



Insights into the spatiotemporal component of feeding ecology: an isotopic approach for conservation management sciences

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ABSTRACT

Aim Bringing new approaches to trace spatiotemporal variations in animals' feeding ecology, which is fundamental for wildlife management and conservation since the accessibility of animals to trophic resources plays a key role in the dynamics of populations and metapopulations.

Location Western Mediterranean coast.

Methods The analysis of naturally occurring stable isotopes constitutes an exceptional approach to assess variations in the trophic ecology of species within the spatiotemporal dimensions. Here, we examined the spatiotemporal heterogeneity in resource exploitation of a nuisance and overpopulated gull species with a great feeding plasticity, the Yellow-legged gull *Larus michahellis*, by measuring the stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) and sulphur ($^{34}\text{S}/^{33}\text{S}$, $\delta^{34}\text{S}$) in different wing feathers.

Results Tracing isotopic changes among feathers in relation to moulting sequence, we showed that isotopic analyses on first primary feathers were good indicators of breeding trophic ecology, while others, such as eighth secondary feathers, reflected the feeding behaviour during the non-breeding period. This knowledge was then used to report on estimations of seasonal feeding patterns throughout the year for seven gull populations along the Western Mediterranean coast.

Main conclusions The high diversity in the exploitation patterns of the foraging habitats found in the study area both at spatial and at temporal scale indicated the opportunistic feeding behaviour of Yellow-legged gulls. Proposed isotopic approach has revealed as a useful tool to evaluate spatiotemporal variations in the feeding ecology of populations which may become clue for dealing with the management of wild species, not only nuisance populations but also endangered species. We finally pointed out the relevance of the isotopic variability among individuals to infer diet diversity and food availability of a given population, thereby allowing demographic forecasts when trophic resources vary in abundance.

Keywords

Feather biogeochemistry, *Larus michahellis*, mixing modelling, nuisance populations, stable isotope signatures (carbon-13, nitrogen-15, sulphur-34), Yellow-legged gull.

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INTRODUCTION

Metapopulation dynamics are highly dependent on the availability and accessibility of populations to trophic resources

throughout their distribution ranges but also along the annual cycle (Oro *et al.*, 2004; Bowler & Benton, 2005). Our ability to trace variations in the trophic ecology of wild populations both at the spatial and at the temporal scale is therefore clue for

several key fields of applied ecology, such as the conservation of endangered species and the management of nuisance and overpopulated species. Most studies driven to investigate the heterogeneity of resource exploitation by wild species have focused on the spatial dimension (i.e. variations in the dietary composition of different populations; Barrett *et al.*, 2007). However, research into seasonal variation in foraging ecology is currently scarce but essential to ascertain when these species are most vulnerable or when populations are most likely to be food-constrained (Feare, 1991; Martin *et al.*, 2007).

Among constraints of conventional tools in tracing nutrient used by wild populations, it is worth noting the short time period over which dietary information can be inferred (< 1 day) (Duffy & Jackson, 1986; Votier *et al.*, 2003; Jordan, 2005). Thus, most of our knowledge on their food preferences and feeding habits is restricted to the breeding period when animals are particularly accessible as they are linked to burrows or nests for pairing, incubation or the care of young. In this regard, the analysis of stable isotopes on specific animal tissues offers an exceptional opportunity to trace the trophic ecology of populations throughout the annual cycle. In particular, determining the isotopic composition of keratinous tissues from a large variety of organisms (such as hair, whiskers, nails, scutes and feathers) are particularly advantageous in this field, as during their formation, structural elements are incorporated into these tissues mainly directly from the daily dietary intake and remaining chemically inert afterwards (Hobson, 2008). Therefore, with the appropriate knowledge about their formation, specific portions of such keratinous tissues can be sampled at any time of the year to reconstruct the diet at specific time periods (e.g. Hedd & Montevecchi, 2006).

Several vertebrates are currently considered as nuisance species worldwide because their populations have become oversized owing to their flexible, opportunistic and gregarious nature, which makes them highly adapted to living in man-modified habitats. The negative effects of these overabundant species on human interests and endangered species (Smith & Carlile, 1993; Vidal *et al.*, 1998) have resulted in an increasing demand on managers to reduce and even revert their population trends. In this sense, limiting the accessibility and availability to trophic resources is considered the most effective management measure to control wild populations, rather than culling programs on eggs, young, juveniles or even adults, using poison or shooting (Goodrich & Buskirk, 1995; Oro *et al.*, 1995; Bosch *et al.*, 2000). Therefore, given that conservation management decisions concerning the modification of the carrying capacity of an ecosystem usually have high economical costs, efficient management should be based on precise and exhaustive knowledge of the resources exploited by each population. This approach implies not only determining the composition of primary diet but also exploring alternative food resources as well as temporal variations in the exploitation of these resources to foresee dietary changes that derive from management policies on restrictions.

In particular, gulls *Larus* spp. have been extensively studied as a potential superabundant species throughout Australia,

North America and Europe because their oversized populations cause negative effects on human interests and endangered species (Smith & Carlile, 1993; Vidal *et al.*, 1998). Here, we focused on an overabundant gull species, namely the Yellow-legged gull *Larus michahellis*, that breeds in the Mediterranean region, and it is the most common and widespread seabird in the Western Mediterranean. Its population reaches at least 120,000 nesting pairs and increases by up to 10% per year (Thibault *et al.*, 1996; Vidal *et al.*, 1998), likely as a result of its ability to exploit human derived trophic resources such as garbage and fishery discards (Bosch *et al.*, 1994; Ramos *et al.*, 2009b). In this regard, two European Union Action Plans (the EU Landfill Directive http://ec.europa.eu/environment/waste/landfill_index.htm, and the Reform of the Common Fisheries Policy <http://ec.europa.eu/fisheries/reform/>) are currently under development with the aim to decrease the accessibility of this species to these two trophic resources. Here, we presented a novel isotope approach directed to investigate the spatio-temporal heterogeneity of resource exploitation by gull populations, which is expected to aid authorities in the development of suitable management actions. By determining the isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) of feathers formerly moulted during the breeding and non-breeding seasons, we characterized seasonal variations in the diet of the Yellow-legged gull at seven colonies where these birds are assumed to use different proportions of marine, freshwater, terrestrial and garbage resources (Ramos *et al.*, 2009b). We examined the spatiotemporal heterogeneity of exploited resources on the basis of a management study and discussed the applicability of the proposed isotope approach for management and biodiversity conservation purposes.

METHODS

Moult phenology and sampling strategy

The study was carried out during the breeding season of 2004 in seven colonies with a variable density of breeding pairs and feeding habits (Table 1; Ramos *et al.*, 2009b) along the Western Mediterranean coast of Spain (Fig. 1). The study was based on feather sampling, because as a single unit, one feather grows relatively rapid and has determinate growth. Therefore, once the moulting pattern and time of feather formation of a species is well-known, analyses on specific individual feathers provide unique isotopic information of a very concrete spatiotemporal period, regardless of the sampling date (Hobson, 2008).

Large gulls start to moult wing feathers at the end of the breeding season, during the late-chick-rearing stage in mid-May, and this process lasts for about 6 or 7 months (personal observation; Ingolfsson, 1970; Baker, 1993). Primary renewal is simple and descends from the most proximal to the most distal feather, i.e. from the 1st (P1) to 10th primary (P10; Fig. 2a; Baker, 1993). Secondary feathers are shed in two waves: one starting with the most proximal feather (S23) soon after the start of the primary moult and then progressing slowly

Table 1 Main informative parameters of studied breeding sites.

Locality site	Distance from human settlements (in km)	Number of breeding pairs	Fishing vessel activity around each area (2007)			References	
			Relative estimation	Number of vessels	Gross tonnage		
Medes Is. (42°0'N, 3°13'E)	Protected islands off the coast of a tourist resort	0.9	6500	Moderate-high	392	8956	Bosch <i>et al.</i> , 2000
Ebro Delta (40°40'N, 0°45'E)	Isolated peninsula in a Natural Park	7.5	6000	High	358	11,195	Oro <i>et al.</i> , 2006
Columbretes Is. (39°54'N, 0°41'E)	Isolated archipelago in a Marine Reserve	55	450	High	245	9248	Oro <i>et al.</i> , 2006
Sa Dragonera Is. (39°35'N, 2°19'E)	Protected island the coast of a tourist resort	0.8	c. 4500	Moderate	440	3897	Daniel Oro personal communication
Benidorm Is. (38°30'N, 0°08'W)	Protected island off the coast of a tourist resort	3	650	Moderate-high	307	13,126	Martínez-Abraín <i>et al.</i> , 2002
Mazarrón Is. (37°33'N, 1°16'W)	Island off the coast of a tourist resort	0.5	900	Low	230	3719	García-Morell & Escribano, 2005
Alborán Is. (35°55'N, 3°04'W)	Remote island in a Marine Reserve	75 (Spanish coast)/ 50 (North African coast)	300	High	310	10,272	Paracuellos & Nevado, 2003

Fishing vessel information for each area was taken from http://ec.europa.eu/fisheries/index_en.htm.

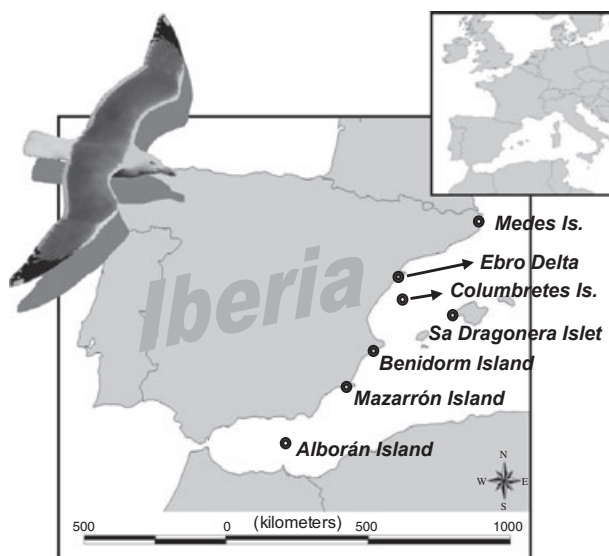


Figure 1 Map of the Iberian Peninsula (South West Europe) indicating the Western Mediterranean breeding colonies of the Yellow-legged gull *Larus michahellis* included in the study.

outwards, the other beginning with the most distal secondaries (S1) after the primary moult is about half completed and then progressing inwards (Fig. 2a; Ingolfsson, 1970).

To ensure the replacement sequence of remiges in the species, during the previous breeding season, we first collected and analysed stable isotope signatures of carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and sulphur ($\delta^{34}\text{S}$) of three primary feathers (1st, 5th and 10th; P1, P5 and P10, respectively) and four

secondaries (1st, 8th, 12th and 16th; S1, S8, S12 and S16, respectively; Fig. 2a) from 14 Yellow-legged gulls captured during the incubation period on Mazarrón Island. As moult is symmetrical between wings (Hedenström & Sunada, 1999), we minimized the effect of an exhaustive feather sampling on the flight performance of birds by removing feathers alternately from the left and right wings. Second, during the early breeding season of 2004, we collected the 1st primary (P1) and the 8th secondary (S8) from 12 to 21 adult gulls at each locality (total $n = 113$, see Table 3 for sampling size per locality) as representative feathers of breeding and non-breeding seasons, respectively. Finally, in five of the seven colonies (Medes Islands, Ebro Delta, Columbretes Islands, Mazarrón Island and Alborán Island), we collected spontaneous regurgitations from chicks ($n = 356$) and discarded fish from vessels (unpublished isotopic data from Alborán discards, Carola Sanpera).

Laboratory and statistical analyses

Feathers were washed in a 0.25 M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contaminants, dried in an oven at 60 °C to constant mass and ground to a fine powder in a freezer mill (Spex Certiprep 6750; Spex Industries Inc., Metuchen, NJ, USA) operating at liquid nitrogen temperature. Once regurgitated items were identified and classified according to their origin (marine, brackish and freshwaters, crops and terrestrial environments, and refuse sites; Bosch *et al.*, 1994), we selected the most well-preserved samples and performed isotope analyses to examine potential intercolony differences at prey isotope levels as well as to perform the mixing modelling. Before isotopic analysis, all

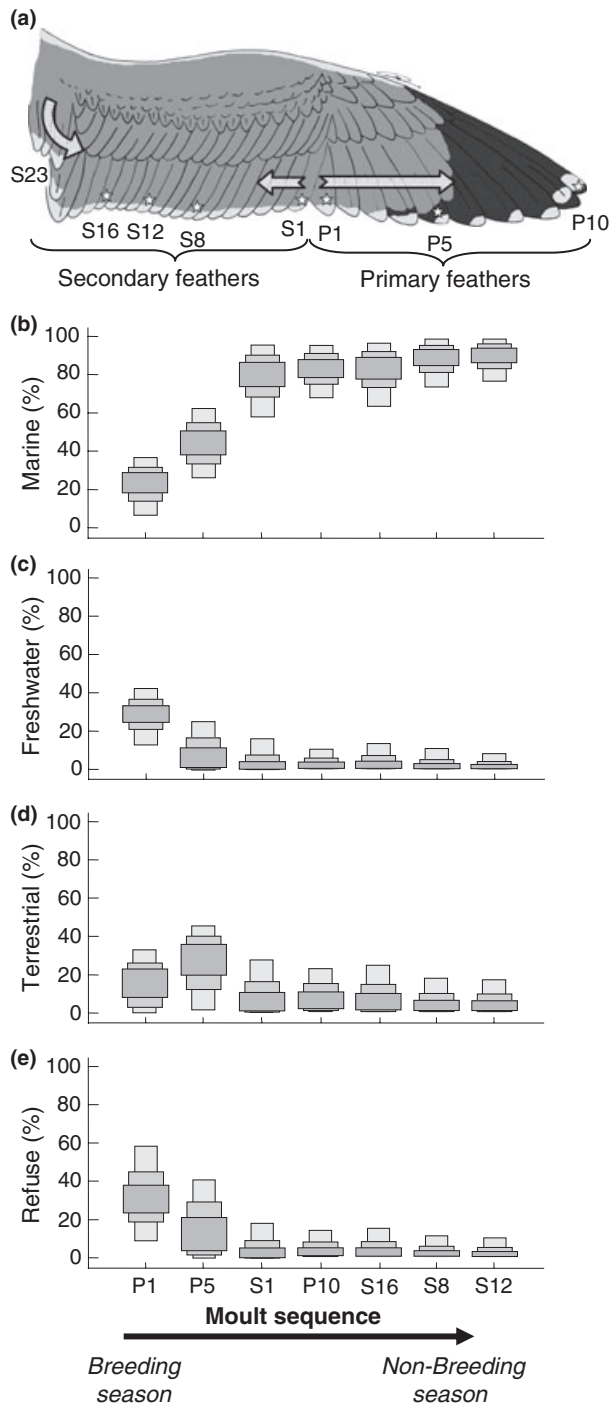


Figure 2 Wing feather moult pattern of the Yellow-legged gull and estimated diet from isotopic composition of some of its feathers. (a) Main moult pattern (white arrows) and selected feathers analysed for stable isotopes (stars) are shown in the wing scheme. Marine (b), freshwater (c), terrestrial (d) and refuse (e) resource consumption (95%, 75% and 50% Bayesian credibility intervals) estimated by Stable Isotope Analysis in R mixing modelling and based on C, N and S isotopic signatures of 1st, 5th, and 10th primary (P1, P5, P10) and 1st, 8th 12th and 16th secondary (S1, S8, S12, S16) feathers from 14 Yellow-legged gulls sampled on Mazarrón Island. Feathers are sorted following the moult sequence defined by Ingólfsson (1970) and Baker (1993).

Table 2 Results from linear mixed models on stable isotopes of C, N and S fitted to the series of moulting feathers in 14 Yellow-legged gulls breeding on Mazarrón Island.

Mean \pm SE (95% CI)	Random parameters	
	Interindividual variance	Residual variance
$\delta^{13}\text{C}$	0.378	
P1 -19.27 ± 1.21 (-19.79 ; -18.75)		0.577
P5 -18.09 ± 0.77 (-18.65 ; -17.53)		0.739
S1 -17.16 ± 1.04 (-17.75 ; -16.57)		0.868
P10 -17.58 ± 0.62 (-17.91 ; -17.25)		0.006
S16 -17.45 ± 0.81 (-17.81 ; -17.08)		0.090
S8 -16.94 ± 0.75 (-17.40 ; -16.49)		0.349
S12 -17.23 ± 0.60 (-17.53 ; -16.91)		0.184
$\delta^{15}\text{N}$	0.300	
P1 11.52 ± 1.48 (10.95; 12.08)		0.836
P5 11.83 ± 0.78 (11.35; 12.30)		0.498
S1 12.51 ± 0.74 (12.08; 12.94)		0.349
P10 12.46 ± 0.58 (12.16; 12.76)		0.013
S16 12.54 ± 0.68 (12.23; 12.86)		0.053
S8 12.66 ± 0.54 (12.34; 12.97)		0.055
S12 12.64 ± 0.50 (12.40; 12.88)		0.028
$\delta^{34}\text{S}$	2.574	1.793
P1 13.39 ± 3.10 (12.28; 14.50)		
P5 15.24 ± 2.69 (14.13; 16.35)		
S1 17.82 ± 1.67 (16.71; 18.93)		
P10 18.01 ± 1.75 (16.90; 19.12)		
S16 17.91 ± 2.36 (16.80; 19.02)		
S8 17.97 ± 1.97 (16.86; 19.08)		
S12 18.25 ± 1.67 (17.45; 19.06)		

Estimated means (\pm SE and 95% confidence intervals; lower and upper limits, $n = 14$) are used to show fixed feather effects. Estimated variance parameters show individual random effects and residual heterogeneity (overall for $\delta^{34}\text{S}$, feather specific for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Feathers are sorted following the moult sequence defined by Ingólfsson (1970) and Baker (1993).

food samples were freeze-dried and ground in a freezer mill. To reduce variability caused by isotopically lighter lipids (Logan *et al.*, 2008), lipids were removed from all dietary samples by means of several chloroform-methanol (2:1) rinses, following the Folch extraction method (Folch *et al.*, 1957).

Subsamples of powdered material were weighed to the nearest μg , placed in tin capsules and crimped for combustion for C, N and S isotope determination. Isotopic analyses were carried out at the Serveis Científico-Tècnics of the University of Barcelona (Spain) by means of a Thermo-Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyzer coupled to a Delta-C isotope-ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany) and applying IAEA standards (IAEA CH6, IAEA CH7 and USGS 24 for C, IAEA N1, IAEA N2 and IAEA NO3 for N, and IAEA-S1, IAEA-S2 and IAEA-S3 for S) inserted every 12 samples to calibrate the system. Stable isotope ratios were expressed in the standard δ -notation relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$), atmospheric N_2 ($\delta^{15}\text{N}$) and troilite

Table 3 Mean least square estimations (\pm SE and 95% confidence intervals; lower and upper limits) on stable isotopes of C, N and S from linear mixed models for summer and winter feathers (P1 and S8, respectively) of Yellow-legged gulls in the seven colonies ordered according to increasing mean values.

		1st primary (P1)		8th secondary (S8)	
		<i>n</i>	Mean \pm SE (95% CI)		Mean \pm SE (95% CI)
$\delta^{13}\text{C}$					
Sa Dragonera Is.	1	12	-19.97 ± 0.14 (-20.24; -19.70)		-19.15 ± 0.32 (-19.78; -18.52)
Medes Is.	2	13	-19.35 ± 0.11 (-19.57; -19.12)		-18.68 ± 0.29 (-19.25; -18.11)
Mazarrón Is.	2 3	15	-19.20 ± 0.14 (-19.48; -18.91)	*	-17.61 ± 0.28 (-18.17; -17.04)
Benidorm Is.	3	12	-18.61 ± 0.16 (-18.92; -18.30)	*	-17.52 ± 0.29 (-18.10; -16.94)
Ebro Delta	3	20	-18.54 ± 0.18 (-18.89; -18.20)		-18.08 ± 0.24 (-18.56; -17.60)
Columbretes Is.	4	21	-17.90 ± 0.08 (-18.05; -17.74)		-18.35 ± 0.22 (-18.78; -17.91)
Alborán Is.	5	20	-16.78 ± 0.09 (-16.96; -16.60)		-16.55 ± 0.25 (-17.05; -16.05)
$\delta^{15}\text{N}$					
Sa Dragonera Is.	1	12	10.70 ± 0.17 (10.37; 11.03)		11.20 ± 0.31 (10.60; 11.81)
Medes Is.	1 2	13	11.10 ± 0.20 (10.71; 11.49)		11.29 ± 0.31 (10.68; 11.89)
Mazarrón Is.	2 3	15	11.56 ± 0.18 (11.20; 11.91)	*	13.06 ± 0.28 (12.50; 13.62)
Benidorm Is.	3 4	12	12.10 ± 0.14 (11.82; 12.38)		12.65 ± 0.26 (12.13; 13.17)
Columbretes Is.	4	21	12.30 ± 0.09 (12.11; 12.48)		12.39 ± 0.21 (11.99; 12.80)
Ebro Delta	4 5	20	12.82 ± 0.21 (12.41; 13.24)		12.86 ± 0.25 (12.37; 13.36)
Alborán Is.	5	20	13.34 ± 0.09 (13.16; 13.53)		13.42 ± 0.23 (12.96; 13.88)
$\delta^{34}\text{S}$					
Medes Is.	1	13	10.52 ± 0.45 (09.63; 11.41)	*	14.10 ± 0.90 (12.32; 15.89)
Mazarrón Is.	1	15	11.21 ± 0.49 (10.23; 12.18)	*	17.05 ± 0.88 (15.31; 18.80)
Sa Dragonera Is.	1 2	12	12.52 ± 0.75 (11.03; 14.01)		12.63 ± 1.14 (10.38; 14.88)
Benidorm Is.	2 3	12	15.28 ± 0.59 (14.11; 16.46)		17.47 ± 0.94 (15.61; 19.33)
Ebro Delta	3	20	16.27 ± 0.52 (15.24; 17.29)		15.05 ± 0.72 (13.62; 16.48)
Columbretes Is.	4	21	18.63 ± 0.28 (18.07; 19.19)	*	14.93 ± 0.67 (13.60; 16.26)
Alborán Is.	4	20	18.86 ± 0.10 (18.66; 19.07)		18.52 ± 0.70 (17.13; 19.91)

Superindices denote groups of colonies not significantly different for their breeding values (P1 feather). Asterisks denote significant differences between the two feather types (P1 and S8) within a colony.

of the Canyon Diablo Meteorite ($\delta^{34}\text{S}$). Replicate assays of standards indicated analytical measurement errors of $\pm 0.1\%$, $\pm 0.2\%$ and $\pm 0.2\%$ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively.

We used linear mixed models (LMM) to analyse isotopic values in the feathers (Littell *et al.*, 1996). To build a set of competing models, we considered several fixed and random effects. Colony and feather type were the two fixed factors considered. As one feather (P1) was moulted at the breeding area whereas the second feather (S8) was moulted during the winter time when individuals are not linked to their breeding area, differences among colonies are expected not to be equal for both feathers; thus, an interaction term was also evaluated to fit the data. Individual random effect was included always in the models to account for the dependence among feathers of the same individual. Isotopic variance among individuals is related to individual heterogeneity in resources use (Bearhop *et al.*, 2004) and its value can change among colonies (because resource availability changes). Because this, we evaluated models both, with an only one common individual random effect and with the random term interacting with the colony (i.e. seven different individual random effects, one for each colony). A parameter corresponding to residual variance was always estimated. By the same argument used earlier, spatial

heterogeneity when moulting S8 can increase variability in the isotopic signal; thus, we also evaluated models including two different residual variances, one for each feather type. Model selection was done using Akaike information criteria (Burnham & Anderson, 2002; Johnson & Omland, 2004). Posterior pair-wise comparisons between groups (colonies and/or feathers) were made using Hochberg's approach (Hochberg, 1988) to maintain the overall error type I at 0.05. Q-Q plots were used to inspect graphically normality of residuals from fitted models and to ensure the model adequacy. For the individual series of feathers sampled on Mazarrón Island, only four competing models were considered, defined by a fixed feather effect and differences among feathers in residual variance. The data analysis was generated using SAS/STAT software (Version 9.1.3 of the SAS System for Windows, copyright 2002–2004; SAS Institute Inc., Cary, NC, USA).

We estimated the foraging habitats exploited by each locality during summer and winter by applying a Bayesian three-isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) four end-points (marine, brackish and freshwaters, crops and terrestrial environments, and refuse sites) mixing models to our isotopic values (SIAR: Stable Isotope Analysis in R; Parnell *et al.*, 2008). Isotopic end-points were typified through the isotopic composition of regurgitated items

Table 4 Parameter estimates from linear mixed models fitted to summer (P1) and winter (S8) feathers in Yellow-legged gulls from seven colonies.

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
Fixed effects	Mean \pm SE	Mean \pm SE	Mean \pm SE
Intercept	-18.68 \pm 0.29	11.29 \pm 0.31	14.10 \pm 0.90
Feather			
S8 (ref)			
P1	-0.66 \pm 0.29	-0.19 \pm 0.29	-3.58 \pm 0.81
Colony			
Medes (ref)			
Ebro Delta	0.60 \pm 0.37	1.58 \pm 0.40	0.94 \pm 1.15
Columbretes	0.33 \pm 0.36	1.11 \pm 0.37	0.83 \pm 1.12
Sa Dragonera	-0.47 \pm 0.43	-0.08 \pm 0.43	-1.47 \pm 1.45
Benidorm	1.17 \pm 0.41	1.36 \pm 0.40	3.36 \pm 1.30
Mazarrón	1.07 \pm 0.40	1.78 \pm 0.42	2.95 \pm 1.26
Alborán	2.13 \pm 0.38	2.14 \pm 0.38	4.42 \pm 1.14
Feather by colony			
P1 \times Ebro Delta	0.20 \pm 0.37	0.15 \pm 0.36	4.80 \pm 1.01
P1 \times Columbretes	1.11 \pm 0.37	0.09 \pm 0.37	7.28 \pm 1.03
P1 \times Sa Dragonera	-0.15 \pm 0.43	-0.32 \pm 0.42	3.47 \pm 1.19
P1 \times Benidorm	-0.43 \pm 0.41	-0.36 \pm 0.41	1.40 \pm 1.15
P1 \times Mazarrón	-0.93 \pm 0.40	-1.32 \pm 0.40	-2.27 \pm 1.11
P1 \times Alborán	0.44 \pm 0.39	0.11 \pm 0.38	3.93 \pm 1.08
Random effects	Variance	Variance	Variance
Interindividual			
Medes	0.052	0.336	2.456
Ebro Delta	0.625	0.883	6.367
Columbretes	0.019	0.004	1.483
Sa Dragonera	0.119	0.152	6.570
Benidorm	0.190	0.056	4.283
Mazarrón	0.202	0.310	3.495
Alborán	0.055	0.000	0.000
Residual			
P1	0.110	0.179	0.217
S8	1.091	0.972	8.888

For fixed effects, estimated means and their standard error (\pm SE) are shown. For individual random effects and residual heteroscedasticity, variance estimates are shown.

which were adjusted to account for diet to feather isotopic discrimination (Table 5). Preys' C, N and S concentrations, which were substantially different among dietary endpoints (one-way ANOVA; $F_{3,77} = 13.27$, $F_{3,77} = 29.46$ and $F_{3,77} = 9.03$ respectively, all $P < 0.001$), were also incorporated to the model. We assumed that prey isotopic signatures do not vary temporally either between the early winter and the breeding season or between years (see Discussion).

RESULTS

Although we detected a certain degree of variability, C, N and S isotopic signatures of the sequence-sorted feathers of gulls on Mazarrón Island showed similar patterns among individuals.

Fitted models (see Table S1 in Supporting Information) showed a significant feather effect on mean isotopic signatures for all three isotopes, while different residual heterogeneity among feather types was relevant for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (Table 2). The estimated means from the fitted model for this colony showed that P1 had the lowest mean signature in the feather series for all three isotopes. Increasing mean values were observed throughout the moult sequence, with intermediate values in P5 and S1 feathers. The latest feathers moulted, i.e. S8 and S12, showed the highest isotopic values (Table 2). In both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ models, the residual variance of P1, P5 and S1 was greater than the individual random effect, while S8, P10, S12 and S16 feathers had similar isotopic values and showed lower residual heterogeneity compared with the individual effect (Table 2).

Using model information criteria, we evaluated several models to determine isotopic patterns between summer and winter feathers (P1 and S8) and among the seven colonies (see Fig. S1 for an overall isotopic landscape and Table S2 for Akaike statistics, both in Supporting Information). According to Akaike weights, the same model was selected to fit the data for all three isotopes. The model included the fixed effects for feather and colony as well as their interaction (14 parameters), a different individual random parameter for each colony (seven parameters), and different residual variance for each feather (two parameters). Fixed effects cannot be directly interpretable because the presence of significant interaction between them and mean values defined by colony and feather combined should be used instead (Table 3). In general, feathers from birds on the Alborán and Columbretes Islands had the highest isotopic values, whereas those from Medes and Sa Dragonera Islands showed the lowest values (Table 3). Significant differences between summer and winter feathers were detected in several colonies, although only on Mazarrón Island did all three stable isotopes differ consistently (Table 3). Birds in the colonies on Benidorm and Mazarrón Islands showed lower $\delta^{13}\text{C}$ signatures in their P1 than in S8 feathers. Similarly, specimens from the Medes and Mazarrón Islands showed lower $\delta^{34}\text{S}$ signatures in P1 feathers compared to S8, whereas in the Columbretes and Alborán Islands, $\delta^{34}\text{S}$ signatures in P1 feathers were the highest (Table 3). It is worth noting that residual variability was much greater for S8 than for P1 feathers (Table 4) for all the three isotopes. Random individual effects were noticeably lower in the colonies on the Columbretes and Alborán Islands than in the others. This observation indicates higher homogeneity in isotopic signatures among individuals. Conversely, the Ebro Delta showed the greatest variability between individuals (Table 4).

Regarding potential differences in baseline isotopic values among colonies (see Hebert *et al.*, 1999), we analysed the signatures of the food types collected along the Iberian Mediterranean coast. Isotopic signatures of the four food types were homogeneous among localities, except that of discarded fish from the Alborán Island, which differed significantly in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ ($F_{\text{WELCH } 4,19} = 45.80$, $F_{\text{WELCH } 4,19} = 17.31$,

Table 5 Summary of mean isotopic values (\pm SE) for the main kind of food resources exploited by Yellow-legged gulls (obtained from chick regurgitates and fishery discards).

Prey class	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	Source
Marine					
Medes	13	-18.36 ± 0.28	9.18 ± 0.43	17.30 ± 0.23	
Ebro Delta	9	-18.18 ± 0.57	9.57 ± 0.51	17.31 ± 0.37	
Columbretes	11	-18.55 ± 0.42	9.44 ± 0.36	17.65 ± 0.52	
Mazarrón	6	-18.40 ± 0.31	9.71 ± 0.96	17.83 ± 0.70	
mean	39	-18.38 ± 0.41	9.46 ± 0.55	17.48 ± 0.48	
Alborán	11	-16.42 ± 0.43	7.98 ± 0.49	18.92 ± 0.31	
Consumer-diet discrimination					
<i>Larus michahellis</i> -marine fish		$\Delta\delta^{13}\text{C} = 0.9$	$\Delta\delta^{15}\text{N} = 1.7$	$\Delta\delta^{34}\text{S} = 1.9$	Ramos <i>et al.</i> , 2009b
Freshwater					
Mazarrón*	4	-18.87 ± 0.69	9.91 ± 2.81	10.12 ± 0.89	
Consumer-diet discrimination					
<i>Larus delawarensis</i> -freshwater food		$\Delta\delta^{13}\text{C} = 0.3$	$\Delta\delta^{15}\text{N} = 3.0$	$\Delta\delta^{34}\text{S} = 1.3\ddagger$	Hobson & Clark, 1992; France & Peters, 1997; Peterson <i>et al.</i> , 1985
Terrestrial					
Medes	3	-18.43 ± 2.76	10.84 ± 5.28	6.76 ± 0.62	
Ebro Delta	6	-17.87 ± 1.77	11.03 ± 3.17	6.70 ± 0.74	
Mazarrón	2	-21.85 ± 4.89	15.88 ± 3.02	7.94 ± 1.72	
Mean	11	-18.38 ± 1.75	11.92 ± 3.00	6.97 ± 1.05	
Consumer-diet discrimination					
<i>Sylvia borin</i> -mealworms		$\Delta\delta^{13}\text{C} = 2.7$	$\Delta\delta^{15}\text{N} = 4.0$	$\Delta\delta^{34}\text{S} = 1.3\ddagger$	Hobson & Bairlein, 2003; Peterson <i>et al.</i> , 1985
Refuse tips					
Medes	5	-22.04 ± 1.63	4.82 ± 1.26	5.40 ± 1.81	
Ebro Delta	2	-19.91 ± 0.04	6.01 ± 1.64	7.02 ± 1.02	
Mazarrón	5	-22.00 ± 1.08	5.98 ± 2.26	7.92 ± 1.93	
Mean	12	-21.67 ± 1.44	5.50 ± 1.74	6.72 ± 2.03	
Consumer-diet discrimination					
<i>Catharacta skua</i> -beef \ddagger		$\Delta\delta^{13}\text{C} = 2.2$	$\Delta\delta^{15}\text{N} = 5.0$	$\Delta\delta^{34}\text{S} = 1.3\ddagger$	Bearhop <i>et al.</i> , 2002; Peterson <i>et al.</i> , 1985

Isotopic signatures used in the mixing models are shown as global means when found to be homogeneous among localities (only marine resources from Alborán Island were found to be significantly different, see Results). Diet-tissue isotope-discrimination factors ($\Delta\delta^{13}\text{C}$, $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{34}\text{S}$ in ‰) between consumer's feathers and different food sources were extracted from the literature.

*Freshwater invertebrates were found extensively only on Mazarrón Is.

‡Refuse tips were mainly composed by meat wastes.

†Peterson *et al.*, 1985 reported 1.3 ‰ as a general $\Delta\delta^{34}\text{S}$.

$F_{\text{WELCH } 4,19} = 46.37$ respectively, all $P < 0.001$; only *post hoc* Tamhane's multiple comparison test for Alborán marine prey were significantly different from all other localities; Table 5 and Fig. S2). When applying mixing models, as isotopic signatures on gull prey did not show variation among localities, we assumed baseline signatures to be homogeneous in the study area, except for the marine resources for the population on the Alborán Island, for which we used only unpublished isotopic data collected by colleagues on anchovies (*Engraulis encrasicolus*) sampled around the Island.

SIAR mixing models estimated similar dietary contributions in both seasons for the Ebro Delta and Alborán populations (Fig. 3). Dramatic changes in feeding patterns were predicted for the Mazarrón Island population, although these variations between seasons were also relevant in gulls in the Medes, Columbretes, Sa Dragonera and Benidorm colonies. Models assumed that the main food items for the Columbretes Islands

colony during the breeding season were from marine environments. However, the contribution of this prey decreased by < 50% during the non-breeding season when freshwater prey and refuse became more common. Refuse was the main source of food for the gull colonies on the Medes and Sa Dragonera Islands during the summertime, comprising approximately half of their diet (42.1% and 53.7%, respectively). The consumption of refuse was also relevant in Benidorm and Mazarrón Islands at that time, representing the 20.7% and the 32.6% of their diet, respectively. However, the use of refuse in these four colonies decreased during the non-breeding season, especially in the population on the Mazarrón Island (Fig. 3d). In contrast, the exploitation of marine resources during the non-breeding season increased in all these four colonies (Fig. 3a). Particularly for the Mazarrón Island colony, for which we recorded temporal changes in diet through the moult sequence, the use of refuse and freshwater prey decreased from breeding to non-

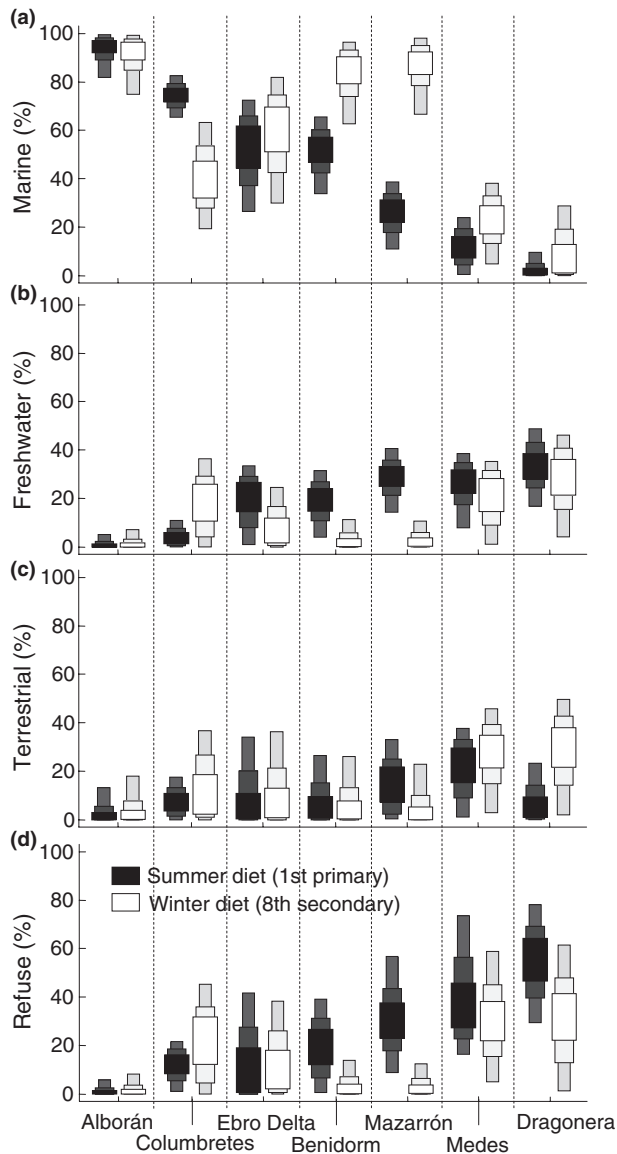


Figure 3 Seasonal estimations of marine (a), freshwater (b), terrestrial (c) and refuse (d) resources computed by Stable Isotope Analysis in R (95, 75 and 50% Bayesian credibility intervals) and based on C, N and S isotopic signatures of 1st primary (P1) and 8th secondary (S8) feathers of Yellow-legged gulls from seven Western Mediterranean colonies. Sample size for Alborán Is. $n = 20$, Columbretes Is. $n = 21$, Ebro Delta $n = 20$, Benidorm Is. $n = 12$, Mazarrón Is. $n = 15$, Medes Is. $n = 13$ and Sa Dragonera Is. $n = 12$.

breeding seasons while marine prey gained great relevance in the diet during the non-breeding season (Fig. 2).

DISCUSSION

Tracing feeding ecology through the moulting sequence

On the basis of the moulting pattern of the Yellow-legged gull (Baker, 1993), stable isotope signatures differ among wing

feathers, thereby allowing us to track dietary changes throughout half a year, from mid-May to early November (see Methods). Stable isotope analysis on feathers moulted in different periods (P1: chick-rearing period and P10, S8, S12 and S16: non-breeding period) indicated that the population on the Mazarrón Island changed dietary preferences between seasons, feeding abundantly on marine, freshwater and refuse resources during the breeding season (see also chicks' diet in Ramos *et al.*, 2009c) but mainly consuming fish during wintertime (Fig. 2). Feathers moulted during the interperiod between the breeding and non-breeding seasons (P5 and S1) revealed that freshwater prey was absent from the diet at that time while refuse scraps gradually gave way to marine prey (Fig. 2). The fact these interperiod feathers present intermediate isotopic values between feathers formerly moulted in the breeding and non-breeding seasons and the observation that their isotopic variability is not particularly wide (see residual variances in Table 2) strongly suggest that the general feeding pattern change gradually within the population. Here, we illustrate that changes in dietary preferences of the Yellow-legged gull can be tracked throughout the moult sequence and therefore throughout the annual cycle by analysing stable isotopes on specific feathers. In particular, isotopic analysis on P1 feathers of these gulls was a good indicator of breeding trophic ecology, while other feathers such as S8 clearly reflected the feeding behaviours during the non-breeding period. We also pointed out here the importance of ensuring the time formation of sampled tissues when aiming to determine summer or winter ecologies (Hobson, 2008) as some feathers could represent feeding events during the interperiods.

Characterizing spatiotemporal dietary patterns using stable isotopes

The great isotopic diversity among populations of Yellow-legged gulls revealed a wide range of feeding strategies among the colonies (see also Hebert *et al.*, 2008). In addition to that variability among colonies, stable isotopes also varied considerably between breeding and non-breeding feathers in some of the colonies, i.e. while some populations maintained their dietary preferences between seasons (e.g. Ebro Delta and Alborán Is.), others changed their feeding habits drastically (e.g. Mazarrón Is.). This spatial and temporal variability in stable isotope signatures in gull feathers may reflect the spatiotemporal availability of some of the resources exploited by the Yellow-legged gull and the feeding plasticity and opportunistic foraging behaviour of this species, which feeds on the most abundant local food sources (Vidal *et al.*, 1998; Duhem *et al.*, 2005). Indeed, we found that most gull populations near human settlements (Table 1), such as Medes, Sa Dragonera, Benidorm and Mazarrón Islands, used refuse sites during the breeding season. However, tourism in most resorts along the Iberian Mediterranean peaks in the summer and decreases thereafter. Consequently, refuse availability decreases in the non-peak periods, and gulls adapt their

feeding strategies by exploiting alternative local resources or alternatively forage further afield. This scenario seems to be the case for these four populations, which showed a considerable decrease in refuse consumption during the non-breeding season (Fig. 3d).

In contrast, remote breeding populations, such as those on the Alborán and Columbretes Islands, feed mainly on marine prey during the breeding season. According to the optimal foraging theory, for these remote populations on minute and uninhabited archipelagos in the middle of large oceanic water masses, the mainland is too distant from their breeding area to be used for chick provisioning with continental prey (Duhem *et al.*, 2005; Ramos *et al.*, 2009b). Consequently, while breeding, both populations of gulls fed offspring and themselves mainly fish (Fig. 3a), which they easily obtained from the fisheries operating in the area (Arcos *et al.*, 2001; Ramos *et al.*, 2009b). However, while estimated dietary preferences for the Alborán population indicate that the most relevant food source for the non-breeding season was also marine prey, the estimated diet for gulls on the Columbretes Islands for this period suggested that they probably spent the winter on the coast, consuming a greater proportion of continental resources. Therefore, local movements of gull populations between seasons should be considered when dealing with issues regarding gull management (Sol *et al.*, 1995; Martínez-Abraín *et al.*, 2002).

The finding that residual variance (variability not explained by the fitted models) was greater in the feathers grown during the non-breeding season (S8) indicated a more diverse diet and greater foraging opportunities at that time than in the summer, when most birds are confined to a limited foraging area close to the breeding colony. Similarly, the most remote and isolated colonies, i.e. the Columbretes and Alborán Islands, tend to show the lowest interindividual variability in the three isotopes analysed. This fact agrees with the niche width interpretation of such variability (Newsome *et al.*, 2007) which should be narrow in these colonies with few dietary alternatives. Conversely, the Ebro Delta, an area with a wide range of available resources (Ramos *et al.*, 2009b) is showing the greatest individual random effect (see Table 4). Therefore, isotopic variability among individuals of a single population could also be used as a good estimator of diverse habitat exploitation strategies and food availability for a given population (Bearhop *et al.*, 2004).

Finally, the observation that fishery discards collected around the Alborán Island differed in isotopic signatures from those from the other sampled sites may be attributable to the influence of the Atlantic Ocean, which conditions the isotope composition present in the local food webs (Pantoja *et al.*, 2002; Gómez-Díaz & González-Solís, 2007). Thus, it is important to ensure the geographic homogeneity of prey signatures before applying a mixing model, especially when the study area is relatively large (Hebert *et al.*, 1999). Although we did not find significant differences in stable isotopes at prey level among the other localities, we cannot rule out completely that intercolony differences in stable isotopes of gulls could be

partially explained by geographic differences at baseline isotope levels. However, the assumption of isotopic homogeneity along the studied area (excepting for Alborán Island) is also supported by the fact that those potential geographic differences are much smaller than differences among disparate prey types, i.e. isotopic differences between prey from different origin (marine, terrestrial or freshwater) are usually greater than geographic variations in stable isotopes at a mid-local scale (Bearhop *et al.*, 2004).

The management perspective

In general, food availability is a determinant factor of population dynamics (Sol *et al.*, 1995; Oro *et al.*, 2004). In the case of gulls, food sources derived from human activities, such as refuse dumps and fishery discards, are usually abundant and relatively predictable, thereby increasing the carrying capacity of the ecosystem and allowing gulls to improve breeding success and adult survival (Pons, 1992). In this regard, the two European Union Action Plans which are currently under development (see Introduction) seek to decrease the availability of both garbage and fishery discards. On the basis of our findings which estimated in *c.* eighty percent the total usage of these two resources, we consider that these management strategies will lead to generalized reductions in Yellow-legged gull populations throughout the study area but also throughout the whole distribution range of this species (Bosch *et al.*, 2000; Brooks & Lebreton, 2001). Breeding success in gull populations such as those on the Medes and Sa Dragonera Islands will be drastically affected by a decrease in refuse dump availability, while the breeding success of colonies on the Columbretes, Benidorm and Alborán Islands will be influenced mainly by the optimization of fishery techniques, which will reduce the amount of discards. In contrast, adult and fledging survival out of the breeding season will be particularly affected in the Benidorm, Mazarrón and Alborán populations as few alternative food sources to fishery discards seem to be available at that time. For all the other study sites (Medes, Ebro Delta, Columbretes and Sa Dragonera), population reductions are also expected during the non-breeding season, although these decreases may be softened by the presence of alternative local food resources, such as freshwater or terrestrial prey, which will be more intensely exploited in the future (Duhem *et al.*, 2005; Hebert *et al.*, 2008). As a result of these sudden reductions in food availability and considering the opportunistic feeding behaviour of the species, we foresee that new and unpredicted trophic resources could be exploited in the near future by these gulls. Under such circumstances, we also predict an increase in conflict within Yellow-legged gull colonies in the relatively short-term but also with endangered species breeding nearby, thereby raising a conservation concern (Goodrich & Buskirk, 1995; Martos & Johnson, 1996; Vidal *et al.*, 1998; Martínez-Abraín *et al.*, 2003; Votier *et al.*, 2004; García-Morell & Escibano, 2005; Oro *et al.*, 2005).

CONCLUSIONS

Although several recent studies have applied stable isotope analysis on distinct wing feathers to elucidate migratory movements (Furness *et al.*, 2006; Ramos *et al.*, 2009a; Quillfeldt *et al.*, 2010), to our knowledge, this is the first study using an isotopic approach to address in detail specific seasonal estimations of the feeding patterns of species. Precise information on feeding strategies throughout the annual cycle is required for foreseeing population dynamics of most species (Feare, 1991; Brooks & Lebreton, 2001). Here, we provide evidence that stable isotope analysis on specific feathers can be used to determine the feeding ecology of a species throughout its annual cycle. Feathers are often replaced in a predictable manner along the annual cycle (Bridge, 2006) and thus provide an excellent opportunity to study exhaustively avian ecology through stable isotopes. Although our study was based on birds and feathers sampling, for other non-avian species, specific portions of keratinous tissues, such as hair, whiskers, nails or scales (Hobson *et al.*, 1996; Reich *et al.*, 2007), sampled at a particular time during their annual cycle, could also be used to provide relevant information about breeding and wintering ecology; however, appropriate validations should be considered. We also pointed out the relevance of the isotopic variability among individuals at population level to infer diet diversity (i.e. niche width) and food availability of a given population, thereby allowing demographic forecasts of the impact of trophic resource constraints, for instance. On the context of the current willing to reduce the growth of these nuisance gull populations, we pointed out the spatiotemporal plasticity in the feeding ecology of the species and highlight the convenience of staggering these restrictions in Yellow-legged gull's trophic resources to preserve the trophic web parsimony. Finally, although our work was based on the management of a problematic species, it is obvious that the isotopic methodology to establish food preferences as well as the output of its conclusions is also suitable for conservation studies of endangered species. We propose therefore that isotopic analyses and the trophic information derived from them are powerful ecological tools which can enormously contribute to conservation management sciences.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Dual stable-isotope biplots of (a) nitrogen-carbon, (b) nitrogen-sulphur, and (c) sulphur-carbon in P1 and S8 feathers from seven colonies as well as in their main prey.

Figure S2 Stable isotope signatures of carbon (a), nitrogen (b) and sulphur (c) of both baseline prey and gull summer feathers at five of the studied colonies.

Table S1 Candidate models evaluated to fit the isotopic data in seven feathers from Mazarrón Island.

Table S2 Candidate models evaluated to fit the isotopic data in P1 and S8 feathers from seven colonies.

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BIOSKETCH

Raül Ramos is interested in the ecological applications of intrinsic biogeochemical markers into those diverse fields such as epidemiology, feeding ecology, moulting and migration studies, and pollution assessments. This study is part of his PhD Thesis titled *Tracing bird ecology throughout feathers*. More recently, his interests have expanded into several field involving life-history traits of long-lived seabirds such as their demography constraints and immune system acquisition.

Author contributions: R.R., F.R. and L.J. conceived the ideas; R.R. and F.R. collected the data; R.R., F.R., J.L.C. and L.J. analysed the data; and R.R. and F.R. led the writing.

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