $P = 0.005$; winter time: period, $F_{1,54} = 0.01$, $P = 0.946$).

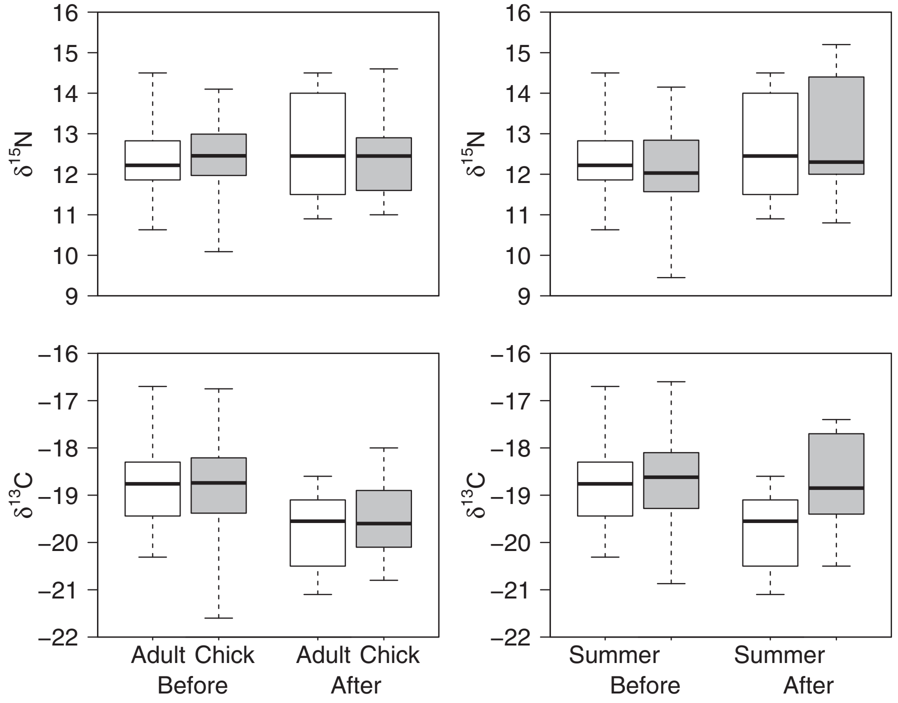

Figure 4. Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ (‰) of adult feathers collected in Ulia, before and after closure of the landfills. Median, first and third quantiles are represented and the whiskers extend to the most extreme data point.

In 2016, we observed significant differences in $\delta^{13}C$ between seasons, but not between sexes ($\delta^{13}C$: season, $F_{1,31} = 24.06$, $P < 0.001$, sex, $F_{1,28} = 0.05$, $P = 0.826$; colony, $F_{2,28} = 0.79$, $P = 0.466$; $\delta^{15}N$: season, $F_{1,31} = 0.11$, $P = 0.747$, sex, $F_{1,28} = 0.01$, $P = 0.922$; colony, $F_{2,28} = 0.25$, $P = 0.779$). No significant interactions were detected between any of the factors ($\delta^{13}C$: colony-by-season, $F_{2,26} = 0.17$, $P = 0.843$; colony-by-sex, $F_{2,26} = 0.62$, $P = 0.545$; season-by-sex, $F_{1,26} = 0.64$, $P = 0.430$; $\delta^{15}N$: colony-by-season, $F_{2,26} = 0.33$, $P = 0.724$; colony-by-sex, $F_{2,26} = 0.28$, $P = 0.760$; season-by-sex, $F_{1,26} = 0.02$, $P = 0.904$). Interestingly, mixing models (Fig. 5) showed diet to have a higher proportion of terrestrial prey in summer (summer: *c.* 40%; winter: <10%), whereas marine prey made the largest contribution in winter (from *c.* 40% to *c.* 60%), when there was also a slightly higher consumption of landfill resources (from *c.* 20% to *c.* 40%).

In all three colonies, isotopic niches of chicks showed < 0.001% overlap between the period before and after landfill closures, and trophic niche values were lower before than after landfill closures (Table 2). In Ulia in 2015, the value of isotopic niche width of chicks was higher than for adults, but the two overlapped for more than 50% of the area (Table 2). In 2016, adults had a higher value of isotopic niche width in winter than in summer in two of the three colonies, and isotopic niche overlap between seasons was < 25% (Table 2).

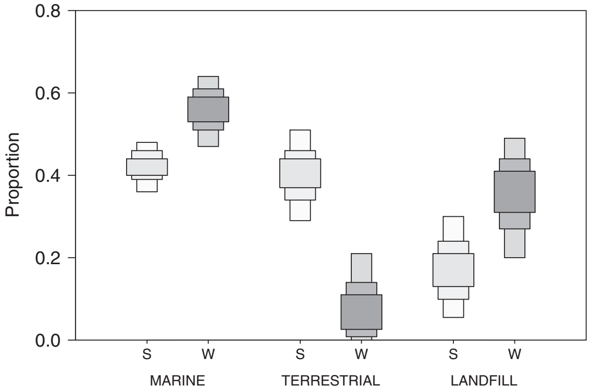

Figure 5. The 'siar' results (± 95, 75 and 50% credible intervals) showing source contribution to adult diet in summer (S, light grey) and winter (W, dark grey) in 2016 in the colonies of Getaria, Santa Clara and Ulia.

Table 2. SEAc overlap area between chicks sampled in different periods (2007–2009 and 2014–2016) and between adults sampled in 2016 in different seasons (winter and summer), differentiated by colony. Area of each SEAc is also indicated (Area 1 refers to the first component of each comparison and Area 2 to the second).

	Overlap SEAc Area 1 – Area 2 (% of Area 1)	Overlap SEAc Area 2 – Area 1 (% of Area 2)	Area 1	Area 2
Chicks				
Getaria 2007–2009 – Getaria 2014–2016	< 0.01 (< 0.001%)	< 0.01 (< 0.001%)	0.53	0.71
Santa Clara 2007–2009 – Santa Clara 2014–2016	< 0.01 (< 0.001%)	< 0.01 (< 0.001%)	0.81	1.45
Ulia 2007–2009 – Ulia 2014–2016	< 0.01 (< 0.001%)	< 0.01 (< 0.001%)	0.56	0.54
Adults				
Getaria 2016 winter – Getaria 2016 summer	0.36 (10.28%)	0.36 (24.46%)	3.49	1.47
Santa Clara 2016 winter – Santa Clara 2016 summer	0.01 (0.25%)	0.01 (0.73%)	3.68	1.28
Ulia 2016 winter – Ulia 2016 summer	< 0.01 (< 0.001%)	< 0.01 (< 0.001%)	0.93	1.01
Ulia (2015 diet): Adults – Chicks	0.51 (50.62%)	0.51 (73.58%)	1.01	0.69

DISCUSSION

By using stable isotopes of δ^{15}N and δ^{13}C we detected significant differences in the Yellow-legged Gull resource use between the periods of high (2007–2009) and low (2014–2016) landfill-food availability associated to the closure of several open-air landfills within the region. In 2014–2016 we observed a reduction in the estimated consumption of food from landfills as compared with 2007–2009, confirming our hypothesis that the contribution of these sites to the diet has decreased. The use of two isotopes (δ^{15}N and δ^{13}C) instead of three (these two and δ^{34}S) as used in a previous analysis (Arizaga *et al.* 2013a) did not affect our results during the period 2007–2009, or also very likely in 2014–2016. This is because δ^{34}S can discriminate between different sources of marine prey (Moreno *et al.* 2010), but the post-landfill period did not seem to be characterized by a higher consumption of marine prey, but instead an increase in prey of terrestrial origin. Nonetheless, we must consider that sea spray can reduce δ^{34}S in terrestrial prey along coastal areas (Hobson *et al.* 2015), and hence its contribution to the diet may be underestimated. The discriminatory power of δ^{34}S is context-related, and for instance Moreno *et al.* (2010) found in northwestern Iberia that δ^{34}S made a difference in populations where different marine species contributed to their diet; however, δ^{34}S will not help to distinguish between different terrestrial sources. So overall it must be acknowledged that there is some degree of uncertainty in diet assessments, especially during the post-landfill period.

Landfill sites still seem to play a role in the trophic ecology of our study gulls, a fact confirmed by findings from GPS tracking of adults during the breeding season (Arizaga *et al.* 2017). Overall, our results suggest that a number of supposedly closed landfill sites are still providing gulls with a source of food, and/or that deterrence measures are not sufficient to prevent gulls from accessing the landfill. That said, we cannot reject the possibility that a part of such ‘landfill resources’ assessed by ‘siar’ models could in fact be accounted for by the gulls scavenging in built-up areas, as food items taken from such zones would probably show isotopic signatures similar to those found in food from landfills. However, preliminary data from GPS-tracked birds in the post-landfill period have shown only a marginal use of built-up areas and therefore landfills, so the sea and fields are the most likely foraging habitats in our study population (Arizaga *et al.* 2017, J. Arizaga unpubl. data). In particularly, *c.* 60% of the GPS-positions were in the colony, 12% in fields (which would fit with the ‘siar’ models suggesting a high consumption of earthworms), 6.5% in landfills, 4% in the sea and 18% in other habitat types (Arizaga *et al.* 2017). Over 90% of locations in ‘other habitats’ corresponded to roosting places (e.g. on industrial unit roofs, riverbanks known to be used to rest). Therefore, it is likely that food consumption in built-up areas, where similar food would be consumed as on landfills, was small, although some uncertainty remains and future research should determine with more accuracy the relative contribution of prey from different terrestrial resources on the population’s diet. The potential relevance of food taken from built-up areas must be here emphasized and compared with other habitats which include fields (as a source of earthworms, in particular during summer) or landfills, without excluding other habitat types that could remain still unidentified or that could be used more intensively in the near future.

The observed increase in the consumption of prey of terrestrial origin (mainly earthworms; N. Zorrozua pers. obs.) compensated for the decrease in food availability from landfill sites, whereas there was no apparent change in the consumption of marine resources. Payo-Payo *et al.* (2015) also detected a diet shift in gulls from Dragonera Island (Mediterranean) after landfill foraging sites became unavailable, although in that case there was a notable increase in the consumption of marine prey. Given the high nutritional quality of marine prey (Annett & Pierotti 1999), the lack of any increase in the use of such prey as an alternative foraging resource in our region is interesting, but the causes underlying this pattern remain unclear. Fish discards in harbours or at sea are still known to be common within the region (N. Zorrozua pers. obs.). Fishing harbours in the study region showed similar activities during 2007–2009 (with a mean ± sd annual amount of fish discharged of 31 794 ± 6167 t, source: Basque Government) and 2014–2016 (26 522 ± 970 t, source: Basque Government). So a reduction in the availability of marine food resources is an unlikely explanation for why gulls in the study region did not increase their consumption of marine prey after landfill closure.

Moreover, we observed that gull resource use was colony-dependent, confirming previous findings (Arizaga *et al.* 2013a). Even though the study colonies were quite close to each other (< *c.* 20 km), our results indicate that resource use differs among sites. Specifically, the colony of Getaria, located near one of the main fishing harbours of Gipuzkoa, was found to feed more on marine prey, probably in association with fishing discards. Differences in isotopic signatures found between Santa Clara and Ulia, two colonies situated very close to each other (< 5 km), support local variations in resource use. Although Yellow-legged Gulls are known to be generalists, some trophic differences between breeding colonies have been reported elsewhere (Duhem *et al.* 2005, Ramos *et al.* 2009a, Moreno *et al.* 2010) and the same happens with other species (Schmutz & Hobson 1998, O'Hanlon *et al.* 2017). In this way, neighbouring breeding colonies can show differences in diet due to segregation of foraging ranges between neighbouring colonies (Wakefield *et al.* 2015).

We also observed significant isotopic differences between winter and summer seasons in the period of low availability of food from landfill sites. During 2007–2009 in the one colony for which we have data for adults, the contribution of terrestrial prey to the diet was very low, and the abundant supply of food from fishing discards and landfills throughout the year probably led to little variation in their diet across seasons, but we do not know whether this was also true in the other two colonies. Foraging changes associated with season have also been reported in some Mediterranean Yellow-legged Gull colonies (Ramos *et al.* 2011) and in other avian species as well (e.g. Beeston *et al.* 2005, Sanchez *et al.* 2005, Romanowski & Zmihorski 2009, Martins *et al.* 2013, Yoshikawa & Osada 2015). Currently, with the shortage of landfill resources, diet reconstruction suggests that adults forage on a higher proportion of terrestrial prey during summer but, interestingly, not in winter, possibly because earthworms then become less available. In support of the notion that the gulls indeed forage on earthworms in summer are the findings that breeding birds at Ulia equipped with GPS-tags made considerable use of fields and grasslands around the colony (Arizaga *et al.* 2017). In winter, gulls were found to continue to forage on a high proportion of landfill food as well as on marine prey, which can probably be explained by them travelling to foraging sites situated further away that cannot be used in summer when adults are restricted in foraging distance/time because of chick-rearing (Gaston 2004). The greater values of isotopic niche width observed in winter compared with summer in two of the three colonies supports this idea. It seems that terrestrial prey was able to compensate for the lack of landfill food in summer but not in winter. Another possibility is that adults might vary their resource use in the breeding period to provide their offspring with higher quality prey (Annett & Pierotti 1989). In line with this rationale, the lack of niche overlap between the seasons may indicate that different prey types are consumed in summer and winter. Likewise, Egunez *et al.* (2018) observed higher use of landfills outside the breeding period. Future analyses of GPS-tracking will help to disentangle whether movement patterns in winter are in line with the expected increased exploitation of landfills and/or fishing harbours/vessels and (possible) longer foraging distances.

Unlike other Yellow-legged Gull populations, where chicks are fed with different food to that consumed by adults (e.g. Moreno *et al.* 2010), we observed no differences between age-classes, which seems to indicate that adults in our region probably do not discriminate between feeding for provisioning and self-maintenance. Even though our sampling was not designed to estimate the feeding ecology for chicks and adults of the same nest, we note that if the diet were to vary substantially between age-classes, we would still find differences at a colony level, as found in other studies (Moreno *et al.* 2010, Alonso *et al.* 2015). The lack of trophic differences between chicks and adults in the Basque area, where this study was carried out, seems to be a persistent phenomenon, given that it was also not found in 2007–2009 when more landfill sites were still open. The absence of a trophic difference between chicks and adults could also be due to our population not being able to switch to the preferred chicks' prey, or that our resolution (with only three resource categories) was too coarse to pick up a difference, whereas studies with a finer resolution might still find a difference.

The recent closure of several landfills, as would be expected, could also have consequences for traits other than diet. For example, Galarza (2015) reported a decline in breeding pairs of *c.* 40% over a period of 8 years (2007–2014) in a nearby colony (Izaro, *c.* 50 km from the nearest studied colony), a fact he attributed to a decrease of almost 90% in the amount of refuse discharged into the landfills surrounding that colony. In the medium or long term, our study colonies would also be expected to suffer population declines, especially when all the local landfills are completely closed. Moreover, landfills have been reported to affect several parameters such as breeding success or survival in many species worldwide (e.g. Pons 1992, Tortosa *et al.* 2002, Romano *et al.* 2006, Oro *et al.* 2013, Steigerwald *et al.* 2015, Plaza & Lambertucci 2017), but the impact of reduced foraging opportunities on landfill sites on these traits remains unknown for our study colonies as they have not yet been assessed.

Although we were not able to observe directly the use these gulls make of their local habitat, it can be deduced that they foraged more on fields once the landfills were closed, although, as far as we know, no conflicts with human activities have been registered so far. In addition, even though the consumption of resources categorized as marine and landfill was demonstrated here, we cannot distinguish whether the marine resources were obtained from harbours or the open sea, or whether gulls were foraging in urban areas, where the resources they would obtain are probably similar to those in landfills. Further studies tracking their movements are needed to gain complementary and detailed information about habitat use. Moreover, the change observed in trophic ecology may imply the consumption of new prey items.

The present work has been conducted on the premise that potential prey items consumed by Yellow-legged Gull and their baseline isotopic values have remained the same during the study period. It would be important in future studies to check whether diet includes any new prey items not exploited before. However, and independently of the type of prey consumed, changes in δ^{15}N and δ^{13}C values before and after the landfill closure were found, so evidently a change in diet has occurred between the two periods.

In conclusion, stable isotope analyses revealed a decrease in consumption of food from landfills following closure, which during the summer was mainly compensated for by a higher intake of prey of terrestrial origin (possibly earthworms), although not in winter. Unlike the period when landfill sites were still open, when no seasonal variation in prey consumption was observed, landfill closure resulted in changes in foraging ecology depending on season. Additionally, these results suggested that a wider range of resources was exploited during winter. Future studies should focus on the extent to which foraging constraints vary between seasons, and the potential consequences of this on the dynamics and territory use of the Yellow-legged Gull and other species which also forage on landfills. Indeed, the observed impact of landfill closure on gulls could also affect other taxa which rely on these sites for foraging.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. The 'siar' results (mean ± 95% credible intervals) for the chicks of three colonies (Ulia, Getaria and Santa Clara) and three years (2007, 2008, 2009). Red, results using two isotopes (δ^{13}C and δ^{15}N); blue, results using three isotopes (δ^{13}C, δ^{15}N and δ^{34}S).

Table S1. Isotopic signatures of δ^{13}C and δ^{15}N (mean ± 95% confidence interval) of chicks and adults in relation to colony of origin and year.