L'estudi de l'ecologia de les aus a través de les seves plomes

Aplicacions ecològiques dels biomarcadors intrínsecs

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Ecological applications of intrinsic biogeochemical markers

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L'ESTUDI DE L'ECOLOGIA DE LES AUS A TRAVÉS DE LES SEVES PLOMES: APLICACIONS ECOLÒGIQUES DELS BIOMARCADORS INTRÍNSECS

TRACING BIRD ECOLOGY THROUGH FEATHERS: ECOLOGICAL APPLICATIONS OF INTRINSIC BIOGEOCHEMICAL MARKERS

Memòria presentada pel llicenciat en Biologia **Raül Ramos i Garcia** per a optar al grau de **Doctor** per la **Universitat de Barcelona**

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Prefaci: la importància de les plomes

"千里送而毛"

"Recórrer mil milles i presentar una ploma com a regal"

Hi ha una antiga expressió xinesa sobre l'art de fer regals. Durant la dinastia Tang, era habitual que els terratinents locals per mostrar el seu respecte a l'Emperador, li oferissin meravellosos regals. Un terratinent li va encomanar a un dels seus servents anomenat *Mian Bogao* portar dos cignes a l'emperador. *Mian* es va posar camí cap a la distant capital imperial amb els dos cignes. En el camí es va trobar amb un llac. Llavors va tenir la brillant idea de posar els cignes a l'aigua perquè nedessin una estona i d'aquesta manera, podrien netejar-se el plomatge que se'ls hi havia embrutat en el seu llarg viatge. Estava segur que l'emperador apreciaria molt més dos cignes blancs i nets, més que els bruts que ara tenia.

Però tan aviat quan *Mian* va deixar les aus a l'aigua, els ingrats cignes varen arrencar el vol i aviat els va perdre de vista. Només unes quantes plomes va romandre a la vora del llac. *Mian* es va entristir al moment i es preguntà que presentaria llavors a l'emperador. Es va posar les plomes a la butxaca i amb tristesa es va tornar a posar en camí.

En arribar al palau imperial, va veure que l'emperador estava envoltat de missatgers que li presentaven meravellosos regals. Quan va arribar el seu torn, *Mian* va oferir una ploma de cigne al emperador amb aquest poema:

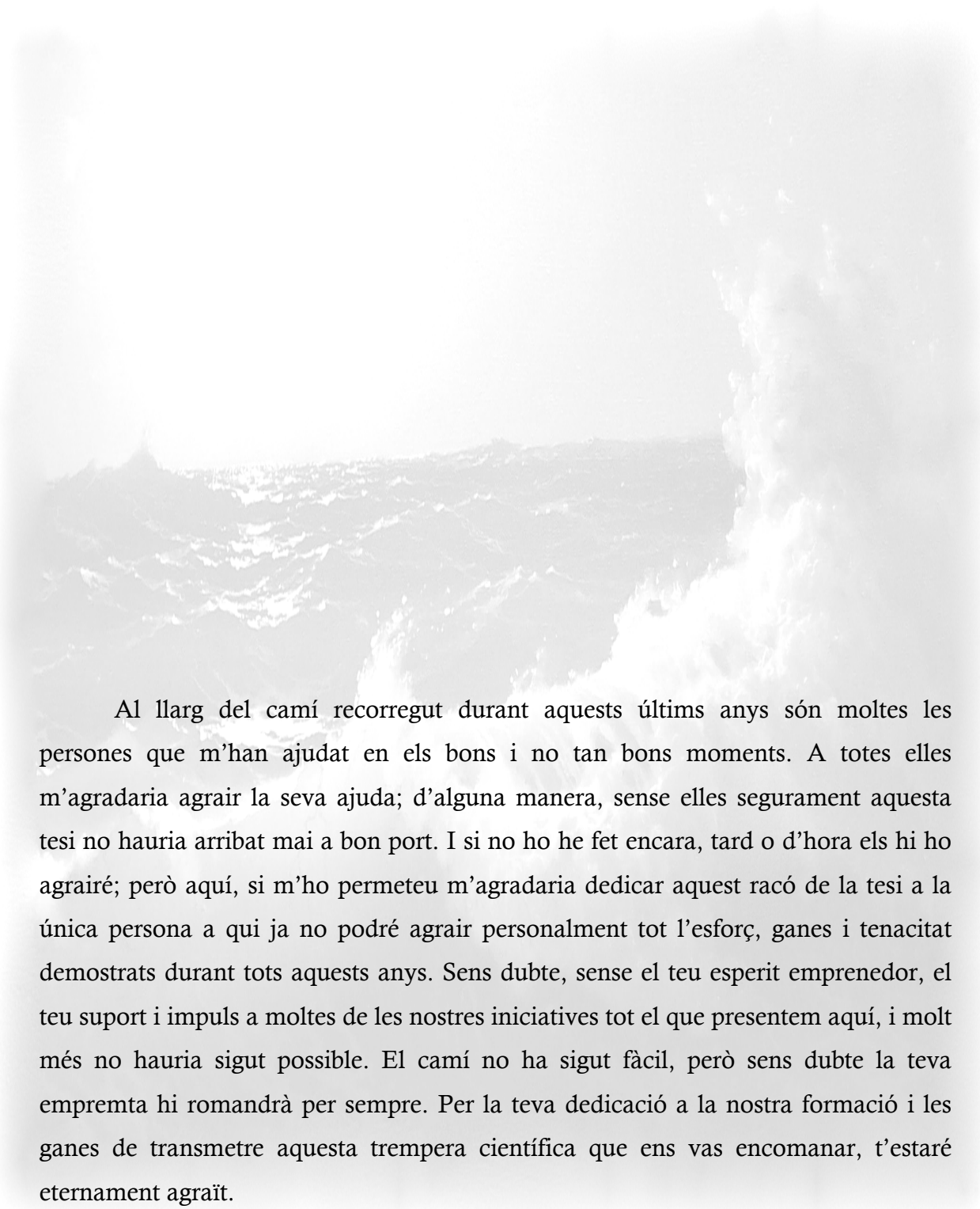*"He viatjat milers de milles per mostrar-vos el meu respecte.*
Però en el meu camí, he perdut els vostres cignes al llac.
Us demano disculpes Altesa, però us juro que el meu respecte per l'emperador és genuí"

L'emperador va quedar impressionat per la sinceritat de *Mian* que es va declarar satisfet amb aquell present. Aquest incident és recordat en la cultura xinesa amb la dita *qiān lǐ sòng ér máo*, literalment, "recórrer mil milles i presentar una ploma com a regal"; que significa que és la intenció i no el regal el que realment importa.

Agraïments

Al llarg del camí recorregut durant aquests últims anys són moltes les persones que m'han ajudat en els bons i no tan bons moments. A totes elles m'agradaria agrair la seva ajuda; d'alguna manera, sense elles segurament aquesta tesi no hauria arribat mai a bon port. I si no ho he fet encara, tard o d'hora els hi ho agrairé; però aquí, si m'ho permeteu m'agradaria dedicar aquest racó de la tesi a la única persona a qui ja no podré agrair personalment tot l'esforç, ganes i tenacitat demostrats durant tots aquests anys. Sens dubte, sense el teu esperit emprenedor, el teu suport i impuls a moltes de les nostres iniciatives tot el que presentem aquí, i molt més no hauria sigut possible. El camí no ha sigut fàcil, però sens dubte la teva empremta hi romandrà per sempre. Per la teva dedicació a la nostra formació i les ganes de transmetre aquesta trempera científica que ens vas encomanar, t'estaré eternament agraït.

Allà on siguis Xavier, ...

... aquest trosset de ciència, és per a tu.

Contingut

L'estudi de l'ecologia de les aus a través de les seves plomes: aplicacions ecològiques dels biomarcadors intrínsecs

Raül Ramos

Resum Aquells aspectes relacionats amb la conservació de la fauna salvatge són particularment rellevants en recerca, com són la delimitació de l'ecologia tròfica i dels moviments espacials de les espècies o de determinades poblacions. Aquest coneixement és particularment necessari en ambients marins on les activitats humanes i els consegüents canvis globals tenen un gran impacte, generant grans fluctuacions en moltes poblacions d'organismes marins. En aquest treball explorem l'ús de l'anàlisi dels biomarcadors intrínsecs com signatures d'isòtops estables o concentracions d'elements traça en l'avaluació de l'ecologia tròfica i dels hàbitats d'alimentació de les espècies, així com en el seguiment dels moviments d'espècies migratòries. En primer lloc, aquest estudi aporta un nou coneixement a l'actual sobre els processos d'integració dels elements en els teixits animals, posant l'accent en el diferent comportament dels isòtops estables i alguns elements traça com el mercuri, seleni o el plom. Mentre els isòtops estables són directament transferits des de la dieta als teixits durant la seva formació (origen exogen), els elements traça es mobilitzen des de diversos òrgans interns, on s'emmagatzemen (origen endogen). En segon lloc, hem demostrat el valor de les anàlisis isotòpiques en diferents tipus d'estudi, des d'estudis de dieta i zones d'alimentació, fins a l'estudi de diferents processos relacionats amb el cicle anual de les aus, com són la migració i la muda. Hem proporcionat clares evidències que els isòtops estables de determinats teixits funcionen com una empremta que roman inert al llarg del temps, i que poden ser usats per a estudiar l'ecologia d'alimentació de la fauna salvatge, així com per seguir els seus moviments en el medi marí. Les diferències isotòpiques entre diferents tipus de preses o entre regions oceàniques distants s'integren en el teixit d'un determinat individu, amb la qual cosa l'anàlisi isotòpic d'aquest teixit pot indicar la dieta seguida quan aquest teixit es va formar o, alternativament, la regió en què es va formar. D'altra banda, també hem demostrat que per a les espècies migratòries que es mouen entre zones amb signatures isotòpiques basals diferents i per a aquelles espècies amb una dieta diferencial al llarg de l'any, les similituds i les diferències isotòpiques entre les diferents plomes es poden utilitzar per avaluar la fenologia de muda d'aquestes plomes en relació al cicle anual de les aus. Finalment, els resultats d'aquest estudi no només permeten constatar la solidesa dels marcadors biogeoquímics en els estudis d'ecologia animal, sinó que també aporten noves prespectives en diversos camps com l'epidemiologia, la gestió i conservació del medi, els estudis migratoris, o els estudis d'impacte ambiental de la contaminació.

Introducció i antecedents

Tenim clares evidències que les activitats humanes i els canvis globals resultants tenen un gran impacte en particular sobre els ecosistemes marins (Halpern et al. 2008), cosa que desencadena sovint en greus fluctuacions poblacionals de molts organismes marins. Aquells aspectes relacionats amb la conservació de la vida marina que afecten negativament la seva dinàmica poblacional són doncs especialment rellevants en recerca. No obstant això, no només les amenaces derivades de l'activitat humana que redueixen les poblacions de fauna marina tenen un interès creixent en el món de la conservació. Una comprensió més profunda del per què algunes poblacions marines esdevenen sobreabundants és cada cop més necessari. Per tant, qualsevol estratègia de gestió efectiva en els ambients marins requereix d'una acurada avaluació de les causes en la disminució poblacional d'espècies marines amenaçades, però també del per

què en l'augment en les poblacions d'algunes espècies problemàtiques.

Aquests augments demogràfics en el medi marí s'han atribuït a diversos factors, com ara la reducció de depredadors que regulen l'estructuració de les xarxes tròfiques o la creixent disponibilitat de nutrients i recursos d'origen antròpic (des d'aigües eutrofitzades fins a restes d'abocadors o descarts de pesqueries industrials; Vidal et al. 1998; Purcell et al. 2007). L'augment dels conflictes entre aquestes expansions demogràfiques i diversos interessos humans, particularment aquells relacionats amb la salut pública (per exemple, floracions de meduses o la disseminació d'enteropatògens per part d'aus marines), destaquen la importància de comprendre les interaccions de cadenes alimentàries, així com l'origen dels recursos tròfics d'aquests organismes. D'altra banda, els episodis de contaminació, com ara vessaments de cru i les activitats de pesca massiva, com la pesca amb palangre, són responsables de la mort directa de centenars de milers de vertebrats marins arreu del món, la qual cosa porta a una disminució de la major part d'espècies de taurons, tortugues, dofins, foques i aus marines (Jennings et al. 2001; Lewison et al. 2004; Pauly et al. 2005; BirdLife International 2008). Per tant, delimitar l'ecologia tròfica i delinear els moviments espacials d'aquestes poblacions permetrà avaluar la interacció espacial amb aquestes activitats humanes, coneixement indispensable per a qualsevol gestió eficaç en la conservació del medi marí.

La importància de l'ecologia tròfica en estudis de gestió de la conservació

Per una banda, un coneixement precís de la composició de la dieta és necessària en diverses àrees clau de l'ecologia aplicada, com ara la conservació d'espècies en perill d'extinció o la gestió d'espècies problemàtiques (Thomas 1972; Garrott et al. 1993). La recerca sobre la variació espaciotemporal de l'ecologia tròfica i les interaccions entre els hàbitats explotats i les activitats humanes, són indispensables per a comprendre quan les espècies són més vulnerables o quan les poblacions poden ser més sensibles a accions com ara el descast selectiu (Fear 1991; Martin et al. 2007). D'altra banda, en utilitzar la fauna salvatge pel biomonitoreig de la contaminació ambiental, un coneixement precís sobre els seus hàbits d'alimentació és també necessari per a interpretar correctament el significat dels nivells de contaminants observats (Becker 2003). Finalment, en avaluar el paper de la fauna silvestre com a reservoris i difusors d'agents infecciosos, descripcions precises de la dieta per cada població són també necessàries per comprendre plenament l'origen de la prevalença del patogen (Daszak et al. 2000; Blanco et al. 2006).

En general, la disponibilitat d'aliments és un factor determinant en la dinàmica poblacional de la majoria de les espècies, així com també del seu èxit reproductor (Oro et al. 2006). En aquest sentit, les fonts d'aliment derivades de l'activitat humana solen ser abundants i relativament predictibles, fet que augmenta la capacitat de càrrega d'un ecosistema i permet una millora en l'èxit reproductor de la fauna salvatge, i probablement, de la seva supervivència (Pons 1992; Purcell et al. 2007). Durant les últimes dècades, moltes espècies de vertebrats han augmentat les seves poblacions com a conseqüència de l'augment d'aquests recursos tròfics (Garrott et al. 1993), i en la majoria dels casos, aquestes comunitats han esdevingut superpoblades i sovint problemàtiques. Aquest excés de població s'atribueix a la flexibilitat, oportunisme, i a la naturalesa gregària de determinades espècies que les fa altament adaptades a viure en hàbitats modificats per l'home (Fear 1991). En particular, les gavines *Larus* sp. han sigut àmpliament estudiades com a una espècie potencialment superabundant en nombroses localitats arreu del món (Steele i Hockey 1990; Belant et al. 1993; Vidal et al. 1998; Bertellotti et al. 2001). El seu impacte negatiu sobre diversos interessos humans, com ara aeroports o dipòsits d'aigua (Monaghan et al. 1985; Dolbeer et al. 1997), i les molèsties i efectes de seva depredació sobre espècies en perill d'extinció (Thomas, 1972; Oro et al. 2005) han fet que les autoritats de gestió ambiental sovint hagin pres part activa en el control de la dinàmica poblacional d'aquestes gavines. En aquest sentit, qualsevol mesura de gestió per controlar eficientment les poblacions sobredimensionades haurien de centrar-se a limitar la disponibilitat de recursos tròfics, reduint així la producció poblacional (Fear 1991; Kilpi i Ost 1998). Per tant, l'establiment de les preferències tròfiques d'aquestes poblacions és essencial alhora de prendre decisions en la seva gestió, facilitant la predicció de canvis i conseqüències en la dinàmica poblacional de fauna salvatge.

La contaminació de les xarxes tròfiques marines és avui en dia de gran interès ambiental a causa dels creixents nivells de contaminació dels ecosistemes marins provinents de diferents fonts antròpiques (Halpern et al. 2008). Les emissions i abocaments de contaminants, però, no són uniformes en l'espai i les diferències en els nivells de contaminants en els organismes marins són difícils d'estudiar perquè la composició d'espècies canvia en els diferents oceans (Becker 2003). En aquest sentit, els depredadors marins situats a les parts altes de la xarxa tròfica poden resultar ser molt bons indicadors de la dinàmica espaciotemporal dels contaminants ja que moltes d'aquestes espècies tenen àmplies distribucions de cria i duen a terme llargues migracions (Monteiro i Furness 1995). Aquestes característiques ofereixen l'oportunitat de comparar nivells de contaminants de poblacions remotes, així

com els nivells de les zones de cria amb les d'hivernada. D'altra banda, degut a la seva abundància, àmplia distribució i hàbits d'alimentació oportunistes, les gavines poden ser emprades per a mesurar aquests nivells de contaminació en ambients influïts per l'home (Sanpera et al. 2007). Pel que fa a la salut humana, les gavines poden resultar particularment útils pel biomonitoreig de contaminants, ja que es beneficien principalment dels mateixos recursos consumits pels éssers humans, és a dir, descarts pesquers provinents de pesqueries i restes de carn procedents d'abocadors. A més, els seus estesos hàbits d'alimentació relacionats amb el consum de deixalles els han convertit en un blanc fàcil alhora de trobar culpables de deteriorar la salut ambiental a través de la contaminació fecal d'aigua potable o d'aigües d'esbarjo (Benton et al. 1983; Lévesque et al. 2000). Així doncs, tant les anàlisis de contaminants com els estudis microbiològics en aquestes espècies carronyaires són especialment rellevants per a les ciències que aborden la salut pública. No obstant això, d'entre els factors que poden contribuir més a la càrrega corporal de contaminants o a la presència de patògens en la fauna salvatge, és l'ecologia tròfica i els hàbits d'alimentació els que s'han descrits com a més rellevants (González-Solís et al. 2002; Becker et al. 2002; Broman et al. 2002). Així doncs, quan la fauna marina és utilitzada per al biomonitoreig del nivell de contaminants, o alhora d'avaluar el risc que les gavines poden suposar per a la salut pública, els hàbitats d'alimentació i les relacions tròfiques de cada població han de ser també determinats amb precisió per interpretar correctament després el significat dels contaminants observats o els nivells de patògens (Furness i Camphuysen 1997; Becker 2003).

La importància d'entendre els moviments de la fauna marina

Tan important com determinar els principals recursos alimentaris de la fauna marina és el de localitzar les seves principal zones d'alimentació en els diferents moments del seu cicle vital. Diverses amenaces antropogèniques, com la sobrepesca, la captura incidental, la contaminació o l'escalfament global posen en risc la vida de milions de vertebrats marins durant els seus moviments anuals. Per tant, la comprensió de la dinàmica espaciotemporal d'aquests vertebrats és essencial per a determinar on i quan aquests animals estan més exposats als impactes humans. En aquest sentit, un coneixement precís sobre els moviments de llarga distància de les espècies migratòries que es mouen a través de les fronteres geopolítiques és d'extrema urgència per a desenvolupar mesures comuns i eficaces de conservació en les diferents jurisdiccions (Marra et al. 2006; Martin et al. 2007).

Els grans depredadors pelàgics com ara els petrells i baldrigues són animals de vida llarga, amb un retard en la seva maduresa sexual, amb una alta supervivència adulta i una relativa baixa taxa de reproducció (Warham 1990; Brooke 2004). Per tant, qualsevol factor addicional que augmenti la taxa de mortalitat adulta té un fort impacte negatiu en la dinàmica d'una població i de l'espècie en el seu conjunt. Avui en dia, la pesca amb palangre és considerada l'amenaça global més greu a la qual s'enfronten les aus marines, provocant una disminució d'efectius en la majoria de les seves poblacions (Brothers et al. 1999; BirdLife International 2008). La captura incidental d'aus marines és una causa emergent d'interès en la pesca amb palangre, no només per l'augment de la preocupació mediambiental sobre la mortalitat d'aus marines, però també per l'augment en les pèrdues d'esquer viu dels palangrers, i per la conseqüent reducció en l'eficiència de les arts de pesca (Weimerskirch et al. 1997). Per tant, un coneixement exacte de les zones d'alimentació utilitzades per les poblacions d'ocells marins, així com la comprensió dels seus moviments estacionals semblen obligatoris per a mitigar l'impacte d'aquestes activitats humanes en el medi marí. Aquesta informació no només ha d'aportar nous punts de vista en la gestió de la pesca amb palangre, sinó també en el disseny dels parcs eòlics a mar obert o en l'avaluació dels efectes dels vessaments de cru en els ecosistemes marins.

Eines tradicionals per a l'estudi de l'ecologia espacial i tròfica de les aus

Durant aquests anys, l'ecologia espacial i tròfica s'han abordat bàsicament a través de metodologies convencionals que han proporcionat informació essencial en molts aspectes. No obstant això, la precisió d'aquests tècniques tradicionals alhora d'estudiar l'ecologia tròfica i els trets migratoris de les espècies sovint s'ha demostrat limitada a causa de diversos inconvenients, biaixos en els mostreigs o restriccions econòmiques (Barrett et al. 2007; Hobson i Norris 2008). Estudis de dieta basats en observacions de camp estan sovint esbiaixats envers les preses més aparents. Normalment, en els estudis de dieta que analitzen continguts estomacals o regurgitats, les preses són difícils d'identificar, ja que sovint estan parcialment digerides. Per tant, aquests estudis solen estar esbiaixats envers aquells tipus de preses més resistents a la digestió, el que du sovint a una sobreestima en la reconstrucció final de la dieta (González-Solís et al. 1997; Votier et al. 2003). A més, aquestes metodologies proporcionen només una visió episòdica de la dieta d'un individu, ja que cada mostra representa només un petit àpat en la dieta d'un individu i no proporciona cap tipus d'informació sobre els recursos utilitzats en el passat. Per tant, un seguiment exhaustiu en el temps

Requadre 1: Què és un isòtop estable?

Diversos elements químics mostren variacions en el seu pes atòmic, com a conseqüència de tenir un nombre diferent de neutrons en el seu nucli. Tot i això, les seves característiques físiques i químiques no canvien substancialment. Cada classe de pes de cada element es coneix com a isòtop, i se'ls anomena estables, ja que no es degraden amb el pas del temps (no radioactius; Hoefs 2004).

Hi ha molts elements amb múltiples formes isotòpiques estables, però només els relacionats amb la biosfera (plantes, animals), la hidrosfera (aigua), i l'atmosfera (gasos) són comunament utilitzats en recerca ecològica, és a dir, els de carboni, nitrogen, sofre, hidrogen i oxigen (West et al. 2006). La forma més abundant de carboni és l'isòtop ^{12}C (98,90%), però també hi ha un isòtop pesat, ^{13}C, menys representat (1,10%). El nitrogen també presenta dues formes isotòpiques, el ^{14}N, sent l'isòtop més comú (99,63%), i el ^{15}N que només ocorre en una petita proporció (0,37%). La forma més abundant de sofre és el ^{32}S (95,02%), però hi ha altres tres isòtops, ^{34}S (4,21%), ^{33}S (0,75%), i una proporció molt petita de ^{36}S (0,02%). El deuteri (^{2}H), amb un protó i un neutró en el seu nucli, és l'isòtop estable d'hidrogen més pesat amb una baixa abundància natural (0,02% en els oceans), mentre que l'hidrogen més comú (^{1}H) al nucli no hi conté neutrons. Finalment, l'isòtop més abundant de l'oxigen a l'atmosfera és ^{16}O (99,76%); ^{17}O i ^{18}O es presenten només en petites proporcions (0,04% i 0,20%, respectivament; Faure i Mensing 2005).

Els espectròmetres de masses són instruments que proporcionen una estimació molt precisa de la relació entre els isòtops més pesats i els més lleugers en una mostra desconeguda respecte a uns estàndards internacional. Les relacions isotòpiques s'expressen convencionalment com a valors δ en parts per mil (‰) d'acord amb la notació delta següent:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1.000$$

on X (‰) és ^{13}C, ^{15}N, ^{34}S, ^{2}H (també D, de deuteri) o ^{18}O i R és la proporció corresponent a ^{13}C/^{12}C, ^{15}N/^{14}N, ^{34}S/^{32}S, ^{2}H/^{1}H o ^{18}O/^{16}O de la mostra i dels materials de referència. $R_{standard}$ de ^{13}C és Pee Dee belemnites (PDB), per a ^{15}N és el nitrogen atmosfèric (AIR), per a ^{34}S és troilite del meteorit Diablo Canyon (CDT) i ^{2}H i ^{18}O de Viena és l'estàndard mitjà d'aigua oceànica (V-SMOW, Werner i Brand 2001). Atès que aquests estàndards internacionals són arbitraris, algunes proporcions d'isòtops són positives (és a dir, més enriquit en l'isòtop pesat en relació a l'estàndard) mentre que altres són negatives (és a dir, més empobrit en l'isòtop pesat).

esdevé llavors necessari per a obtenir informació fiable sobre els hàbits alimentaris d'una determinada població (Votier et al. 2001; Jordan 2005). Finalment, com que la majoria d'espècies són difícils de mostrejar fora de la temporada de cria, especialment aquelles espècies lligades als ambients marins, molts dels estudis de la dieta basats en mètodes convencionals només se centren en les preferències alimentàries del període de cria, obviant per complet el període no reproducció (però vegeu Dalerum i Angerbjörn 2005). No obstant això, com que les variacions estacionals en la dieta poden ser claus alhora d'explicar certes tendències en les dinàmiques d'una població, avaluacions precises de la utilització temporal i espacial dels recursos alimentaris sovint són essencials per a una fructífera gestió de la conservació o per biomonitoreigs de qualsevol tipus (Stewart et al. 1994; Fuller i Sievert 2001; Becker 2003).

Tradicionalment, els patrons i les rutes migratòries dels animals han estat estudiades a través de marcadors extrínsecs, marcant o anellant individus (Berthold et al. 2003; Hobson i Norris 2008). Aquests estudis es basen en tècniques de captura-recaptura que esperen recapturar individus marcats prèviament en altres localitats; tot i que aquests mètodes solen presentar molt baixes taxes de recuperació (menys del 0,01%). Aquests tècniques tradicionals requereixen un gran esforç de seguiment a través del temps (de diverses dècades) per a

obtenir informació fiable sobre els moviments migratoris dels animals (Berthold et al. 2003). D'aquesta manera, les àrees d'hivernada i principals rutes migratòries han estat més o menys definides per a diverses espècies terrestres (Elphick 2007; Newton 2008), tot i que aquestes metodologies tradicionals han tingut menys èxit en els ambients marins. A causa de l'evident reduïda recuperació d'individus a mar obert durant l'hivern, el coneixement del grau de connectivitat entre les distants poblacions de cria i els quarters d'hivernada de les espècies migratòries marines és encara insuficient. Malgrat els avenços recents en tècniques de seguiment via satèl·lit que permeten esmenar parcialment la manca actual de coneixement en la migració marina, aquests estudis solen limitar-se a uns pocs individus sovint seguits només durant curts períodes de temps a causa de limitacions logístiques i econòmiques (Luschi et al. 1998; Block et al. 2001; Bonfil et al. 2005; González-Solís et al. 2007; Semmens et al. 2007).

Com a resultat d'aquestes desavantatges i limitacions en el coneixement de l'ecologia espacial i tròfica, hi ha un creixent interès per a usar biomarcadors intrínsecs, com ara els isòtops estables (Requadre 1) o la concentració d'elements traça, tan per a determinar els hàbits d'alimentació com per a identificar i vincular els diferents llocs de cria i d'hivernada d'una gran varietat d'espècies en el medi marí (Cherel et al. 2000; Semmens et al. 2007;

Requadre 2: Què fa els isòtops estables rellevants en estudis de dieta?

Tot i que les propietats de les formes isotòpiques no canvien dràsticament entre elles, les diferents característiques termodinàmiques produeixen petites diferències en la cinètica de les reaccions bioquímiques on estan implicades (White 2001; Hoefs 2004). Aquestes diferències en les formes isotòpiques produeixen un enriquiment o un empobriment dels isòtops pesats en relació als isòtops lleugers. Un enriquiment succeeix quan l'isòtop estable més pesant s'acumula en el producte (en relació amb el substrat), mentre que l'isòtop més lleuger és preferiblement eliminat. D'altra banda, un empobriment es produeix quan s'afavoreix l'isòtop més lleuger. Els processos d'enriquiment i d'empobriment es coneixen en general amb el nom de fraccionament o de discriminació (Post 2002).

Hobson i Clark (1992) van descriure com a fraccionament els canvis en el senyal isotòpic entre la dieta i els teixits dels consumidors com a conseqüència de dos factors principals, l'assimilació bioquímica selectiva de components de la dieta respecte les diferents signatures isotòpiques, i la discriminació isotòpica. La funció que descriu aquesta relació és la següent:

$$D_t = D_d + \Delta_{dt}$$

on D_t és la signatura isotòpica de teixit del consumidor, D_d és la signatura isotòpica de la dieta, i Δ_{dt} és el factor de fraccionament isotòpic entre la dieta i el teixit del consumidor. Des del punt de vista ecològic, a mesura que l'energia es transmet al llarg dels ecosistemes, el fraccionament isotòpic té lloc, resultant en alteracions de les signatures isotòpiques dels consumidors respecte de la seva dieta. És a dir, que aquest fraccionament isotòpic es produeix en cada nivell tròfic, i se suma al llarg de les cadenes tròfiques. Per tant, una vegada considerades les assumpcions necessàries, l'anàlisi d'isòtops estables esdevé una valuosa eina en la reconstrucció quantitativa dels fluxos d'elements al llarg de les xarxes tròfiques, que poden ajudar a respondre preguntes rellevants en ecologia tròfica (Gannes et al. 1997; Bearhop et al. 2004).

Rooker et al. 2008; Caut et al. 2008). Tot i que els marcadors biogeoquímics intrínsecs no ofereixen el detall taxonòmic que ofereixen per exemple l'anàlisi de regurgitats o la precisió geogràfica proporcionada pels marcadors extrínsecs (com ara la recuperació d'una anella o el seguiment via satèl·lit), la seva anàlisi evita la majoria dels biaixos i limitacions relacionades amb les tècniques convencionals. Per exemple, alhora d'avaluar l'ecologia tròfica a través de l'anàlisi d'isòtops estables o de les concentracions d'elements traça en un determinat teixit, només els elements assimilats durant la digestió, són considerats, evitant així la majoria dels biaixos relacionats amb la digestibilitat de les preses (Gannes et al. 1997). A més, a diferència de l'anàlisi de regurgitats on cada mostra representa només un fenomen d'alimentació, l'anàlisi de marcadors biogeoquímics intrínsecs proporciona un resum de la dieta seguida durant un període de temps per un determinat individu (Post 2002). Pel que fa als moviments migratoris, l'ús de marcadors biogeoquímics intrínsecs permet incrementar també el coneixement actual en la dinàmica migratòria de moltes espècies, ja sigui perquè les anàlisis biogeoquímiques es poden realitzar de manera extensiva amb relativament poques limitacions, o bé perquè la recuperació posterior dels animals no és necessària (Hobson 1999). A més, l'ús de marcadors biogeoquímics intrínsecs és particularment avantatjós en aquest camp de l'ecologia, ja que cap altre marcador intrínsec (dades biomètriques o anàlisis genètiques) pot proporcionar informació sobre les zones d'hivernada (Hobson i Norris 2008).

Interès ecològic dels marcadors biogeoquímics

Normalment, els elements i formes isotòpiques assimilades a través de la dieta s'incorporen i són fraccionats en els diferents teixits animals. A mesura que l'energia és transferida a través dels ecosistemes, el fraccionament dels elements se succeeix, resultant-ne alteracions de les signatures isotòpiques dels consumidors en relació a la seva dieta (Hobson i Clark 1992). Aquest fraccionament és ben definit quan es considera l'anàlisi d'isòtops estables (Requadre 2), per bé que també ocorre quan alguns elements traça com el mercuri són considerats (Honda et al. 1987; Cabana i Rasmussen 1994; Becker et al. 2002). Aquesta amplificació es produeix a cada nivell tròfic, i se suma al llarg de les cadenes tròfiques, el que permet d'alguna manera ser utilitzat per a investigar les preferències alimentàries dels consumidors, així com per a determinar l'amplada de seu nínxol tròfic (Hobson et al. 1994; Post 2002). No obstant això, cal tenir en compte que a gran escala, les concentracions biogeoquímiques d'aquestes xarxes tròfiques poden diferir inherentment a nivell basal, emmascarant qualsevol relació entre consumidors i preses (Gannes et al. 1997). Així doncs, des d'un punt de vista tròfic pot resultar ser inapropiat utilitzar les concentracions dels elements traça i les signatures isotòpiques dels grans consumidors com a mitjanes absolutes per comparar-les directament entre zones remotes (Requadre 3). Molt probablement, aquesta limitació aviat s'esmenarà amb l'ús de les anàlisis isotòpiques de composició específica (CSIA, compound-specific isotopic analyses) d'aminoàcids tròfics versus d'origen (Lorrain et al. 2009). No obstant això, aquesta variabilitat geogràfica en els nivells basals, també pot ser utilitzada per a seguir els moviments animals al llarg de zones remotes

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Requadre 3: Què poden dir-nos els isòtops estables?

A escala local, tres isòtops estables (generalment els de C, N i S) són comunament mesurats per a establir els orígens alimentaris i les relacions tròfiques en estudis de dieta (Peterson i Fry 1987). Els isòtops estables de C (δ^{13}C) estan presents en les tres macromolècules tròfiques, proteïnes, greixos i hidrats de carboni, el que pot arribar a reflectir les diverses fonts d'aliments dels consumidors en els seus teixits. Els isòtops estables de N (δ^{15}N) en els teixits dels consumidors només reflecteixen el metabolisme proteic de la dieta, ja que el nitrogen és bàsicament absent en lípids i carbohidrats. El sofre en els teixits dels consumidors és només present en aminoàcids tals com la cisteïna i la metionina. Per tant, l'anàlisi d'isòtops estables de S (δ^{34}S) també representa el flux proteic de dieta. Típicament, els isòtops de C i S permeten el seguiment de l'origen dels elements que s'incorporen a les xarxes tròfiques (Krouse i Herbert 1988; Hobson et al. 1994). Grans diferències entre els δ^{34}S d'ambients marins i terrestres, converteixen a aquests isòtops en una eina particularment útil alhora de detectar i distingir entre fonts d'aliments heterogènies (Peterson et al. 1985). En canvi, els valors de δ^{15}N solen utilitzar-se per a inferir informació sobre interaccions entre xarxes tròfiques i per mostrar l'estatus tròfic de les espècies (Vanderklift i Ponsard 2003).

    A gran escala, degut principalment a la variabilitat geogràfica en l'estructuració de xarxes tròfiques o en les peculiaritats biogeoquímiques inherents a cada zona, moltes de les proporcions d'isòtops estables poden ser utilitzades com a simples marcadors bioquímics de la zona on el teixit d'un consumidor es va formar (Kelly et al. 2008; Hobson i Norris 2008). L'abundància natural dels isòtops estables d'hidrogen i d'oxigen (δ^{2}H i δ^{18}O, respectivament) són particularment emprats en els estudis de migració de la fauna silvestre. Tot i que el carboni, hidrogen i oxigen són presents en les tres macromolècules que componen la dieta, el repte més interessant aquí és el fet que una part d'aquests isòtops dels teixits del consumidor deriven de l'aigua presa o de l'aire inhalat. Com que les molècules d'aigua que contenen els isòtops més lleugers d'hidrogen i d'oxigen són més probablement evaporades i precipitades, extensos gradients isotòpics lligats al cicle global de l'aigua han sigut descrits, permetent ràpides assignacions geogràfiques de la formació de determinats teixits dels consumidors (Bowen et al. 2005). A més de les δ^{2}H i δ^{18}O, altres signatures isotòpiques, com les de δ^{13}C i δ^{34}S també varien geogràficament, per tant a nivell global aquestes signatures proporcionen una empremta geogràfica extremadament valuosa per al seguiment de moviments migratoris.

(Hobson 1999; Webster et al. 2002). Típicament per a les espècies migratòries de llarga distància, les zones de cria i d'hivernada estan separades per milers de quilòmetres de distància. Les diferents concentracions isotòpiques i elementals de les xarxes tròfiques situades en aquestes zones remotes poden ser identificades d'alguna manera en els teixits d'aquests animals migratoris, i permetre a la vegada traçar la seva migració. Això té especial rellevància en la biologia de la conservació, ja que ofereix noves eines per a l'estudi de la connectivitat migratòria entre poblacions d'una mateixa espècie (Hobson 2005a). No obstant això, aquesta variabilitat geogràfica dels marcadors biogeoquímics en els ambients marins és encara poc coneguda.

    La selecció del teixit a analitzar és una qüestió molt important en el disseny de qualsevol estudi ecològic basat en l'anàlisi de marcadors biogeoquímics. El període espaciotemporal integrat pels marcadors biogeoquímics intrínsecs depèn del creixement i la taxa de renovació dels teixits analitzats (Rubenstein i Hobson 2004). Els teixits amb una alta taxa de renovació solen integrar elements biogeoquímics i isòtops estables incorporats en un passat relativament recent, però els teixits que són renovats lentament tendeixen a integrar elements i formes isotòpiques d'un llarg període de temps (Hobson i Clark 1992). Els teixits queratinosos d'una gran varietat d'organismes, com

ara el pèl, les barbes de les balenes, ungles, escates o les plomes són especialment avantatjosos en recerca ecològica (Hobson i Clark 1992; Bearhop et al. 2003; Reich et al. 2007). En primer lloc, el seu mostreig és especialment recomanable en estudis que tracten amb espècies amenaçades o en perill d'extinció, ja que són els teixits més simples, menys molestos i no letals alhora de mostrejar per a estudis biogeoquímics. No obstant això, més rellevant és que, durant la formació d'aquest tipus de teixits, els elements estructurals s'incorporen directament des de la ingesta diària, restant químicament inerts un cop formats. El posterior anàlisi biogeoquímic d'aquests teixits queratinosos reflecteix doncs la composició de la dieta del lloc on van créixer i on van ser formats (Hobson 2005b). Entre ells, però, les plomes tenen un valor excepcional, ja que a més dels avantatges anteriorment citats, com una sola unitat, una ploma creix relativament ràpid i té un creixement determinat i definit. A més, els patrons de muda són estacionalment predictibles i bastant constants al llarg del temps; així doncs anàlisis específiques en determinades plomes poden proporcionar valuosa informació biogeoquímica d'un període espaciotemporal molt concret, independentment de la data del mostreig de les plomes (Hobson 2005b; Inger i Bearhop 2008). Per tant, per a aquelles espècies d'aus amb el patró de muda i el temps de formació de les plomes coneguts,
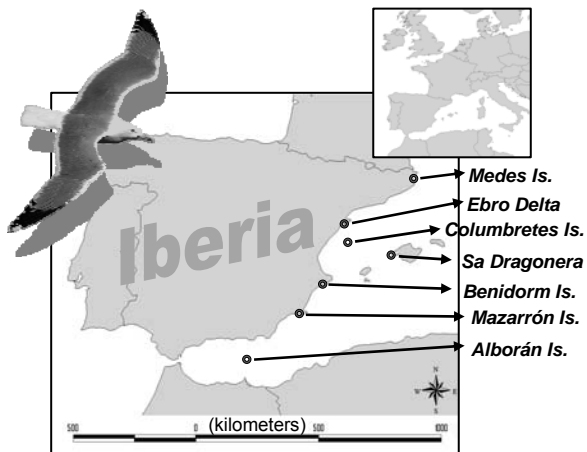
Fig. 1 Mapa de la Península Ibèrica amb les colonies de cria de gavià de potes grogues *Larus michahellis* incloses en aquest estudi.

plomes específiques poden ser mostrejades en qualsevol moment de l'any per examinar els seus hàbits alimentaris i la ingesta de metalls pesats en determinats períodes de temps (Hobson 2008). Complementàriament, considerant que els nivells basals d'elements biogeoquímics varien geogràficament i que són bastant consistents entre anys, a través de l'anàlisi de determinades plomes d'un sol individu els investigadors poden obtenir informació simultània de les diferents àrees en què aquestes plomes s'han format, com poden ser la zona de cria i d'hivernada (Cherel et al. 2000; Hobson et al. 2004). Per tant, mitjançant l'anàlisi dels marcadors bioquímics intrínsecs de determinades plomes d'aus migratòries, el nostre coneixement actual sobre una amplia gamma d'afers migratoris que afecten als ocells pot millorar enormement (Hobson 2008).

No obstant això, la nostra comprensió de les estratègies de muda sol veure's reduïda degut al coneixement insuficient dels patrons de muda, especialment en relació amb els moviments migratoris de les espècies. Això és particularment greu en les aus marines, probablement degut als seus hàbits pelàgics que les fan generalment inaccessibles durant el període no reproductor, quan en general aquestes aus muden la majoria de les seves plomes (Bridge 2006). És per això, la major part del nostre coneixement sobre els patrons de muda de les aus marines es limita a la temporada de cria, quan les aus són fàcilment accessibles als investigadors

Fig. 2 Localitats de cria i d'hivernada de la baldriga cendrosa *Calonectris diomedea*. Principals àrees d'alimentació de les baldrigues cendroses al final del període reproductor, entre agost i octubre (llegendes en groc), període on la majoria de les baldrigues cendroses muden la primera primàvia i durant l'època d'hivernada, entre desembre i gener (llegendes en blau cel), quan la majoria de les bandrigues muden la vuitena secundària. Els rangs d'activitat són derivats de l'anàlisi *kernel* que abarca des del 5 (tons clars) fins al 90% (tons foscos) de les localitzacions validades. El nombre d'aus incloses en cada regió es mostra en parentesi. Les localitats de mostreig es mostren amb creus. Fotografia cortesia d'Albert Cama.

(Weimerskirch 1991; Monteiro i Furness 1996). Observacions directes a alta mar des de vaixells han proporcionat informació valuosa sobre la muda d'aquestes aus durant els períodes entre cries successives, ja que les aus poden ser observades mudant activament les seves plomes de vol (Brown 1990; Camphuysen i Van Der Meer 2001). En aquest sentit, però, resultats més robustos i un coneixement més precís poden ser obtinguts a partir de l'anàlisi biogeoquímic de les plomes d'aquelles espècies amb patrons de muda poc coneguts (Camphuysen i Van Der Meer 2001). Com que els elements traça i les formes isotòpiques s'integren en les plomes en el moment de la seva formació, les similituds en composició biogeoquímica entre les plomes d'un mateix individu pot revelar on aquestes plomes han sigut formades i per tant es poden arribar a dilucidar seqüències de muda d'acord amb els moviments migratoris de les espècies.

Objectius de l'estudi, espècies objecte d'estudi i recerca específica

L'objectiu principal d'aquesta tesi doctoral és el de brindar noves prespctives en l'ús de les anàlisis biogeoquímiques en la gestió i conservació de la fauna silvestre. La nostra recerca se centra en l'ús i l'aplicabilitat de la composició biogeoquímica de plomes per entendre diferents aspectes de l'ecologia tròfica i espacial de les aus marines en el medi marí.

En el primer bloc hem explorat el valor de les signatures d'isòtops estables alhora d'avaluar l'ecologia tròfica d'espècies problemàtiques. Per a aquesta part de la recerca vam triar com a espècie model el gavià de potes grogues *Larus michahellis* (Fig. 1). Aquesta espècie ha augmentat les seves poblacions de manera exponencial en les últimes quatre dècades i avui en dia és considerada una espècie plaga a causa del seu impacte negatiu en aeroports, ciutats, embassaments, camps de cultiu, en la pesca i sobre espècies protegides (Thomas 1972; Mudge i Ferns 1982; Monaghan et al. 1985; Dolbeer et al. 1997; Vidal et al. 1998; Oro et al.
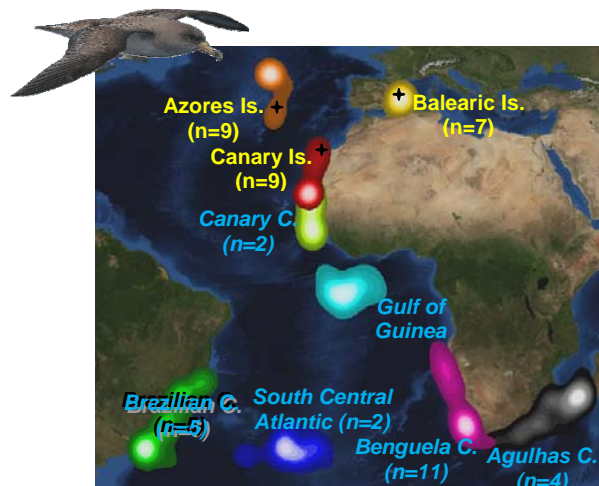
2005). Aquest augment demogràfic ha sigut atribuït a diversos factors, entre ells la protecció de determinades àrees, la disponibilitat creixent de recursos alimentaris d'origen humà, així com la gran capacitat de les gavines per adaptar-se a ambients alterats per l'home (Pons 1992; Bosch et al. 1994; Belant 1997). Els objectius específics d'aquest bloc han sigut (1) definir les preferències alimentàries a través de la tipificació isotòpica de la dieta com una eina per a una ràpida i precisa avaluació de l'ecologia tròfica, (2) entendre el component espaciotemporal de la dieta d'aquesta espècie superabundant a través de l'anàlisi isotòpica, i finalment (3) explorar les possibles relacions entre els hàbits d'alimentació insalubres dels gavians i la propagació de determinats enteropatògens.

En el segon bloc, hem explorat el valor dels marcadors biogeoquímics intrínsecs alhora d'entendre la dinàmica dels contaminants en el medi marí, així com les grans migracions que duen a terme els vertebrats en aquest medi. En aquesta part de la recerca, hem triat com a espècie model la baldriga cendrosa *Calonectris diomedea* (Fig. 2). Es tracta d'un petrell pelàgic i colonial que cria de manera discreta en caus i escletxes d'illes i illots al llarg de tota la seva distribució de cria, visitant el niu únicament durant les nits més fosques del període reproductor. Du a terme llargues i ràpides migracions transoceàniques des de les localitats de cria situades a la Mediterrània i a la Macaronèsia fins a les zones d'hivernada, situades a les principals zones de surgència de l'Atlàntic central i sud (Mougin et al. 1988; Ristow et al. 2000; González-Solís et al. 2007). Dues vegades a l'any, centenars de milers d'aquests baldrigues viatgen desenes de milers de quilòmetres a través de l'equador entre les localitats de cria i d'hivernada, incrementant d'aquesta manera la seva susceptibilitat enfront les amenaces derivades de l'activitat humana. En particular, la baldriga cendrosa és una de les aus marines més afectades per les pesqueries quan aquestes aus proven d'alimentar-se de l'esquer dels palangres. Així doncs, aquests espècie esdevé cada vegada més amenaçada comparada amb altres espècies d'aus marines simpàtriques (Cooper et al. 2003). Els objectius específics d'aquest segon bloc varen ser (4) entendre el procés d'integració isotòpica en les plomes d'acord amb les pautes migratòries i de muda de l'espècie, (5) determinar el valor dels marcadors biogeoquímics intrínsecs en el seguiment de les migracions oceàniques, i per últim (6) explorar l'ús de plomes d'aus marines per avaluar els nivells de contaminants en els ambients marins.

Bloc I. L'estudi dels patrons d'espaciotemporals en ecologia tròfica: el cas d'una espècie problemàtica, el gavià de potes grogues *Larus michahellis*

Capítol 1. Definint les preferències alimentàries d'una espècie superabundant durant el període reproductor
● R. Ramos, F. Ramírez, C. Sanpera, L. Jover, X. Ruiz (2009) Diet of yellow-legged gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. *Journal of Ornithology* 150: 265-272
● R. Ramos, F. Ramírez, C. Sanpera, L. Jover, X. Ruiz (2009) Feeding ecology of yellow-legged gulls *Larus michahellis* in the Western Mediterranean: a comparative assessment using conventional and isotopic methods. *Marine Ecology Progress Series* 377: 289-297

Capítol 2. Comprenent el component espaciotemporal de l'ecologia tròfica d'espècies oportunistes
● R. Ramos, F. Ramírez, J.L. Carrasco, L. Jover (2009) Understanding annual feeding ecology from the isotopic composition of feathers: applications in the management of a problematic gull species. En preparació

Capítol 3. Avaluant el paper dels hàbits d'alimentació dels ocells en la salut ambiental
● R. Ramos, M. Cerdà-Cuéllar, F. Ramírez, L. Jover, X. Ruiz (2009) The influence of insalubrious diets in avian enterobacteria prevalence: the exploitation of refuse sites by gulls and implications for environmental health. Enviat a *Environmental Microbiology*

Bloc II. L'estudi de diferents trets migratoris al llarg dels oceans: el cas d'una au marina pelàgica, la baldriga cendrosa *Calonectris diomedea*

Capítol 4. Esbrinant els patrons migratoris i de muda d'espècies discretes
● R. Ramos, T. Militão, J. González-Solís, X. Ruiz (2009) Moulting strategies of a long-distance migratory seabird: the Mediterranean Cory's Shearwater *Calonectris diomedea diomedea*. *Ibis* 151: 151-159
● R. Ramos, J. González-Solís, X. Ruiz (2009) Linking isotopic and migratory patterns in a pelagic seabird. *Oecologia* 160: 97-105

Capítol 5. Entenent les migracions oceàniques a través dels marcadors biogeoquímics intrínsecs
● R. Ramos, J. González-Solís, J.P. Croxall, D. Oro, X. Ruiz (2009) Understanding oceanic migrations with intrinsic biogeochemical markers. *PLoS ONE* 4: e6236

Capítol 6. Avaluant els nivells de contaminants en els ambients marins a través de migrants transoceànics

● R. Ramos, J. González-Solís, M.G. Forero, R. Moreno, E. Gómez-Díaz, X. Ruiz, K.A. Hobson (2009) The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters. *Oecologia* 159: 345-354

Informe del director

El doctorant **Raül Ramos Garcia** presenta en la seva tesi doctoral titulada "**L'estudi de l'ecologia de les aus a través de les seves plomes: aplicacions ecològiques dels biomarcadors intrínsecs**", tota una sèrie de treballs de gran qualitat científica, publicats la major part d'ells en revistes científiques internacionals de gran prestigi incloses en el *Science Citation Index*. Passo a detallar a continuació la contribució científica que ha realitzat el doctorant en cada un dels articles, així com els seu factor d'impacte (*Thomson Institute for Scientific Information*):

● Diet of yellow-legged gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps
R. Ramos, F. Ramírez, C. Sanpera, L. Jover, X. Ruiz (2009)
Journal of Ornithology 150: 265-272
Factor d'impacte (2008): 1,465
Disseny del treball: **R.R.**, F.R., C.S., L.J., X.R.
Mostreig i anàlisi de mostres: **R.R.**, F.R., L.J.
Redacció científica: **R.R.**, X.R.

● Feeding ecology of yellow-legged gulls *Larus michahellis* in the Western Mediterranean: a comparative assessment using conventional and isotopic methods.
R. Ramos, F. Ramírez, C. Sanpera, L. Jover, X. Ruiz (2009)
Marine Ecology Progress Series 377: 289-297
Factor d'impacte (2008): 2,631
Disseny del treball: **R.R.**, F.R., C.S., L.J., X.R.
Mostreig i anàlisi de mostres: **R.R.**, F.R., L.J.
Redacció científica: **R.R.**, X.R.

● Understanding annual feeding ecology inferred from isotopic composition of feathers: applications to a problematic species from a management perspective.
R. Ramos, F. Ramírez, J.L. Carrasco, L. Jover (2009)
En preparació
Factor d'impacte: -
Disseny del treball: **R.R.**, F.R., L.J.
Mostreig i anàlisi de mostres: **R.R.**, F.R., J.L.C., L.J.
Redacció científica: **R.R.**, L.J.

● The influence of insalubrious diets in avian enterobacteria prevalence: the exploitation of refuse sites by gulls and implications for environmental health.
R. Ramos, M. Cerdà-Cuéllar, F. Ramírez, L. Jover, X. Ruiz (2009)
Enviat a *Environmental Microbiology*
Factor d'impacte: -
Disseny del treball: **R.R.**, M.C.-C., F.R., L.J., X.R.
Mostreig i anàlisi de mostres: **R.R.**, M.C.-C., F.R., L.J.
Redacció científica: **R.R.**, M.C.-C., X.R.

● Moulting strategies of a long-distance migratory seabird: the Mediterranean Cory's Shearwater *Calonectris diomedea diomedea*.
R. Ramos, T. Militão, J. González-Solís, X. Ruiz (2009)
Ibis 151: 151-159
Factor d'impacte (2008): **1,443**
Disseny del treball: **R.R.**, J.G.-S., X.R.
Mostreig i anàlisi de mostres: **R.R.**, T.M., J.G.-S.
Redacció científica: **R.R.**, J.G.-S., X.R.

● Linking isotopic and migratory patterns in a pelagic seabird.
R. Ramos, J. González-Solís, X. Ruiz (2009)
Oecologia 160: 97-105
Factor d'impacte (2008): **3,008**
Disseny del treball: **R.R.**, J.G.-S., X.R.
Mostreig i anàlisi de mostres: **R.R.**, J.G.-S.
Redacció científica: **R.R.**, J.G.-S., X.R.

● Understanding oceanic migrations with intrinsic biogeochemical markers.
R. Ramos, J. González-Solís, J.P. Croxall, D. Oro, X. Ruiz (2009)
PLoS ONE 4: e6236
Factor d'impacte: -
Disseny del treball: **R.R.**, J.G.-S., J.P.C., D.O., X.R.
Mostreig i anàlisi de mostres: **R.R.**, J.G.-S., D.O.
Redacció científica: **R.R.**, J.G.-S., X.R.

● The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters.
R. Ramos, J. González-Solís, M.G. Forero, R. Moreno, E. Gómez-Díaz, X. Ruiz, K.A. Hobson (2009)
Oecologia 159: 345-354
Factor d'impacte (2008): **3,008**
Disseny del treball: **R.R.**, J.G.-S., M.G.F., X.R.
Mostreig i anàlisi de mostres: **R.R.**, J.G.-S., M.G.F., R.M., E.G.-D., X.R., K.A.H.
Redacció científica: **R.R.**, J.G.-S., M.G.F., X.R.

De la mateixa manera informo que cap dels coautors participants en els articles que componen aquesta tesi han utilitzat, implícitament o explícita cap d'aquests treball per a l'elaboració de la seva pròpia tesi doctoral.

Barcelona, a 21 de setembre de 2009
Signat:

Dr. Lluís de Jover i Armengol
Departament de Salut Pública,
Facultat de Medicina, Universitat de Barcelona

L'estudi de l'ecologia de les aus a través de les seves plomes: aplicacions ecològiques dels biomarcadors intrínsecs
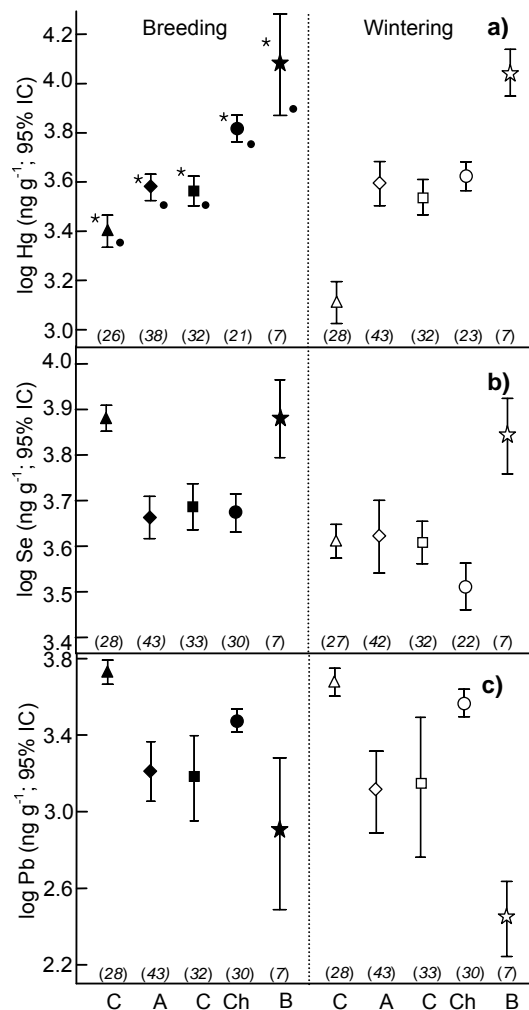
Raül Ramos

L'ús d'anàlisis biogeoquímiques en ecologia animal ha augmentat enormement en els darrers anys i s'ha convertit en una eina rellevant en l'estudi de l'ecologia tròfica i la dinàmica espaciotemporal de moltes espècies (Newsome et al. 2007; Hobson i Norris 2008). En aquest estudi hem mostrat com els marcadors biogeoquímics intrínsecs i els isòtops estables en particular, poden contribuir i proporcionar grans avenços en diversos camps, tals com l'epidemiologia, la gestió de la conservació, estudis de seguiment de les migracions, o mesures de pol·lució. No obstant això, diversos aspectes relacionats amb la naturalesa i l'origen dels teixits analitzats són factors clau en l'èxit de qualsevol treball d'investigació basat en isòtops estables o en concentracions d'elements traça, requerint-se sovint validacions prèvies (Hobson i Clark 1992; Gannes et al. 1997; Hobson i Bairlein 2003). En recerca ornitològica, a causa de les seves característiques peculiars, les plomes són el teixit més àmpliament analitzat biogeoquímicament d'entre tots els possibles (vegeu la Introducció). No obstant això, la interpretació correcta d'aquests resultats basats en la composició biogeoquímica de plomes normalment necessita d'un coneixement precís dels processos d'integració elemental, d'alguns coneixements bàsics sobre la dieta, les zones d'alimentació i el comportament migratori de les espècies, així com dels processos de la muda d'aquestes espècies (és a dir, la fenologia de substitució de les plomes en relació amb el cicle anual de les aus).

Avenços en el coneixement de la integració biogeoquímica en les plomes

En utilitzar les anàlisis biogeoquímiques com marcadors intrínsecs, es presisa d'un coneixement profund de la integració dels isòtops i elements traça des de les diferents xarxes tròfiques als diferents teixits animals. Els nostres resultats suggereixen que la dinàmica metabòlica dels diferents contaminants
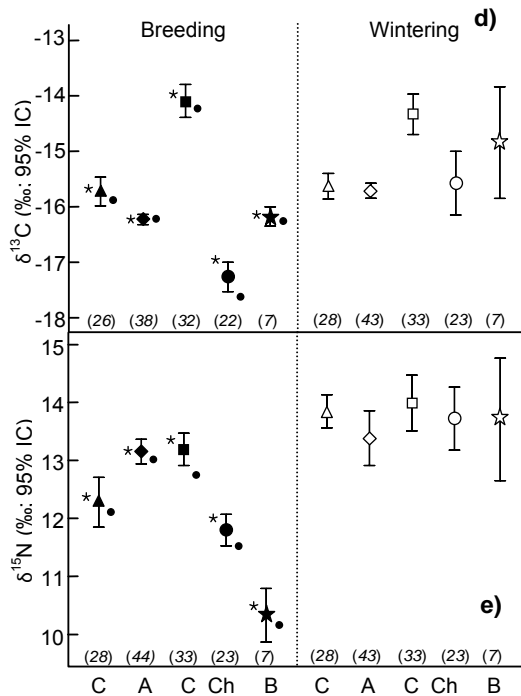

Fig. 3 Mitjanes i intervals de confiança del 95% de les concentracions de contaminants (a, b i c per al Hg, Se i Pb, respectivament) i dels isòtops estables de carboni i de nitrogen (d i e, respectivament) en les primeres primàries i vuitenes secundàries (símbols plens i buits, respectivament) de la baldriga de Cap Verd i la baldriga cendrosa (Cap Verd (CV): *triangles*, Açores (A): *rombes*, Canàries (C): *quadrats*, Chafarines (Ch): *cercles* i Balears (B): *estrelles*). Els valors mitjans de mascles (*asteriscs*) i femelles (*punts*) es mostres per a cada colònia quan les diferències sexuals foren significatives o marginal significatives. Les mides mostrals s'indiquen entre parèntesis (*n*).

Fig. 3 Continuació

no és homogènies entre sí, i que els elements traça i els isòtops estables s'integren en els teixits animals de diferent manera. D'una banda, trobàrem que mentre que les diferències en els nivells basals al llarg de la geografia influiren les concentracions de Se i Pb en plomes, l'ecologia tròfica de les aus també jugà un paper important alhora d'explicar les concentracions de Hg (Fig. 3). A més, vàries limitacions reproductives entre mascles i femelles, com són la posta d'ous o l'esforç diferencial en la criança dels polls, també afectaren les concentracions de Hg (Fig. 4). D'altra banda,
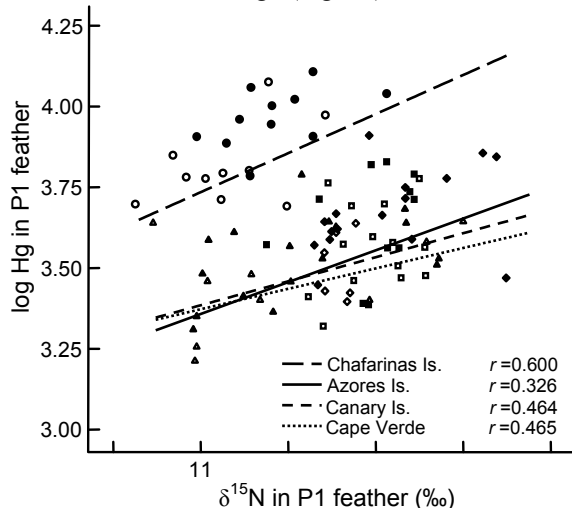

Fig. 4 Relació entre els isòtops estables de nitrogen i la concentració de mercuri en les primeres primàries. Les regressions lineals es mostren per a cada localitat de cria per separat, Cap Verd: *triangles*, Açores: *rombes*, Canàries: *quadrats* i Chafarinas: *cercles*. Mascles i femelles s'indiquen amb símbols plens i buits, respectivament.

analitzant la composició biogeoquímica de plomes d'animals seguits amb sistemes de teledetecció, vàrem demostrar que mentre les signatures d'isòtops estables de les plomes reflectiren un origen exogen, és a dir, que són immediatament transferits de la dieta a les plomes durant el procés muda (Hobson 1999), la composició elemental de les plomes indicà un origen endogen dels elements traça, és a dir, que són parcialment mobilitzats des de diversos òrgans on s'emmagatzemen (Goede 1991; Furness 1993; Taula 1). Com a conseqüència, la interpretació de les concentracions elementals dels teixits formats en una determinada temporada s'ha de fer amb precaució, ja que aquests valors podrien estar reflectint l'exposició a aquests elements durant un altre període. Aquests resultats posen de manifest el comportament diferencial en l'acumulació i la dinàmica d'excreció dels diferents contaminants, així com també entre les signatures isotòpiques i els elements traça (Hobson 2008).

Avenços en el coneixement de l'ecologia tròfica des d'una perspectiva isotòpica

Alhora de delimitar les preferències alimentàries de les espècies, la majoria dels estudis es basen mostrejos de polls, principalment degut a la incapacitat dels pollets per a volar el que els fa fàcilment accessibles als investigadors en les localitats de cria. Això en facilita la captura, manipulació i control d'individus, així com la possibilitat d'aplicar estratègies específiques de mostreig, com ara el remostreig. A més, tant la facilitat per a obtenir mostres de preses relativament ben conservades a través dels regurgitats de polls com el fet que els regurgitats són les anàlisis convencionals de dieta menys esbiaixades, converteix els regurgitats de polls en l'eina més popular entre els estudis de la dieta (González-Solís et al. 1997; Barrett et al. 2007).

El nostre objectiu aquí va ser trobar mètodes apropiats per a proporcionar informació fiable sobre l'ecologia tròfica d'una determinada espècie. En primer lloc, vàrem reconstruir la dieta dels polls de gavià de potes grogues al llarg del període de cria (tres mostreigs consecutius) mitjançant l'anàlisi de regurgitats (Fig. 5). Els nostres resultats van mostrar que les deixalles humanes, ja sigui d'abocadors o els descarts pesquers, van ser el principal component alimentari en la dieta dels polls. A més, es van analitzar les signatures isotòpiques de carboni, nitrogen i sofre en plomes de polls de gavià, així com en les seves principals preses. A través de les signatures isotòpiques usant models de mescla, vàrem obtenir una caracterització independent de les proporcions alimentàries dels diferents recursos emprats en diferents localitats de cria de gavians (des d'hàbitats marins, ambients salobres i d'aigua dolça, ambients terrestres i camps de cultius fins a abocadors). En comparar ambdós mètodes (anàlisi

Taula 1 Classificació discriminant basada en la biogeoquímica de les plomes

	Stable isotopes P1	Stable isotopes S8	Element analysis P1	Element analysis S8
Breeding colonies				
Original data				
Azores Is. (n=9)	100.0	44.4	66.7	66.7
Balearic Is. (n=7)	100.0	57.1	71.4	100.0
Canary Is. (n=9)	100.0	66.7	88.9	88.9
Total (n=25)	100.0	56.0	76.0	84.0
Cross-validation				
Azores Is. (n=9)	100.0	22.2	66.7	55.6
Balearic Is. (n=7)	100.0	28.6	57.1	85.7
Canary Is. (n=9)	100.0	55.6	88.9	55.6
Total (n=25)	100.0	36.0	72.0	64.0
Wintering sites				
Original data				
Benguela C. (n=11)	63.6	90.9	45.5	36.4
Brazil-Falklands C. (n=5)	80.0	100.0	60.0	40.0
Agulhas C. (n=4)	75.0	100.0	75.0	50.0
Canary C. (n=2)	100.0	100.0	100.0	100.0
SC Atlantic (n=2)	100.0	100.0	50.0	100.0
Total (n=24)	75.0	95.8	58.3	50.0
Cross-validation				
Benguela C. (n=11)	54.5	63.6	27.3	27.3
Brazil-Falklands C. (n=5)	20.0	60.0	40.0	0.0
Agulhas C. (n=4)	25.0	100.0	25.0	50.0
Canary C. (n=2)	100.0	100.0	100.0	100.0
SC Atlantic (n=2)	100.0	0.0	0.0	50.0
Total (n=24)	50.0	66.7	33.4	33.4

Les taxes de classificació correcta (%) obtingudes mitjançant l'anàlisi d'isòtops estables (δ^{13}C, δ^{15}N, δ^{34}S, δ^{2}H i δ^{18}O) i les concentracions d'element traça (Se, Pb i Hg) en plomes d'estiu (P1) i d'hivern (S8). Les anàlisis discriminants van ser validades usant el procediment *jackknife*. La zona d'hivernada del golf de Guinea no va ser inclosa en aquesta anàlisi, ja que només va ser visitada per un sol ocell.

tradicional de regurgitats i models de mescla d'isòtops estables), vàrem trobar una concordança general en els principals patrons alimentaris, per exemple, en l'ús dels recursos marins i d'escombraries (Fig. 6). No obstant això, algunes de les proporcions estimades pels models isotòpics no s'ajustaren als valors esperats calculats a partir de l'anàlisi de regurgitats. Alguns dels valors de consum d'invertebrats (tant d'aigua dolça com terrestres) estimats van ser majors en el model de mescla respecte l'anàlisi de regurgitats. Aquest resultat suggereix que els models de mescla d'isòtops estables poden reflectir millor el consum de determinats recursos que normalment queden subestimats pels mètodes tradicionals. És a dir, que
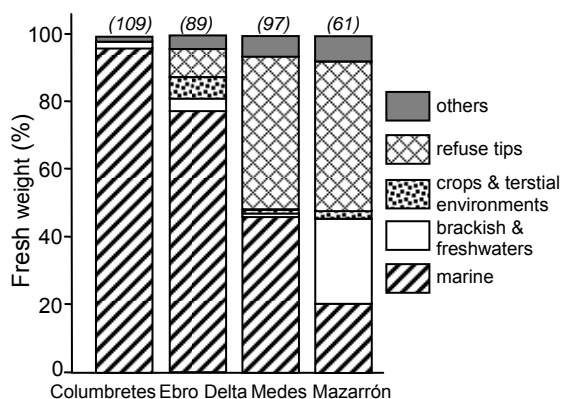
l'anàlisi de regurgitats sol subestimar la importància de preses petites i toves, com ara els invertebrats, mentre que les preses més grans i més difícils de digerir queden sobreestimades (Duffy i Jackson 1986).

Vàrem trobar també que els isòtops difereixen en la seva capacitat de determinar els diferents recursos alimentaris. D'acord amb estudis anteriors, els nivells de δ^{13}C i δ^{34}S van ser més grans a mesura que el consum de preses marines augmentava (France i Peters 1997; Knoff et al. 2002). No obstant

Fig. 5 Percentatges de consum en pes fresc d'acord amb els principals hàbitats d'alimentació dels polls de gavià de potes grogues *Larus michahellis* (mida mostral entre parèntesis).
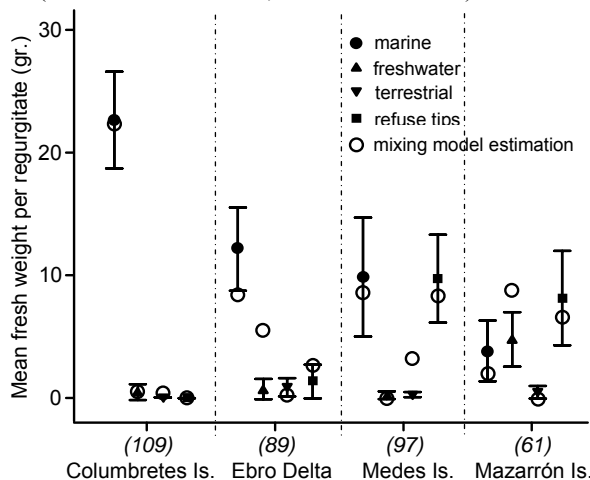

Fig. 6 Mitjana de pes fresc per regurgitat (95% IC) dels diferents hàbitats d'alimentació calculats a partir del mostreig directe de la dieta (anàlisis de regurgitats; mida de la mostra entre parèntesis). Els pesos de cada hàbitat d'alimentació estimats pel model de mescla també s'indiquen.

Taula 2 Resum dels valors mitjans isotòpics (± SE) per als diferents recursos tròfics explotats pels gavians de potes grogues (obtinguts a partir dels regurgitats de polls i de descarts pesquers) i la seva significació (correcció de *Welch*, P-valor) en les diferències entre les colònies

Prey class		n	$\delta^{13}C$ (‰)	P	$\delta^{15}N$ (‰)	P	$\delta^{34}S$ (‰)	P
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	0.445	9.46 ± 0.55	0.249	17.48 ± 0.48	0.132
	Alborán	11	-16.42 ± 0.43	<0.001	7.98 ± 0.49	<0.001	18.92 ± 0.31	<0.001
Freshwater invertebrates	Mazarrón[a]	4	-18.87 ± 0.69	-	9.91 ± 2.81	-	10.12 ± 0.89	-
Terrestrial invertebrates	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	0.667	11.92 ± 3.00	0.350	6.97 ± 1.05	0.718
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	0.010	5.50 ± 1.74	0.597	6.72 ± 2.03	0.232

[a] els invertebrats d'aigua dolça només van ser trobats abundantment a Mazarrón
Les signatures isotòpiques utilitzades en els models de mescla s'indiquen com a mitjana global quan es trobaren homogènies entre localitats (només els recursos marins de l'illa d'Alborán mostraren diferències significatives).

això, només les signatures isotòpiques del sofre varen diferir prou entre els diferents tipus de preses com per a ser considerades un bon indicador de l'origen de dieta (dietes continentals i terrestre versus dietes basades en recursos marins; Taula 2). També es va trobar que les signatures de $\delta^{15}N$ s'empobrien com a resultat del consum d'escombraries provinents d'abocadors degut principalment a la simplicitat de la xarxa tròfica implicada en aquest recurs (Hebert et al. 1999).

En resum, els models de mescla varen corroborar que les reconstruccions alimentàries basades en regurgitats estan esbiaixades envers les preses més aparents. No obstant això, més important va ser que vàrem construir un model isotòpic fiable basat en el mostreig de plomes per a una ràpida assignació dels recursos tròfics utilitzats per a poblacions o espècies

que solen ser oportunistes en els seus hàbits d'alimentació i que per tant, són capaces de canviar ràpidament la seva dieta. Tot i que aquests resultats estan basats en mostres provinents de polls, el resultat d'aquest estudi obre noves portes a l'estudi de diferents aspectes tròfics de tota la població d'una espècie, inclosos els juvenils i adults, posant de relleu d'alguna manera la viabilitat del mostreig de polls per a obtenir un coneixement bàsic de la dieta d'una espècie, així com per a fomentar el seu estudi en determinades validacions metodològiques.

Avenços en el coneixement dels patrons de muda dels ocells

Les plomes dels polls però, no proporcionen cap tipus informació sobre la dieta fora del període de
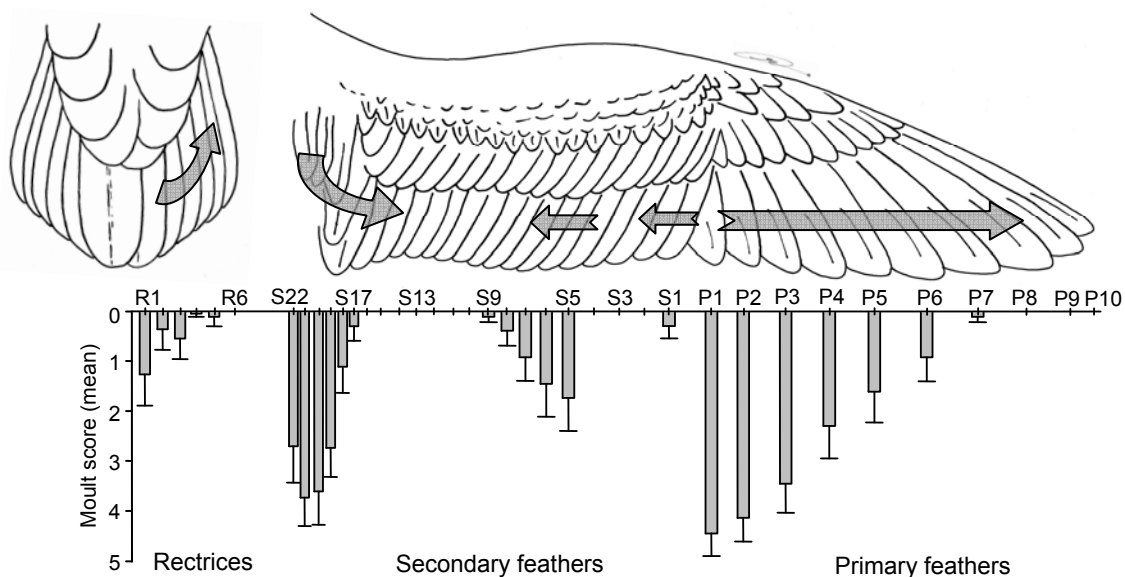

Fig. 7 Esquema de l'ala i de la cua de 32 baldrigues cendroses (17 mascles i 15 femelles) capturades incidentalment per un palangrer el 5 d'octubre en aigües catalanes (NW Mediterrani). El principal patró de muda es descriu amb fletxes de color gris i per a cada ploma s'indiquen les puntuacions mitjanes de muda (+ IC 95%).
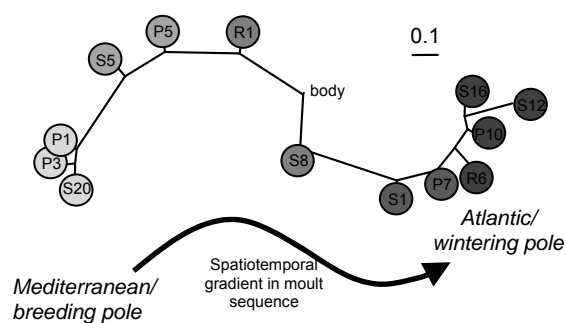
Fig. 8 Relació biogeoquímica entre diferents plomes de baldriga cendrosa. L'arbre *neighbour-joining* mostra les similituds entre plomes de 20 baldrigues cendroses en base a les seves signatures de δ¹³C, δ¹⁵N i δ³⁴S. L'arbre de similituds està basat en les distàncies euclídies per a cada parell de plomes; la longitud de la barra superior representa 0,1 unitats de distància.

cria, la qual cosa dificulta el coneixement global sobre diferents aspectes de l'ecologia anual de les espècies, per exemple, dels hàbitats d'alimentació explotats durant la temporada no reproductora o dels moviments migratoris. En aquest sentit, les plomes de les aus adultes són molt valuoses ja que els patrons de muda són estacionalment predictibles i bastant consistents en el temps. Per tant, les anàlisis d'isòtops estables en determinades plomes poden proporcionar informació isotòpica d'un període únic i concret, independentment de la data de mostreig (Hobson 2005b), sent especialment adequats per a avaluar patrons estacionals en la dieta i moviments migratoris. No obstant això, un coneixement precís sobre els patrons de muda i el temps de la formació de les plomes de cada espècie és indispensable.

Com a activitat d'alta demanda energètica, la muda sol ocórrer durant la temporada no reproductora, quan les aus són generalment inaccessibles (Marshall 1956; Bridge 2006; Edwards 2008). No obstant això, observacions de muda poden ser obtingudes d'espècimens morts recollits durant el període d'entre cries, atropellats, ferits, capturats incidentalment per pesqueries o trobats morts per casualitat. Aquests animals doncs, recollits en diferents períodes, permeten obtenir un patró de muda fiable, detallat i complet, fins i tot fora del període reproductor. Aquí hem avaluat la muda de les ales, cua i plomes del cos d'un centenar de baldrigues cendroses capturades accidentalment per palangrers catalans durant tot l'any. Els nostres resultats van revelar una estratègia de muda fins ara desconeguda per a l'espècie al llarg de la superfície alar (Fig. 7). A més a més, informació precisa sobre la muda de les espècies pot obtenir-se fàcilment mitjançant l'anàlisi de la composició isotòpica de plomes específiques. Basant-nos en les signatures isotòpiques de vàries plomes, vàrem demostrar que determinades plomes situades al llarg de la superfícies alar diferien en la seva composició i es subdividien en dos grups corresponents a les plomes mudades durant el període de cria i d'hivernada (Figs 8 i 9). En el cas dels migrants de llarga distància, les diferències isotòpiques entre els tipus
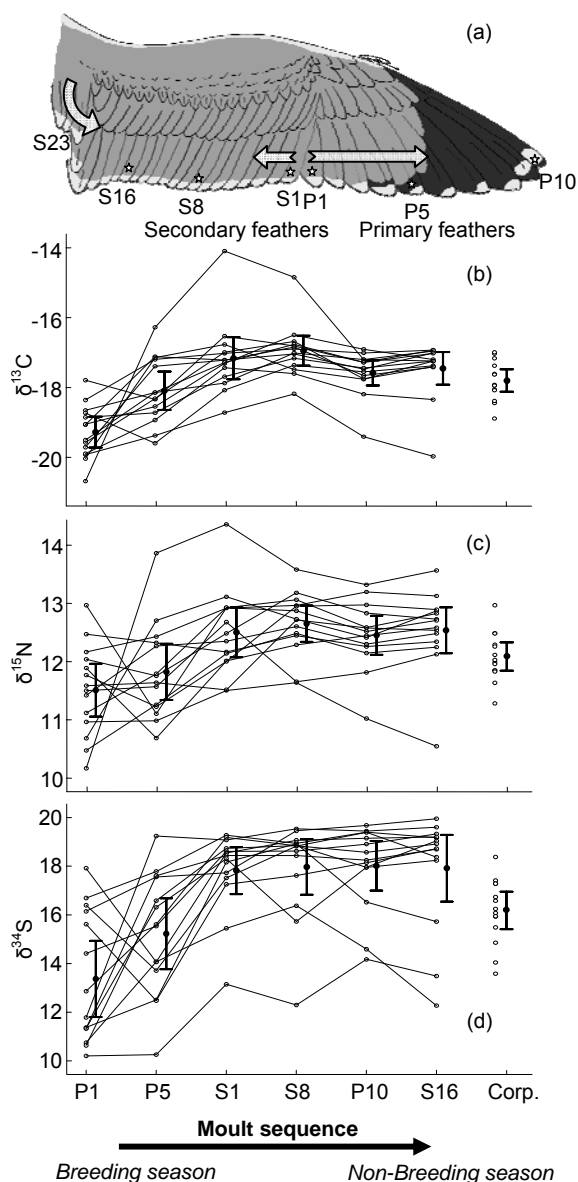

Fig. 9 Patró de muda alar del gavià de potes grogues i la composició isotòpica d'algunes de les seves plomes. a) El principal patró de muda (fletxes blanques; Ingolfsson 1970; Olsen i Larsson 2004) i les plomes seleccionades per a l'anàlisi d'isòtops estables (estrelles) es mostren en l'esquema de l'ala. També es mostren les signatures de carboni b), nitrogen c) i sofre d) de les 1ª, 5ª i 10ª primàries (P1, P5, P10) i de les 1ª, 8ª i 16ª secundàries (S1, S8, S16) de 14 gavians. Les plomes es classificaren segons la seqüència de muda definida per Ingolfsson (1970) i per Olsen i Larsson (2004). Cada línia connecta els valors isotòpics de les plomes d'un mateix individu. Les signatures isotòpiques individuals d'algunes plomes corporals es mostren per separat (Corp). Les mitjanes i els IC del 95% CI són representats com a barrer d'error per a cada ploma.

de plomes poden sorgir a causa d'una dieta diferencial entre estacions o bé perquè les signatures isotòpiques basals entre les xarxes tròfiques marines de les zones de cria i d'hivernada són dispars (Pantoja et al. 2002; Cherel et al. 2007; Cherel i Hobson 2007). En el cas de les espècies residents, les diferències isotòpiques entre les plomes es deuen a un diferent comportament d'alimentació entre les estacions, ja que els moviments de dispersió a hivern, en general són relativament curts.

Independentment del motiu de les diferències, les anàlisis isotòpiques de les plomes ens poden ajudar a identificar els patrons muda i el període en què cada ploma és mudada. Per tant, el nostre coneixement dels patrons de muda de les aus podria incrementar enormement mitjançant l'anàlisi de la composició isotòpica de plomes (Cherel et al. 2000; Cherel et al. 2006).

Connectant l'ecologia tròfica i la càrrega microbiològica de la fauna salvatge

Una vegada que un model isotòpic està descrit i configurat per a determinar dietes específiques, gran varietat d'estudis poden dur-se a terme amb més comoditat, salvant els biaixos de les metodologies convencionals, i guanyant en simplicitat en el mostreig i rapidesa en l'anàlisi. Per exemple, basant-nos en les signatures d'isòtops estables de C, N i S vàrem caracteritzar la dieta dels polls de gavià de potes grogues, relacionant a continuació la càrrega d'enterobacteris zoonòtics amb el grau d'explotació de recursos provinents d'abocador. Els nostres resultats suggeriren que les colònies de gavines situades prop d'assentaments humans, i que en gran part s'alimenten d'escombraries i deixalles, són més susceptibles a contribuir en major o menor grau a un deteriorament de la salut pública. En particular, els enterobacteris *Campylobacter* van ser menys freqüentment aïllats en aquelles colònies de gavians allunyades de poblacions humanes i que s'alimenten principalment de recursos marins, mentre que en poblacions que carronyegen en abocadors les prevalences d'aquest enterobacteri van ser majors. Més important encara, vàrem relacionar de forma individual el grau de consum de restes d'abocador amb la incidència de *Campylobacter* spp., és a dir que els polls que són alimentats amb restes

d'abocador eren més propensos a ser portadors de *Campylobacter* que els que són alimentats exclusivament amb peix (Fig. 10a). D'altra banda, les majors prevalences de *Salmonella* spp. van ser observades en aquelles colònies més properes als assentaments humans així com entre els volantons més àmpliament alimentats amb restes d'abocador (Fig. 10c), tot i que finalment ni les diferències entre localitats, ni tampoc entre els individus van resultar ser estadísticament significatives. Igualment, encara que les relacions específiques de *Salmonella* serovar Typhimurium i *Campylobacter jejuni* (dos dels enterobacteris més patogènics en salut humana) amb els hàbits d'alimentació de les gavines no van ser significatives, d'alguna manera pensem que els nostres resultats aporten una mica de llum a aquesta qüestió (Fig. 10b, d). Aquí, mostrem com el coneixement alimentari proporcionat per l'anàlisi d'isòtops estables en el context de l'ecologia animal pot aplicar-se amb confiança en estudis epidemiològics basats en malalties infeccioses emergents d'aus salvatges (Reed et al. 2003).

Estudiant l'ecologia tròfica anual dels ocells

Vàrem utilitzar també el model isotòpic del polls de gavià de potes grogues per a augmentar el coneixement de l'ecologia tròfica anual i els possibles canvis estacionals en la dieta dels adults. Amb un adequat coneixement de la muda (vegeu més amunt), els models de mescla basats en la composició isotòpica de determinades plomes permeteren investigar l'estratègia alimentària de cada individu i de cada població durant tot l'any. Anàlisis isotòpiques de les primeres primàries (P1) dels gavians de potes grogues van resultar ser uns bons indicadors de l'ecologia tròfica durant el
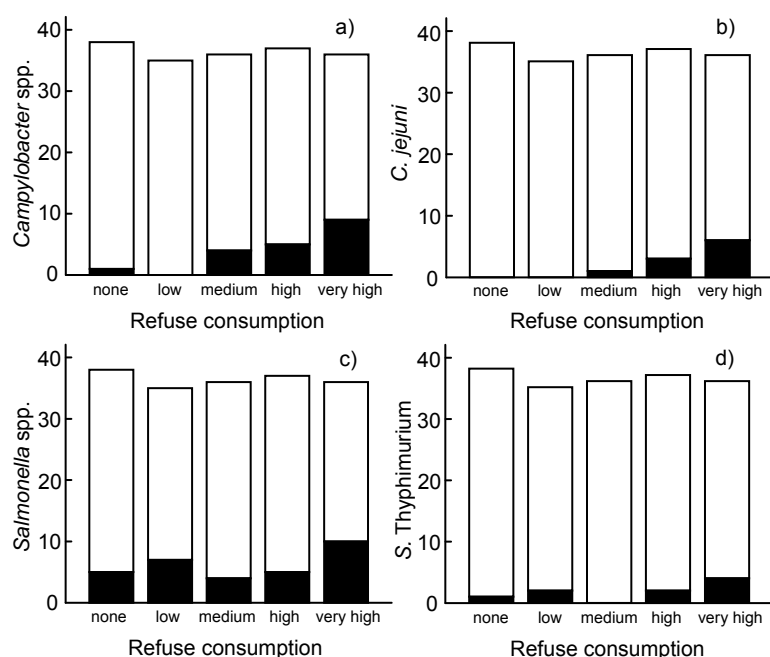

Fig. 10 Prevalença d'enterobacteris en polls de gavià de potes grogues en funció del consum de restes d'abocador a la costa del Mediterrani ibèric. El nombre de casos positius per a *Campylobacter* spp. (a), *Campylobacter jejuni* (b), *Salmonella* spp. (c) i *Salmonella enterica* subsp. *enterica* serotip Typhimurium (d) es mostren en negre en funció del consum de restes d'abocador. El nombre de negatius per als enterobacteris es mostra en blanc. Les categories de l'eix de les X representen els quintils (*n*=182; cap=38, baix=35, mig=36, alt=37, molt alt=36) d'acord amb el percentatge individual de consum de deixalles estimat a partir de models isotòpics de mescla.

Taula 3 Mitjanes percentuals en l'ús dels hàbitats del gavià de potes grogues estimats a partir dels models isotòpics de mescla per a diferents plomes específiques

locality	feather	n	Estimated foraging habitat (%)			
			marine	freshwater	terrestrial	refuse
Mazarrón (seq.)	P1	14	41.56±16.80	18.94±4.53	1.06±3.97	38.44±15.23
	P5	14	56.91±17.43	4.74±6.44	13.85±19.72	24.51±18.39
	S1	14	67.25±12.91	3.71±4.43	23.12±15.96	5.91±9.88
	S8	14	63.69±12.23	4.76±3.54	28.08±13.00	3.47±7.07
	P10	14	71.39±13.58	5.17±5.67	18.39±12.19	5.05±11.27
	S16	14	65.52±12.74	6.64±5.09	22.83±12.35	5.02±13.30
	Corp.	14	66.24±7.56	1.17±3.16	16.50±11.04	16.09±13.03
Medes Is.	P1	13	25.58±12.19	12.63±7.80	3.47±8.75	58.32±12.24
	S8	13	47.51±20.42	8.98±11.94	11.12±11.76	32.39±15.44
Ebro Delta	P1	20	53.94±15.14	20.47±9.64	6.58±13.48	19.01±19.51
	S8	20	53.04±20.39	12.98±13.09	17.40±16.97	16.58±22.58
Columbretes Is.	P1	21	82.62±9.04	5.73±3.86	5.47±6.03	6.17±9.84
	S8	21	51.40±18.40	12.85±8.88	15.81±14.96	19.95±22.15
Sa Dragonera Is.	P1	12	31.80±7.78	22.00±1.69	0	46.20±7.77
	S8	12	41.91±19.82	14.69±7.78	7.37±11.55	36.03±24.10
Benidorm Is.	P1	12	61.26±19.58	12.97±5.86	0.92±3.17	24.85±16.24
	S8	12	62.30±11.85	7.94±4.27	20.31±11.54	9.44±19.06
Mazarrón Is.	P1	15	33.04±17.10	13.79±9.09	1.81±3.98	51.37±12.27
	S8	15	59.72±11.94	11.80±6.80	21.95±10.13	6.53±16.85
Alborán Is.	P1	20	75.65±9.95	8.02±8.87	8.03±6.37	7.65±8.02
	S8	20	78.09±11.73	3.18±7.21	10.88±7.53	7.85±8.88

Diferents plomes mudades al llarg del cicle anual del gavià es mostren només per a les aus de l'illa de Mazarrón (mostreig previ). Els usos dels hàbitats (%) estimats per al període reproductor i per al no reproductor (P1 i S8, respectivament) es mostren per a cada localitat.

període de la cria, mentre que les vuitenes secundàries (S8) reflectiren el comportament alimentari del període no reproductor. Els isòtops estables va revelar una disparitat en les estratègies d'alimentació entre les poblacions de gavià de potes grogues en l'àrea d'estudi (vegeu també Hebert et al. 2008; Taula 3). A més, mentre algunes poblacions van mantenir les seves preferències alimentàries en les diferents estacions, altres van canviar els seus hàbits alimentaris en forma dràstica. Aquesta gran diversitat en els patrons d'explotació dels diferents recursos al llarg de l'àrea d'estudi i la facilitat i la rapidesa amb què els gavians varen canviar la seva dieta entre períodes posaren de manifest d'alguna manera el comportament altament oportunista d'alimentació de l'espècie (Vidal et al. 1998; Hebert et al. 2008). Així, aquesta plasticitat espaciotemporal en l'ecologia tròfica del gavià ha de ser considerada per les autoritats encarregades de la gestió de la conservació quan es disposen a reduir o limitar el creixement de certes poblacions problemàtiques. Aquí, presentàrem proves clares que l'anàlisi d'isòtops estables en plomes específiques pot ser utilitzat per a determinar l'ecologia tròfica de les aus durant tot el seu cicle anual, brindant noves oportunitats per a la gestió de poblacions problemàtiques, però també en la conservació d'espècies en perill d'extinció. Finalment, el fet que els descarts pesquers recol·lectats als voltants de l'illa d'Alborán (la localitat més remota de totes) fossin isotòpicament diferents dels descarts de la resta de localitats incloses en l'estudi (Taula 2) va
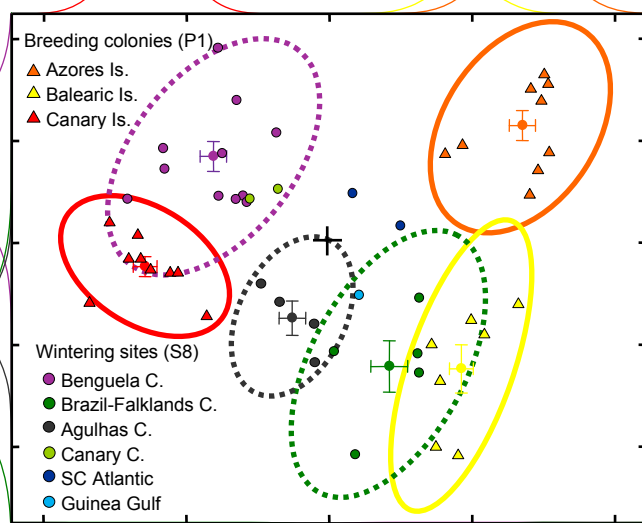

Fig. 11 Composició isotòpica de les plomes d'estiu i d'hivern de la baldriga cendrosa. Anàlisi de components principals (*PCA*) de les signatures d'isòtops estables de carboni ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sofre ($\delta^{34}S$), hidrogen ($\delta^{2}H$) i oxigen ($\delta^{18}O$) a la primera primària (P1) i vuitena secundària (S8; triangles i cercles, respectivament) mudades respectivament en les localitats de cria i en els quarters d'hivernada. L'eix de les X representa el *PC1* (59,0%), mentre que l'eix Y representa el *PC2* (21,1%); tots dos eixos estan dividits unitàriament amb els zeros a la intersecció central. El·lipses gaussianes bivariades (95% de probabilitat de la mitjana de la població) i corbes de distribució normal també es representen.

posar de manifest la importància d'assegurar l'homogeneïtat geogràfica en les signatures isotòpiques de les preses abans d'aplicar qualsevol model de mescla, especialment si l'àrea d'estudi considerada és relativament gran (Gannes et al. 1997; Hebert et al. 1999).

Esbrinant assumptes migratoris en el medi marí

En general, les poblacions reproductores d'aus marines s'estenen al llarg dels oceans, separades sovint per diversos milers de quilòmetres, i situades per tant, en diferents règims oceanogràfics (Longhurst 1998; Fig. 2). En aquest sentit, les primeres primàries (P1) de baldriga cendrosa mudades durant el període de cria (vegeu més amunt) diferiren en la seva composició isotòpica entre les diferents localitats de cria (Fig. 11). Aquestes diferències isotòpiques en les plomes primàries ens varen permetre assignar qualsevol individu a una relativament restringida àrea de cria (classificació correcte del 100%). A l'hivern, les baldrigues cendroses va viatjar a la zona central i sud de l'Atlàntic, concentrant-se en una de les sis àrees d'hivernada associades amb les corrents oceàniques de Benguela, Brasil-Malvines, Agulhas, Canàries, i amb el sud de l'Oceà Atlàntic Central i el golf de Guinea (Fig. 2). Com que cada zona té les seves pròpies peculiaritats oceanogràfiques (Longhurst 1998), diferències geogràfiques en els diferents elements biogeoquímics eren d'esperar. De fet, les signatures isotòpiques registrades en diversos estudis locals duts a terme en aquests sectors tropicals i subtropicals de l'oceà Atlàntic indiquen l'existència de gradients isotòpics permanents a nivell basal (Sholto-Douglas et al. 1991; Matsuura i Wada 1994; Schwamborn 1997; Fischer et al. 1998). En concordança amb aquests treballs, les signatures

isotòpiques de les plomes secundàries (S8) de baldrigues adultes mudades durant el període hivernal també diferiren entre les principals àrees d'hivernada (Fig. 11). En concret, la baldriga cendrosa del Mediterrani (*Calonectris diomedea diomedea*) hiverna principalment en dues d'aquestes àrees (Fig. 12): al nord-est de l'Atlàntic tropical, associada amb la corrent sud de Canàries i a la zona oriental de l'Oceà Atlàntic Sud, associada amb la corrent de Benguela (Ristow et al. 2000; González-Solís et al. 2007). En base als resultats isotòpics de plomes mudades a l'hivern (P10) de 20 baldrigues seguides amb geolocalitzadors, dos grups isotòpicament distints d'aquestes aus van ser identificats (Fig. 13), presumiblement corresponents a les dues principals zones d'hivernada a l'Atlàntic. Tot i que la nostra anàlisi de les plomes va reflectir clarament la migració de la baldriga cendrosa, cada isòtop per separat va contribuir de manera diferent a explicar aquest patró de migració. Si bé les signatures de N va marcar la migració del Mediterrani a l'Atlàntic (i viceversa), els valors isotòpics de C i S indicaren les diferents províncies oceàniques de l'Atlàntic, proporcionant globalment una empremta geogràfica que permet el seguiment dels moviments migratoris a través de les grans masses oceàniques (Fig. 13). Aquest resultat posa de relleu el potencial ús dels isòtops estables per al seguiment dels moviments animals en el medi marí. En conclusió, els nostres resultats en estudis de migració indiquen que les signatures isotòpiques de regions oceàniques distants es poden integrar a les plomes d'una determinada au i poden indicar la regió en què cada ploma va ser mudada, proporcionant noves oportunitats per a la identificació de zones de cria d'animals marins, així com quarters d'hivernada. Aquest enfocament dóna una nova visió sobre els estudis de dinàmica
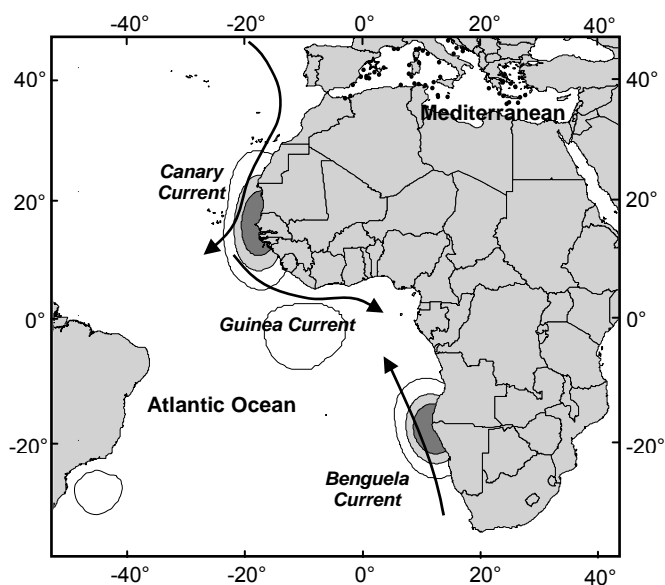

Fig. 12 Zones d'hivernada de 10 baldrigues cendroses del Mediterrani *Calonectris diomedea diomedea* (Balears *n*=8 i Chafarinas *n*=2) seguides amb geolocalitzadors. Les àrees d'hivernada d'aquestes aus se situaren a les corrents de Benguela (*n*=4) i Canàries (*n*=4), a la regió de confluència Brazil-Malvines (*n*=1) i al golf de Guinea (*n*=1; González-Solís et al. 2007; Daniel Oro i Jacob González-Solís no publicat). Distribució de cria (punts sòlids) i principals zones d'hivernada derivades de l'anàlisi *kernel* que abarca el 95% (blanc), el 75% (gris) i el 50% (gris fosc) de les localitzacions filtrades definits respectivament per Thibault et al. (1997) i González-Solís et al. (2007). La ubicació d'unes altres 20 baldrigues capturades per palangrers catalans es mostra amb una estrella. Les principals corrents oceàniques que afecten les àrees d'hivernada també es mostren convenientment adaptades de Brown et al. (1989).
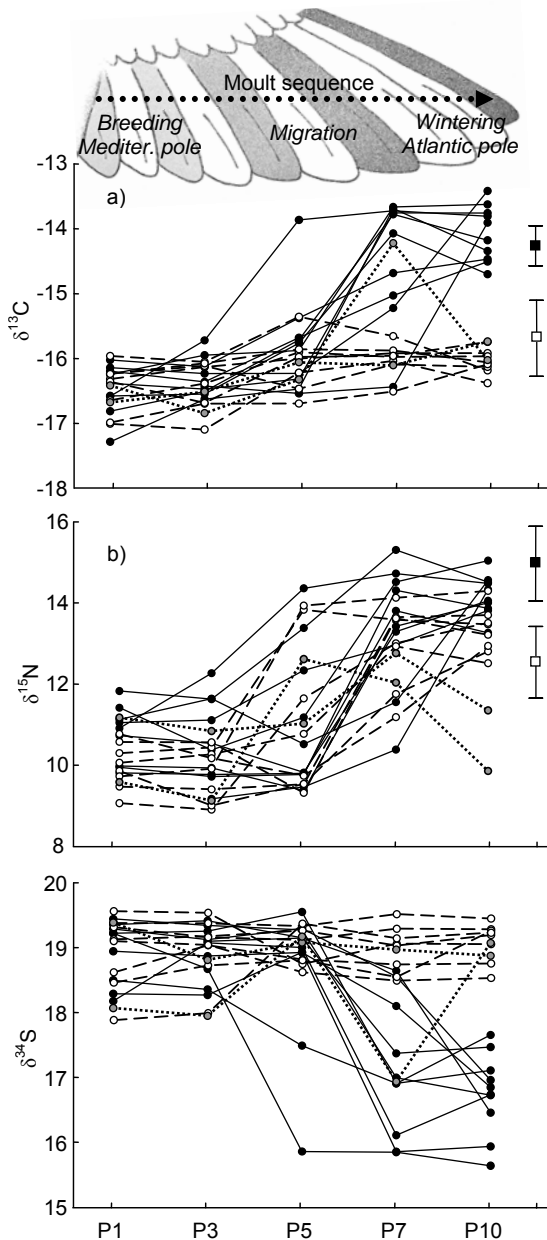
Fig. 13 Signatures isotòpiques de carboni a), nitrogen b) i sofre c) de les 1ª, 3ª, 5ª, 7ª i 10ª primàries (P1, P3, P5, P7 i P10, respectivament) de 20 baldrigues cendroses del Mediterrani. Cada línia connecta els valors isotòpics de les plomes d'un mateix individu. Atès que els valors isotòpics de la 10ª primària se segreguen en dos grups, presumiblement corresponents a les dues principals zones d'hivernada (representades a la Figura 12; corrents de Benguela i Canàries), els individus de cada grup es representen amb punts negres i línies contínues o amb punts blancs i línies discontínues. Dos individus que, inesperadament, mudaren la darrera ploma (P10) un cop a les zones de cria es mostren amb punts grisos i línies de punts. Els valors isotòpics de C i N (mitjana ± IC 95%) de plomes mudades en aquestes dues àrees d'hivernada d'altres espècies d'aus marines s'indiquen com a referència (Ref.). Els quadrats negres corresponen a mascarells del Cap *Morus capensis* alimentant-se a la corrent de Benguela (Jaquemet i McQuaid 2008); mentre que els quadrats blancs corresponen a baldrigues de Cap Verd *Calonectris edwardsii* que principalment s'alimenten a la corrent Sud de Canàries (Gómez-Díaz i González-Solís 2007).

mudades a finals de l'època de cria representa fidelment els elements adquirits en les localitats de cria. Per tant, aquestes característiques permeten una avaluació integradora dels nivells de contaminants de vastes zones en les àrees de cria, en lloc de fonts específiques regionals de contaminants (Walsh 1990; González-Solís et al. 2002). En el nostre cas, les baldrigues cendroses passen de mitjana 243 dies en les àrees de cria, però només 80 dies a les zones d'hivernada (González-Solís et al. 2007), convertint-se així en excel·lents integradors bioacumulatius dels nivells basals de les àrees de reproducció (Taula 1). Es va explorar la variabilitat geogràfica dels metalls pesants en plomes de baldriga de fins a cinc arxipèlags remots i es van relacionar amb a les diferències geogràfiques en les emissions i descàrregues d'aquests metalls, així com amb l'ecologia tròfica de les aus (com es mostra amb els valors de δ^{15}N). Les diferències en les concentracions de Se i Pb entre colònies van ser degudes principalment als alts valors d'aquests elements en les baldrigues de Cap Verd (Fig. 3b, c), que probablement són el resultat dels elevats nivells basals d'aquests dos elements en aquesta zona de l'Atlàntic (Cutter i Cutter 1995; Helmers 1996). Arribàrem a la conclusió que les concentracions de Se i Pb foren afectades principalment pels aports locals de les corrents de surgència i per la deposició atmosfèrica, ressaltant la importància dels nivells basals com a factor rellevant que influeix en la dinàmica d'aquests dos elements en xarxes tròfiques locals. En el cas del Hg, els nivells més alts es van trobar en els individus de les colònies del Mediterrani (Fig. 3a, Illes Balears i Chafarinas). Aquest resultat està probablement relacionat amb les emissions i els abocaments d'aquest contaminant a Europa, fet que genera uns relativament alts nivells de Hg al Mediterrani comparats amb els de l'Atlàntic, com ja està descrit anteriorment en un bon nombre d'estudis sobre grans depredadors marins (Renzoni et al. 1986; Andre et al. 1991; Lahaye et al. 2006). No obstant això, el Hg presentà

migratòria, de connectivitat migratòria, d'identificació de l'origen d'un individu, de l'avaluació de l'impacte humà en poblacions remotes en el medi marí, i dels canvis en la distribució dels animals tan en les seves àrees d'hivernada, com en les zones de reproducció.

Avaluant el nivell de contaminants en el medi marí

Com s'ha assenyalat abans, contràriament al que passa en l'acumulació i el comportament d'excreció dels isòtops estables, els elements traça adquirits en una temporada poden ser transferits a teixits formats en una altra temporada. No obstant això, especialment per a aquelles espècies amb llargues èpoques de reproducció i relativament curts períodes d'hivernada, la composició elemental de les plomes

una dinàmica més complexa que la del Se o la del Pb, com ho demostra la seva associació addicional amb les signatures d'isòtops estables i per les diferències en els seus nivells entre els sexes. Els nivells de Hg durant el període reproductor van ser associats als valors individuals de $\delta^{15}N$ dins de cada localitat (Fig. 4), indicant que els processos de biomagnificació, no només es produeixen entre les espècies a través de la xarxa tròfiques (per exemple Honda et al. 1987), sinó també entre els individus a un nivell intraespecífic. Finalment, tot i que els nivells de contaminants de les colònies incloses en el nostre mostreig diferiren entre ells, aquests valors varen ser generalment similars als publicats anteriorment per a la mateixa espècie a la Mediterrània i Oceà Atlàntic Central (Renzoni et al. 1986; Monteiro et al. 1999). Així, tot i l'actual preocupació ambiental sobre la creixent contaminació d'origen antròpic dels oceans, vàrem trobar que els nivells de metalls pesants i de Se en les plomes d'aquestes aus marines no foren particularment diferents als publicats fa una dècada (Thompson et al. 1992; Elliott et al. 1992 ; Sanpera et al. 2000; Arcos et al. 2002).

L'estudi de l'ecologia de les aus a través de les seves plomes: aplicacions ecològiques dels biomarcadors intrínsecs

Raül Ramos

● *Disparitat en la integració dels marcadors biogeoquímics intrínsecs en les plomes*: Vàrem demostrar que mentre les signatures d'isòtops estables de les plomes reflectiren un origen exogen, és a dir, que són immediatament transferits des de la dieta a les plomes quan es produeix la seva muda, els elements traça de les plomes poden indicar un origen endogen d'aquests elements, és a dir, que són parcialment mobilitzats des de diversos òrgans on s'emmagatzemen. En conseqüència, la interpretació de les concentracions d'elements traça dels teixits formats durant un determinat període s'ha de fer amb precaució, ja que aquests valors podrien estar reflectint l'exposició a aquests elements durant un període anterior.

● *Robustesa dels isòtops estables en estudis de dieta*: Les signatures isotòpiques proporcionaren una visió integradora de les dietes assimilades en comptes de la informació puntual obtinguda a partir dels recursos ingerits. Mentre que l'anàlisi de la dieta convencional requereix un control exhaustiu al llarg del temps per a obtenir informació fiable, l'anàlisi d'isòtops estables només requereix d'un simple mostreig. A més, l'anàlisi directa de la dieta produeix lleugeres sub- i sobreestimacions en comparació amb les estimacions isotòpiques, principalment derivades de la digestibilitat diferencial de les preses. Així, tot i que no varen assolir la precisió taxonòmica obtinguda amb l'anàlisi directa de la dieta, les signatures d'isòtops estables i l'ús dels models de mescla varen resultar ser eines extremadament valuoses per a una ràpida i fiable avaluació de l'ecologia tròfica de determinades poblacions.

● *L'empremta de les dietes oportunistes*: Les signatures de $\delta^{34}S$ discriminaren entre l'origen marí i salobro-terrestre dels recursos alimentaris, mentre

que un $\delta^{15}N$ empobrit va ser el resultat de l'explotació de recursos provinents d'abocadors. Aquest patró isotòpic ofereix una empremta general alhora d'estudiar l'ecologia alimentària de les poblacions o espècies que són altament oportunistes en els seus hàbits d'alimentació.

● *Els isòtops estables com a traçadors oceànics*: Els resultats dels estudis de migració indicaren que les signatures isotòpiques de regions oceàniques distants poden integrar-se el teixit d'un individu determinat i poden indicar la regió en què aquest teixit va ser format. Si bé les signatures de N va marcar la migració entre el Mediterrani i l'Atlàntic, els valors isotòpics de C i S indicaren les diferents províncies oceàniques de l'Atlàntic, proporcionant una empremta geogràfica global per al seguiment dels moviments migratoris. Aquest resultat posa de relleu el potencial ús dels isòtops estables alhora de seguir els moviments animals en el medi marí.

● *L'homogeneïtat geogràfica en les signatures de les preses*: El fet que determinats tipus de presa en la localitat més remota mostressin signatures isotòpiques diferents a les de les preses d'altres localitats incloses en el mostreig, van posar de manifest la importància de garantir i assegurar l'homogeneïtat geogràfica de les signatures isotòpiques basals abans d'aplicar qualsevol model de mescla, especialment si l'àrea d'estudi considerada és relativament gran.

● *Els isòtops estables com a eines innovadores de gestió*: Hem proporcionat clares evidències que l'anàlisi d'isòtops estables en plomes específiques pot ser utilitzat per determinar l'ecologia tròfica de les aus durant tot el seu cicle anual, fins i tot durant els períodes en què els animals no són accessibles als investigadors. També vàrem relacionar

positivament el grau d'explotació de restes d'abocador (estimat a partir de l'anàlisi d'isòtops estables) amb a la presència d'enterobacteris zoonòtics a nivell individual, fet particularment rellevant per a la salut pública i ambiental. Així doncs, els nostres resultats varen destacar que les anàlisis d'isòtops estables i una de les seves aplicacions ecològiques, els models de mescla poden proporcionar fantàstiques oportunitats a les autoritats de gestió per investigar diferents aspectes relacionats amb l'ecologia tròfica de les espècies.

● *Els isòtops estables en estudis de muda*: Vàrem demostrar que les semblances i les diferències isotòpiques entre els diferents tipus de plomes poden ser utilitzades per a avaluar la fenologia de substitució de plomes en relació amb el cicle anual de les aus. Per tant, tant per a espècies migratòries que es mouen entre diferents regions isotòpiques, com per a aquelles espècies amb una dieta diferencial entre estacions, el coneixement dels seus patrons de muda pot augmentar enormement mitjançant l'anàlisi de la composició isotòpica de les plomes.

● *Dinàmiques metabòliques diferents entre contaminants*: D'una banda, les diferències en els nivells basals entre les diferents àrees geogràfiques explicaren les concentracions de Se i Pb observades en les plomes. D'altra banda, tot i que els nivells basals també afectaren a les concentracions de Hg de les plomes, l'ecologia tròfica de les aus també semblà tenir un paper rellevant alhora d'explicar la seva variabilitat.

● *L'actual estatus de la contaminació dels oceans des del punt de vista de les aus marines*: Tot i l'actual preocupació ambiental sobre la creixent contaminació d'origen antròpic dels oceans, vàrem trobar que els nivells de metalls pesants i de Se en les plomes d'aus marines no foren particularment diferents als publicats fa una dècada.

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Bloc I:

L'estudi dels patrons d'espaciotemporals en ecologia tròfica: el cas d'una espècie problemàtica, el gavià de potes grogues Larus michahellis

Aproximació isotòpica de l'ecologia tròfica d'una espècie de gavina superabundant, per a la qual vàrem dissenyar un model estadístic basat en els isòtops estables de les plomes per a calcular a nivell individual els índexs de la contribució relativa de cada tipus de presa a la seva dieta. Hem proporcionat clares evidències que les signatures d'isòtops estables i una de les seves aplicacions ecològiques, els models de mescla resulten instruments extremadament útils per a una ràpida i precisa avaluació de l'ecologia tròfica de les espècies o de determinades poblacions. Aquesta metodologia es presenta aquí en el context de l'ecologia aplicada, per a una correcta gestió de les dinàmiques poblacionals d'espècies problemàtiques, però també en un context epidemiològic, on les preferències alimentàries d'algunes poblacions han de ser definides amb precisió.

Capítol 1:

Definint les preferències alimentàries d'una espècie superabundant durant el període reproductor

Presentem aquí un estudi descriptiu de dieta d'una espècie de gavina problemàtica arreu de la costa del Mediterrani occidental. Mitjançant l'anàlisi convencional de dieta, basada en més de 350 regurgitats, vàrem estudiar el component espaciotemporal en l'ús de diferents hàbitats d'alimentació explotats per les gavines de potes grogues durant la temporada de cria. Utilitzant l'heterogeneïtat de la dieta subministrada als polls com a estimador de la variabilitat dels hàbitats d'alimentació d'una determinada població, es van avaluar la presència i la importància de recursos tròfics alternatius a les preses provinents d'abocadors i de descarts pesquers. Aquests resultats podrien preveure i aclarir alguns esdeveniments futurs així com possibles canvis en la dinàmica poblacional d'aquestes gavines, com a conseqüència de les decisions de gestió adoptades recentment (la Directiva sobre abocadors de la Unió Europea i el Pla d'Acció Europeu per garantir la sostenibilitat de la pesca al Mediterrani).

Presentem aquí un estudi comparatiu entre mètodes convencionals i isotòpics alhora de descriure la dieta d'una espècie problemàtica a la conca mediterrània. Es varen determinar les contribucions dels recursos marins, terrestres, d'aigua dolça i els dels abocadors en polls de gavià de potes grogues, utilitzant dues metodologies diferents. Els mètodes convencionals basats en regurgitats i l'anàlisi d'isòtops estables en plomes de polls van ser comparats i avaluats, concloent que ambdós coincidien en termes generals, tot i que l'anàlisi convencional subestimava lleugerament la proporció de preses petites, tals com els invertebrats terrestres o d'aigua dolça.

ORIGINAL ARTICLE

Diet of Yellow-legged Gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps

Raül Ramos · Francisco Ramírez · Carolina Sanpera · Lluís Jover · Xavier Ruiz

Abstract In recent decades, the Yellow-legged Gull (*Larus michahellis*) has become a problematic species in many Mediterranean countries, mainly because it interferes with human interests. However, this gull also has a negative impact on several other bird species, many of which are classified as endangered. Two different European Union Action Plans are currently under development with the aim of decreasing the availability of food derived from human activities, such as garbage and fishery discards, which are considered to be the main causes of the super-populations of this gull. Here, we describe the diet of Yellow-legged Gull chicks, with particular emphasis on establishing the dependence of each population on refuse dumps, in order to forecast changes in gull population dynamics in response to the management decisions being implemented. We sampled four colonies along the Western Mediterranean in Spain: the Medes Islands, the Ebro Delta, the Columbretes Islands, and Mazarrón Island. To elucidate their feeding ecology and to avoid obtaining a discrete estimation from a single sampling, we collected regurgitates from each colony three times throughout the chick-rearing period. Slightly differential feeding habits were observed between chick age classes. Younger chicks in all four colonies tended to be consistently provisioned with smaller prey such as invertebrates. Distinct uses of several foraging habitats among localities were observed. In particular, the use of refuse dumps was common and abundant in two of the colonies: the Medes and Mazarrón Islands. As a consequence of current management strategies, generalized reductions in Yellow-legged Gull populations and increases in the consumption of alternative food resources to those of fishery discards and refuse scraps are expected. Finally, we predict that decreased food availability will force some gulleries to increase predation on endangered species, thereby raising a conservation concern.

Keywords Dietary analysis · Feeding ecology · Fishery discards · Landfill management · Regurgitate

X. Ruiz: deceased on 27 April 2008.

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Introduction

Over the last several decades, many vertebrate species have increased in abundance as a result of habitat changes resulting from human activity (Garrott et al. 1993). Most of the communities of these species are overpopulated. This overpopulation is attributed to their flexible, opportunistic, and gregarious nature, which makes them highly adapted to living in habitats modified by man. In particular, gulls have been extensively studied as a potential superabundant species in numerous localities around the world (Belant et al. 1993; Bertellotti et al. 2001; Steele and Hockey 1990; Vidal et al. 1998).

In the Mediterranean basin, populations of the Yellow-legged Gull (*Larus michahellis*) have greatly increased over the last four decades and have become problematic in

this region (Vidal et al. 1998). Like other gull species, the Yellow-legged Gull is considered a pest because of its negative impact on airports, cities, reservoirs, arable land, and fisheries (Dolbeer et al. 1997; Monaghan et al. 1985; Mudge and Ferns 1982). In other cases, this gull species disturbs, displaces, or even predates on other, often protected, species (Furness and Monaghan 1987; Oro et al. 2005; Swennen and Van de Meer 1992; Thomas 1972). Most of these effects can be attributed to overpopulated gulleries that have arisen from the scavenging capacity of this bird on increasing food resources derived from human activities (Furness et al. 1992), particularly garbage but also fishery discards (Bosch et al. 1994; Mudge and Ferns 1982; Pons 1992).

In general, food availability is a determinant factor of population dynamics and also the breeding success of most species (Oro et al. 2006). In this respect, food sources derived from human activities, such as refuse dumps, are usually abundant and relatively predictable, thereby increasing the carrying capacity of an ecosystem and allowing gulls to improve breeding success and probably survival (Pons 1992). Although the removal of these food resources produces a decrease in the number of breeding pairs, the production per pair is not affected and birds breed successfully without having access to nearby refuse dumps (Kilpi and Öst 1998). In this regard, the European Union Landfill Directive (1999/31/EC) aims to reduce the amount of biodegradable municipal waste sent to landfills by up to 40% of the 1995 level by 2020 (http://ec.europa.eu/environment/waste/landfill_index.htm). Moreover, in October 2002, the European Union also adopted an Action Plan to ensure the sustainability of fisheries in the Mediterranean (http://ec.europa.eu/fisheries/cfp/2002_reform_en.htm). Most of these measures focus on preventing catches of unwanted fish to achieve biologically, environmentally, and economically sustainable fisheries.

Thus, establishing the dietary preferences of several gull populations will facilitate the prediction of changes in and consequences for gull population dynamics. Moreover, any other management measure to effectively control populations of gulls should focus on limiting resource availability during a sensitive season (i.e., the breeding period), thereby reducing the production of a population (Kilpi and Öst 1998). Overall, management decisions are usually costly (Thomas 1972) and should be based on an accurate knowledge of the feeding habits and resources exploited by each gull population.

Dietary analyses to assess feeding habits in birds have several limitations that hamper the accuracy of results (Duffy and Jackson 1986; González-Solís et al. 1997), such as biases of distinct magnitude depending on the type of food sample analyzed. Diet studies based on direct observations are usually biased towards the most

conspicuous prey, and prey from gut contents or pellets can be difficult to identify as items are often considerably or completely digested. Among the food sampling methods available, regurgitates are the least biased and most reliable for describing diet composition (González-Solís et al. 1997; Seefelt and Gillingham 2006). However, regurgitate analysis provides only episodic information on feeding habits, i.e., each sample represents only a short collection of specific feeding events in the diet of an individual and does not provide information on resources used in the past.

In this study, we analyzed more than 350 chick regurgitates from four Yellow-legged Gull colonies along the Western Mediterranean coast of Spain. We sampled three age classes of chicks throughout the chick-rearing period to: (1) study the differential use of resources by each chick class; (2) explore the spatial heterogeneity in exploited resources on the basis of potential nearby feeding habitats; and (3) predict population dynamic changes in these colonies as well as in other populations with similar feeding patterns in response to the implementation of future management strategies (see above).

Methods

Study area

The study was carried out in four colonies along the Iberian Mediterranean coast during the chick-rearing period in 2004. From north to south, the colonies sampled were: the Medes Islands, the Ebro Delta, the Columbretes Islands, and Mazarrón Island (Fig. 1). Relevant information about the location of the colonies and the activity of fishing vessels near each area is given in Table 1.

Gull sampling and regurgitate analysis

We visited each colony three times during the chick-rearing period. In each visit, we sampled preferentially chicks of a similar age class. Following this sampling strategy, we sought to check for possible age-differential feeding but also tried to obtain a whole and robust estimation of feeding habits throughout the chick-rearing period. We sampled a single chick from each brood to avoid pseudoreplication of parents feeding the same prey to their offspring. We measured bill, head, and tarsus length to the nearest mm using digital calipers. Previous studies have shown that bill and tarsus length of gulls grow linearly with age (Coulson et al. 1981; Greig et al. 1983; Werschkul 1979). Because we sampled chicks that differed slightly in age, we used a principal components analysis (PCA) with bill, head, and tarsus length as variables to classify them
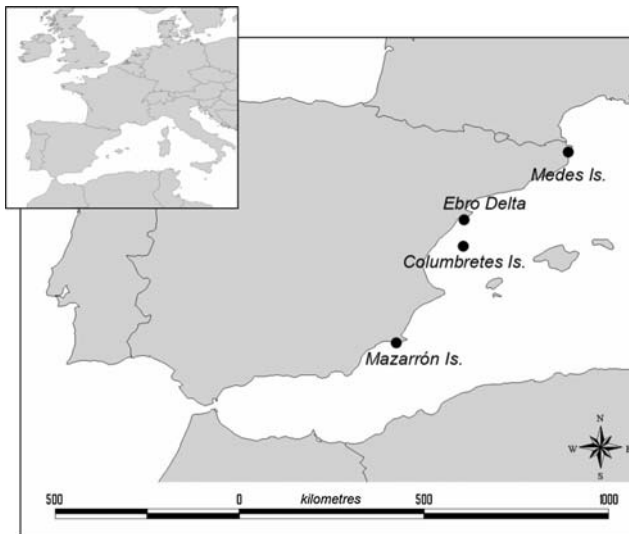
Fig. 1 Map of the Iberian Peninsula. Colony sites (*filled circles*) of the Yellow-legged Gull (*Larus michahellis*) included in the study along the Spanish Western Mediterranean coast are indicated

into three age groups. The PCA generated a continuous variable of global size component, which we artificially trichotomized into discrete categories (Bennett and Owens 2002). Chicks belonging to the first category (first age) were up to 1 week old, the second category (second age) included 2- and 3-week-old chicks and the third category (third age) included chicks just before fledging (between 4 and 5 weeks old).

Food samples were collected as spontaneous regurgitations ($n = 356$) from chicks when they were handled for measuring. Each regurgitate was placed in a sealed plastic bag and kept frozen until the laboratory analysis. Regurgitates were weighed and their contents identified to the

order level using standard reference guides. Prey was assigned to six categories on the basis of the foraging habitats where they were captured (Bosch et al. 1994): marine prey (distinguishing between pelagic and benthonic fish); brackish and freshwater; crops and terrestrial environments; refuse tips; and others. The presence of each category in each locality is represented by its relative biomass (the total biomass of the category/mass of all samples). Diet analyses were based on foraging habitats as these could be intuitively compared with the gull's trophic ecology whereas little could be inferred from the taxonomic point of view (Cooper et al. 1990).

Statistical analysis

To compare the use of foraging habitats among localities and age classes, we evaluated the heterogeneity of the exploited habitats inferred from the regurgitate analysis using indexes of diversity (Duffy and Jackson 1986). Habitat heterogeneity was estimated using the Shannon–Weaver index (Keylock 2005; Pielou 1967; Shannon and Weaver 1949):

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where p_i is the proportion of biomass belonging to individuals in the ith species or category (in our case, each foraging habitat). Indexes of diversity (H') were calculated using the biomass of each foraging habitat and were paircompared following Hutcheson's procedure (Zar 1996) among colonies and among ages. A Bonferroni correction using the sequential Holm's procedure (Holm 1979) was applied to maintain an overall error type I of 0.05 in the 18 simultaneous multiple comparisons.

Table 1 Main informative parameters of the breeding sites of the Yellow-legged Gull (*Larus michahellis*)

	Locality site	Distance from human settlements (km)	Number of breeding pairs	Fishing vessel activity around each area			References
				Relative estimation	Number of vessels	Gross tonnage	
Columbretes Is. (39°54′N, 0°41′E)	Isolated archipelago in a Marine Reserve	55.0	450	High	329	9,844	Oro et al. (2006)
Ebro Delta (40°40′N, 0°45′E)	Isolated peninsula in a Natural Park	7.5	6,000	High	520	11,440	Oro et al. (2006)
Medes Is. (42°0′N, 3°13′E)	Islands off the coast of a tourist site	0.9	6,500	Moderate–high	579	9,517	Bosch et al. (2000)
Mazarrón Is. (37°33′N, 1°16′W)	Island off the coast of a tourist site	0.5	900	Low	277	4,156	García-Morell and Escribano (2005)

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

Results

In the Columbretes Islands, the feeding pattern of the second and third age classes did not differ. However, a small proportion of freshwater invertebrates was found in the diet of the first age class (Tables 2, 3) but not in the older ones. Chicks from the Ebro Delta showed changes in the feeding pattern throughout the chick-rearing period, although in Mazarrón Island the diet differed only between the youngest and oldest chicks (Table 3). In both localities, a higher proportion of small invertebrates (both from terrestrial and freshwater habitats) was found in the smaller chicks, while the presence of resources from refuse dumps increased in the third age class (Table 2). In the Medes Islands, the second age class differed from the others while no differences were found between the first and third groups (Table 3). Although no consistent feeding patterns were observed throughout the chick-rearing period in the four colonies, a greater proportion of smaller prey, both from brackish and freshwater (Mazarrón Is. and Columbretes Is.) and from crops and terrestrial environments (Ebro Delta and Medes Is.), was detected in the diets of younger chicks (Table 2).

In spite of the significant differences among age classes in most localities, we grouped all regurgitate samples to compare the chicks' diet among colonies to obtain a global assessment of foraging preferences throughout the breeding season in each locality (Fig. 2; Table 2). Dietary heterogeneity showed a gradient among the colonies, with the birds on Mazarrón Island exhibiting the highest value. The index for the Medes Islands was slightly lower, followed by the Ebro Delta, whereas birds from the Columbretes Islands showed the lowest degree of dietary heterogeneity (Table 2). When pair-compared, all the indexes differed (Table 3).

Overall, gulls used three main foraging habitats to feed their chicks: pelagic prey, refuse dumps, and brackish and freshwater ecosystems (Fig. 2). These categories represented 97.6, 87.4, 85.5, and 89.6% of total biomass in the Columbretes Islands, the Ebro Delta, the Medes Islands and Mazarrón Island, respectively. Pelagic fish samples occurred in all four localities, whereas those from refuse dumps were present in the diets of three. However, waste had a considerable relevance only in the Medes Islands (45.4%) and Mazarrón Islands (43.8%), being less important in the Ebro Delta (8.5%). As expected, the regurgitates

Table 2 Diet of Yellow-legged Gull chicks of different age categories

	n	Foraging habitat						$H' \pm SE$
		Pelagic prey	Benthonic prey	Brackish and freshwater	Crops and terrestrial	Refuse tips	Others	
Columbretes Is.								
1st age	28	88.9	0.0	7.2	0.0	0.0	3.9	0.42 ± 0.015
2nd age	38	96.3	0.9	2.6	0.0	0.0	0.2	0.19 ± 0.009
3rd age	42	96.7	0.4	0.0	0.0	0.0	2.8	0.16 ± 0.007
Total	108	95.7	0.6	1.9	0.0	0.0	1.7	0.22 ± 0.019
Ebro Delta								
1st age	36	56.4	2.5	4.2	29.0	3.9	4.1	1.16 ± 0.018
2nd age	29	82.8	4.4	6.2	0.4	0.0	6.3	0.66 ± 0.018
3rd age	24	75.9	1.0	2.2	0.2	18.7	2.0	0.74 ± 0.022
Total	89	74.7	2.6	4.2	6.0	8.5	4.1	0.95 ± 0.007
Medes Is.								
1st age	36	21.0	0.0	0.0	7.5	56.9	14.5	1.12 ± 0.016
2nd age	35	27.8	0.0	0.0	0.6	62.3	9.3	0.90 ± 0.014
3rd age	27	51.3	14.0	1.8	0.1	31.0	1.9	1.13 ± 0.022
Total	98	39.2	7.2	0.9	1.2	45.4	6.1	1.18 ± 0.006
Mazarrón Is.								
1st age	14	15.5	0.0	48.5	0.0	28.7	7.3	1.19 ± 0.040
2nd age	30	23.4	0.0	25.8	2.2	43.5	5.2	1.29 ± 0.021
3rd age	17	15.5	0.0	19.4	4.0	48.1	13.0	1.35 ± 0.038
Total	61	20.3	0.0	25.5	2.6	43.8	7.8	1.33 ± 0.010

The first age category included 1-week-old chicks, the second 2- to 3-week-old chicks and the third 4- to 5-week-old chicks. Values are given in % of fresh weight (biomass) in relation to age classes and on the basis of foraging habitats. Diversity indexes and their standard errors are also shown

Table 3 Diversity pair-comparisons among age categories and colonies

Age-comparisons	t statistic	df	P value
Columbretes Is.			
1st–2nd	13.57	48	<0.001*
1st–3rd	15.76	42	<0.001*
2nd–3rd	2.21	75	0.030
Ebro Delta			
1st–2nd	19.58	64	<0.001*
1st–3rd	29.05	58	<0.001*
2nd–3rd	8.95	53	<0.001*
Medes Is.			
1st–2nd	10.50	70	<0.001*
1st–3rd	−0.43	52	0.672
2nd–3rd	−9.05	48	<0.001*
Mazarrón Is.			
1st–2nd	−2.17	21	0.041
1st–3rd	−2.96	30	0.006*
2nd–3rd	−1.53	27	0.139
Colony comparisons			
Columbretes–Ebro Delta	−36.37	139	<0.001*
Columbretes–Medes	−48.39	132	<0.001*
Columbretes–Mazarrón	−51.56	158	<0.001*
Ebro Delta–Medes	−23.48	180	<0.001*
Ebro Delta–Mazarrón	−29.48	116	<0.001*
Medes–Mazarrón	−12.16	105	<0.001*

* Significant differences at an overall error type I of 0.05
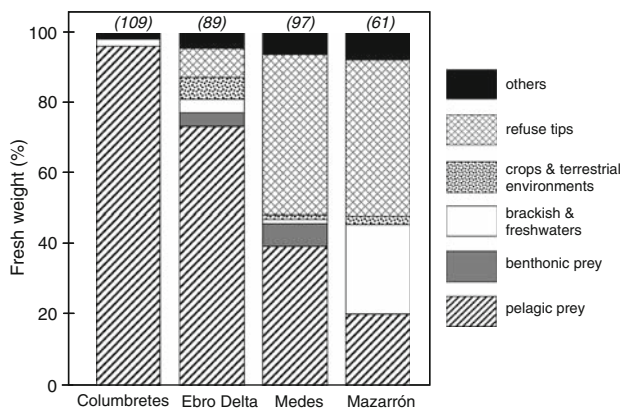
Fig. 2 Fresh weight percentages of prey on the basis of the main foraging habitats in regurgitates of Yellow-legged Gull chicks

collected from the Columbretes Islands did not include food from refuse dumps and most of the food items came from the marine environment (96.3%). Prey from brackish and freshwater habitats were relevant only in regurgitates from chicks inhabiting Mazarrón Island (25.5%).

Discussion

Regurgitation analyses showed that food sources related to human activities, such as refuse or fishery discards, were the main dietary components of our sample of chicks, as reported in the study by Duhem et al. (2003). These sources comprised more than 85% of the total prey biomass in three localities, whereas in the fourth (Mazarrón Island), this value reached 65% (Fig. 2). These values are similar to those reported in a previous study by Bosch et al. (1994) for the colonies on the Medes Islands and in the Ebro Delta. Moreover, our findings are consistent with data from other colonies in the Western Mediterranean (Vidal et al. 1998) indicating that the exploitation of these food resources is maintained over time and space. Therefore, owing to the generalist and opportunistic feeding habits of the Yellow-legged Gull, it is reasonable to assume that the availability of these resources makes a considerable contribution to the expansive dynamics of its populations in the Mediterranean. This opportunistic behavior and high feeding adaptability are relevant factors to take into account when assessing population dynamics or the management of pest species (Thomas 1972).

The information from chick regurgitates showed substantial differences between localities, particularly in the use of the marine habitat. These differences can be explained by the availability per capita (i.e., related to the colony size) of this food resource in each colony as well as by the presence of alternative food sources that are easier to obtain than fish, such as garbage from refuse dumps (Bertellotti et al. 2001). According to the optimal foraging theory, one can expect birds to feed in a way that maximizes their energy intake (Schoener 1971) as well as that of their chicks during the breeding period. Garbage consisting mainly of chicken, pork, and beef scraps has a high energetic value per meal and high fat and protein content per gram (Pierotti and Annet 1991). This observation together with the ease of obtaining this food could explain the high proportion of this food resource in diets when refuse dumps are abundant and close to breeding colonies (in both the Medes Is. and Mazarrón Is.). The lower proportion of garbage in the diet of the chicks sampled in the Ebro Delta, compared to those from the Medes Is., might be related to lower availability of this resource to the colony in the Ebro Delta, as there are five times as many refuse dumps in area around the Medes Islands than in the Ebro Delta (Bosch et al. 1994). According to the optimal foraging theory, for the gulls on the Columbretes Islands, the mainland is too far away from their breeding area to be used for chick provisioning. Consequently, these gulls feed their offspring mainly with fish (see Duhem et al. 2005), which can be obtained from the fisheries operating in the area (Arcos et al. 2001) or even from sub-surface predators (Oro 1995).

Thus, on the Columbretes Islands the Yellow-legged Gull competes not only for space but also for food (Oro et al. 2006), thereby limiting the size of the colony.

Prey from crops and terrestrial environments (mostly small invertebrates) were common in the diet of the chicks sampled in the Ebro Delta, although their relevance decreased with age (Table 2). Similarly, brackish and freshwater prey (also mostly represented by small invertebrate larvae of Syrphidae) was of considerable importance in Mazarrón Island and especially abundant in smaller chicks. The presence of these two prey types in the chicks' diet indicates the opportunistic behavior of Yellow-legged Gulls, as well as the proximity of this food resource to the breeding site. However, the observation that these small prey were specially abundant in younger chicks might be attributed to the need to provide small food items that chicks can easily swallow and digest or to requirements to increase the feeding rates of these chicks during this period (Pedrocchi et al. 1996). Supporting this idea, the diet of these younger chicks was found to show greater heterogeneity than that of older nestlings, and it was also constant in the sampled colonies (Table 2).

Two European Union Action Plans are currently under development and seek to decrease the availability of food derived from human activities to gulleries, such as garbage and fishery discards (see "Introduction"). These management decisions should be taken into account when forecasting changes in gull population dynamics. In this regard, presumed drastic reductions are expected in most Yellow-legged Gull colonies. Reduced availability of fishery discards or decreased access to refuse dumps will broaden the trophic niche of these birds, thereby leading to an increased consumption of alternative food sources, such as those from terrestrial habitats (Duhem et al. 2005), when available and relatively close to the colony. The trophic niche width, measured as the heterogeneity of the foraging habitats exploited, provides a suitable approach to measure the feeding plasticity and opportunism of a species (La Mesa et al. 2000) and could be used as an estimator of the number of distinct foraging opportunities the species has in each locality. Our study suggested that the gull populations on the Medes and Mazarrón Islands will be the most affected by a decrease in refuse dump availability, while birds from the Ebro Delta and the Columbretes Islands will be influenced mainly by the optimization of fishery techniques, which will reduce the amount of discards. However, the colonies on Mazarrón Island and in the Ebro Delta have alternative food resources nearby, such as freshwater or terrestrial invertebrates, which could be more intensely exploited in the future. Drastic reductions in the gull population are expected on the Medes Islands, as their population holds one of the greatest densities of breeding pairs (Bosch et al. 2000) which depends mainly on these

two foraging habitats during the whole chick-rearing period. In addition, as a consequence of the reduction of the carrying capacity of the ecosystem, we can predict increasing conflicts in the relatively short-term within Yellow-legged Gull colonies but also with endangered species breeding nearby, e.g., European Storm Petrels (*Hydrobates pelagicus*), Audouin's Gulls (*Larus audouini*), Greater Flamingos (*Phoenicopterus ruber*) and several species of herons (García-Morell and Escribano 2005; Vidal et al. 1998). Interactions with protected species may range from increasing disturbance to active persecution and predation on eggs, chicks, and even adults (Martínez-Abraín et al. 2003; Oro and Martínez-Abraín 2007).

Here, we addressed the spatiotemporal component in the use of distinct foraging habitats by Yellow-legged Gulls during the breeding season. Consistent with the opportunistic behavior of the species, several foraging habitats were identified depending on their availability and proximity to the colony. Using chick diet heterogeneity as an estimator of variability of the feeding habitats exploited, we can evaluate the presence and relevance of alternative food resources to refuse dumps and fishery discards. These results may help us to predict the effects of recent management decisions on gull population dynamics. Generalized reductions in Yellow-legged Gull populations are expected over its whole distribution range. Furthermore, the consumption of alternative food sources to fishing discards and refuse scraps, when available, will gain importance. In addition, a reduction in feeding resources will force some gulleries to increase predation on other species, some of which are endangered, with consequent conservation concern. The results and predictions presented here elucidate future scenarios which should be considered by management authorities in the relatively short term.

Zusammenfassung

Nahrungsangebot für die Küken der Mittelmeermöwe *Larus michahellis* entlang der spanischen westlichen Mittelmeerküste: die Bedeutung von Mülldeponien

Die Mittelmeermöwe *Larus michahellis* ist in den letzten Jahrzehnten zu einer Problemart in den Mittelmeerländern geworden, hauptsächlich wegen ihrer Interaktion mit den Interessen der Menschen, aber auch mit anderen Arten, die normalerweise geschützt sind. Momentan werden zwei verschiedene Aktionspläne der Europäischen Union entwickelt, die versuchen, die Verfügbarkeit von Futter zu reduzieren, das durch menschliche Aktivität anfällt (z.B. Müll und Fischereiabfälle) und als Hauptursache für die übergroßen Möwenpopulationen angesehen wird. Das Ziel

dieser Arbeit war, die Nahrung von Küken der Mittelmeermöwe zu beschreiben und insbesondere die Abhängigkeit der Populationen von Abfall zu ermitteln, um Änderungen in der Populationsdynamik der Möwen absehen zu können, die durch diese Management-Entscheidungen zustande kommen. Vier Kolonien entlang des westlichen Mittelmeers wurden beprobt: Medes-Inseln, Ebrodelta, Columbretes-Inseln und Mazarrón-Insel. Um die Nahrungsökologie der Möwen aufzuklären und es zu vermeiden, eine diskrete Schätzung von nur einer einzigen Beprobung zu erhalten, haben wir in jeder Kolonie hervorgewürgtes Futter dreimal während der Kükenaufzuchtsperiode gesammelt. Wir fanden unterschiedliche Nutzungen verschiedener Nahrungssuchhabitate an den vier Standorten. Die Nutzung von Mülldeponien war üblich und häufig in zwei der untersuchten Kolonien, auf den Medes-Inseln und der Mazarrón-Insel. Es konnte eine leichte Tendenz beobachtet werden, unterschiedlich alte Küken unterschiedlich zu füttern. In allen vier untersuchten Kolonien gab es die Tendenz, dass jüngere Küken durchweg mit kleinerer Beute, z.B. Invertebraten, versorgt wurden. Diese Ergebnisse könnten helfen zu verstehen, welche Auswirkungen die gegenwärtigen Management-Entscheidungen auf zukünftige Ereignisse und Veränderungen in der Populationsdynamik der Möwen haben werden.

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Feeding ecology of yellow-legged gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods

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ABSTRACT: Accurate determination of feeding habits using only conventional dietary analysis is usually difficult, due to both biases caused by prey digestibility differences and to the discrete aspect of the sampling. However, combining conventional methods with stable isotope analysis provides an integrated view of the assimilated diet. Here, we measured stable isotope ratios of carbon (δ^{13}C), nitrogen (δ^{15}N) and sulphur (δ^{34}S) in feathers of yellow-legged gull *Larus michahellis* nestlings from 4 colonies along the western Mediterranean coast, where the gulls consume different proportions of marine, terrestrial and garbage resources. We collected and analysed chicks' regurgitates in each colony, thereby determining the isotopic signatures in a significant sample of prey. By applying a mixing model to our isotopic values, we compared the information provided by the 2 methodologies. According to direct prey sampling and the particular conditions of the breeding areas, populations with more enriched δ^{34}S values consumed a diet richer in marine prey, with lower δ^{15}N signatures indicating garbage consumption. The main pattern resulting from the mixing model agreed with direct diet sampling. However, estimated proportions for small-sized prey were controversial, which indicates that small, soft prey items might be underestimated by regurgitate analysis. We conclude that stable isotope signatures and the use of mixing models are useful tools for the rapid assessment of feeding ecology in certain populations. Solving analytical biases should be considered in future feeding studies, thereby saving time and minimising bird disturbance by using an isotopic methodology.

KEY WORDS: Carbon-13 · Mixing models · Nitrogen-15 · Regurgitate · Sulphur-34

INTRODUCTION

Accurate knowledge of diet composition is mandatory in several key areas of applied ecology, such as the management of problematic species and the use of a species to monitor the environmental impact of pollutants. The direct sampling method of reconstructing diets suffers from several drawbacks that hamper the applicability of results (Duffy & Jackson 1986,

González-Solís et al. 1997, Barrett et al. 2007). Diet studies based on field observations are biased toward the most conspicuous prey. Prey are difficult to identify from gut contents or regurgitates, which are often partially digested (but see Cherel et al. 2007). Moreover, such studies might also be biased toward prey types that are more resistant to digestion, leading to overestimation in the final reconstructed diet (González-Solís et al. 1997, Votier et al. 2003) or proving inapplicable

because of the intrinsic characteristics of some food types (e.g. pieces of meat and garbage taken at refuse dumps). These methodologies provide only an episodic view of an individual's diet, since each sample represents only a specific feeding event (Votier et al. 2001). Thus, to obtain reliable information about a population's feeding habits, exhaustive monitoring over time is needed (Jordan 2005). However, conventional approaches are still required when we need to identify the taxonomic group of prey consumed. Regurgitated food appears as the least biased method for describing diet composition (González-Solís et al. 1997).

The use of stable isotope analysis (SIA) in animal ecology has increased enormously in recent years and has become an important tool to study trophic ecology (Hodum & Hobson 2000, Post 2002). Although SIA does not provide the taxonomic detail achieved by conventional dietary analysis, it avoids prey digestibility biases because it only takes into account assimilated food. Moreover, it provides a ready-made integrated estimate because the assimilated diet for a certain period of time is summarised, depending on the tissue analysed (Hobson 1999). Stable isotopes of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and sulphur ($^{34}S/^{32}S$, $\delta^{34}S$) are used primarily to determine sources of primary production and are useful to trace the input of these elements into food webs (Krouse & Herbert 1988, Richards et al. 2003). In particular, the use of $\delta^{34}S$ is recommended when the origin of the diet is heterogeneous (Peterson et al. 1985). Stable isotopes of nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) are indicators of food web interactions, and the trophic positions of species as consumers are typically enriched in ^{15}N relative to their food (Post 2002, Vanderklift & Ponsard 2003). If isotopic signatures in the tissue of a consumer and its main dietary sources are known and adjusted by the fractionation factors between the tissue and each of the sources (Gannes et al. 1998), we can apply mixing models to obtain an index of the relative contribution made by each kind of prey to the consumer's diet, as long as prey are isotopically distinct (Schwarcz 1991).

Populations of several species of gulls (*Larus* spp.) have increased dramatically throughout Australia, North America and Europe in the past few decades (Smith & Carlile 1993, Vidal et al. 1998). These demographic increases have been attributed to several factors (Pons 1992, Bosch et al. 1994, Belant 1997), such as the protection from human disturbance, the increasing availability of anthropogenic food from both periurban open-air refuse dumps and industrial fisheries, and the great ability of gulls to adapt to human-altered environments.

We studied the diet of yellow-legged gulls *Larus michahellis*. This species breeds around the Mediterranean basin, and is the most common and widespread seabird of the western Mediterranean, where its population reached ~120 000 nesting pairs (Pérennou et al. 1996), increasing up to 10% yr^{-1} (Thibault et al. 1996, Vidal et al. 1998). As a result of its increasing population, the yellow-legged gull has become problematic throughout its Mediterranean range. In most cases it is considered a pest because of its interactions with human populations in airports, cities, arable lands or fisheries. In addition, it interacts with other species, usually under protection, which can be disturbed, predated and displaced by the gulls from their breeding areas (Vidal et al. 1998, Oro & Martínez-Abraín 2007).

On the other hand, owing to its abundance, wide distribution and opportunistic feeding habits, the yellowlegged gull might also be used to measure the effects of pollution in the Mediterranean area. This is another important aspect of applied ecology in the case of this species, as it benefits from the same resources consumed by humans, as indicated by its use of refuse dumps and fishery discards. Therefore, if this species is to be used for biomonitoring, the foraging habitats and trophic relationships for every population must be determined to correctly interpret the meaning of the observed pollutant levels (Furness & Camphuysen 1997, Becker 2003, Sanpera et al. 2007).

Our main objective was to isotopically characterise ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) the diet of yellow-legged gull populations at different localities where they are believed to consume different proportions of marine, terrestrial and garbage resources. We used an approach based on the feeding ecology of nestlings alone. This ensured that the tissues analysed (feathers) were formed exclusively from the dietary inputs received at the colony site (Sanpera et al. 2007), thus avoiding potential problems caused by the seasonal movements of adult populations. Secondly, as few papers have combined direct dietary analysis and indirect mixing models based on isotopic signatures, we aimed to compare results from these 2 approaches and to find appropriate tracers to isotopically define the different food resources exploited by gulls.

MATERIALS AND METHODS

Study area and sampling strategy. The study was carried out in 4 colonies along the Iberian Mediterranean coast during the chick-rearing period in 2004. From north to south, the sampled colonies were in the Medes Islands, the Ebro Delta, the Columbretes Islands, and Mazarrón Island (Fig. 1). These colonies were chosen because we expected their diets to differ and their stable isotopes to differ accordingly. The Medes Islands (42° 0′ N, 3° 13′ E) lie 900 m off the coast near a series of tourist towns, and they hold one of the

Fig. 1. *Larus michahellis.* Iberian Peninsula (southwest Europe), indicating breeding colonies included in the study. Photo shows a yellow-legged gull chick begging one of its parents for food

largest colonies of yellow-legged gulls in the Mediterranean (ca. 6650 pairs after several cullings; Bosch et al. 2000). According to Bosch et al. (1994), their diet is mainly composed of garbage from refuse dumps and some fishery discards. About 6000 pairs live in the Ebro Delta colony (Guinart et al. 2004), located on the Peninsula de la Banya (40° 40′ N, 0° 45′ E), a protected area of salt marshes within the Ebro Delta Natural Park, where fishing is intense (Pedrocchi et al. 2002). Previous studies have indicated that the gulls' diet is mainly composed of fishery discards and, to a lesser extent, garbage (Bosch et al. 1994). The Columbretes Islands (39° 54′ N, 0° 41′ E) are a volcanic archipelago, lying 55 km east off the coast of Castelló. No previous detailed studies were available, but owing to the islands' great distance from the mainland, these birds were expected to feed mainly on fish during the breeding season. Their colony size is the smallest of the 4 colonies sampled, with about 400 breeding pairs (Martínez-Abraín et al. 2003). Mazarrón Island (39° 33′ N, 1° 16′ W) also lies immediately opposite a large tourist town, which is especially well populated during summer. No previous studies on the diet of this colony were available; however, based on previous observations, we expected to find a mixture of garbage and terrestrial prey caught in the very intensive crop fields surrounding the town, as well as some fishery discards.

Each colony was visited 3 times throughout the chick-rearing period. During each visit, a single chick was sampled from each brood to avoid pseudoreplication due to parents feeding the same prey to their offspring. Food samples were collected as spontaneous regurgitations (n = 356) from chicks when they were handled for marking and measuring. The regurgitates were individually placed in sealed plastic bags and stored at −20°C until laboratory analysis. On the last visit only, when fledglings were available, 6 to 8 growing scapular feathers were collected from each bird (n = 107). Scapular feathers from chicks grow slowly and constantly (R. Ramos pers. obs.); thus, we hypothesised that they represent the entire feather-growth period.

Laboratory procedure and stable isotope analysis. In the laboratory, every regurgitate was weighed and identified to the order level using standard reference guides (Ruiz 1985). They were then assigned to 5 categories according to foraging habitat (see Bosch et al. 1994): (1) marine, (2) brackish and fresh waters, (3) crops and terrestrial environments, (4) refuse tips, mainly including food scraps and (5) other. The importance of the different categories in each colony was represented by its percentage biomass. Dietary analyses focused on foraging areas because our previous observations had indicated that differences in prey signatures were different enough to guarantee good discrimination using stable isotope methodology.

Feathers were washed in a 0.25 M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contamination, dried in an oven at 60°C to constant mass, and ground to a fine powder in a freezer mill (Spex Certiprep 6750) operating at liquid nitrogen temperature. Additionally, from all available prey we took a sample of the main prey items consumed in each locality to analyse their isotopic signatures. Before isotopic analysis, food samples were freeze-dried and ground to a fine powder in the freezer mill. To reduce variability due to isotopically lighter lipids, which may have a particular influence on carbon isotope ratios (Attwood & Peterson 1989, Hobson & Welch 1992), lipids were removed by the Folch extraction method (Folch et al. 1957) with several chloroform-methanol (2:1) rinses.

Subsamples of 0.4 mg of feather powder for carbon and nitrogen and about 3.5 mg for sulphur analyses were weighed to the nearest μg, placed into tin capsules and crimped for combustion. Samples were oxidised in a Flash EA1112 coupled to a stable isotope mass spectrometer Delta C through a Conflo III interface (ThermoFinnigan), where the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values were determined.

Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1)$$

where X (‰) is ^{13}C, ^{15}N or ^{34}S, and R is the ratio of corresponding element (^{13}C/^{12}C, ^{15}N/^{14}N or ^{34}S/^{32}S), in sample or standard. The standard values were Pee Dee Belemnite for ^{13}C, atmospheric nitrogen for ^{15}N and troilite from the Canyon Diablo Meteorite for ^{34}S. The isotopic ratio mass spectrometry facility at the Serveis Científico-Tècnics of the University of Barcelona (Spain) applies IAEA standards inserted every 12 samples to calibrate the system. Replicate assays of standards indicated measurement errors of ±0.1, ±0.2 and ±0.2‰ for carbon, nitrogen and sulphur, respectively, but these are likely to be underestimates of true measurement error for complex organics like feathers.

Isotopic mixing models and statistical analysis. Relative indices of the different food sources were estimated for each population using δ^{13}C, δ^{15}N and δ^{34}S mean values in a concentration-weighted mixing model. When isotope values differ substantially among the sources, a concentration-weighted model is recommended (Phillips & Koch 2002). Following Phillips & Koch (2002), we used the ISOCONC 1.01 model suitably modified to use 4 food sources and 3 stable isotope signatures. To apply mixing models, isotopic values for food sources must be adjusted by appropriate factors (Δdt) to account for trophic fractionation (Gannes et al. 1998). The fractionation factor for marine fish and feathers was established from Columbretes Islands data, as only marine food resources were used there. We used Δdt values from the literature for the other food categories (Table 1). When negative values near 0 were generated by the mixing model, percentages were readjusted for each locality, setting the most negative value to 0 and recomputing other percentages according to the original proportions given by the model.

Intercolony differences in biomass percentages of prey were identified using a Kruskal-Wallis test followed by a Mann-Whitney pair-comparison test with Bonferroni adjustment. Normality checks for distributions of values of δ^{13}C, δ^{15}N and δ^{34}S were made using Q-Q plots. No severe deviations from normality were found, and we used parametric tests throughout. Because variances were found to be heterogeneous, isotopic differences between localities were analysed using 1-way analysis of variance (ANOVA) with Welch's correction. Tamhane's test procedure was used in *a posteriori* pairwise comparisons. Spearman correlations were used to examine relationships between isotope data and measurements of marine prey consumption at the colony level. To compare the 2 approaches, we represented the single values derived from mixing models (converted to weights using the mean weight of all regurgitates) with the error bars for each foraging habitat (with mean and 95% confidence intervals) computed from conventional dietary analysis. Statistical analysis was carried out using SPSS 15.0.

RESULTS

Diet analysis

According to diet biomass percentages (Fig. 2), the gulls used 3 main foraging habitats: marine environments, refuse tips and brackish and freshwater marshes. Total regurgitates from these 3 habitats respectively represented 98.2, 90.3, 92.7 and 89.6% of total biomass in the Columbretes, Ebro Delta, Medes and Mazarrón colonies. Prey from marine environments occurred in diets at all 4 localities, while food from refuse tips was only present in the diets at 3 sites. However, only garbage was relevant in the Medes site (45.4%) and the Mazarrón site (43.8%), and it was poorly represented in the Ebro Delta site (8.5%). As expected, the Columbretes population did not exhibit

Table 1. Diet-tissue isotope-fractionation factors (Δ^{13}C, Δ^{15}N and Δ^{34}S) between consumers' feathers and different food sources extracted from the literature

| Prey class | Consumer–diet | Diet-tissue discrimination | | | Source |
		Δ^{13}C (‰)	Δ^{15}N (‰)	Δ^{34}S (‰)	
Marine	*Larus michahellis*–marine fish	0.9	1.7	1.9	The present study
Freshwater invertebrates	*Larus delawarensis*–freshwater food	0.3	3.0	1.3[a]	Hobson & Clark (1992) France & Peters (1997) Peterson et al. (1985)
Terrestrial invertebrates	*Sylvia borin*–mealworms	2.7	4	1.3[a]	Hobson & Bairlein (2003) Peterson et al. (1985)
Refuse tips	*Catharacta skua*–beef[b]	2.2	5.0	1.3[a]	Bearhop et al. (2002) Peterson et al. (1985)

[a]Peterson et al. (1985) reported 1.3‰ as a general value for Δ^{34}S
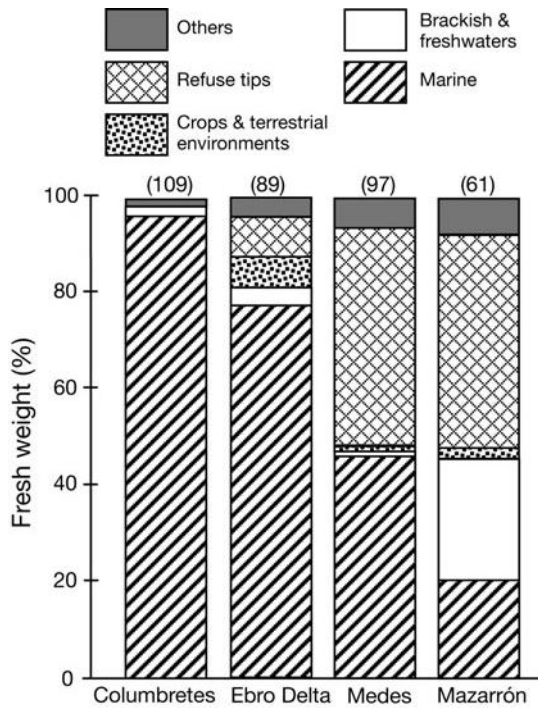[b]Samples from refuse tips were mainly composed of meat waste

Fig. 2. *Larus michahellis.* Fresh weight percentages of prey according to the main foraging habitats indicated in regurgitates of yellow-legged gull chicks (sample size in brackets)

food from refuse tips, with most food coming from the sea (96.3%). Significant differences were found between biomass percentages of marine prey (Kruskal-Wallis, $H_3 = 101.16$, p < 0.001) in the 4 localities. All of them were compared and were significantly different, except the Ebro Delta–Medes and Medes–Mazarrón pairs. On the other hand, prey from brackish and freshwater habitats were only common in regurgitates from Mazarrón (25.5%; Fig. 2).

Stable isotope analysis and mixing models

Values of $\delta^{13}C$ in feathers showed significant differences between localities ($F_{\text{Welch } 3,23} = 69.06$, p < 0.001; Fig. 3a,b). Post hoc comparisons showed that Columbretes chicks had the highest $\delta^{13}C$ values (–17.49 ± 0.18; n = 42). Ebro Delta and Medes Islands chicks showed intermediate values (–18.19 ± 0.32; n = 23 and –18.37 ± 0.49; n = 22, respectively), which were not significantly different, whereas Mazarrón chicks (–18.76 ± 0.61; n = 20) showed the lowest values, which were different from those of the Ebro Delta but not different from those of the Medes colony. At the colony level, we found a high, significantly positive correlation between mean $\delta^{13}C$ values and marine prey
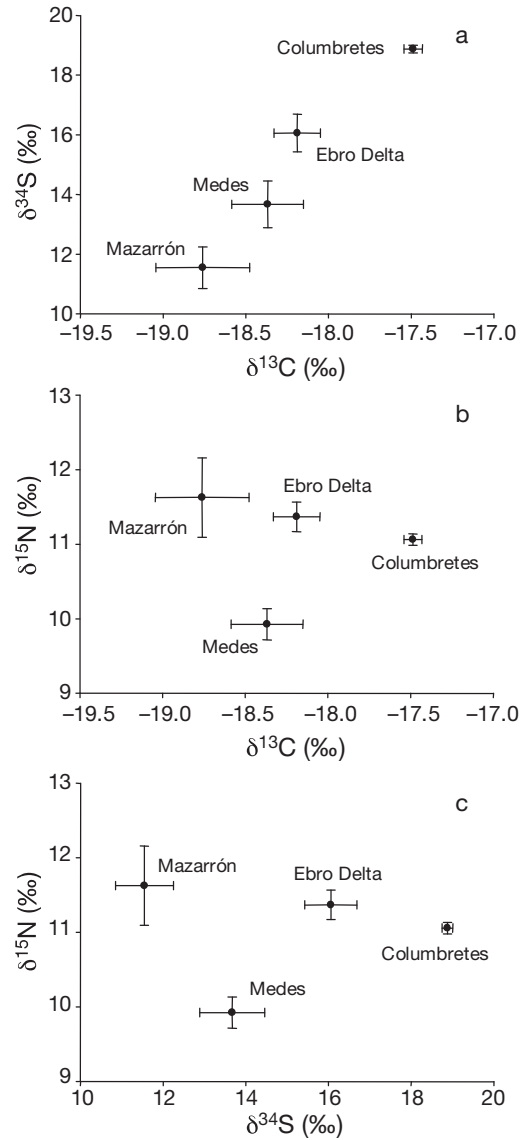
Fig. 3. *Larus michahellis.* Mean (95% CI) (a) sulphur-carbon, (b) nitrogen-carbon and (c) nitrogen-sulphur isotope signatures in feathers of fledglings in relation to the breeding colony (sample size is 42, 23, 22 and 20 for the Columbretes, Ebro Delta, Medes and Mazarrón colonies, respectively)

consumption ($r_S = 1.00$, p < 0.001, n = 4). Feathers also exhibited significant differences between localities in $\delta^{15}N$ ($F_{\text{Welch } 3,23} = 44.08$, p < 0.001; Fig. 3b,c). Post hoc comparisons showed that values from Columbretes (11.06 ± 0.25), Ebro (11.37 ± 0.46) and Mazarrón (11.63 ± 1.14) were similar, whereas values from the Medes site (9.92 ± 0.47) were significantly lower. Mean $\delta^{15}N$ values exhibited no relationship with consumption of fish ($r_S = -0.40$, p < 0.60, n = 4). The $\delta^{34}S$ values of feathers differed between localities ($F_{\text{Welch } 3,23} = 222.46$, p < 0.001; Fig. 3a,c) in the same direction

Table 2. Summary of mean isotope values (± SE) for the main kind of resources found in chick regurgitates and their significance (p values) in differences among colonies

Prey class	n	$\delta^{13}C$ (‰)	p	$\delta^{15}N$ (‰)	p	$\delta^{34}S$ (‰)	p
Marine	29	−18.38 ± 0.73	0.113	9.64 ± 0.97	0.808	16.93 ± 1.52	0.214
Freshwater invertebrates[a]	4	−18.87 ± 0.69	–	9.91 ± 2.81	–	10.12 ± 0.89	–
Terrestrial invertebrates	11	−18.38 ± 1.75	0.667	11.92 ± 3.00	0.350	6.97 ± 1.05	0.718
Refuse tips	12	−21.67 ± 1.44	0.010	5.50 ± 1.74	0.597	6.72 ± 2.03	0.232

[a]Freshwater invertebrates were only found extensively on Mazarrón Island

apparent in $\delta^{13}C$ values, although with a higher discrimination power. The post hoc Tamhane's procedure exhibited 4 significantly different groups. Feathers from Columbretes (18.88 ± 0.42) exhibited higher $\delta^{34}S$ values than those of Mazarrón (11.56 ± 1.49). The Ebro Delta and Medes sites had intermediate values (16.06 ± 1.46 and 13.67 ± 1.77, respectively). A strong positive correlation was found between mean $\delta^{34}S$ values and marine prey (mostly fish) consumption (r_S = 1.00, p < 0.001, n = 4).

Related to potential differences in baseline isotope signatures among colonies (see Hebert et al. 1999), no consistent intercolony differences were found in the main food categories for $\delta^{13}C$, $\delta^{15}N$ or $\delta^{34}S$ values (see Table 2 for significance values). Values of $\delta^{13}C$ and $\delta^{15}N$ for garbage were significantly lower than those for both fish and invertebrates ($F_{Welch\ 3,14}$ = 15.33, p < 0.001 and $F_{Welch\ 3,12}$ = 19.08, p < 0.001, respectively; post hoc Tamhane's multiple comparison test, p < 0.05), whereas $\delta^{34}S$ showed a different pattern: signatures of refuse tips and terrestrial invertebrates were lower

than those of freshwater invertebrates. Freshwater invertebrates showed lower $\delta^{34}S$ values than those of fish ($F_{Welch\ 3,14}$ = 227.54, p < 0.001; post hoc Tamhane's multiple comparison test, p < 0.05).

Carbon, nitrogen and sulphur concentrations were significantly different in the different food sources (1-way ANOVA; $F_{3,55}$ = 15.23, $F_{3,55}$ = 23.30 and $F_{3,55}$ = 4.61 respectively, all p < 0.001), justifying the use of a concentration-weighted mixing model (see Phillips & Koch 2002; Fig. 4). The model indicated that most of the prey consumed in Columbretes came from marine environments. For the Ebro Delta colony, the model estimated that half of the food was from marine sources, almost a third was freshwater invertebrates and a small amount was from refuse tips (15.3%). For the Medes site, the importance of both marine prey and garbage was around 40%, whereas terrestrial invertebrates represented almost 20% of ingested food. Finally, for the Mazarrón site, the mixing model indicated freshwater environments to be the most exploited food source (46%), although garbage (34.4%) and marine prey (19.6%) were also significant.

DISCUSSION

Feeding ecology of gulls

Isotope signatures as well as the information obtained from chick regurgitates showed substantial differences among localities, particularly in the exploitation of marine environments (Figs. 2 & 4). These differences can be explained by the availability per capita (i.e. related to the colony size) of this food resource, as well as by the presence of other alternative foods easier to obtain than marine prey, such as garbage from refuse tips. Refuse, consisting primarily of chicken, pork, and beef scraps, is easy to obtain if it is abundant and close to the breeding colony, and it has high values in energy per meal and fat and protein per gram (Pierotti & Annet 1991). This explains its high proportion in diets of gull colonies with nearby dense human populations, such as those at the Medes and Mazarrón sites. The lower proportion of refuse in the
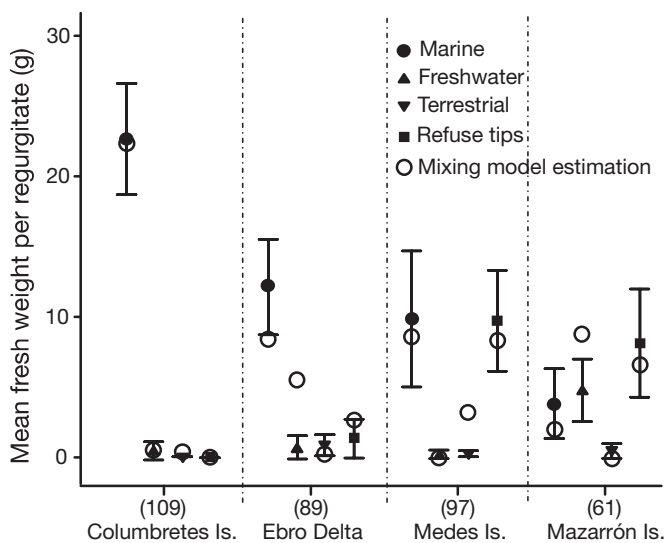

Fig. 4. Mean fresh weight per regurgitate (95% CI) of different foraging habitats computed from direct diet sampling (sample size in brackets). Mean weights of each foraging habitat estimated by the mixing model are also indicated

diet of the Ebro Delta birds compared to those from Medes might be related to its lower availability. Indeed, refuse tips in the Medes Islands are 5 times more abundant than those of the Ebro Delta (Bosch et al. 1994). At Columbretes, the mainland is too far away, and consequently chicks are mainly fed marine fish collected by the parents (i.e. through association with sub-surface predators; Oro 1995) or from fishery discards of the fishing fleet operating in this area (R. Ramos pers. obs.; Oro et al. 1996).

Both methodologies showed that prey related to human activities, such as garbage or fish discards (Witt et al. 1981), were the main components of chick diets in the sampled colonies, comprising more than 80% of the total diet biomass in 3 localities, while at the other site (Mazarrón) this value was 60 to 65% (Figs. 2 & 4). On the other hand, nearby environments, such as freshwater sources or crops, were also exploited during the breeding season, especially in those populations associated with continental environments. However, their relevance mainly depends on the study methodology chosen, which suggests some drawbacks (see below). This heterogeneity in foraging habitats, exploited both within and among populations, according to their availability indicates the generalist and opportunistic feeding habits of this species (see Bosch et al. 1994) and emphasises the importance of a technique allowing a quick assessment of yearly changes in every population.

Isotopic tracers in feeding ecology

As we did not find differences in the isotopic signatures within each of the main prey categories among localities, the considerable isotopic variation among feathers from different colonies was related to differences in diet composition (see Hebert et al. 1999). In agreement with previous studies, we found that $\delta^{13}C$ and $\delta^{34}S$ levels were higher as the consumption of marine prey increased (France & Peters 1997, Knoff et al. 2002). However, only sulphur signatures differed enough among all prey types (Table 2) to be considered a useful tracer to assess the origin of the food consumed (see Hobson 1999). On the other hand, carbon signatures of the main prey types were rather similar, except garbage values, suggesting that the correlation between $\delta^{13}C$ and the consumption of marine prey could be a secondary effect derived from garbage consumption, since the proportions of both resources in the diet are roughly complementary. Thus, $\delta^{34}S$ seems to have a greater discrimination power (Fig. 3a) that allowed us to distinguish between continental-terrestrial and marine-based diets even better than with $\delta^{13}C$ (but see Knoff et al. 2002). Therefore, $\delta^{34}S$ can be used

as an integrated measurement of marine food consumption throughout the nestling growth period. We found that samples from refuse tips provided the lowest values for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (Table 2) and consequently, the Medes and Mazarrón sites, due to the consumption of garbage by gulls, showed the lowest values for these isotopes (Fig. 3). We expected $\delta^{15}N$ values from refuse tips to be the least enriched owing to a short food chain (Hebert et al. 1999). Accordingly, $\delta^{15}N$ values of the Medes colony were significantly lower than those from other localities (Fig. 3b,c), since almost half of its dietary biomass came from refuse tips (Fig. 2). Nevertheless, it should be noted that at the Mazarrón site, although almost half of the chicks' dietary biomass also came from refuse tips, $\delta^{15}N$ values were relatively high (Fig. 3b,c). This unexpected high value can be explained by taking into account that freshwater invertebrates show high $\delta^{15}N$ values (Table 2) and that a moderate consumption of that resource might substantially increase $\delta^{15}N$ values of consumers (Vanderklift & Ponsard 2003). We would not have known this had we not collected direct as well as indirect samples. The fact that these invertebrates were mostly involved in food webs based on dead organic matter might explain their high $\delta^{15}N$ signatures (Ponsard & Arditi 2000)

Comparing diet analysis and mixing models

The main pattern resulting from the mixing model values was in agreement with the direct method to determine diets, although some of the estimated weights provided by mixing models did not fit into confidence intervals computed from regurgitate analysis (Fig. 4). Whereas the relative weights of marine and garbage resources agreed between both methodologies, estimations for some of the invertebrates (both from freshwater and terrestrial environments) were higher in the mixing model than in the regurgitate analysis. Indeed, regurgitate analyses underestimate the importance of small and soft prey items, such as invertebrates, while larger and harder prey are overestimated (Duffy & Jackson 1986, Hobson & Clark 1992), suggesting that isotopic results are necessary to fully describe diet.

Despite some problems found with the mixing model, probably derived from inaccurate fractionation assumptions (Phillips & Koch 2002), it helped us to estimate diet composition with minimum disturbance to the birds (Hodum & Hobson 2000, Ben-David & Schell 2001). Moreover, mixing models overcome problems of over- or underestimations related to digestibility of prey items (Croxall et al. 1997), as only assimilated food is considered. However, other prob-

lems arose, with fractionation factors being crucial. In this respect, very few papers have reported the fractionation between a consumer's tissue and a particular kind of dietary resource; most studies have measured fractionation factors according to whole diet without taking into account different diet components (but see Hobson & Bairlein 2003, Pearson et al. 2003). To solve the problem regarding the lack of precise fractionation factors, future experimental studies should be carried out to account for different kinds of diets as well as different consumer tissues, although difficulties in obtaining experimental diets based on only a single resource, like invertebrates, are well known.

Another restriction imposed by the mixing model approach was related to the taxonomic precision of the results, which are rather coarse compared to the exhaustive detail achieved by direct dietary analysis. For instance, as noted above, we would not have resolved the importance of freshwater contributions without direct samples. However, the information provided by stable isotopes about the feeding ecology of a generalist species was enough to obtain a general idea of the use of different foraging habitats exploited by these birds.

CONCLUSIONS AND APPLICATIONS

In the present study we have shown that the combined analysis of isotopic signatures of carbon, nitrogen and sulphur in fledgling feathers of yellow-legged gulls can characterise the different proportions of feeding resources from marine habitats, brackish and freshwater environments, crops and refuse tips. In particular, δ^{34}S signatures discriminated among marine and freshwater-terrestrial origins of dietary resources, while a depleted δ^{15}N resulted from the exploitation of refuse tips. Although the taxonomic detail achieved by direct dietary analysis was not obtained, mixing models have become useful in the study of feeding ecology of populations or species that are highly opportunistic in their feeding habits and hence able to rapidly change the resources used. This is particularly relevant to correctly interpreting comparisons among populations about pollutant levels accumulated, or in ascertaining the provenance of such pollutants. However, exhaustive captive-feeding experiments should be carried out to determine the specific fractionation factors between a consumer's tissue and its diet. We stress the use of feathers in the study of the feeding ecology because feathers are sufficiently powerful and easy to sample and could be particularly useful in studies of endangered species by minimising disturbance to sampled populations.

Isotope signatures provide an integrative view on assimilated diets instead of the discrete information obtained from ingested resources. Whereas conventional dietary analysis requires exhaustive monitoring over time to obtain reliable information, SIA only needs single sampling depending on the tissue available. Moreover, direct dietary analysis produced slight under- and overestimations derived from the differential digestibility of prey. Thus, the information generated by isotopes, especially if calibrated from analysis of direct samples, is a reliable method to determine the importance of certain exploited resources.

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Capítol 2:

Comprenent el component espaciotemporal de l'ecologia tròfica d'espècies oportunistes

Mesurant els ràtios d'isòtops estables en plomes de gavià mudades en diferents períodes al llarg del seu cicle anual, vàrem caracteritzar els hàbits d'alimentació (entre marins, d'aigua dolça, recursos terrestres i restes d'abocador) durant l'època de cria i durant el període no reproductor per a diferents poblacions de gavià de potes grogues al llarg de la Mediterrània Occidental. D'aquesta manera, es van descriure els patrons estacionals d'alimentació per a diferents poblacions de gavians, així com canvis en la dieta al llarg de l'any desconeguts fins aleshores.

Understanding annual feeding ecology from the isotopic composition of feathers: applications in the management of a problematic gull species

Raül Ramos · Francisco Ramírez ·
Josep Lluís Carrasco · Lluís Jover

Abstract   Forecasting changes in population dynamics requires access to precise dietary information recorded throughout the year. In this context, stable isotope analysis on specific animal tissues offers an exceptional approach to study the trophic ecology of a given species over time. Here we examined the spatiotemporal heterogeneity in exploited resources of a problematic bird species, namely the Yellow-legged gull *Larus michahellis.* We also provide management authorities with a useful tool for the rapid and precise assessment of the feeding ecology of this species in order to confidently predict changes in population dynamics. We measured the stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) in Yellow-legged gull feathers moulting throughout the annual cycle. We then characterized the feeding habits (among marine, freshwater, terrestrial and garbage resources) during the breeding and non-breeding seasons of seven gull populations along the Western Mediterranean coast. We demonstrate that isotopic analyses on first primary feathers are good indicators of breeding trophic ecology, while other feathers, such as eighth secondaries, clearly reflect the feeding behaviour during the non-breeding period. We report on estimations of seasonal feeding patterns as well as unknown dietary changes throughout the year for several Yellow-legged gull populations. The high diversity in the exploitation patterns of the foraging habitats in the study area as well as the ease and rapidity with which Yellow-legged gulls changed their diets indicates the opportunistic feeding behaviour of this species. This spatiotemporal plasticity in the tropic ecology of this gull should be taken into consideration by conservation management authorities when planning to reduce or restrict the growth of problematic populations. Here we demonstrate the utility of stable isotope analysis on specific tissues to determine the feeding ecology of the Yellow-legged gull throughout the annual cycle and provide further insight into the management of problematic populations but also the conservation of endangered species.

Introduction

Precise knowledge of diet composition is mandatory in several key fields of applied ecology, such as the conservation of endangered species and the management of problematic and overpopulated species. Research into seasonal variation in foraging ecology and interactions between exploited habitats and human activities is 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).

However, in general, animals are usually accessible for biological research for only part of the year. Given that during the breeding season adult animals can be found in nests or burrows for pairing, incubation or the care of young, most of our knowledge of food preferences and feeding habits is usually restricted to this period and much less dietary and foraging information is available for the rest of the year (Barrett et al. 2007). Conventional tools to reconstruct diets on the basis of regurgitates have several drawbacks, which are related mainly to the variation in digestibility of prey, and thus hamper the accuracy of results (Duffy & Jackson

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1986; González-Solís et al. 1997; Votier et al. 2003). Moreover, such studies usually require exhaustive monitoring over time to obtain reliable data on the feeding ecology of a species, as each sample represents only a specific feeding event in the diet of one specimen (Jordan 2005; Votier et al. 2001).

In this context, recent years have witnessed a dramatic increase in the use of isotopic analyses to infer the trophic ecology of species. Such analyses are an alternative to traditional dietary approaches (Post 2002). Although isotopic studies do not provide the taxonomic detail achieved by conventional dietary analysis, they are advantageous as they consider only the food assimilated and offer an integrative estimation of the diet of a species (Gannes, O'Brien & Martínez 1997). In addition, for birds for which the main moult pattern and time of feather formation are known, analyses of stable isotopes on specific feathers are particularly appropriate to assess seasonal dietary patterns. Once formed, feathers become chemically inert, and thus reflect diet during the moulting period. Therefore, when moulting patterns are known, particular feathers can be sampled at any time of the year to examine feeding habits in given periods (Hobson 2008). In this regard, stable isotopes of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and sulphur ($^{34}S/^{32}S$, $\delta^{34}S$) are used mainly to determine sources of primary production and are useful to trace the input of these elements into foodwebs (Hobson 1999; Krouse & Herbert 1988). In contrast, stable isotopes of nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) are indicators of foodweb interactions and the trophic positions of species, as consumers are typically enriched in ^{15}N compared to their food (Post 2002; Vanderklift & Ponsard 2003).

Over the last few decades many vertebrate species have increased in abundance as a result of habitat changes caused by anthropogenic disturbances (Feare 1991). Most of these species become overabundant because of their flexible,

opportunistic and gregarious nature, which makes them highly adapted to living in man-modified habitats. In particular, gulls *Larus* spp. have been extensively studied as a potential superabundant species throughout Australia, North America and Europe as they largely interact with human interests or endangered species (Smith & Carlile 1993; Vidal, Medail & Tatoni 1998). The behaviour of gulls in this regard is attributed to their great capacity to benefit from increasing food resources derived from human activities, particularly garbage but also fishery discards (Belant 1997; Bosch, Oro & Ruiz 1994; Pons 1992).

Traditionally, problematic bird populations have been controlled by culling programs on eggs, chicks or even adults, using poison or shooting (Bosch et al. 2000; Feare 1991). However, limiting the availability of resources has proved to be the most effective management measure to control bird populations (Bosch et al. 2000; Oro, Bosch & Ruiz 1995). 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 in order to forecast dietary changes that derive from management policies on restrictions.

Here we focused on the on a problematic bird, namely the Yellow-legged gull *Larus michahellis*. This species breeds in the Mediterranean region and is the most common and widespread seabird in the Western Mediterranean, where its population reaches at least 120,000 nesting pairs and increases by up to 10% per year (Thibault et al. 1996; Vidal, Medail & Tatoni 1998). Using isotope analyses

Table 1 Main informative parameters of the breeding sites of Yellow-legged gull *Larus michahellis*

	Locality site	Distance from human settlements (km)	Number of breeding pairs	Fishing vessel activity around each area (2007)			References
				Relative estimation	Number of vessels	Gross tonnage	
Medes Is. (42º0'N, 3º13'E)	Protected islands off the coast of a tourist resort	0.9	6500	Moderate-high	392	8,956	Bosch et al. 2000
Ebro Delta (40º40'N, 0º45'E)	Isolated peninsula in a Natural Park	7.5	6000	High	358	11,195	Oro et al. 2006
Columbretes Is. (39º54'N, 0º41'E)	Isolated archipelago in a Marine Reserve	55	450	High	245	9,248	Oro et al. 2006
Sa Dragonera Is. (39º35'N, 2º19'E)	Protected island the coast of a tourist resort	0.8	ca. 4500	Moderate	440	3,897	Daniel Oro personal communication
Benidorm Is. (38º30'N, 0º08'W)	Protected island off the coast of a tourist resort	3	650	Moderate-high	307	13,126	Martínez-Abraín et al. 2002
Mazarrón Is. (37º33'N, 1º16'W)	Island off the coast of a tourist resort	0.5	900	Low	230	3,719	García-Morell & Escribano 2005
Alborán Is. (35º55'N, 3º04'W)	Remote island in a Marine Reserve	75 from coast of Spain, 50 from coast of NAfrica	300	High	310	10,272	Paracuellos and Nevado 2003

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

($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), we characterized the summer and winter diet of the Yellow-legged gull at several sites where these birds are assumed to use different proportions of marine, freshwater, terrestrial and garbage resources (Ramos et al. 2009c). We explored the spatiotemporal heterogeneity in exploited resources of this problematic species according to the potential nearby feeding habitats. Our ultimate aim was to be able to predict population dynamic changes around the Western Mediterranean according to the coming European Union management measures (see the discussion).

Methods

Study area and sampling strategy

The study was carried out in seven colonies with a variable density of breeding pairs and feeding habits (Table 1) along the Western Mediterranean coast of Spain (Ramos et al. 2009c). From North to South, the colonies sampled were as follows: the Medes Islands, the Ebro Delta (Catalonia), Sa Dragonera Islet (Balearic Islands), the Columbretes Islands, Benidorm Island (Valencia), Mazarrón Island (Murcia) and Alborán Island (Almería; Fig. 1).

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 six or seven months (personal observation; Ingolfsson 1970; Olsen & Larsson 2004). 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 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). Generally speaking, the moult of seabird body

Fig. 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.

feathers is thought to take place during several periods throughout the annual cycle (Allard et al. 2008; Ramos, González-Solís & Ruiz 2009).

To ensure the replacement sequence of remiges in the species, we collected and analyzed stable isotope signatures of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) of three primary feathers (1st, 5th and 10th; P1, P5 and P10, respectively), three secondaries (1st, 8th and 16th; S1, S8 and S16, respectively; Fig. 2a), as well as some breast feathers from 14 Yellow-legged gulls captured during the incubation period on Mazarrón Island. As moult is symmetrical between wings (Ramos et al. 2009a), we minimized the exhaustive feather sampling on the flight performance of birds by removing feathers alternately from the left and right wings. Secondly, during the following early breeding season when adults were incubating eggs, we collected the 1st primary (P1) and the 8th secondary (S8) from 12 to 21 adult gulls at each locality (total *n*=113) 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, C. Sanpera). Each food sample was placed in a sealed plastic bag and kept frozen until laboratory analysis.

Sample preparation and laboratory analysis

The feathers were washed in a 0.25M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contaminants, dried in an oven at 60ºC to constant mass and ground to a fine powder in a freezer mill (Spex Certiprep 6750; Spex Industries Inc., Metuchen, New Jersey, USA) operating at liquid nitrogen temperature. Regurgitate items were assigned to four categories on the basis of the foraging habitat (Bosch, Oro & Ruiz 1994): a) marine, b) brackish and freshwaters, c) crops and terrestrial environments, and d) refuse tips. We selected the most well-preserved samples and performed isotope analyses to examine potential intercolony differences at baseline isotope levels. Before isotopic analysis, all food samples were freeze-dried and ground in a freezer mill. To reduce variability caused by isotopically lighter lipids (Attwood & Peterson 1989; Hobson & Welch 1992), lipids were removed from all dietary samples by means of several chloroform-methanol (2:1) rinses, following the Folch extraction method (Folch, Lees & Sloane-Stanley 1957).

Subsamples of 0.4 mg of feather powder for C and N and about 3.5 mg for S analyses were weighed to the nearest μg, placed in tin capsules and crimped for combustion. The samples were oxidized in a Flash EA1112 coupled to a Delta C stable
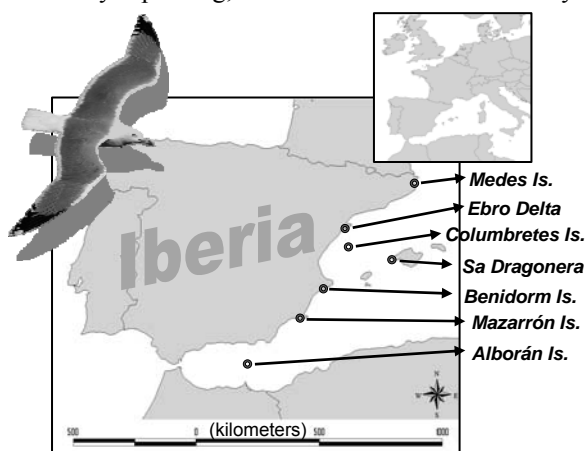
isotope mass spectrometer through a Conflo III interface (ThermoFinnigan, Bremen, Germany), which was used to determine the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X (‰) is ^{13}C, ^{15}N or ^{34}S, and Rs is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$, related to the standard values. $R_{standard}$ for ^{13}C is Pee Dee Belemnite (PDB); for ^{15}N, atmospheric nitrogen (AIR); and for ^{34}S, troilite of the Canyon Diablo Meteorite (CDT). The isotopic ratio mass spectrometry facility at the "Serveis Científic-Tècnics" of the "Universitat de Barcelona" (Spain) applied international standards that were inserted every 12 samples to calibrate the system and compensate for drift over time.

Statistical analysis

We used linear mixed models (LMM) with normal link to analyze isotopic values in the feathers (Littell et al. 1996). Feather type and colony were included as fixed effects. Individual random effect was included to account for the dependence among feathers of the same individual. Residual heterogeneity and interactions between effects were also evaluated. Model selection was done using Akaike information criteria (Johnson & Omland 2004). Posterior pair-wise comparisons 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.

Since C, N and S concentrations of the food sources analyzed were substantially different (one-way ANOVA; $F_{3,77}= 13.27$, $F_{3,77} = 29.46$ and $F_{3,77} = 9.03$ respectively, all $P < 0.001$), we estimated the

foraging habitat used by each bird during summer and winter by applying concentration-weighted mixing models to our isotopic values (Phillips & Koch 2002). We developed the model ISOCONC 1.01 (Phillips & Koch 2002) to use four food sources and three stable isotope signatures. Calculated percentages were readjusted when negative values were generated by the model, thus making zero the most negative value and recalculating other percentages according to original proportions given by the model (Ramos et al. 2009b). To account for trophic fractionation between diet and consumer tissue (Gannes, del Rio & Koch 1998), isotopic values of food sources were adjusted by the appropriate fractionation factors taken from the literature (see Ramos et al. 2009b). When applying the model, we assumed that prey isotopic signatures do not vary temporally either between the early winter and the breeding season or between years (Hobson 2008).
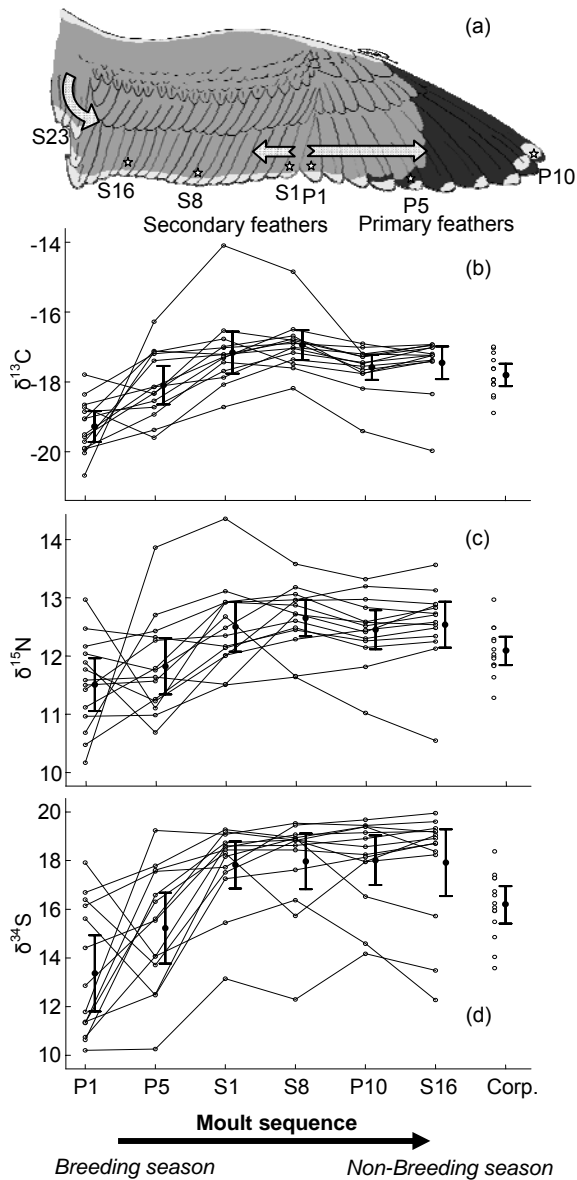
Results

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 (Fig. 2bcd). Fitted models showed a significant feather effect on mean isotopic signatures for all three isotopes, while residual heterogeneity among feather types was relevant only for $\delta^{13}C$ and $\delta^{15}N$ 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, P10 and S16 showed the highest isotopic values (Fig. 2). Body feathers showed intermediate isotopic values and did not show significant differences from

Table 2 Results from linear mixed models fitted to the series of moulting feathers in Yellow-legged gulls breeding on Mazarrón Is.

	$\delta^{13}C$			$\delta^{15}N$			$\delta^{34}S$		
Estimated means	mean	95% CI		mean	95% CI		mean	95% CI	
P1	-19.27	-19.79	-18.75	11.52	10.95	12.08	13.39	12.28	14.50
P5	-18.09	-18.65	-17.53	11.83	11.35	12.30	15.24	14.13	16.35
S1	-17.16	-17.75	-16.57	12.51	12.08	12.94	17.82	16.71	18.93
S8	-16.94	-17.40	-16.49	12.66	12.34	12.97	17.97	16.86	19.08
P10	-17.58	-17.91	-17.25	12.46	12.16	12.76	18.01	16.90	19.12
S16	-17.45	-17.81	-17.08	12.54	12.23	12.86	17.91	16.80	19.02
Corp.	-17.79	-18.22	-17.37	12.08	11.71	12.45	16.15	15.04	17.26
Random parameters									
	variance			variance			variance		
Individual effect	0.378			0.300			2.574		
Residual							1.793		
P1	0.577			0.836					
P5	0.739			0.498					
S1	0.868			0.349					
S8	0.349			0.055					
P10	0.006			0.013					
S16	0.090			0.053					
Corp.	0.267			0.193					

Estimated means (with 95% Confidence Interval; lower and upper limits) are used to show fixed feather effects. Estimated variance parameters show individual random effects and residual heterogeneity among feathers when present

Fig. 2 Wing feather moult pattern of the Yellow-legged gull and isotopic composition of some of its feathers. a) Main moult pattern (white arrows; Ingolfsson 1970; Olsen & Larsson 2004) and selected feathers analyzed for stable isotopes (stars) are shown in the wing scheme. Carbon b), nitrogen c) and sulphur d) stable isotope signatures of 1st, 5th, and 10th primary (P1, P5, P10) and 1st, 8th and 16th secondary (S1, S8, S16) feathers from 14 Yellow-legged gulls sampled on Mazarrón Is. Feathers are classified following the moult sequence defined by Ingolfsson (1970) and Olsen and Larsson (2004). Each line connects the isotopic values of feathers from the same individual. Individual stable isotope signatures of some breast feathers are shown separately (Corp.). Mean and 95% CI are represented as error bars for each feather type.

from Medes and Sa Dragonera Islands showed the lowest values (Fig. 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 $\delta^{13}C$ signatures in their P1 than in S8 feathers. Similarly, specimens from the Medes and Mazarrón Islands showed lower $\delta^{34}S$ signatures in P1 feathers compared to S8, whereas on the Columbretes Islands $\delta^{34}S$ signatures in P1 feathers were the highest (Table 3). Residual variance was much greater for S8 than for P1 feathers (Appendix 2). 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 (Appendix 2).

Regarding potential differences in baseline isotopic values among colonies (see Hebert et al. 1999), we analyzed 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 $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ ($F_{WELCH\ 4,19} = 45.80$, $F_{WELCH\ 4,19} = 17.31$, $F_{WELCH\ 4,19} = 46.37$ 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 4). 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. Mixing models estimated similar diet contributions in both seasons for the Ebro Delta, Sa Dragonera and Alborán populations (Table 5). Dramatic changes in feeding patterns were predicted for the Mazarrón Island population, although these variations between seasons were also notorious in gulls in the Medes, Columbretes and Benidorm colonies. Models assumed that the main

P5 and S1 in any of the stable isotopes. In both the $\delta^{13}C$ and $\delta^{15}N$ models, the residual variance of P1, P5 and S1 was greater than the individual random effect, while S8, P10 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. The selected models for all three isotopes included the fixed effects for feather and colony as well as their interaction, the individual random effect that interacted with the colony, and the residual heterogeneity between the two feathers (see Appendix 1). Fixed effects were not easily interpretable due to the presence of significant interaction between them. In general, feathers from birds on the Alborán and Columbretes Islands had the highest isotopic values whereas those

Table 3 Mean parameter estimation (with 95% Confidence Intervals; lower and upper limits) from linear mixed models for summer and winter feathers (P1 and S8, respectively) of Yellow-legged gulls in the seven colonies

			1st primary (P1)			8th secondary (S8)	
	Locality	Estimated mean	95% CI lower	95% CI upper	Estimated mean	95% CI lower	95% CI upper
$\delta^{13}C$	Medes Is.		-19.35	-19.57	-19.12	-18.68	-19.25	-18.11
	Ebro Delta		-18.54	-18.89	-18.20	-18.08	-18.56	-17.60
	Columbretes Is.		-17.90	-18.05	-17.74	-18.35	-18.78	-17.91
	Sa Dragonera Is.		-19.97	-20.24	-19.70	-19.15	-19.78	-18.52
	Benidorm Is.	*	-18.61	-18.92	-18.30	-17.52	-18.10	-16.94
	Mazarrón Is.	*	-19.20	-19.48	-18.91	-17.61	-18.17	-17.04
	Alborán Is.		-16.78	-16.96	-16.60	-16.55	-17.05	-16.05
$\delta^{15}N$	Medes Is.		11.10	10.71	11.49	11.29	10.68	11.89
	Ebro Delta		12.82	12.41	13.24	12.86	12.37	13.36
	Columbretes Is.		12.30	12.11	12.48	12.39	11.99	12.80
	Sa Dragonera Is		10.70	10.37	11.03	11.20	10.60	11.81
	Benidorm Is.		12.10	11.82	12.38	12.65	12.13	13.17
	Mazarrón Is.	*	11.56	11.20	11.91	13.06	12.50	13.62
	Alborán Is.		13.34	13.16	13.53	13.42	12.96	13.88
$\delta^{34}S$	Medes Is.	*	10.52	9.63	11.41	14.10	12.32	15.89
	Ebro Delta		16.27	15.24	17.29	15.05	13.62	16.48
	Columbretes Is.	*	18.63	18.07	19.19	14.93	13.60	16.26
	Sa Dragonera Is		12.52	11.03	14.01	12.63	10.38	14.88
	Benidorm Is.		15.28	14.11	16.46	17.47	15.61	19.33
	Mazarrón Is.	*	11.21	10.23	12.18	17.05	15.31	18.80
	Alborán Is.		18.86	18.66	19.07	18.52	17.13	19.91

Asterisks denote significant differences (*p*-value <0.05) between the two feather types

food items for the Columbretes Island colony during the breeding season was from marine environments. However, the contribution of this environment decreased by up to 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, Sa Dragonera and Mazarrón Islands during the summertime, comprising approximately half of their diet (58.3, 46.2 and 51.4%, respectively). However, the use of refuse decreased during the non-breeding season, especially in the population on the Mazarrón Islands. The consumption of refuse also decreased during winter in the Benidorm Island colony. In contrast, the exploitation of discards from fishing vessels during the non-breeding season increased in the colonies on the Medes, Benidorm and Mazarrón Islands. Particularly for the latter, for which we

recorded temporal changes in diet through the moult sequence, the use of refuse and freshwater prey decreased over time, while marine but also terrestrial prey gained relevance in the diet during the non-breeding season (Fig. 2 & Table 5).

Discussion

Tracing feeding ecology through the moulting sequence

On the basis of the moulting pattern of the Yellow-legged gull (Baker 1993; Olsen & Larsson 2004), stable isotope signatures differ among wing feathers, thereby allowing us to track dietary changes throughout the year, from mid-May to early November. Feathers moulted during the chick-rearing period (P1) showed the greatest signatures

Table 4 Summary of mean isotopic values (± SE) for the main kind of food resources exploited by Yellow-legged gulls (obtained from chick regurgitates and fishery discards)

Prey class	Locality	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Marine	Columbretes	11	-18.55 ± 0.42	9.44 ± 0.36	17.65 ± 0.52
	Ebro Delta	9	-18.18 ± 0.57	9.57 ± 0.51	17.31 ± 0.37
	Medes	13	-18.36 ± 0.28	9.18 ± 0.43	17.30 ± 0.23
	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
Freshwater invertebrates	Mazarrón[a]	4	-18.87 ± 0.69	9.91 ± 2.81	10.12 ± 0.89
Terrestrial invertebrates	Ebro Delta	6	-17.87 ± 1.77	11.03 ± 3.17	6.70 ± 0.74
	Medes	3	-18.43 ± 2.76	10.84 ± 5.28	6.76 ± 0.62
	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
Refuse tips	Ebro Delta	2	-19.91 ± 0.04	6.01 ± 1.64	7.02 ± 1.02
	Medes	5	-22.04 ± 1.63	4.82 ± 1.26	5.40 ± 1.81
	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

[a] freshwater invertebrates were found extensively only on Mazarrón Is.

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)

consistently for all three stable isotopes and revealed that the colony on the Mazarrón Island fed abundantly on marine, freshwater and refuse resources during the breeding season (Table 5). Feathers moulted during the non-breeding period (S8, P10 and S16) indicated that this population changed dietary preferences radically between seasons, mainly consuming fish and terrestrial invertebrates at that time (Table 5). In addition, isotopic homogeneity among non-breeding feathers of this colony revealed that this feeding pattern was constant throughout the long moulting period during the non-breeding season (Fig. 2 & Table 2). Finally, feathers moulted during the interperiod between the breeding and non-breeding seasons (P5 and S1) revealed that freshwater prey was absent from the diet while refuse scraps gradually gave way to marine and terrestrial prey (Table 5). The presence of individual intermediate isotopic values between both periods and the observation that isotopic

Fig. 3 Mean carbon a), nitrogen b) and sulphur c) stable isotope signatures (95 % CI) of 1st primary (P1) and 8th secondary (S8) feathers of Yellow-legged gulls from seven Western Mediterranean colonies.

variability in these feathers (see 95% Confidence Interval in Fig. 2 & Table 2) is not particularly different to those feathers formerly moulted during the breeding period strongly suggest that the overall feeding pattern change gradually within the population. Here we demonstrate that dietary preferences of the Yellow-legged gull can be tracked throughout the moult sequence and therefore throughout the annual cycle by analyzing stable isotopes on specific feathers. In particular, isotopic analysis on P1 feathers of these gulls were good indicators of breeding trophic ecology, while other feathers such as S8 clearly reflected the feeding behaviours during the non-breeding period.

Similarly to feathers moulted between breeding and non-breeding periods (P5 and S1), the isotopic signatures of several breast feathers showed intermediate values between the seasons (Fig. 2 & Table 2). However, the additional reduced variability of body-feather isotopic values compared with that of P5 and S1 (Table 2) indicated the mixture of breeding and non-breeding body feathers within the same specimen when sampling, instead of the moulting of body feathers during the interperiod between seasons. Therefore, our results corroborated the findings that gull body feathers are partially moulted in both breeding and non-breeding periods and that isotopic analyses of several body feathers can provide a reliable average value for each individual for the entire year (but see Arcos et al. 2002;Phillips et al. 2009).

Characterizing spatiotemporal dietary patterns using stable isotopes

The great isotopic variability among populations of Yellow-legged gulls revealed a wide range of foraging 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 (Ebro Delta, Sa Dragonera and Alborán), others changed their feeding habits drastically (Mazarrón). 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 predates the most abundant local food source (Duhem et al. 2005;Vidal, Medail & Tatoni 1998). Indeed, we found that most gull populations near human settlements used refuse sites during the breeding season (Medes, Sa Dragonera, Benidorm and Mazarrón Islands). 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 the Medes, Benidorm and Mazarrón populations, which showed a considerable decrease in refuse consumption during the non-breeding season. However, in the Sa Dragonera area, the larger resident population in Majorca and the favourable climate of the Balearic Islands, which allows a longer tourist season, may also allow gulls access to refuse outside the breeding season.

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 terrestrial prey (Duhem et al. 2005;Ramos et al. 2009c). Consequently, while breeding, both populations of gulls fed offspring and themselves mainly fish (Table 5), which they easily obtained from the fisheries operating in the area (Arcos, Oro & Sol 2001;Ramos et al. 2009c). 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 (Martínez-Abraín et al. 2002;Sol, Arcos & Senar 1995).

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 isotopes present in the local food webs (Gómez-Díaz & González-Solís 2007;Pantoja et al. 2002). 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).

The management perspective

In general, food availability is a determinant factor of population dynamics (Oro et al. 2006). 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, two European Union Action Plans (the European Union Landfill Directive [1999/31/EC] http://ec.europa.eu/ /environment/waste/landfill_index.htm, and the Reform of the Common Fisheries Policy http://ec.europa.eu/fisheries/cfp/2002_reform_en.ht m) are currently under development. These seek to decrease the availability of garbage and fishery discards (Ramos et al. 2009c). On the basis of our findings, 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). By limiting the availability of food derived from human activities, the trophic niche of these birds will widen, thereby leading to an increased use of alternative food sources, such as those from terrestrial and freshwater habitats (Duhem et al.

Table 5 Breeding- and non-breeding-mean habitat usage (P1 and S8, respectively, in %, ± SE) of Yellow-legged gulls estimated by isotopic mixing models

| Locality | Feather | n | Estimated foraging habitat (%) | | | |
			Marine	Freshwater	Terrestrial	Refuse
Medes Is.	P1	13	25.58±12.19	12.63±7.80	3.47±8.75	58.32±12.24
	S8	13	47.51±20.42	8.98±11.94	11.12±11.76	32.39±15.44
Ebro Delta	P1	20	53.94±15.14	20.47±9.64	6.58±13.48	19.01±19.51
	S8	20	53.04±20.39	12.98±13.09	17.40±16.97	16.58±22.58
Columbretes Is.	P1	21	82.62±9.04	5.73±3.86	5.47±6.03	6.17±9.84
	S8	21	51.40±18.40	12.85±8.88	15.81±14.96	19.95±22.15
Sa Dragonera Is.	P1	12	31.80±7.78	22.00±1.69	0	46.20±7.77
	S8	12	41.91±19.82	14.69±7.78	7.37±11.55	36.03±24.10
Benidorm Is.	P1	12	61.26±19.58	12.97±5.86	0.92±3.17	24.85±16.24
	S8	12	62.30±11.85	7.94±4.27	20.31±11.54	9.44±19.06
Mazarrón Is.	P1	15	33.04±17.10	13.79±9.09	1.81±3.98	51.37±12.27
	S8	15	59.72±11.94	11.80±6.80	21.95±10.13	6.53±16.85
Alborán Is.	P1	20	75.65±9.95	8.02±8.87	8.03±6.37	7.65±8.02
	S8	20	78.09±11.73	3.18±7.21	10.88±7.53	7.85±8.88
Mazarrón (seq.)	P1	14	41.56±16.80	18.94±4.53	1.06±3.97	38.44±15.23
	P5	14	56.91±17.43	4.74±6.44	13.85±19.72	24.51±18.39
	S1	14	67.25±12.91	3.71±4.43	23.12±15.96	5.91±9.88
	S8	14	63.69±12.23	4.76±3.54	28.08±13.00	3.47±7.07
	P10	14	71.39±13.58	5.17±5.67	18.39±12.19	5.05±11.27
	S16	14	65.52±12.74	6.64±5.09	22.83±12.35	5.02±13.30
	Corp.	14	66.24±7.56	1.17±3.16	16.50±11.04	16.09±13.03

Estimated habitat usage throughout the moult sequence of birds on Mazarrón Is. is also shown

2005). Breeding success in gull populations such as those on the Medes, Sa Dragonera and Mazarrón 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 Medes and Alborán populations as few alternative food sources to fishery discards and refuse are available. For all the other study sites (Ebro Delta, Columbretes, Sa Dragonera, Benidorm and Mazarrón), 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 invertebrates, which will be more intensely exploited in the future (Duhem et al. 2005;Hebert et al. 2008). Finally, as a result of the reduction in food availability, 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 (Sanz-Aguilar et al. 2009;Vidal, Medail & Tatoni 1998).

In addition, the lower isotopic variability of feathers from individuals in the breeding than the non-breeding season and in some remote populations, such as those on the Columbretes and Alborán Islands compared to the Ebro Delta (see Appendix 2), indicate that food availability and diverse habitat exploitation strategies can also be inferred from stable isotope variance. The finding that individual variability was greater in the feathers of gulls in the non-breeding seasons 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, showed the least individual variability in the three isotopes analyzed. This observation is attributed to few dietary alternatives on those colonies (Ramos et al. 2009c). Therefore, isotopic variability among individuals of a single population could also be used as a good estimator of diet diversity and food availability for a given population, thereby allowing rapid forecast of the impact of trophic resource constraints.

To our knowledge, this study is the first to apply stable isotope analysis on distinct wing feathers to address specific seasonal estimations of the feeding patterns of a problematic bird species. Precise information on feeding strategies in the breeding and non-breeding season is mandatory for forecasting changes in population dynamics (Brooks & Lebreton 2001;Feare 1991). Here we provide evidence that stable isotope analysis on specific feathers can be used to determine the feeding ecology of the Yellow-legged gull throughout its annual cycle. We propose that the information derived from this type of analysis will contribute to the management of problematic populations, but also to the conservation of endangered species. Feathers are often replaced in a predictable manner along the annual cycle (Bridge 2006) and thus provide an excellent opportunity to study avian ecology through stable isotopes. For other non-avian species, specific portions of keratinous tissues, such as hair, whiskers, nails, scales (Estrada, Lutcavage & Thorrold 2005;Hobson et al. 1996;Reich, Bjorndal & Bolten 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. Finally, the high diversity in the exploitation patterns of the foraging habitats throughout the study area and the ease and rapidity with which Yellow-legged gulls adapted their feeding strategies demonstrates the opportunistic nature of this species (Hebert et al. 2008;Vidal, Medail & Tatoni 1998). Thus, this spatiotemporal plasticity in the tropic ecology of the gull should be considered by conservation management authorities when planning to reduce or restrict the growth of problematic gull populations.

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Appendix 1 Models evaluated to fit the data corresponding to the three isotopes analyzed in two types of feathers (P1 and S8) from birds in the seven colonies and their corresponding Akaike's Information Corrected Criterion (AICC)

Fixed effects	Random effects	Residual variance	δ^{13}C AICC	δ^{15}N AICC	δ^{34}S AICC
colony, feather, colony by feather	individual	common	638.1	637.3	1096.4
colony, feather, colony by feather	individual	by feather	588.4	618.2	1082.6
colony, feather, colony by feather	individual by colony	common	623.5	623.9	1090.3
colony, feather, colony by feather	individual by colony	by feather	575.7	601.3	1062.5
colony, feather	individual by colony	by feather	605.8	618.5	1159.0

Appendix 2 Parameter estimates from GLMMs fitted to summer (P1) and winter (S8) feathers in Yellow-legged gulls from seven colonies

		δ^{13}C	δ^{15}N	δ^{34}S
Fixed effects		mean ± SE	mean ± SE	mean ± SE
Intercept		-18.68 ± 0.29	11.29 ± 0.31	14.10 ± 0.90
Feather	S8 (ref)			
	P1	-0.66 ± 0.29	-0.19 ± 0.29	-3.58 ± 0.81
Colony	Medes (ref)			
	Ebro Delta	0.60 ± 0.37	1.58 ± 0.40	0.94 ± 1.15
	Columbretes	0.33 ± 0.36	1.11 ± 0.37	0.83 ± 1.12
	Sa Dragonera	-0.47 ± 0.43	-0.08 ± 0.43	-1.47 ± 1.45
	Benidorm	1.17 ± 0.41	1.36 ± 0.40	3.36 ± 1.30
	Mazarrón	1.07 ± 0.40	1.78 ± 0.42	2.95 ± 1.26
	Alborán	2.13 ± 0.38	2.14 ± 0.38	4.42 ± 1.14
Feather by Colony	P1*Ebro Delta	0.20 ± 0.37	0.15 ± 0.36	4.80 ± 1.01
	P1*Columbretes	1.11 ± 0.37	0.09 ± 0.37	7.28 ± 1.03
	P1* Sa Dragonera	-0.15 ± 0.43	-0.32 ± 0.42	3.47 ± 1.19
	P1*Benidorm	-0.43 ± 0.41	-0.36 ± 0.41	1.40 ± 1.15
	P1*Mazarrón	-0.93 ± 0.40	-1.32 ± 0.40	-2.27 ± 1.11
	P1*Alborán	0.44 ± 0.39	0.11 ± 0.38	3.93 ± 1.08
Random effects				
		variance	variance	variance
	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 (± SE) are shown. For random effects and residual heteroscedasticity, variance estimates are shown

Capítol 3:

Avaluant el paper dels hàbits d'alimentació dels ocells en la salut ambiental

Presentem aquí les prevalences de *Campylobacter* i *Salmonella* en una espècie de gavina problemàtica arreu de la conca del Mediterrani. Utilitzant l'anàlisi d'isòtops estables per a caracteritzar la dieta dels polls de gavià de potes grogues, vàrem demostrar que la ocurrència de bacteris enteropatògens es relacionava positivament amb el grau d'explotació dels abocadors. Els nostres resultats suggereixen doncs, que les colònies d'aus properes a assentaments humans i que en gran part s'alimenten de deixalles i escombraries, poden ser més susceptibles a deteriorar la salut ambiental i pública.

The influence of insalubrious diets in avian enterobacteria prevalence: the exploitation of refuse sites by gulls and implications for environmental health

**Raül Ramos · Marta Cerdà-Cuéllar · Francisco Ramírez ·
Lluís Jover · Xavier Ruiz**

Abstract Wild animals are reservoirs of *Campylobacter* and *Salmonella*, the most notorious bacterial agents causing human enteric diseases worldwide. Despite this fact, there are no published results of investigations that relate the feeding habits and health conditions of wild animals to their microbiological carriage. For this purpose, we have studied three gulleries along the North-Eastern Iberian coast, with a varying degree of dependence on refuse sites as a food resource, which may determine differential bacterial incidence, as well as health status of birds. We found that *Campylobacter* occurrence in chicks is directly related to the degree of exploitation of refuse tips by their parents. Re-infection within the colony seems to be the most likely explanation for the high *Salmonella* values observed as no dietary relationship was found. We have also found that both *Campylobacter* and *Salmonella* do not affect the body condition of chick gulls, enabling the potential dispersal of pathogenic enterobacteria over large geographical areas, via fledgling or adult movements. Differential ecological constraints between *Campylobacter* and *Salmonella*, the relevance of avian feeding ecology on enterobacteria incidence and the likely asymptomatic infection of these bacteria on wild

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birds enables one to comment on the establishment of specific epidemiological measures to preventively limit the spread of these enteropathogens.

Introduction

Campylobacter and *Salmonella* spp. are the leading cause of zoonotic enteric infections in developed and developing countries, and their incidence is increasing even in countries with adequate public health surveillance (W.H.O. Scientific Working Group 1980; Oberhelman and Taylor 2000; Friedman et al. 2001). Despite the health impact of these enteropathogenic bacteria, their epidemiology remains poorly understood and the full epidemiological pathways leading to infection in humans have not yet been elucidated.

Well known modes of transmission to humans include physical contact with domestic animals, person-to-person spread and consumption of contaminated food and water (W.H.O. Scientific Working Group 1980). In addition, wild animals might also play a significant role in the epidemiology of enterobacteria (Refsum et al. 2002; Bogomolni et al. 2008). For instance, the role of wild birds in the bacteriological deterioration of drinking and recreational water reservoirs is well documented, causing increases in the levels of pathogenic microorganisms by faecal contamination (Benton et al. 1983; Lévesque et al. 2000). Furthermore, due to their ability to fly freely and to cover long distances during annual movements, wild-living birds are suspected of functioning as effective dispersers of disease via the aforementioned faecal contamination on pastures and surface waters throughout the world (Reed et al. 2003).

Seagulls in particular, due to their scavenging feeding habits, are one of the most documented carriers of such enterobacteria (Kapperud and Rosef 1983; Monaghan et al. 1985; Cízek et al. 1994; Broman et al. 2002). Although feeding habits related to garbage and sewage are largely assumed throughout the literature to increase the risk of microbiological infection on wildlife (Lévesque et al. 2000; Broman et al. 2002), to our knowledge no studies prove this assumption by combining dietary analysis and microbiological carriage determination. The increasing number of studies concerning seagulls and environmental public health are also partially due to the fact that populations of several species of gulls *Larus* spp. have increased dramatically throughout Australia, North America and Europe during the past several decades (Smith and Carlile 1993; Vidal et al. 1998). These geographic expansions have been attributed generally to factors such as the protection from human disturbance, the increasing availability of anthropogenic food from both peri-urban open-air refuse sites and industrial fisheries, as well as the great ability of gulls to adapt to anthropic environments (Pons 1992; Bosch et al. 1994).

In this study, we aimed to evaluate the effect of insalubrious feeding habits on enterobacteria prevalences of seagulls and, at a wider scale, to foresee the potential effect of this behaviour on environmental public health. For this purpose, we took samples from gull chicks from three gulleries of Yellow-legged gull *Larus michahellis* located in the North-Eastern region of the Iberian Peninsula, with a varying degree of trophic dependence on refuse sites. Gull chicks were samples in order to ensure that bacterial prevalences are due to the dietary inputs received at the colony site. Samples were analysed in order to (a) assess the prevalence of the most epidemiologically relevant *Campylobacter* and *Salmonella* spp. in Yellow-legged gulls; (b) to explore the relationship between the incidence of these enteropathogens and the gulls' feeding habits, and the risk that this may pose to public health; and finally, (c) to shed light on the infectious effect of such bacteria on gull health by assessing the Yellow-legged gulls as a carrier, with or without manifestation of disease.

Methods

Study area and sampling strategy

The study was carried out in the main Yellow-legged gull colonies along the North-Eastern Iberian coast during the chick-rearing period in 2005. From North to South the three sampled colonies were: Medes Islands (Is.), Ebro Delta and Columbretes Is. (Fig. 1). The Medes Is. (42º0'N, 3º13'E) are 900m

off the coast of a series of tourist towns. This area holds one of the largest and most densely populated colonies of Yellow-legged gulls in the Mediterranean (*ca.* 6650 pairs after several cullings). According to previous studies, the diet of Medes Is. gulls is mainly composed of garbage from refuse sites and some fishery discards (Bosch et al., 1994; Ramos et al., 2009). The Ebro Delta colony holds about 6000 pairs of Yellow-legged gulls and it is located at the Peninsula de la Banya (40º40'N, 0º45'E). The Ebro Delta is a protected area of salt marshes within the Ebro Delta Natural Park, where fishing vessel activity is also important. Previous studies indicated that the gulls diet here is mainly composed of fishery discards and, to a lesser proportion, of garbage and other terrestrial resources (Bosch et al. 1994; Ramos et al. 2009). The Columbretes Is. (39º54'N, 0º41'E) is a volcanic archipelago, which lies 55 km East off the coast of Castelló. Owing to the relatively large distance to mainland, birds in this area are found feeding mainly on marine resources during the chick-rearing period (Ramos et al. 2009). The colony size is the smallest of the three sampled colonies, comprising of approximately 400 breeding pairs.

To avoid pseudo-replication, a single fledgling (aged 30-40 days) from each brood was sampled during the late chick-rearing period. Each fledging was captured, measured, weighed and marked. In order to aid the evaluation of chick body condition, bill-head and tarsus lengths were measured to the nearest mm using digital calipers and wing length using a wing rule. We collected 6-8 growing scapular feathers from each bird as well as some food samples spontaneously regurgitated, for stable isotope analysis of carbon, nitrogen and sulphur (C, N and S). As scapular feathers from chicks grow slowly and constantly throughout the chick-rearing period (R. Ramos, personal observation), isotopic composition of such feathers integrated the chicks diet throughout the time period studied (see below). Regurgitates were individually placed in sealed plastic bags and kept frozen at -20ºC until laboratory analysis. After ensuring chicks had regurgitated most of their stomach contents, fledglings weights were obtained with a spring balance with a precision of 10g. For bacterial isolation, duplicate cloacal swabs from each chick were taken and placed in Amies charcoal medium (Deltalab, Barcelona, Spain), transported to the laboratory and cultured within 2-3 days after sampling (n = 182). All sampled fledglings were apparently healthy at the time of sampling, although no detailed pathological examination was attempted.
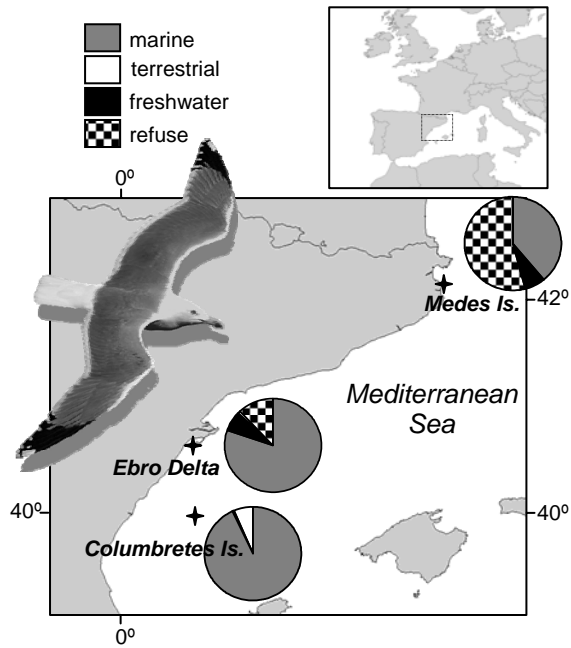
Evaluating chick body condition

Fig. 1 Map locations and foraging habitat exploitation of the Yellow-legged gull colonies sampled in the study along the Western Mediterranean. Foraging habitat exploitation percentages estimated by individual isotopic mixing models are represented as colony mean in circle diagrams.

Body mass is one of the most commonly measured variables used to evaluate avian health status (Tella et al. 2001). Since fledgling body mass may vary depending on feeding rates, we ensured chicks were weighed with empty stomachs, reducing the potential effect of recently ingested food on body mass. Body mass is also influenced by age and size. Therefore, we calculated a Principal Component Analysis (PCA) composite body size index based on head-bill, tarsus and wing lengths and then regressed the body size against mass to derive residuals as a body condition index.

Bacterial isoation and identification

Salmonella spp. isolation: Each sample was enriched overnight in buffered peptone water (BPW,Oxoid) at 37°C. Next, a selective enrichment in Rappaport-Vassiliadis broth (Oxoid) at 37°C for 24-48 h was performed, and then subcultured on xylose-lysine-desoxycholate agar (XLT4, Merck). Presumptive *Salmonella* colonies (black H_2S precipitating colonies) were streaked on MacConkey agar (Oxoid) and lactose negative isolates were confirmed with the Mucap test kit (Biolife) and the API 20E system. *Salmonella* spp. isolates were serotyped at the National Reference Centre for Animal Salmonellosis (Algete, Madrid, Spain).

Campylobacter spp. isolation: Cotton swabs were plated onto *Campylobacter* blood-free Selective agar (Oxoid) and incubated at 42°C under microaerobic (85% N_2, 10% CO_2, 5% O_2) conditions. The plates

were checked for growth of *Campylobacter*-like colonies after 48 h of incubation. From each plate with growth of suspected *Campylobacter*, two to three colonies were isolated and further investigated. Isolates showing inability to grow on blood agar under aerobic conditions at 37°C, gram-negative seagull-shaped cells under light microscopy, and positive reactions in catalase and oxidase tests, were regarded as *Campylobacter* spp. Identification to species level was carried out with a species-specific multiplex PCR for *C. jejuni*, *C. coli* and *C. lari* (Chuma et al. 2000).

Food and feather procedures and stable isotope analysis

Under laboratory conditions, regurgitates were weighed and prey, that have been consumed by the birds, were identified to the order level using standard reference guides. Based on the foraging habitat usually exploited by gulls, described by Bosch et al. (1994), regurgitate samples were assigned to four categories: a) marine; b) brackish and fresh waters; c) crops and terrestrial environments; and d) refuse sites. A random sample of the main prey types in each category (*n* = 5) was taken from different colonies in order to analyse the isotopic signatures of each prey type. Before isotopic analysis, food samples were freeze dried and ground to a fine powder in a freezer mill (Spex Certiprep 6750; Spex Inc., Metuchen, New Jersey, USA) operating at liquid nitrogen temperature. To reduce variability due to isotopically lighter lipids, which may particularly influence the carbon isotopes ratio (Hobson and Welch 1992), lipids were removed from food samples by the Folch's extraction method (Folch et al. 1957) with several chloroform-methanol (2:1) rinses. Afterwards, feathers were washed in a 0.25 M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contamination, dried in an oven at 60ºC to constant mass, and ground to a fine powder in the freezer mill.

From both powdered-feather and powdered-food samples, subsamples, weighed to the nearest µg, were taken as follows: 0.4 and 0.5mg for feathers and food samples respectively, for C and N analysis and 3.5 and 8.0mg for sulphur analysis. All samples were placed into tin capsules and crimped for combustion. Samples were oxidized in a Flash EA1112 coupled to a stable isotope mass spectrometer Delta C through a Conflo III interface (ThermoFinnigan, Bremen, Germany), where the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values were determined. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X (‰) is ^{13}C, ^{15}N or ^{34}S and R are the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$,

related to the standard values. $R_{standard}$ for ^{13}C is Pee Dee Belemnite, for ^{15}N is atmospheric nitrogen and for [34]S is troilite of the Canyon Diablo Meteorite. Isotopic ratio mass spectrometry facility at the Serveis Científico-Tècnics of University of Barcelona (Spain) applies IAEA standards inserted every 12 samples to calibrate the system. Replicate assays of standards indicated measurement errors of ± 0.1 for C, ± 0.2 for N and ± 0.2 ‰ for S. However, these errors are likely to be underestimates of true measurement error for complex organics such as feathers.

Isotopic mixing models and statistical analyses

Relative indexes of the different food sources were estimated for every individual, using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ mean values in a concentration-weighted mixing model. Because the elemental concentrations of C, N and S are significantly different between the four sources considered (one-way ANOVA; $F_{3,16}$ = 19.64, $F_{3,16}$ = 27.93 and $F_{3,16}$ = 4.41 respectively, all p < 0.01), a concentration-weighted model is recommended (Phillips and Koch 2002). For this study, we adapted the Phillips & Koch ISOCONC 1.01 model (2002), to analyse four food sources and three stable isotope signatures. In order to apply mixing models, isotopic values for food sources must be adjusted by appropriate fractionation factors (Δ_{dt}) to account for trophic fractionation (Gannes et al 1998). The fractionation factor for marine fish and feathers was established from Columbretes Is. data as only marine food resources were used there. Other Δ_{dt} values were obtained from the literature (Peterson et al. 1985; Hobson and Clark 1992; Hobson and Bairlein 2003). When negative values near zero were generated by the mixing model, percentages were readjusted for each locality by setting the most negative value to zero and re-computing other percentages according to original proportions given by the model (Ramos et al 2009).

To estimate prevalence values exact, confidence intervals for proportions were used. Relationships in contingency tables have been evaluated through Pearson χ^2 statistics, using exact p-values because some table cells have low expected values. McNemar test was used to compare paired probabilities, also using exact p-values. Binary logistic regression models were fitted using conditional maximum likelihood estimators to introduce locality as strata, and using Monte Carlo re-sampling techniques to estimate standard errors and p-values. Statxact-7 and LogXact-6 were used to carry out the statistical analysis.

Results

We analysed the occurrence of *Campylobacter* and *Salmonella* spp. (Table 1) and estimated the individual diet (presented as overall population

means in Fig. 1) of 182 gull chicks, sampled on three different localities. Overall, *Salmonella* spp. were isolated from 31 birds (17.0% ; CI95%: 11.9 to 23.1%) and *Campylobacter* spp. were recovered from 19 (10.4%; CI95%: 6.6 to 15.7%) of all samples collected (Table 1). Prevalence of both bacteria were not significantly different both in Ebro Delta and Medes Is. (McNemar's test, p = 0.69 and p = 0.85, respectively), but *Salmonella* infection was more probable than *Campylobacter* infection in Columbretes Is. (McNemar's test, p = 0.02). Overall, no relationship was found between *Campylobacter* and *Salmonella* infections (Fisher's exact test, p = 0.25; only one bird from Medes Is. was positive for both bacteria), and *Salmonella* prevalence was not significantly greater than *Campylobacter* when considering samples from all three localities pooled (McNemar's test, p = 0.09). *Salmonella* Typhimurium was the most common serotype in Medes and Columbretes Is., followed by *S.* Bredeney (Medes Is.) and *S.* Corvallis (Columbretes Is.), while *S.* Hadar was the most isolated in Ebro Delta. Among the 19 *Campylobacter* isolates, 10 were *C. jejuni* (1 from Columbretes Is. and 9 from Medes Is.); no *C. coli* or *C. lari* were identified (Table 1).

The dietary mixing models based on stable isotope analysis of carbon, nitrogen and sulphur of fledging feathers suggested that on average the majority of food resources for Columbretes Is. came

Table 1 *Campylobacter* species and *Salmonella* serovar carriage rates of faecal samples from Yellow-legged gull chicks sampled throughout three Western Mediterranean colonies.

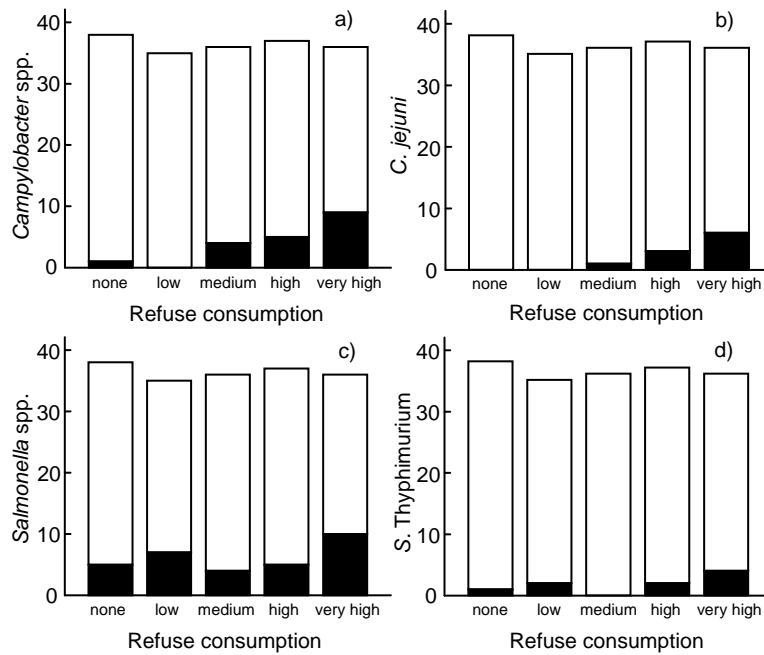|	Columbretes Is.	Ebro Delta	Medes Is.
	(*n*=71)	(*n*=36)	(*n*=75)
Campylobacter			
C. jejuni	1	0	9
C. coli	0	0	0
C. lari	0	0	0
undeterminated	2	2	7
Total of positives	3	2	14
(prevalence)	(4.23%)	(5.56%)	(18.67%)
Salmonella enterica subsp. *enterica*			
Azteca	0	0	1
Bardo	0	0	1
Brandenburg	1	0	0
Bredeney	1	2	2
Corvallis	2	0	0
Derby	2	0	1
Enteritidis	1	0	0
Hadar	2	2	0
Ituri	0	0	1
Lexington	0	0	1
Newport	0	0	2
Paratyphi B	0	0	1
Rissen	0	0	1
Typhimurium	2	1	6
Virchow	1	0	0
1,4, 5,12:i:-	0	0	1
1,4,12:i:-	0	0	1
4,12:i:-	0	0	1
4,5,12:i:-	0	0	1
undeterminated	4	4	4
Total of positives	11	4	16
(prevalence)	(15.49%)	(11.12%)	(21.34%)

Fig. 2 Enterobacteria prevalence on Yellow-legged gull chicks according to refuse consumption on the Iberian Mediterranean coast. The number of positives for *Campylobacter* spp. (a), *Campylobacter jejuni* (b), *Salmonella* spp. (c) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (d) are shown in black according to the refuse consumption. The number of enterobacteria negatives is shown in white. X-categories represented the quintiles (*n*=182; none=38, low=35, medium=36, high=37, very high=36) according to the individual refuse consumption percentage estimated from isotopic mixing models.

from marine environments (93.2%; Fig. 1). For the Ebro Delta, the model estimated that 80.8% of food was marine resources and few from refuse tips (12.1%) and freshwater ecosystems (7.1%). For Medes Is., both marine prey and garbage were well represented with 38.7% and 54.3% of importance, respectively (Fig. 1).

The highest values of *Salmonella* spp. prevalence among fledging gulls were observed in Medes Islands (Table 1) although differences among localities were not statistically significant (χ^2 =1.99, *p* = 0.39). Similarly, prevalence of *Salmonella* Typhimurium did not show any significant geographical variation (χ^2 =2.53, Monte Carlo *p* = 0.36). A conditional logistic model using locality as strata did not find any reliable model to fit *Salmonella* spp. or *Salmonella* Typhimurium prevalences using the consumption of refuse, the body condition index and their possible interaction as dependent variables (LR test = 3.29, Monte Carlo *p* = 0.19; LR test = 0.8, Monte Carlo *p* = 0.71, respectively). On the other hand, *Campylobacter* prevalence was significantly greater in Medes Is. (18.7%), but lower in Columbretes Is. where only three birds (4.2%) were found positive for *Campylobacter* (χ^2 =9,28, *p* = 0.0007; Table 1). A conditional logistic model using locality as strata showed that *Campylobacter* prevalence was positively related to refuse consumption (parameter ± se: 7.19 ± 3.31, *p* = 0.029; Fig. 2) whereas body index condition did not show significant relationship. Similarly, *C. jejuni* infections were more probable in Medes Is. than in the other localities (χ^2 =10.5, Monte Carlo *p* = 0.001) although we failed to find any relationship with refuse consumption or body condition using conditional logistic regression (LR test = 2.41, Monte Carlo *p* = 0.29).

Discussion

Our findings about the overall incidence of *Salmonella* spp. on chick gulls from the Western Mediterranean basin were similar to those described in other studies within the same area (15.4%; Bosch and Muniesa 1996), as well as in the Atlantic Iberian Peninsula (13.0%; Duarte et al. 2002), British Islands (9.5%; Monaghan et al. 1985), Fennoscandinavia (13.0%; Refsum et al. 2002) and Central Europe (11.0 and 19.2%; Glunder et al. 1991; Cízek et al. 1994). However, compared to other studies, the carrier rate of *Campylobacter* spp. among gulls in this study was relatively low (compared with 13.7, 34.0 and 62.0%; Glunder et al. 1991; Broman et al. 2002; Moore et al. 2002), particularly in those colonies with little or no refuse consumption, i.e. Columbretes Is. and Ebro Delta (Table 1). As previously suggested, *Campylobacter* among gulls was less frequently isolated from colonies distant from human populations, and from colonies that feed mainly on marine resources, than among populations which scavenged on refuse sites (Kapperud and Rosef 1983). Thus, although several studies considered thermophilic campylobacters to be avian commensals, i.e. a normal component of the intestinal microbiota of wild birds at moderately high rates (Moore et al. 2002), we found *Campylobacter* incidence was greater in those colonies with greater waste consumption, suggesting that refuse tips act as a source of these enteric bacteria.

Campylobacter and *Salmonella* have been isolated from a variety of ecological sources, although in sealife both pathogens are thought to cause little or no disease (Minette 1986). In addition, several extensive studies on Antarctic seabirds found

them all to be *Salmonella*- and *Campylobacter*-negative, suggesting that seabirds, in general, acquire both bacteria after exposure to human-contaminated environments, or after scavenging on refuse tips and sewage sludge, while wild birds which live away from such environments are unlikely to harbour such enteropathogenic bacteria (Palgrem et al. 2000; Bonnedahl et al. 2004). Therefore, the presence of these bacteria in gull chicks is mainly owing to feeding habits on terrestrial environments, and particularly those related to garbage and sewage (Tizard 2004). In agreement, birds from Medes Is. feeding abundantly on refuse waste showed greater *Campylobacter* bacteria prevalence than those from Columbretes, which fed almost exclusively on fish (Fig. 1). However, chicks from Columbretes Is., showed relatively high prevalence of *Salmonella* but low prevalence of *Campylobacter,* which might be due to differential ecological behaviour between both bacteria. *Salmonella* can persist in the environment for long periods (Literák et al. 1996) and probably survive in the soil of the breeding colony between reproductive periods. On the other hand, *Campylobacter* infection may be restricted to direct transmission, since some abiotic variables such as temperature and aerobic atmosphere (Newell and Fearnley 2003; Sinton et al. 2007), but particularly dehydration negatively affect the survival of *Campylobacter* in the environment (Murphy et al. 2006). Therefore, reinfection within the colony seems to be the most likely explanation for the high *Salmonella* values among colonies, whereas direct feeding of contaminated food by adults seems to be the most likely reason for *Campylobacter* prevalence in chicks (Newell and Fearnley 2003). Supporting this different ecological behaviour in the infection pathways between *Salmonella* and *Campylobacter*, we found no associative relationship between both bacteria within each individual. In addition, constant values of *Salmonella* incidence in gulls throughout Europe (from 10% to 20%) may represent a stable level of carriage, compared to the variable *Campylobacter* prevalence (ranging from 1 to 62%), further corroborating the difference in persistence between these bacteria in the environment.

Two of the most threatening enterobacteria for human health, *Salmonella* serovar Typhimurium and *Campylobacter jejuni* (Tauxe 1997) were the most isolated bacteria in the studied area. However, other bacteria more related to wild avifauna such *C. lari* were surprisingly not detected. These two human enteropathogenic bacteria were found more abundant in Medes Is. (Table 1), where Yellow-legged gulls extensively exploited refuse sites. Although we failed in detecting specific significant relationship of *S.* Thyphimurium and *C. jejuni* with gull's feeding habits, we strongly believe that the results presented here bring some light to this issue (Fig. 2). The overall low number of isolates

throughout the study area and the obvious close association between feeding habits and colony negatively affected the statistical power of the data analysis. Therefore, more extensive studies should be carried out to further assess this specific linkage.

Dispersal range of infectious pathogens is linked to the movement capacity of their infected hosts as well as of their animal reservoirs (Frost 2001). In spite of that fact, there are few published epidemiological studies focusing on the potential effect of bacterial carriage on the health status of wildlife. Presumably, birds with enterobacterial infection or with food limitation were in poorer body condition and they might be negatively affected, especially during the sensitive chick development stage (Tella et al. 2001). However, our results suggested that these bacteria did not affect the body condition of chick gulls, providing some evidence that gulls may merely act as non-affected carriers of these enterobacteria, rather than showing clinical signs of disease. Therefore, as subclinical carriers, there would be no health limitation imposed on Yellow-legged gulls by infection. In turn, the fledgling and adult movement capacity is not hampered and therefore there is potential for the dispersal of pathogenic *Campylobacter* and *Salmonella* over large geographical areas. This could, in part, contribute to the nearly worldwide distribution of both enteropathogens (W.H.O. Scientific Working Group 1980).

We provided some clear evidences here that the feeding exploitation of gulls on resources available near the cities, e.g. meat scraps from refuse sites, can affect their zoonotic enterobacterial carriage rate. Our findings suggest that avian colonies nearby human settlements, and which largely feed on refuse, may be more likely to deteriorate the environment public health. Some ecological differences between *Campylobacter* and *Salmonella* are also suggested to have a role in explaining their prevalences, i.e. that *Salmonella* can survive on the soil over long periods of time, while *Campylobacter* can be more sensitive to environmental factors, suggesting that transmission of *Campylobacter* among gulls could be mainly vertical throughout contaminated food provided by the adults (Newell and Fearnley 2003). These differential ecological constraints between *Campylobacter* and *Salmonella*, the relevance of avian feeding ecology on enterobacteria incidence and the likely asymptomatic infection of these bacteria on wild birds sheds new light onto the establishment of specific epidemiological measures to preventively limit the spread of these enteropathogens.

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Bloc II:

L'estudi de diferents trets migratoris al llarg dels oceans: el cas d'una au marina pelàgica, la baldriga cendrosa Calonectris diomedea
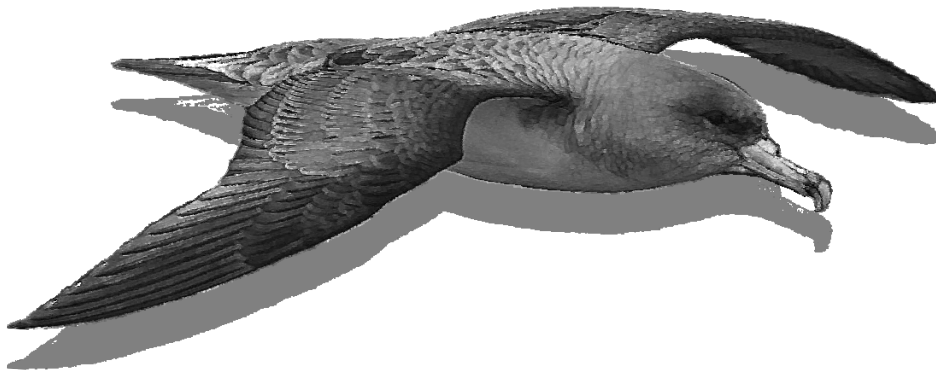
Enfocament multidisciplinar que fusiona patrons de muda, estratègies migratòries, càrrega corporal de contaminants i anàlisis d'isòtops estables sota un sostre comú per a entendre la integració dels marcadors biogeoquímics intrínsecs en els teixits animals, així com l'ús d'aquests marcadors com traçadors dels moviments migratoris en el medi marí. D'aquesta manera, la biogeoquímica dels teixits es presenta com una eina innovadora i amb potencial suficient com per a entendre i resoldre els problemes de conservació als quals s'enfronten molts vertebrats marins.

Capítol 4:

Esbrinant els patrons migratoris i de muda d'espècies discretes

R. Ramos, T. Militão, J. González-Solís, X. Ruiz (2009) Moulting strategies of a long-distance migratory seabird: the Mediterranean Cory's Shearwater *Calonectris diomedea diomedea*. *Ibis* 151: 151-159

Presentem aquí el més entenedor i complet estudi sobre els patrons de muda d'una espècie d'au marina migratòria molt emprada com a espècie model en diferents tipus d'estudis. En particular, vàrem reportar les anàlisis de muda d'un centenar de baldrigues cendroses capturades incidentalment en diferents períodes per vaixells de pesca de palangre al llarg del litoral català. Les diferències en els patrons de muda i la fenologia desacoblada entre els diferents tipus de plomes es discuteixen en relació a l'eficiència de vol i en el context de l'evolució de les estratègies de muda. A més, els patrons de muda i la seva variabilitat entre els individus i els gèneres, així com dins dels propis individus (entre les dues ales i les meitats de la cua) es descriuen en detall.

R. Ramos, J. González-Solís, X. Ruiz (2009) Linking isotopic and migratory patterns in a pelagic seabird. *Oecologia* 160: 97-105

Reportem aquí un estudi exhaustiu dels patrons espaciotemporals dels isòtops estables en relació a la muda d'una au marina migratòria. Presentant els valors d'isòtops estables de carboni, nitrogen i sofre de les plomes de vol de diverses baldrigues cendroses, proporcionàrem clares evidències que les signatures isotòpiques de diferents províncies oceàniques poden integrar-se en les plomes d'un mateix individu i assenyalar la zona on cada ploma ha sigut mudada. Els resultats també varen revelar un patró de muda desconegut fins aleshores per a aquesta espècie pelàgica que muda la majoria de les seves plomes durant l'hivern, quan aquestes aus són inaccessibles per al seu estudi. Aquest estudi confirma doncs, el potencial de l'anàlisi d'isòtops estables com a eina per investigar i seguir la migració d'aus marines a determinades zones d'hivernada. Això té especial rellevància en els estudis de connectivitat migratòria i estratègies de conservació de la fauna marina ja que ofereix noves oportunitats per a estudiar la mortalitat induïda per activitats humanes, com les pesqueries, els vessaments de cru o el canvi climàtic.

Moulting strategies of a long-distance migratory seabird, the Mediterranean Cory's Shearwater *Calonectris diomedea diomedea*

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Seabird moult is poorly understood because most species undergo moult at sea during the non-breeding season. We scored moult of wings, tail and body feathers on 102 Mediterranean Cory's Shearwaters *Calonectris diomedea diomedea* accidentally caught by longliners throughout the year. Primary renewal was found to be simple and descendant from the most proximal (P1) to the most distal (P10) feather. Secondaries showed a more complex moulting pattern, with three different asynchronous foci: the first starting on the innermost secondaries (S21), the second on the middle secondaries (S5) and the latest on the outermost secondaries (S1). Rectrix moult started at a later stage and was simple and descendant from the most proximal feather (R1) expanding distally. Although a few body feathers can be moulted from prelaying to hatching, moult of ventral and dorsal feathers clearly intensified during chick rearing. Different moulting sequences and uncoupled phenology between primary and secondary renewal suggest that flight efficiency is a strong constraint factor in the evolution of moulting strategies. Moreover, moult of Cory's Shearwaters was synchronous between wings and largely asynchronous between tail halves, with no more than one rectrix moulted at once. This result is probably related to the differential sensitivity of wings and the tail on flight performance, ultimately derived from different aerodynamic functions. Finally, Cory's Shearwater females renewed feathers earlier and faster than males, which may be related to the lower chick attendance of females.

Keywords: feather, flight performance, longline fisheries, migration, moult score, seabird bycatch.

Until recently, bird wing-moult studies have been focused largely on understanding potential effects on feather gaps on the wing surface, because moulting can reduce flight efficiency and manoeuvrability, affecting the aerodynamic performance of birds (Tucker 1991, Hedenström & Sunada 1999, Pyle 2005). As gaps can be particularly critical during long-distance flights, we cannot fully understand moulting strategies without knowing the moulting phenology in relation to the migratory movements. However, a renewed interest in the spatiotemporal patterns of moult is flourishing because biogeochemical analyses of feathers are increasingly used in a large variety of studies, such as monitoring heavy metal levels of the ecosystems (e.g. Monteiro & Furness 1995, Eens *et al.*

1999, Becker *et al.* 2002, Becker 2003), studying the trophic structure of bird communities (Kelly 2000, Forero *et al.* 2004), tracing migratory movements (Hobson 2005b, Pérez & Hobson 2006, Hellgren *et al.* 2008) or assigning birds to their breeding origin (Kelly *et al.* 2005, Gómez-Díaz & González-Solís 2007). In these studies, knowing the spatiotemporal patterns of moult is essential because, once formed, feathers are inert and mostly reflect what the bird was feeding on when and where the feather was grown.

As primary feathers were considered the most relevant for wing loading, the majority of the literature on the subject of moult tackles primary renewal (Weimerskirch 1991, Underhill *et al.* 1992, Rothery & Newton 2002). However, moult gaps in the middle of the wing surface, i.e. on secondaries, can have an even greater effect on aerodynamic performance than more distal gaps (Hedenström & Sunada 1999).

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Moreover, as trace elements and isotopic forms can penetrate into different feathers of the same individual depending on where these were grown, there is increasing need to know the detailed moulting pattern of each flight and tail feather as well as body feathers.

Our understanding of moulting strategies is often hampered by an insufficient knowledge of the moulting patterns, especially in relation to migratory movements. This is particularly acute in seabirds, probably because their pelagic habits render them generally inaccessible during the non-breeding season, when most feathers are usually moulted (Marshall 1956, Bridge 2006). Most of our knowledge about seabird moult patterns is restricted to the breeding season, when those birds are readily accessible to investigators (Weimerskirch 1991, Monteiro & Furness 1996). At-sea observations from vessels have provided some valuable information on seabird moult during the interbreeding periods, as birds can be observed actively moulting their flight feathers (Brown 1990, Camphuysen & Van Der Meer 2001). However, more powerful results and precise observations can be obtained from dead specimens, either incidentally bycaught in fisheries or casually found on beaches or at sea (Cooper *et al.* 1991). Such animals, collected at different periods, allow a reliable, detailed and complete moult pattern to be obtained even outside the breeding season.

Here, we present our observations on the moult of wings, tail and body feathers on 102 Mediterranean Cory's Shearwaters *Calonectris diomedea diomedea* accidentally caught by longliners from the prelaying to the post-breeding period, just before migrating from the Mediterranean to the Atlantic. Our main objectives were: (1) to improve the current knowledge of moult pattern of Cory's Shearwater as well as to understand the moulting strategies in relation to the breeding and migrating activities, and (2) to improve the potential use of feathers of a model species, which is increasingly used in studies on trophic ecology, pollution monitoring or migration using biogeochemical analyses of their feathers (e.g. Monteiro & Furness 1995, Gómez-Díaz & González-Solís 2007). Monteiro and Furness (1996) found no flight feather renewal on Cory's Shearwater throughout the first stages of the breeding season (April–August, prelaying to early chick-rearing period) and not until early September were the first primary feathers found to be moulting simply and descendantly. In addition, body feather replacement at breeding locations was established to start at the middle incubation period (mostly in July). However,

rather less is known about moult sequence or timing of other feathers such as rectrices or secondaries. For this purpose, we determined in detail the moulting patterns of flight, tail and body feathers, and the variability of the moulting patterns among individuals and genders as well as within individuals (between left and right halves of the body).

METHODS

Study species

The Cory's Shearwater is a procellariiform which carries out a long and rapid transoceanic migration from its Mediterranean and Macaronesian breeding grounds to the wintering areas in the central and south Atlantic (Mougin *et al.* 1988, Ristow *et al.* 2000, González-Solís *et al.* 2007). In particular, Mediterranean Cory's Shearwaters traverse the Strait of Gibraltar twice a year in large numbers (Tellería 1980, Paterson 1997). Autumn passage to the Atlantic takes place between mid-October and the end of November, peaking on average on 29 October, and inwards movement to the Mediterranean takes place between late February and early April, on average on 2 March (González-Solís *et al.* 2007).

The simplified feather scheme of Cory's Shearwater wings and tail (Fig. 1a) consists of 10 primary feathers (external remiges), 22 secondary feathers (mid and internal remiges) and 12 tail feathers (rectrices). The flying strategy of Cory's Shearwater combines alternatively several wing beats with gliding at the sea-surface level (Rosén & Hedenström 2001).

Sampling strategy

We recorded the moulting stage of 102 Cory's Shearwaters accidentally caught by Mediterranean longliners in Catalonian waters (including French North Catalonia) between 2003 and 2007. Fishermen landed and froze all the specimens the same day they were caught and birds were stored at –20 °C until laboratory analysis. The cooperation of the fishermen was voluntary and non-profit making. Birds were caught during prelaying (between 3 May and 15 May; $n = 13$), incubation (between 15 May and 10 July; $n = 43$), early chick rearing (between 10 July and 15 September; $n = 6$), late chick rearing (between 15 September and 15 October; $n = 38$, 32 of them coming from the same date and location, 5 October 2004), and the post-breeding periods (after 15 October; $n = 2$; Thibault *et al.* 1997).
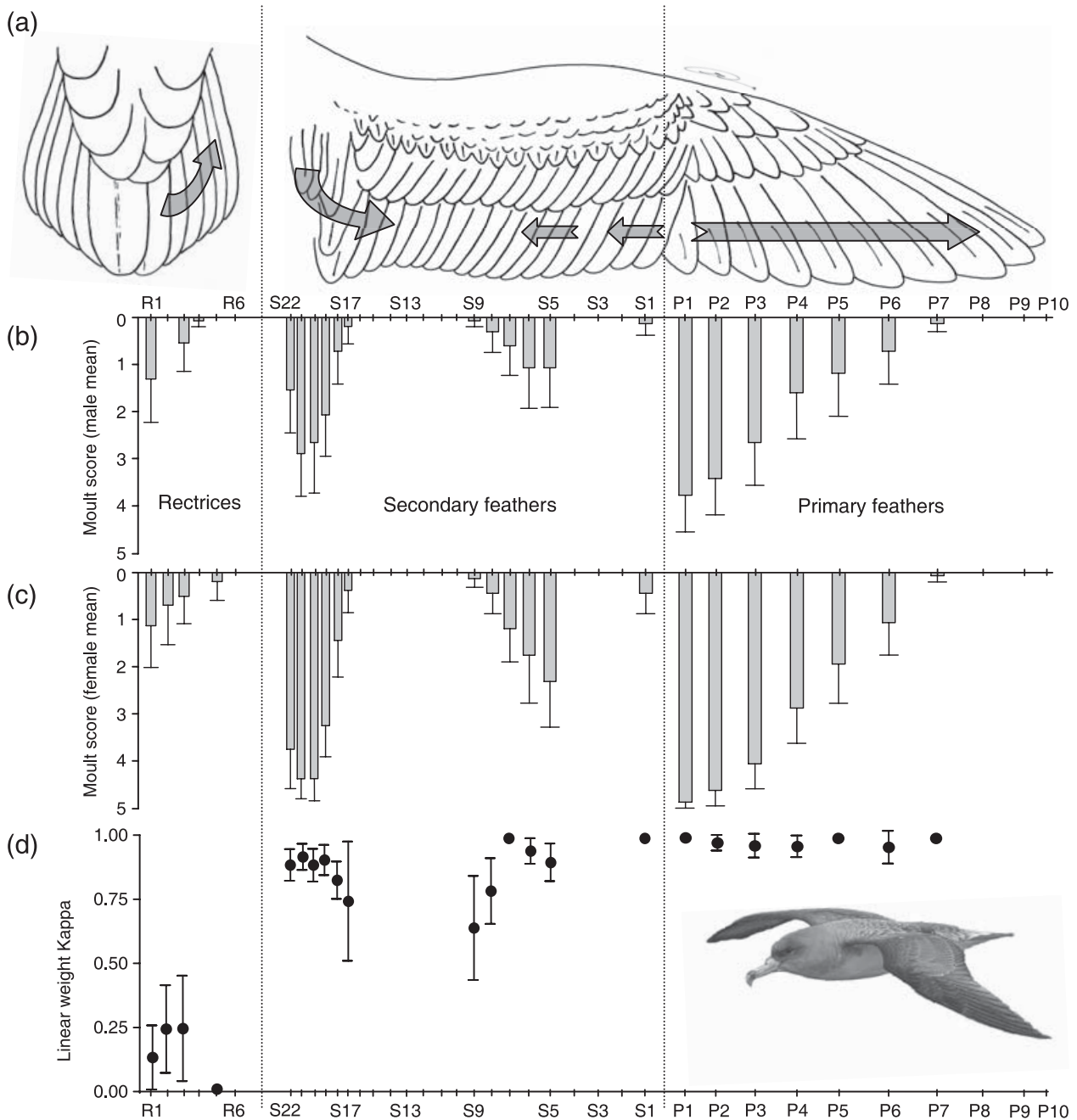
Figure 1. Tail and wing moulting scheme of 32 Cory's Shearwaters (17 males and 15 females) accidentally caught by a longliner on 5 October in Catalonian waters (NW Mediterranean): (a) the main moult pattern is shown with grey arrows, male (b) and female (c) mean scores (+ CI 95%) for each feather, and (d) agreement index (symmetry degree ± CI 95%) between feathers of the two wings and halves of tail. The picture was provided courtesy of Albert Cama.

All birds were sexed by dissection and age determined by measuring the size of the bursa of Fabricius, a dorsal diverticulum of the cloaca that performs an immunosuppressive function in immature birds (Glick 1983). The bursa is greatly reduced in size or absent in adults and it has been used in several studies to separate birds-of-the-year from 1-year-old or older birds (Mercer-Oltjens & Woodard 1987,

Broughton 1994). As in other long-lived Procellariidae, Cory's Shearwaters reach sexual maturity between the 5th and 9th year (Thibault *et al.* 1997). Moult patterns are thought to ultimately depend on several intrinsic individual factors, such as age (Furness 1988, Edwards 2008). To avoid such potential biases, those animals with obvious bursa were considered earlier juveniles (Broughton 1994) and were excluded from the study; only specimens with no bursa trace, which were assumed to be adults, were considered in the study.

We examined the 10 primary (excluding the minute 11th primary), the 22 secondary and the 6 rectrix feathers from each body half. The moult stages of flight feathers were scored as follows: 0 (old feather remaining), 1 (feather missing or in pin), 2 (new feather emerging from the sheath up to one third grown), 3 (new feather between one and two thirds grown), 4 (new feather two thirds to full grown and with remains of waxy sheath at its base) and 5 (new feather fully developed with no trace of waxy sheath at base; Ginn & Melville 2000). Therefore, the fully renewed primaries would score 50, secondaries 110 and rectrices 30. In addition, we scored the proportion of growing feathers on ventral and dorsal surfaces as follows: 0 (no growing feathers), 1 (1–10 growing), 2 (11–50 on ventral and 11–25 on dorsal) and 3 (> 50 on ventral and 25 on dorsal).

To relate moult to energetic status, a body condition index was estimated for each bird by summing the scores of subcutaneous fat (between feathers on breast), intestinal fat (around distal parts of gut; scoring 0 = no fat, 1 = some fat, 2 = fat and 3 = very fat) and condition of pectoral muscle (scoring 0 = strongly emaciated, 1 = emaciated, 2 = moderate condition and 3 = good condition; van Franeker *et al.* 2005).

Statistical analysis

Differences in moult scores between left and right wings and tail halves were evaluated on moulting birds caught at the same location on 5 October (late chick-rearing period; *n* = 32) using the agreement test of Cohen's weighted Kappa in a contingency table for each feather. We checked for normality of the individual moult scores for each type of feather examining Q-Q plots. No severe deviations from normality were found and we used parametric tests throughout. Due to the heterogeneity of variances found, score differences among periods were analysed using one-way ANOVA with Welch's correction followed by Tamhane *post-hoc* pairwise comparisons (Zar 1996).

We analysed sexual differences in 32 birds collected from bycatch on 5 October by comparing their body score values (Mann–Whitney *U*-test) as well as by comparing the number of remaining old feathers (i.e. score = 0) on primary, secondary and rectrix feathers (Student's *t*-test). Spearman correlations were used to examine likely relationships between body condition and the amount of old flight feathers on such birds collected on 5 October. Statistical analysis was carried out using SPSS 15.0 (SPSS 2006).

RESULTS

The 32 birds recovered from bycatch during early October (Fig. 1a–c) showed a simple and descendant primary moult from the innermost (P1) to the outermost feather (P10) from almost all birds showing new or growing P1 (96.9%) to only 9.7% of birds growing P7. No birds were found replacing the most distal primaries, i.e. P8, P9 or P10. Secondaries showed a more complicated moult pattern, with three foci located on the innermost, the middle and the outermost secondaries centred on S21, S5 and S1, respectively (Fig. 1a–c). The S21 focus started moulting at the late breeding season (probably a little bit later than P1) and expanded ascendant and descendant from that feather. In early October, we recorded a new or growing S21 in 90.4%, S5 in 48.4% and S1 in 19.4% of the birds. The renewal of rectrices seemed to be more erratic than wing feathers. In early October, we recorded a new or growing R1 in 38.8% and R3 in 19.4% of birds, but only two birds (6.4%) showed a new R2. Other rectrices were mainly found as old feathers.

In three birds (of 102), we found an extra secondary feather. The presence of the diastataxy phenomenon, which implies the absence of the fifth secondary but the presence of the remaining covert feathers (Humphrey & Clark 1961), is notable for Cory's Shearwater, although it has been previously reported in other procellariiform species (Bostwick & Brady 2002).

Two males were caught in the Mediterranean just before migrating to the Atlantic (early November) and had suspended moult, i.e. presented almost all feathers as either old or completely grown, without any active moulting focus (Table 1). That is, primary feathers were moulted completely up to P5 or P6 and secondaries were renewed from S5 to S7 or S8 and from S18 to the innermost secondary (S22), and only in one of the two birds was one flight feather still actively moulting (S1 feather in both wings).

Table 1. Male (m) and female (f) mean (± se) moult scores of Cory's Shearwaters throughout the breeding period (range in brackets).

Period		n	Ventral	Dorsal	Primary	Secondary	Rectrix
Prelaying	m	5	0	0.2 ± 0.2 (0–1)	0	0	0
(3 May–15 May)	f	8	0.1 ± 0.1 (0–1)	0	0	0.4 ± 0.4 (0–3)*	0
Incubation	m	28	0.7 ± 0.2 (0–3)	0.1 ± 0.1 (0–1)	0	0	0
(15 May–10 July)	f	15	0.3 ± 0.2 (0–3)	0.1 ± 0.1 (0–1)	0	0	0
Early chick rearing	m	3	2.7 ± 0.3 (2–3)	2.3 ± 0.3 (2–3)	0	0	0
(10 July–15 Sept)	f	3	3.0 ± 0 (3–3)	2.0 ± 0.6 (1–3)	3.7 ± 2.3 (0–8)	3.7 ± 2.7 (0–9)	0.7 ± 0.6 (0–2)
Late chick rearing	m	19	2.9 ± 0.1 (2–3)	2.6 ± 0.2 (0–3)	13.9 ± 2.0 (0–29)	15.2 ± 2.6 (0–47)	2.2 ± 0.6 (0–7)
(15 Sept–15 Oct)	f	19	2.7 ± 0.1 (2–3)	2.7 ± 0.1 (2–3)	19.7 ± 1.3 (9–28)	24.0 ± 2.1 (7–39)	2.3 ± 0.5 (0–8)
Post-breeding	m	2	1.0 ± 0 (1–1)	0	27.0 ± 2.8 (25–29)	44.0 ± 5.7 (40–48)	2.5 ± 3.5 (0–5)
(2 November)							
Fully renewed score			**3**	**3**	**50**	**110**	**30**

*Only one bird from 8 May was found replacing the right S4 (score 3). This feather could have been lost accidentally or alternatively it could have been the last feather replaced during a protracted moult.

We explored sexual differences in 32 shearwaters caught on 5 October for the five different types of feathers (Table 1). Ventral, dorsal and rectrix moulting scores did not show significant differences between sexes (ventral: Mann–Whitney $U = 118.0$, $n = 32$, $P = 0.332$; dorsal: $U = 128.0$, $n = 32$, $P = 0.726$; rectrices: $t_{30} = 0.66$, $P = 0.518$). In contrast, the number of new feathers on the wing was significantly greater in females than in males (primaries: $t_{30} = 2.16$, $P = 0.039$; secondaries: $t_{30} = 2.80$, $P = 0.009$; Fig. 1b & 1c).

No substantial differences were found in moult pattern between the right and left wings (Fig. 1d). Cohen's weighted Kappa for primary feathers showed high agreement between wings (mean = 0.98, ranging from 0.96 to 1.0). Secondary feather moult patterns showed a larger variability, but the pattern was mainly consistent between the two wings (mean = 0.88, ranging from 0.65 to 1.0). Tail feathers showed the lowest coefficient of agreement (mean = 0.17, ranging from 0 to 0.26), which is closer to a disagreement than to an agreement pattern (Fig. 1d).

Ventral, dorsal, primary, secondary and rectrix moult scores showed significant differences between defined periods ($F_{\text{WELCH }4,97} = 73.62$, 168.34, 72.62, 57.49 and 12.58, respectively, with all $P < 0.0001$; Table 1). Most birds did not moult ventral and dorsal body feathers during prelaying, incubation and post-breeding periods and *post-hoc* comparisons showed their scores did not differ. In contrast, birds were actively moulting in early and late chick-rearing periods (Table 1). In the chick-rearing period, ventral body feathers scored mainly 3 (almost more than 50 growing feathers found on each bird), whereas

dorsal feathers ranged between 2 and 3 (from 10 to more than 25). Almost no primary, secondary and rectrix feathers were moulted from prelaying to early chick-rearing periods (moult score ~0), but moulting started in the late chick-rearing and intensified in the post-breeding period (Table 1). Finally, no relationship was found between body condition and moult stage ($r_S = 0.08$, $n = 32$, $P = 0.688$).

DISCUSSION

General moult pattern

As previously described, primary renewal was consistently simple and descendant from the most proximal to the most distal feather, i.e. from P1 to P10 (Fig. 1a–c; Brown 1990, Monteiro & Furness 1996). It has been suggested that P2 is the first primary feather to be moulted in some petrel species (Allard *et al.* 2008), but this is not the case in Cory's Shearwaters, which showed greater moulting scores of P1 and which are often found without the P1 when caught in their burrows at the end of the breeding season (pers. obs.). Secondaries showed a more complex moulting pattern, with three asynchronous different foci: the first in time occurring on the innermost secondaries (from S21 and expanding mainly descendant towards distal feathers); the second on the middle (from S5 and expanding proximally); and the latest focus to develop on the outermost secondaries (from S1 expanding proximally; Fig. 1a–c). In contrast to the simple and descendant moulting strategy of primaries, the presence of three asynchronous moulting foci equidistantly located among the 22 secondary feathers indicated that birds avoid

having large feather gaps on the wing surface (Warham 1990, Weimerskirch 1991, Bridge 2006). The first and the most relevant secondary focus during the breeding season was located on the innermost feathers (called tertials by some authors), which are the feathers least involved in the lift surface. The S1 focus was the last to develop, probably to avoid a time overlap with the renewal of the first primary feathers, which are adjacent to the S1, as this would result in a large gap on the middle of the wing. Differences in moulting patterns and uncoupled phenology between primary and secondary renewal suggest that flight efficiency is a strong constraint factor in the evolution of moulting strategies. Rectrix moult started at a later stage, was simple and descendant from the most proximal feather and expanding distally (Fig. 1a–c), and it was probably completed on arrival at the wintering quarters. However, the lack of sampled birds in March, when birds have just returned to the Mediterranean, did not allow us to corroborate whether some individuals replace the last moulted feathers (*e.g.* P10 and R6) after returning to the breeding grounds, as reported for the Atlantic Cory's Shearwater (Monteiro & Furness 1996). Although a few body feathers can be moulted from prelaying to hatching, moult of ventral and dorsal feathers clearly intensified during the chick-rearing period (Table 1). In several seabird studies, it is often considered that body moult occurs mostly in winter (Monteiro & Furness 1995, Thompson & Furness 1995, Arcos *et al.* 2002), although our results suggested that this is not true for Cory's Shearwater where there is a considerable amount of body feather moult during breeding (see also Allard *et al.* 2008). As previously found (Monteiro & Furness 1996), the phenology of ventral body moult does not differ from dorsal body moult and in both cases occurs earlier than flight moult.

The fact that moult schedule overlaps with the end of the breeding season is exceptional, especially in long-distance migratory birds (but see Barbraud & Chastel 1998). Generally, the main energy-demanding activities such as moult, breeding and migration are temporally segregated (Marshall 1956, Hemborg *et al.* 2001, Bridge 2006, Edwards 2008). However, Cory's Shearwaters heavily moult body, wing and tail feathers during the late chick-rearing period. Procellariiform seabirds have relatively the longest breeding period among all avian orders, they have long wings with a relatively high number of secondary feathers and many species perform long-distance migrations. These

traits probably force some albatrosses to carry out biennial breeding (Edwards 2008) as well as spending more than one season to completely replace flight feathers (Weimerskirch 1991). However, in Procellariidae such as Cory's Shearwater, it could compel them to start moulting before migration, so that all feathers can be renewed before the end of the wintering period (Warham 1990, Monteiro *et al.* 1996).

Traditionally, moult and migration have been considered to be incompatible (Payne 1972), not only because of the increased costs of feather synthesis (Lindström *et al.* 1993), but also because of the elevated flight costs of having wing gaps during moult (Hedenström & Sunada 1999). In this respect, it is well known that long-distance migratory birds can delay or suspend their moult until reaching wintering grounds (Lindström *et al.* 1994). This may also be the case for Cory's Shearwaters, as the two birds caught in early November showed signs of suspended moult, just when shearwaters are about to leave the Mediterranean (Tellería 1980, González-Solís *et al.* 2007).

Between-individual variability

In procellariiform species and in seabirds in general, most authors assume body symmetry on moult (Cooper *et al.* 1991, Monteiro & Furness 1996). Very few papers have evaluated moult pattern separately in both hemiparts (see Langston & Rohwer 1995). Moult of Cory's Shearwaters was synchronous between wings and largely asynchronous between tail halves (Fig. 1d), with no more than one rectrix moulted at once. This result is probably related to the differential sensitivity of the wings and tail on flight performance, ultimately derived from different aerodynamic functions (Pennycuick 1987, Thomas 1993). Increased wing asymmetry decreases stability and aerial manoeuvrability, whereas on the tail the effect of reducing the lifting surface is more relevant during manoeuvres (Thomas 1993, Swaddle *et al.* 1996). As pelagic seabirds spend long periods gliding and yawing while foraging, synchrony in wing feather moulting is probably under stronger stabilizing selection than tail feathers.

Moult score analysis showed that moult pattern was quite constant, but that moult phenology is rather variable between individuals. Among 32 Shearwaters caught on 5 October, moulting stage ranged from no moult to several new and moulting

feathers (P1–P7, S1, S5–S9, S17–S22; see ranges in Table 1). The energetic condition of such birds was not related to this variability, although it has been proposed as a determinant factor in several seabird studies (Edwards 2008). Therefore, between-individual variability could be partly explained by breeding stage, as failed breeders usually moult earlier and faster than successful breeders, as reported in several procellariiform species (Hunter 1984, Furness 1988, Brown 1990, Barbraud & Chastel 1998). Similarly, such between-individual moult phenology could ultimately depend on some other intrinsic individual factors such as age (Furness 1988, Edwards 2008). Although juveniles were excluded from the study after examining the bursa of Fabricius, older immatures typically do not show a well-developed bursa (Broughton 1994) and therefore could have been included as adults in the study.

Several procellariiform species show sexual differences in the timing of flight feather moult (Hunter 1984, Weimerskirch 1991, Langston & Rohwer 1995). Sexual differences may result from a balance of differential reproductive constraints and duties between sexes during the whole breeding period (Weimerskirch 1991). In most cases, males renew feathers earlier and faster than females, but Cory's Shearwaters showed the opposite trend. This may result from the differential chick attendance of males and females. Males feed their chicks more often than females (Granadeiro *et al.* 1998), which may leave females with more energy available to advance their wing moult.

Asynchrony in moulting patterns between individuals, but also between sexes, should be taken into account when planning biogeochemical studies based on feathers. The analytical study of feathers opens new opportunities to investigate biogeochemical processes in the environment (Hobson 2005a). However, as moulting phenology is rather variable, the most convenient target feather should be carefully selected to ensure the correct development of the study. Therefore, specific moult studies on migratory birds or detailed examination of feather wear patterns should be considered by future studies to distinguish which feathers are grown before and after migration (e.g. Pyle 2005). For example, in Cory's Shearwaters P1 and S21 can be considered to grow around the breeding area and P10, S13 and R6 on the wintering grounds in almost all cases. In contrast, a mix of body feathers can be considered to include feathers grown in both areas in similar proportions.

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ECOSYSTEM ECOLOGY - ORIGINAL PAPER

Linking isotopic and migratory patterns in a pelagic seabird

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Abstract The value of stable isotope analysis in tracking animal migrations in marine environments is poorly understood, mainly due to insufficient knowledge of isotopic integration into animal tissues within distinct water masses. We investigated isotopic and moult patterns in Cory's shearwaters to assess the integration of different stable isotopes into feathers in relation to the birds' trans-oceanic movements. Specimens of Mediterranean Cory's shearwater *Calonectris diomedea diomedea* caught accidentally by Catalan longliners were collected and the signatures of stable isotopes of C (δ^{13}C), N (δ^{15}N) and S (δ^{34}S) were analysed in 11 wing and two tail feathers from 20 birds, and in some breast feathers. Based on isotopic signatures and moult patterns, the feathers segregated into two groups (breeding and wintering), corresponding to those grown in the Mediterranean or Atlantic regions, respectively. In addition, feathers grown during winter, i.e. moulted in Atlantic waters, were grouped into two isotopically distinct profiles, presumably corresponding to the two main wintering areas previously identified for Mediterranean Cory's shearwater in tracking studies. N signatures mainly indicated the Mediterranean-to-Atlantic migration, whereas C and S signatures differed according to the Atlantic wintering area. Our results indicate that isotopic signatures from distant oceanic regions can integrate the feathers of a given bird and can indicate the region in which

each feather was grown. This study thus underscores how stable isotope analysis can link marine animals to specific breeding and wintering areas, and thereby shed new light on studies involving assignment, migratory connectivity and carry-over effects in the marine environment.

Introduction

While links between winter and summer terrestrial migration patterns have been extensively investigated using stable isotopes, few studies have been conducted on marine animals (Rau et al. 1992; Hobson and Schell 1998; Burton and Koch 1999; Gómez-Díaz and González-Solís 2007; Popp et al. 2007; Rooker et al. 2008). Natural isotopic gradients in baseline values of phytoplankton and particulate organic matter in oceanic masses may eventually be used to track marine animal movements (Rau et al. 1982; Goericke and Fry 1994; Pantoja et al. 2002). However, the geographic variability of isotopic signatures of marine organisms is poorly understood. In addition, a full understanding of which isotopes best reflect changes associated with remote oceanic areas has yet to be achieved.

The stable isotopes of C (^{13}C/^{12}C, δ^{13}C) and S (^{34}S/^{32}S, δ^{34}S) are relevant to ecological applications since they are used to track the input of these elements within foodwebs (Krouse and Herbert 1988; Hobson 2005a). In addition, the stable isotopes of N (^{15}N/^{14}N, δ^{15}N) are indicators of foodweb interactions and the trophic positions of species (Post 2002). Isotopic forms assimilated through diet are fractioned and incorporated into tissues as these tissues are

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formed (Hobson 1999). Since tissues turn over at different rates, each tissue integrates isotopic information over various temporal scales (Podlesak et al. 2005; Quillfeldt et al. 2008). In addition, if animals range over different areas, tissues can also incorporate isotopes over various spatial scales (Inger and Bearhop 2008). Interpreting this process requires an in-depth understanding of the metabolic and replacement rates of the various tissues in relation to migratory movements (Ogden et al. 2004; Cherel et al. 2005). In this respect, bird feathers are valuable because moult patterns are seasonally predictable and fairly consistent over time; specific feathers thus provide isotopic information from a single period, regardless of the sampling date (Hobson 2005b; Inger and Bearhop 2008). Once feathers are formed, their composition does not change but integrate the diet during the period when feathers were grown (Hobson 1999; Ramos et al., in press). Isotopic signatures in summer- and winter-grown feathers may differ for several reasons. Typically for long-distance migrants, foodwebs in breeding and wintering areas are located thousands of kilometres apart and often differ in their baseline isotopic levels (Marra et al. 1998). In addition, birds may feed on different prey or forage in different habitats in summer and winter (Cherel et al. 2006). Therefore, if certain feathers are moulted at different points of the annual cycle, changes in the isotopic profile of the same individual would be expected to reflect different migratory patterns and wintering areas (Minami and Ogi 1997; Cherel et al. 2000).

The main objectives of this study are: (1) to shed light on the relationship between isotopic patterns and migratory movements; and (2) to evaluate the usefulness of δ^{13}C, δ^{15}N and δ^{34}S analyses in linking breeding and wintering water masses of marine animals. In pursuit of these objectives, we chose a pelagic seabird, the Mediterranean Cory's shearwater *Calonectris diomedea diomedea*, for several reasons: (1) it feeds exclusively on nektonic prey, mainly fish and cephalopods; (2) it is a long-distance migratory seabird known to cover a disparate range of oceanographic provinces with significantly different baseline isotopic levels (Gómez-Díaz and González-Solís 2007); (3) like other petrels, it moults in both breeding and wintering areas (Monteiro and Furness 1996; Ramos et al. 2009); (4) since Cory's shearwaters are often accidentally caught by longliners, we were able to collect dead specimens and gather a comprehensive sample of flight and tail feathers for stable isotope analysis.

Materials and methods

Bird species studied and sampling strategy

Cory's shearwater *C. diomedea* is a procellariform species that undertakes long and rapid migrations from its Mediterranean and Macaronesian breeding grounds to its wintering areas in the central and south Atlantic (Mougin et al. 1988; González-Solís et al. 2007). It includes two subspecies: *C. diomedea diomedea* Scopoli breeds on islands in the Mediterranean, while *C. diomedea borealis* Cory breeds in the northeast Atlantic, from the Azores to the Canary archipelagos (Gómez-Díaz et al. 2006). Previous research on tracked Cory's shearwaters identified migration movements from the Mediterranean to two distinct wintering areas: the northeast tropical Atlantic, associated with the southern Canary Current near the confluence of the Guinea Current; and the eastern South Atlantic Ocean, associated with the Benguela Current (Fig. 1; Ristow et al. 2000; González-Solís et al. 2007).

Moulting of wing and tail feathers begins at the peak of breeding season, during the mid-chick-rearing stage in mid-September (Alonso et al., in press; Ramos et al. 2009). The main moult pattern of wing and tail feathers for Cory's shearwater is shown in Fig. 2a (adapted from Ramos et al. 2009). Primary renewal is simple and descends from the most proximal to the most distal feather, i.e. from P1 to P10. Secondaries show a more complex moult pattern, with three different asynchronous foci: the initial focus occurs on the innermost secondaries, also called tertials (from S21 and expanding mainly descendant towards the distal feathers); the second occurs on the middle secondaries (from S5 and expanding proximally); and the third and final focus develops on the outermost secondaries (from S1 expanding proximally). Beginning at a later stage, rectrix moult is simple, descending from the most proximal feathers and expanding distally; it is probably completed upon arrival in the wintering grounds (Monteiro and Furness 1996; Ramos et al. 2009). We collected and analysed stable isotopes from right flight and right tail feathers from ten male and ten female Cory's shearwaters caught by Catalan longliners (western Mediterranean) during the prelaying exodus and incubation period of 2003 and 2004. All birds were sexed by dissection; age was determined by checking the bursa of Fabricius (Glick 1983; Broughton 1994). Only specimens with no bursa were considered for the purposes of this study. We analysed the δ^{13}C, δ^{15}N and δ^{34}S of five primary feathers (1st, 3rd, 5th, 7th and 10th), six secondaries (1st, 5th, 8th, 12th, 16th and 20th), two rectrices (1st and 6th) as well as some breast feathers (Fig. 2a: feathers in grey).

Sample preparation and laboratory analysis

The feathers were washed in a 0.25-M NaOH 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, Metuchen, N.J.) operating at

Fig. 1 Noteworthy migratory events (mean date *in parentheses* and range *in grey*) in the phenology of ten tracked Mediterranean Cory's shearwaters breeding on Balearic ($n = 8$) and Chafarinas Islands ($n = 2$) and wintering in the areas associated with the Benguela ($n = 4$) and Canary ($n = 4$) Currents, Brazil-Falklands confluence region ($n = 1$) and the Guinea Gulf ($n = 1$) (González-Solís et al. 2007; D. Oro and J. González-Solís, unpublished data). Breeding range distribution (*solid dots*) and main wintering areas derived from kernel analyses encompassing 95% (*white*), 75% (*grey*) and 50% (*dark grey*) of filtered locations for Mediterranean Cory's shearwaters defined by Thibault et al. (1997) and González-Solís et al. (2007), respectively. The location of birds caught by Mediterranean longliners is shown by a *star*. The main oceanic currents affecting the wintering areas are also shown, adapted from Brown et al. (1989). Picture courtesy of Albert Cama
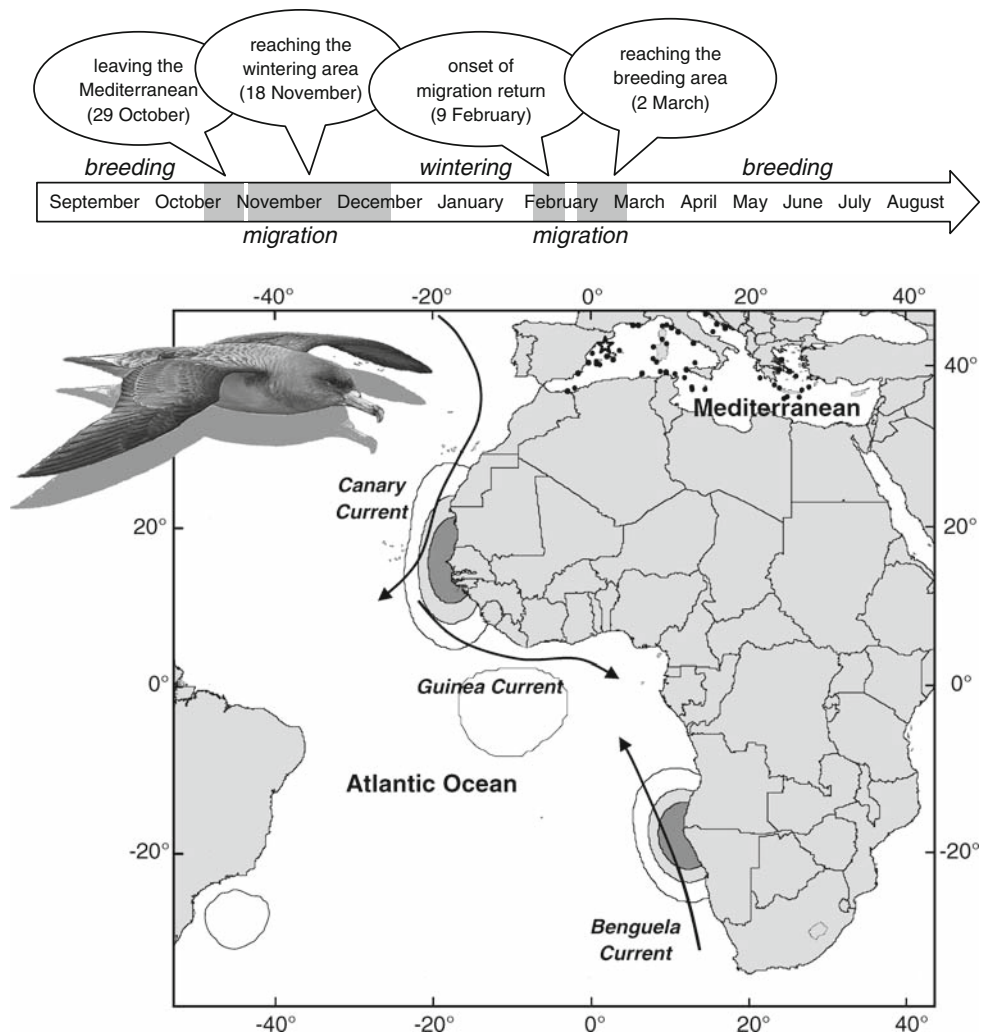
liquid N temperature. Subsamples of feather powder (0.4 mg) were analysed for C and N; 3.5 mg subsamples were analysed for S. The samples were weighed to the nearest microgram, placed into tin capsules and crimped for combustion. The samples were oxidized in a Flash EA1112 coupled to a Delta C stable isotope mass spectrometer through a Conflo III interface (ThermoFinnigan, Bremen, Germany), which was used to determine the δ^{13}C, δ^{15}N and δ^{34}S values. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X (‰) is ^{13}C, ^{15}N or ^{34}S and R is the corresponding ratio ^{13}C/^{12}C, ^{15}N/^{14}N or ^{34}S/^{32}S, related to the standard values. R_{standard} for ^{13}C is Pee Dee belemnite; for ^{15}N, atmospheric N; and for ^{34}S, troilite of the Canyon Diablo Meteorite. The isotopic ratio mass spectrometry facility at the Serveis Científico-Tècnics of the Universitat de

Barcelona (Spain) applied international standards that were inserted every 12 samples to calibrate the system and compensate for any drift over time.

Statistical analysis

To evaluate the extent of growth in various possible breeding and wintering areas, we used the feathers' δ^{13}C, δ^{15}N and δ^{34}S signatures to calculate the Euclidean distance for all pairwise comparisons of the different samples. We then constructed an unrooted tree of similarities (Fig. 2b) from the similarity matrix using the neighbour-joining clustering analysis implemented in the NTSYSpc package, version 2.1 (Rohlf 1997). The feathers' isotopic signatures are shown in Table 1, sorted according to isotopic similarity. The feathers with the highest and lowest mean score values, corresponding to those moulted in breeding and wintering areas, respectively (i.e. P1-P3-S20 and P10-S12-R6, respectively; Fig. 2b) are represented

Fig. 2 Cory's shearwater moult and biogeochemical relationship among feathers. **a** Selected feathers analysed for stable isotopes are shown in a *grey gradient* in the tail and wing scheme. The main moult pattern is shown by the *white arrows* (Ramos et al. 2009). **b** Neighbour-joining tree showing feather relationships based on signatures of stable isotopes of C (δ^{13}C), N (δ^{15}N) and S (δ^{34}S) from 20 Cory's shearwaters. The similarity tree is based on Euclidean pairwise distances among feathers; the *length* of the *scale bar* represents 0.1 units of distance
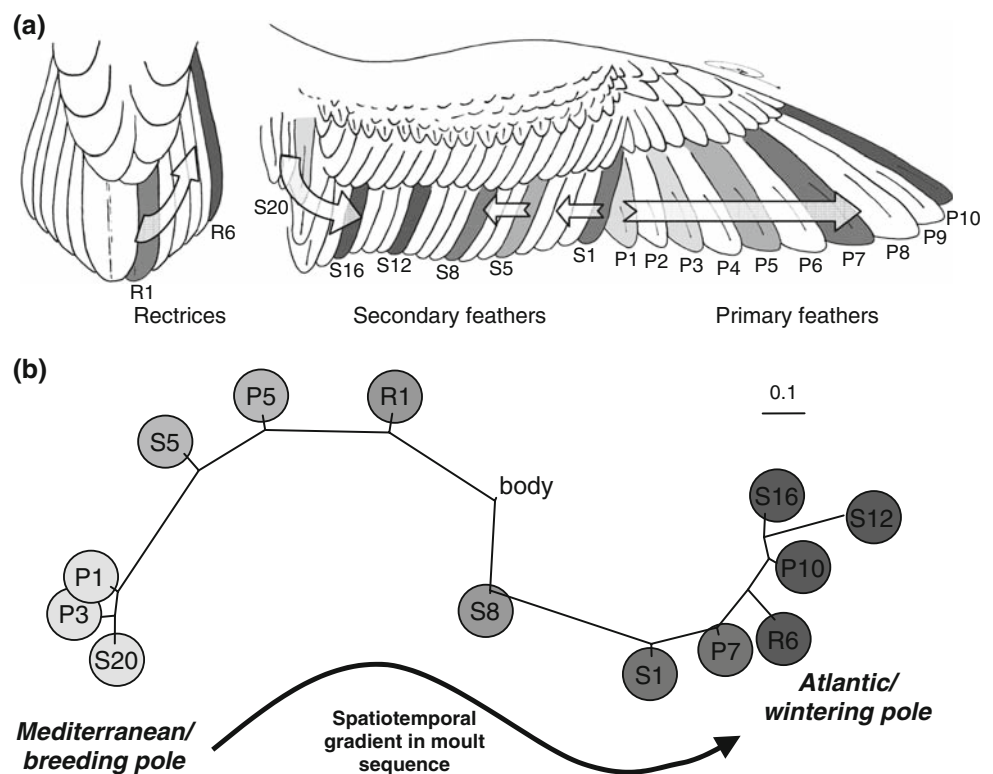
individually in a neighbour-joining tree (Fig. 3). Finally, since primary renewal is simple and descendant, beginning at the end of the chick-rearing period shortly before migration (Monteiro and Furness 1996; Ramos et al. 2009), the isotopic signatures of primary feathers are shown individually for each stable isotope to illustrate isotopic changes during the spatiotemporal moult sequence (Fig. 4).

Although few feeding pattern differences have been found for this species during the breeding season (Ramos et al., in press; Granadeiro et al. 1998), Mann–Whitney tests were used to assess sex-based differences in stable isotope values for each feather type. Sequential Bonferroni adjustment was used to assess significant differences at an overall α-level of 5% (SPSS 2006).

Results

The similarity tree is based on isotopic signatures of different type of feathers, with P1, P3 and S20 grouped in one pole (i.e. the breeding pole) and P10, S12, S16 and R6 grouped in another (i.e. the wintering pole; Fig. 2; Table 1). The P5 and S5 feathers were situated close to the breeding pole, which means that most of those feathers were moulted in the breeding area. However, the P7 and S1 feathers appear to have been moulted mainly in the wintering area since they were next to the wintering pole.

Other feathers, including S8, R1 and body feathers, were situated between the two poles, which suggests that they were moulted almost equally in each area.

Cluster analysis on specific individual feathers (Fig. 3) indicated three clearly defined groups: one consisted of breeding feathers (P1, P3 and S20) and two other groups included most of the winter feathers (P10, S12 and R6), evenly represented. The similarity tree showed a few unexpected winter feathers inside the breeding group (Fig. 3, filled symbols, belonging to two different individuals).

The sequential moult pattern expressed in the primary feathers' δ^{13}C, δ^{15}N and δ^{34}S signatures showed similar values for P1 and P3 among individuals (Table 1); it diverged in two equivalent differentiated groups for P10 already defined in Fig. 3 (Fig. 4, black dots; $n = 10$; δ^{13}C = -14.09 ± 0.43, δ^{15}N = 14.12 ± 0.51, δ^{34}S = 16.76 ± 0.62; white dots, $n = 8$; δ^{13}C = -16.08 ± 0.19, δ^{15}N = 13.06 ± 0.90, δ^{34}S = 19.10 ± 0.30). The two individuals classified with P10 as breeding feathers in Fig. 3 apparently showed breeding isotopic signatures especially in the δ^{15}N values (Fig. 4: grey dots, $n = 2$; δ^{13}C = -15.76 ± 0.01, δ^{15}N = 10.60 ± 1.06, δ^{34}S = 19.06 ± 0.25).

Finally, no significant isotopic differences were found between males and females in any feather types (sequential Bonferroni test).

Table 1 Stable isotope values of C (δ^{13}C), N (δ^{15}N) and S (δ^{34}S) (mean ± SE) of 1st, 3rd, 5th, 7th and 10th primaries, 1st, 5th, 8th, 12th, 16th and 20th secondaries, 1st and 6th rectrices and some body feathers

Moult sequence	Moult area	Feather	n	δ^{13}C	δ^{15}N	δ^{34}S
August		P1	19	−16.50 ± 0.08	10.37 ± 0.17	18.92 ± 0.12
		S20	20	−16.50 ± 0.06	10.22 ± 0.17	19.04 ± 0.11
September	Mediterranean pole	P3	20	−16.40 ± 0.03	10.20 ± 0.22	18.92 ± 0.10
		S5	20	−15.96 ± 0.21	10.64 ± 0.35	18.60 ± 0.24
October		P5	20	−15.95 ± 0.14	11.06 ± 0.39	18.85 ± 0.18
		R1	20	−15.98 ± 0.22	11.64 ± 0.42	18.85 ± 0.20
November	Migration	body	20	−15.77 ± 0.12	12.01 ± 0.16	18.53 ± 0.13
		S8	20	−15.50 ± 0.23	12.26 ± 0.41	18.40 ± 0.27
December		S1	20	−15.35 ± 0.27	12.80 ± 0.35	17.92 ± 0.29
		P7	20	−15.14 ± 0.24	13.05 ± 0.30	17.96 ± 0.27
January	Atlantic pole	R6	20	−15.08 ± 0.26	13.35 ± 0.25	18.21 ± 0.33
		P10	20	−15.07 ± 0.23	13.44 ± 0.26	17.92 ± 0.29
February		S16	20	−15.06 ± 0.27	13.39 ± 0.34	17.71 ± 0.34
		S12	20	−14.92 ± 0.26	13.77 ± 0.24	17.71 ± 0.31

Feathers are sorted according to isotopic similarity (Fig. 2; Euclidean distance for all pairwise comparisons)
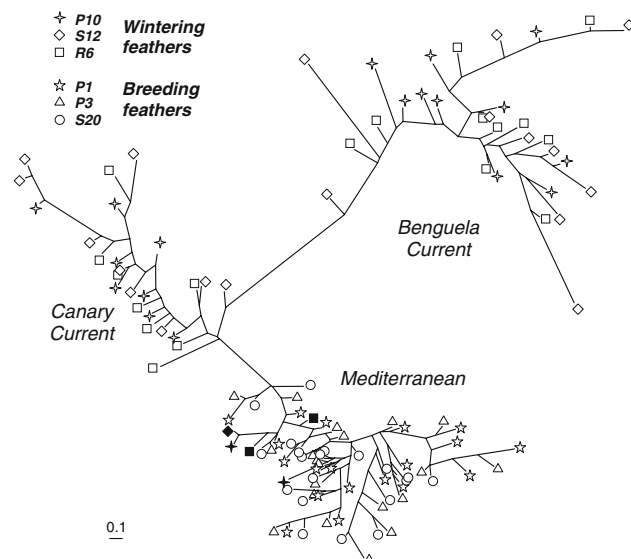
Fig. 3 Neighbour-joining tree showing individual relationships among breeding and wintering feathers (P1-P3-S20 and P10-S12-R6, respectively) based on δ^{13}C, δ^{15}N and δ^{34}S signatures from 20 Cory's shearwaters. The similarity tree is based on Euclidean pairwise distances among feathers; the *length* of the *scale bar* represents 0.1 units of distance. *Filled symbols* correspond to presumed winter-grown feathers with breeding isotopic values. NB: two individuals out of 20 moulted their P10 and R6 in Mediterranean waters (one of them also moulted its S12), i.e. upon returning to the breeding grounds

Discussion

Stable isotope signatures and moult patterns

Our study shows that the stable isotope signatures are clearly related to moult patterns and migration movements. The

species' migration movements are well defined: the autumn migration from the Mediterranean to the Atlantic takes place from mid-October to late November, followed by wintering in two main areas associated with the African continental shelf (Fig. 1; Tellería 1980; Paterson 1997; Ristow et al. 2000; González-Solís et al. 2007). The isotopic composition of the Mediterranean and Atlantic marine foodwebs differs considerably, and these differences can be used to identify the area in which each feather was grown (Pantoja et al. 2002; Gómez-Díaz and González-Solís 2007). Based on this data and on the species' moult pattern, we found that the primary feathers' isotopic signatures changed sequentially from P1 to P10, forming two poles, referred to as the "Mediterranean pole" and "Atlantic pole", respectively (Fig. 2). The signatures for P5 and P7 ranged from one pole to the other, showing an intermediate composition on average and indicating substantial individual variability in the moult area of the middle primaries (Table 1; Fig. 2). Similarly to P1, the secondary feathers' isotopic composition indicated that the innermost feathers (around S20, also known as tertials) were all grown in the Mediterranean before the birds migrated to the Atlantic (Table 1; Fig. 2). In contrast, S12 and S16 were moulted in the Atlantic (Fig. 2). On average, S1, S5 and S8 showed an intermediate composition, again indicating substantial individual variability in the moult patterns (Table 1; Fig. 2). In the case of the rectrices, the isotopic signatures of R1 showed a Mediterranean or Atlantic origin depending on the bird, suggesting that the onset of rectrix moult can occur before or after migration. In contrast, the most distal rectrices (R6) mainly showed an Atlantic origin, indicating that rectrix moult takes place in the winter grounds (Table 1; Figs. 2, 3).

Although Cory's shearwater moult pattern is fairly
uniform from individual to individual (Ramos et al. 2009),
the isotopic variability of certain feathers (i.e. P5, P7, S1,
S5, S8 or R1) suggests that the moult suspension point may
be slightly variable depending on the individual. Moult and
migration have generally been considered to be incom-
patible in the case of long-distance migratory birds (Payne
1972), most of which are thought to delay or suspend their
moult until reaching their wintering grounds (Lindström
et al. 1994). Moult suspension may ultimately depend on
intrinsic individual factors such as age, changes in breeding
phenology, the energy-related condition associated with
their breeding stage and breeding success. Immature birds
or failed breeders usually moult earlier and faster than
successful breeders (Furness 1988; Barbraud and Chastel
1998; Edwards 2008; Alonso et al., in press).

The isotopic signatures from several breast feathers
showed intermediate values between the Mediterranean
and Atlantic regions (Table 1; Fig. 2). Since a number of
body feathers of each individual were sampled for this
analysis, these intermediate values may be attributable to
individual variability in the moult patterns of body feathers
(e.g. some birds moulted all body feathers during breeding
and others in winter) or to a mixture of winter and breeding
feathers within the same specimen. The reduced variability
of body-feather isotopic values compared with that of
feathers moulted in wintering or breeding grounds (e.g. R1,
S8 or S1) indicates that the mixture of winter and breeding
feathers within the same specimen was true (Table 1).
Indeed, body moult is regularly observed throughout the
breeding period (Monteiro and Furness 1996; Ramos et al.
2009); however, our isotopic analyses clearly show that
body moult also occurs during the wintering period, when
the birds are inaccessible. Therefore, our results corrobo-
rate the finding that isotopic analyses of Cory's shearwater
body feathers can provide a reliable average value for the
entire year. Nevertheless, moult patterns can change

radically among species and should thus be carefully
investigated for reliability.

Isotopic signatures and wintering areas

Mediterranean Cory's shearwaters mainly winter in two
specific areas: the northeast tropical Atlantic, associated
with the southern Canary Current and the confluence of the
Guinea Current; and the eastern South Atlantic Ocean,

associated with the Benguela Current (Fig. 1; Ristow et al. 2000; González-Solís et al. 2007). Isotopic signatures reported in several local studies pertaining to the tropical and subtropical Atlantic indicate that permanent isotopic gradients at baseline levels could also occur. In particular, C isotopic signatures in phytoplankton may vary from −18.0 to −20.0‰ around Cape Blanc in the Canary Current; from −20.9 to −21.7‰ around the Guinea Basin (Fischer et al. 1998); from −15.9 to −17.3‰ around the southwest coast of Africa in the Benguela Current (Sholto-Douglas et al. 1991); and from −21.1 to −23.2‰ in the coastal waters off Uruguay and southern Brazil in the Brazil-Falklands/Malvinas Current confluence region (Matsuura and Wada 1994; Schwamborn 1997). Although little such information is available for other elements, these differences suggest that feathers grown in different Atlantic sectors may have distinct isotopic values. Indeed, based on our analyses of winter-grown feathers (P10, S12 and R6; Fig. 2), two isotopically distinct groups of Cory's shearwaters were identified (Figs. 3, 4), presumably corresponding to the two main wintering areas in the Atlantic (Fig. 1). Trophic studies carried out on gannets (*Morus capensis*) breeding in South Africa (along the Benguela Current; Jaquemet and McQuaid 2008) and on Cape Verde shearwaters (*Calonectris edwardsii*) breeding in the Cape Verde archipelago (on the southern Canary Current; Gómez-Díaz and González-Solís 2007) found that C and N isotopic signatures matched the two isotopic groups found in Cory's shearwater winter-moulted feathers (Fig. 4). Based on this comparison, it is reasonable to assume that Cory's shearwaters showing high C and N signatures in the 10th primary feather wintered in the Benguela Current, whereas those with low values for these isotopes wintered in the southern Canary Current (Fig. 4). As further support of our hypothesis, differences in C isotope signatures between both areas were consistent with previous literature on remote Atlantic regions such as these (see above; Sholto-Douglas et al. 1991; Fischer et al. 1998).

Differences among isotopes

Even though our analyses of feathers combining all isotopic signatures clearly reflected the Mediterranean-to-Atlantic migration, as well as the birds' segregation into two main wintering areas, each isotope responded differently to these factors. Signatures of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of feathers grown during the breeding season were rather similar for all specimens, confirming that all specimens moulted their first three primary feathers before leaving the Mediterranean (Fig. 4). In contrast, the $\delta^{13}C$ and $\delta^{34}S$ signatures of the last primaries moulted in the Atlantic formed two distinct groups, with each containing the same

individuals, presumably indicating the two main wintering areas noted previously. However, low $\delta^{13}C$ and high $\delta^{34}S$ signatures in the 10th primary feather (from the group presumably wintering in the southern Canary Current) were similar to those in the first primary feathers grown in the Mediterranean (Fig. 4a, c). Similarity in these signatures would make it impossible to distinguish between the breeding and wintering areas. In this respect, N isotopic values are essential for distinguishing between Mediterranean- and Atlantic-grown feathers. Although the $\delta^{15}N$ signatures of Atlantic-grown feathers did not segregate into two groups, all specimens showed an overall shift towards higher isotopic values compared to the $\delta^{15}N$ signatures of Mediterranean-grown feathers (Fig. 4b). Indeed, the changes in $\delta^{15}N$ signatures between the Atlantic and the Mediterranean indicated that two of 20 specimens moulted their last primary feather once they had returned to the Mediterranean (Fig. 4b; two specimens in grey). Lower $\delta^{15}N$ signatures in the Mediterranean than in the Atlantic were previously reported in particulate organic matter (Pantoja et al. 2002). Therefore, while $\delta^{15}N$ signatures signalled the Mediterranean-to-Atlantic migration, $\delta^{13}C$ and $\delta^{34}S$ values indicated different oceanic provinces within the Atlantic, providing an overall geographic fingerprint for tracking migratory movements.

This study underscores the potential use of stable isotopes to track animal movements in the marine environment. The integration of ocean-specific isotopic forms into the tissues of marine organisms offers new opportunities for identifying marine animals' breeding grounds and oceanic winter quarters. This approach could shed new light on studies involving migratory dynamics, migratory connectivity, origin identification, assessment of human impacts on remote populations, changes in animal distribution and the carry-over effects from distinct wintering areas to breeding grounds in the marine environment.

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Capítol 5:

Entenent les migracions oceàniques a través dels marcadors biogeoquímics intrínsecs

Reportem un estudi pioner en la combinació de marcadors biogeoquímics intrínsecs com els isòtops estables i les concentracions d'elements traça amb dispositius electrònics per al seguiment animal. En particular, utilitzant els coneixements de muda de les aus marines en combinació amb aparells electrònics de seguiment col·locats a les aus, vàrem demostrar que les plomes mudades en diferents regions oceàniques durant els períodes de reproducció i d'hivernació diferien en la seva composició química, el que ens permeté identificar les àrees oceàniques en què aquestes plomes foren formades. Aquest estudi confirma el potencial dels marcadors biogeoquímics intrínsecs com a eines valuoses alhora d'entendre la dinàmica espaciotemporal dels vertebrats marins unint localitats de cria amb àrees específiques d'hivernada, el que pot aportar nous coneixements sobre l'assignació, la connectivitat migratòria i sobre diversos estudis de conservació que afectin la fauna marina.

Understanding Oceanic Migrations with Intrinsic Biogeochemical Markers

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Abstract

Migratory marine vertebrates move annually across remote oceanic water masses crossing international borders. Many anthropogenic threats such as overfishing, bycatch, pollution or global warming put millions of marine migrants at risk especially during their long-distance movements. Therefore, precise knowledge about these migratory movements to understand where and when these animals are more exposed to human impacts is vital for addressing marine conservation issues. Because electronic tracking devices suffer from several constraints, mainly logistical and financial, there is emerging interest in finding appropriate intrinsic markers, such as the chemical composition of inert tissues, to study long-distance migrations and identify wintering sites. Here, using tracked pelagic seabirds and some of their own feathers which were known to be grown at different places and times within the annual cycle, we proved the value of biogeochemical analyses of inert tissue as tracers of marine movements and habitat use. Analyses of feathers grown in summer showed that both stable isotope signatures and element concentrations can signal the origin of breeding birds feeding in distinct water masses. However, only stable isotopes signalled water masses used during winter because elements mainly accumulated during the long breeding period are incorporated into feathers grown in both summer and winter. Our findings shed new light on the simple and effective assignment of marine organisms to distinct oceanic areas, providing new opportunities to study unknown migration patterns of secretive species, including in relation to human-induced mortality on specific populations in the marine environment.

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Introduction

Understanding spatiotemporal dynamics of marine vertebrates is essential to determine when and where animals are exposed to human impacts [1,2]. We have now clear signs that human activities and resulting global changes are having a strong impact on marine ecosystems [3]. Contamination episodes and massive fishery activities, such as oil spills or longlining, are responsible for the direct death of hundreds of thousands of marine vertebrates worldwide, leading to overall population declines of many shark, turtle, dolphin, seal and seabird species [4,5]. Global warming is also inducing changes in the distribution and abundance of marine prey and will therefore affect the distribution and movements of their predators [6,7]. Assessing the spatial interaction between these threats and marine predators will therefore be critical for effective conservation management. In migratory predators, this means not only assessing their distribution and abundance over time, but also their movements between breeding, feeding and wintering areas.

Although recent advances in tracking technology are helping to fill the current gap in marine migration knowledge, studies are usually restricted to a few individuals often tracked for short periods due to logistical and financial constraints [8–13]. As a result, there is increasing interest in using intrinsic markers to identify and link breeding and wintering sites of a large variety of marine predators [10,14,15]. In this regard, biogeochemical intrinsic markers, such as stable isotope signatures or element concentrations, can be particularly useful for studying migration dynamics, as no other intrinsic marker (i.e. biometrics or genetics) can identify wintering areas [16].

Migrating birds with known moulting patterns provide a singular opportunity to validate the utility of biogeochemical markers in the marine environment. As feathers grow, the elements and their isotopic forms assimilated through the diet are incorporated into the keratin structure. Once formed, feathers become metabolically inert, thus integrating the composition of the local food web where feathers were grown [17]. Many studies using biogeochemical markers have recently attempted to link wintering and breeding populations of different bird species along terrestrial environments [18,19]. However, hindered by the difficulty of determining wintering grounds in the open ocean, seabirds and ocean isotopic landscapes have attracted less

attention [20,21]. Nevertheless we can now track seabirds over the entire annual cycle using geolocators, which allows us to relate breeding and wintering areas to the geochemical composition of specific feathers.

Twice a year, millions of seabirds travel tens of thousands of kilometres across the equator to move between wintering and breeding areas, enhancing their susceptibility to threats posed by human activities [2]. As a result, pelagic seabirds are becoming increasingly threatened at a faster rate globally than all other species-groups of birds [5].

Here, we explored the value of using biogeochemical analyses as intrinsic markers to understand long distance movements of vertebrates in the marine environment. To do so, we studied Cory's shearwaters *Calonectris diomedea*, a long-distance migrant that breeds on temperate northeast Atlantic and Mediterranean Islands and winters in major upwelling areas of the Atlantic Ocean [13] (Fig. 1). To elucidate the integration of isotopes and elements from different food webs into their tissues, we first tracked 25 shearwaters over the entire annual cycle using light level geolocators, allowing us to identify both the breeding and the wintering areas for each bird. Second, we determined the stable isotope signatures of carbon (δ^{13}C), nitrogen (δ^{15}N), sulphur (δ^{34}S), hydrogen (δ^{2}H) and oxygen (δ^{18}O) and the elemental concentrations of selenium (Se), lead (Pb) and mercury (Hg) in feathers from the tracked birds moulted at the end of the breeding season and at the wintering grounds [22,23]. Using both sets of information, we demonstrate that isotopic signatures and element composition in feathers reflect the signatures of water masses where they were grown.

Results and Discussion

We found that breeding birds mostly foraged within a few hundreds of kilometres of their colony sites (mean distance to the colony in August: Azores Is.: 380.1±96.7 km; Balearic Is.: 297.0±86.8 km; Canary Is.: 442.7±121.4 km; Fig. 1). Selected populations for this study are separated by several thousand kilometres and located in different oceanographic regimes [24]. In this regard, primary feathers grown at the end of the breeding period differed in their composition among breeding sites in stable isotope signatures (ANOVA: δ^{13} C, $F_{2,14} = 66.6$, $P<0.001$; δ^{15} N, $F_{2,12} = 40.1$, $P<0.001$; δ^{34} S, $F_{2,15} = 146.8$, $P<0.001$; δ^{2} H, $F_{2,14} = 74.0$, $P<0.001$; δ^{18} O, $F_{2,12} = 48.5$, $P<0.001$) as well as in elementary content (ANOVA: Se, $F_{2,14} = 11.8$, $P = 0.001$; Pb, $F_{2,12} = 7.3$, $P = 0.009$; Hg, $F_{2,12} = 12.6$, $P = 0.001$). These differences allowed us to assign any individual to a relatively restricted breeding area using isotopic signature (100% correct classification) and, to a lesser extent, elemental composition (72.0% correct classification) of primary feathers (Table 1). Although the studied populations do not include the entire known breeding distribution for the species [mainly missing the central and eastern Mediterranean; 25], with the results presented here we can assign with
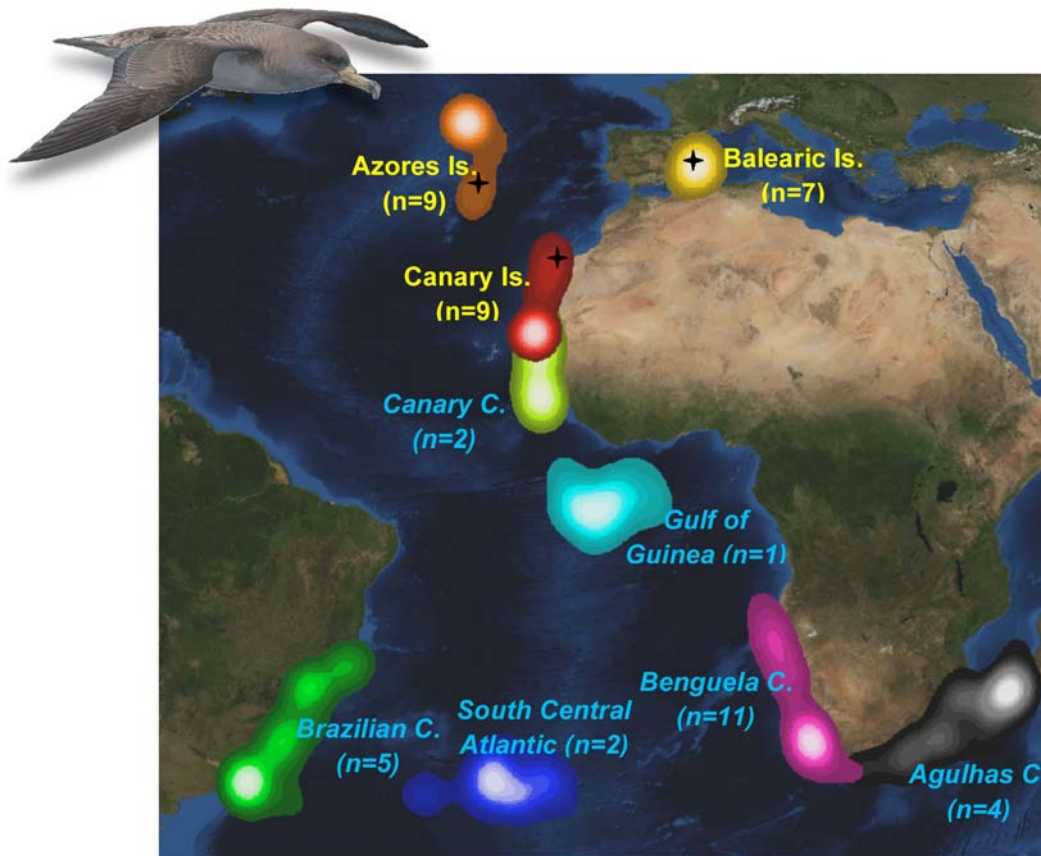

Figure 1. Studied breeding and wintering sites of Cory's shearwater. Main foraging areas of Cory's shearwaters at the end of the breeding season, between August and October (legends in yellow), the period when most Cory's shearwaters grow the first primary feather and during the wintering season, between December and January (legends in light blue), when most shearwaters grow the eighth secondary feather [22,23]. Activity ranges are derived from kernel analyses encompassing from 5 (light tone) to 90% (dark tone) of validated locations. Number of birds included in each area is shown in brackets. Sampling sites are shown with black crosses. Picture courtesy of Albert Cama.
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Table 1. Discriminant classification based on feather biogeochemistry.

	Stable isotopes P1	Stable isotopes S8	Element analysis P1	Element analysis S8
Breeding colonies				
Original data				
Azores Is. (n = 9)	100.0	44.4	66.7	66.7
Balearic Is. (n = 7)	100.0	57.1	71.4	100.0
Canary Is. (n = 9)	100.0	66.7	88.9	88.9
Total (n = 25)	100.0	56.0	76.0	84.0
Cross-validation				
Azores Is. (n = 9)	100.0	22.2	66.7	55.6
Balearic Is. (n = 7)	100.0	28.6	57.1	85.7
Canary Is. (n = 9)	100.0	55.6	88.9	55.6
Total (n = 25)	100.0	36.0	72.0	64.0
Wintering sites				
Original data				
Benguela C. (n = 11)	63.6	90.9	45.5	36.4
Brazil-Falklands C. (n = 5)	80.0	100.0	60.0	40.0
Agulhas C. (n = 4)	75.0	100.0	75.0	50.0
Canary C. (n = 2)	100.0	100.0	100.0	100.0
SC Atlantic (n = 2)	100.0	100.0	50.0	100.0
Total (n = 24)	75.0	95.8	58.3	50.0
Cross-validation				
Benguela C. (n = 11)	54.5	63.6	27.3	27.3
Brazil-Falklands C. (n = 5)	20.0	60.0	40.0	0.0
Agulhas C. (n = 4)	25.0	100.0	25.0	50.0
Canary C. (n = 2)	100.0	100.0	100.0	100.0
SC Atlantic (n = 2)	100.0	0.0	0.0	50.0
Total (n = 24)	50.0	66.7	33.4	33.4

Correct classification rates (%) obtained using stable isotope analysis (δ^{13}C, δ^{15}N, δ^{34}S, δ^{2}H and δ^{18}O) and element concentrations (Se, Pb and Hg) on summer (P1) and winter (S8) feathers. Discriminant analyses were cross validated using jackknife procedures. The Gulf of Guinea wintering area was not included in this analysis because it was visited by only a single bird.
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confidence the geographic origin (at large scale) of these shearwaters using the biogeochemical values of their breeding feathers.

In winter, birds travelled to the central and south Atlantic, concentrating in one of the six wintering areas (Fig. 1) associated with the Benguela (n = 11), Brazil-Falklands (n = 5), Agulhas (n = 4), Canary (n = 2) Currents, with the South Central Atlantic Ocean (n = 2) and with the Gulf of Guinea (n = 1). These oceanic areas were not different from those previously reported for the species [13,26–28]. Since each area has its own distinctive oceanographic features [24], the isotopic signatures of the secondary feathers grown during the wintering period also differed among the main wintering areas (ANOVA: δ^{13}C, $F_{2,7} = 28.1$, $P<0.001$; δ^{15}N, $F_{2,9} = 45.9$, $P<0.001$; δ^{34}S, $F_{2,7} = 34.0$, $P<0.001$; δ^{2}H, $F_{2,8} = 21.3$, $P = 0.001$; δ^{18}O, $F_{2,9} = 2.36$, $P = 0.079$) and could also be used to assign birds to specific wintering oceanic areas (66.7% correct classification; Table 1 and Fig. 2). In contrast, elemental analyses did not differ among wintering sites and showed a low rate of correct assignment (33.4%; ANOVA: Se, $F_{2,7} = 0.7$, $P = 0.54$; Pb, $F_{2,9} = 1.5$, $P = 0.27$; Hg, $F_{2,6} = 1.1$, $P = 0.38$). Indeed, elemental analyses of feathers moulted in wintering areas were more successful in assigning birds to their breeding origin than to their wintering areas (64.0% vs. 33.4% of

correct classification; Table 1). In fact, element concentrations of primary and secondary feathers are rather similar when grouped according to the breeding areas (Table S1). Such results confirm a differential behaviour in the accumulation and excretion dynamics between isotopic ratios and element concentrations [16,21]. Whereas stable isotope signatures of feathers reflect an exogenous origin, *i.e.* they are promptly transferred from the diet to feathers when moulting [29], elemental burdens of feathers may indicate an endogenous origin of elements, *i.e.* they are partially mobilized from various organs where they are stored [30,31]. Consequently, the interpretation of elemetal concentrations of migratory species from tissues formed out of the breeding season should be made with caution because those values could reflect exposures to elements during the breeding season, and vice versa. The deposition of elements acquired at breeding grounds into tissues grown out of the breeding period may be particularly important in species with long breeding seasons and relatively short wintering periods. In our case, Cory's shearwaters spend on average 243 days at the breeding grounds, but only 80 days on the wintering grounds [13].

Geographical variation in composition of tissues grown in distinct oceanic water masses can arise from different sources. Migrating predators may change diet between seasons, resulting in
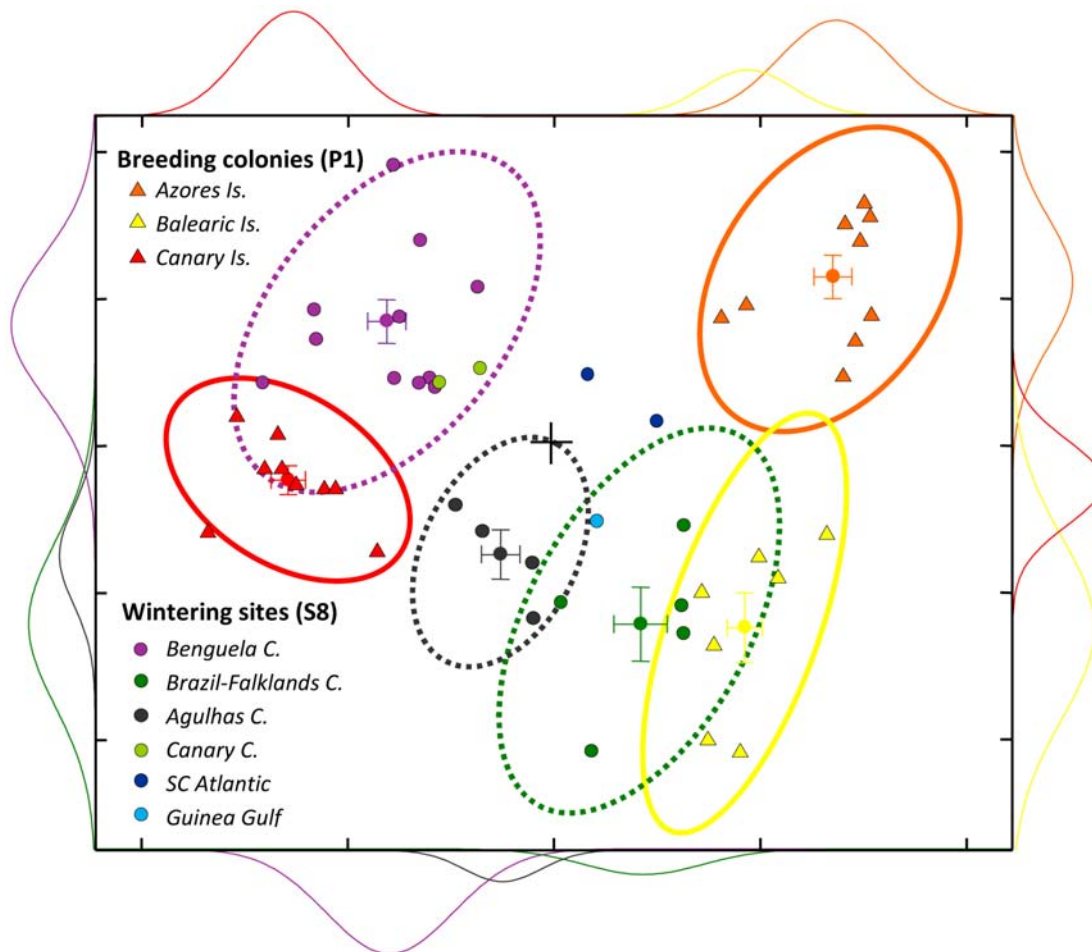
Figure 2. Isotopic composition of summer and winter feathers. Principal Component Analysis (PCA) of stable isotopic signatures of carbon (δ^{13}C), nitrogen (δ^{15}N), sulphur (δ^{34}S), hydrogen (δ^{2}H) and oxygen (δ^{18}O) in first primary (P1) and eighth secondary (S8) feathers (triangles and circles, respectively) of Cory's shearwaters moulted in breeding and wintering areas respectively. X-axis represents PC1 (59.0%) while Y-axis represents PC2 (21.1%); both are unitary divided with zeros on the middle cross-intersection. Gaussian bivariate ellipses (95% probability interval of the mean population) and normal distribution curves are shown.
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differences in trophic level and in isotopic values and element levels incorporated into the tissues [32]. Seasonal differences in foraging behaviour, such as inshore vs. offshore foraging, could also contribute to these differences [14,33]. In some cases, natural biogeochemical gradients have also been described in the marine environment [34,35]. Finally, as many biogeochemicals biomagnify throughout food chains, differential food web complexity among oceanographic systems has been identified as a prime source of geographical variation [36,37], contributing to the characterization of specific oceanic water masses.

In summary, this study showed that by choosing the appropriate tissue, isotope ratios and element composition can be used to assign marine predators to specific oceanic regions used during the breeding or wintering periods. Feathers of long-distance migratory seabirds are often replaced in a predictable manner in different oceanic regions throughout their annual journeys [22,38,39], thus providing excellent opportunities to study their migration through biogeochemical analyses [e.g. 40]. For other non-avian migratory species, specific portions of tissues, such as hair, whiskers, nails, scales [41–44], sampled at a particular time within their annual cycle could also be used to provide biogeochemical information about breeding and wintering areas; however appropriate

validations should be conducted. In organisms with long erythropoiesis and vitellogenesis processes (e.g. reptiles), even corpuscular blood (red blood cells) and yolk eggs can be used for such purposes [45,46]. However, it is also evident that elemental concentrations acquired in one season could be transferred to tissues grown in another season, highlighting the need to consider the carry-over effect of elemental concentrations between distinct oceanic areas. Such results open new insights into migration routes of marine vertebrates and provide an effective tool that can be used to assign marine organisms to specific breeding and wintering areas. This information provides new opportunities to study human-induced mortality caused by activities, such as fisheries, oil spills or climatic changes, on specific populations.

Methods

Ethics Statement

All animals were handled in strict accordance with good animal practice as defined by the current European legislation, and all animal work was approved by the respective regional committees for scientific capture (Consejería de Medio Ambiente del Cabildo de Gran Canaria, Canary Is., Spain; Secretaria Regional do

Ambiente da Região Autónoma dos Açores, Azores Is., Portugal; and Govern Balear, Balearic Is., Spain).

Study design

Transoceanic migrations can currently be investigated using global location sensing (GLS) devices based on recording light levels, which can be deployed on a bird all year-round and will give 2 positions per day with an accuracy of 186 ± 114 km [47]. This method can provide year round information on the location of breeding and wintering sites. In June and July 2002 we deployed 50 geolocators on Cory's shearwaters breeding in three geographically distant areas: Vila Islet (Azores Is.), Pantaleu Islet (Balearic Is., Mediterranean) and Veneguera (Gran Canaria, Canary Is.). After approximately one year we retrieved the GLS loggers and sampled the 1st primary and 8th secondary feathers from those birds that returned, obtaining year-round GLS data and feather samples from 9, 7 and 9 birds, respectively. Feathers were analysed for stable isotopes of carbon, nitrogen, sulphur, hydrogen and oxygen and for elemental concentrations of selenium, lead and mercury.

Sample preparation and laboratory analyses

All feathers were washed in a 0.25 M sodium hydroxide solution, rinsed thoroughly in distilled water to remove any surface contamination, dried in an oven at $60°C$ to constant mass, and ground to a fine powder in a freezer mill (Spex Certiprep 6750; Spex Inc., Metuchen, New Jersey, USA) operating at liquid nitrogen temperature. Subsamples of 0.4 mg of feather powder for carbon and nitrogen, about 3.5 mg for sulphur, and 0.25 mg for hydrogen and oxygen analyses were weighed to the nearest µg, placed into tin and silver capsules and crimped for combustion. Samples were oxidized in a Flash EA1112 and TC/EA coupled to a stable isotope mass spectrometer Delta C and Delta Plus XL, respectively through a Conflo III interface (ThermoFinnigan, Bremen, Germany), where the $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, δ^2H and $\delta^{18}O$ values were determined. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1]\times1000$, where X (‰) is ^{13}C, ^{15}N, ^{34}S ^2H or ^{18}O and R are the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, $^2H/^1H$ or $^{18}O/^{16}O$ related to the standard values. $R_{standard}$ for ^{13}C is Pee Dee Belemnite (PDB), for ^{15}N is atmospheric nitrogen (AIR), for ^{34}S is troilite of the Canyon Diablo Meteorite (CDT) and for ^2H and ^{18}O is Vienna Standard Mean Ocean Water (V-SMOW). The isotopic ratio mass spectrometry facility at the Serveis Científic-Tècnics of Universitat de Barcelona (Spain) applies international standards (IAEA CH$_7$, IAEA CH$_6$ and USGS 24 for C, IAEA N1, IAEA N2 and IAEA NO$_3$ for N and IAEA-S1, IAEA-S2 and IAEA-S3 for S) while Duke Environmental Stable Isotope Laboratory of Duke University (USA) uses internal keratin standards previously calibrated against NIST and IAEA reference materials (CFS, BWB and CHS for H and O; Wassenaar and Hobson 2003), all of them inserted every 12 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of ±0.1, ±0.2, ±0.3, ±1.5 and ±0.1‰ for carbon, nitrogen, sulphur, hydrogen and oxygen respectively but these are likely underestimates of true measurement error for complex organics like feathers.

To determine trace element concentrations, 50 mg of feather powder was digested in 1 ml of nitric acid (69–70%) and 0.5 ml of hydrogen peroxide (30%) using Teflon® bombs during 12 hours at $60°C$. The result of the digestion was diluted into 7 ml of distilled water. Quantitative analysis was performed using the ICP-AES technique (atomic emission spectrometer, Perkin Elmer Optima 3200 RL, Connecticut, USA) at Serveis Científico-Tècnics of Universitat de Barcelona (Spain). Accuracy of analysis was checked by measuring certified reference material (Human Hair CRM 397, Community Bureau of Reference, Commission of the European Community).

Statistical analyses

Element concentrations were log-transformed to achieve normality. Differences among breeding and among wintering populations in stable isotope and element values were tested with one-way ANOVA. Tests among wintering populations do not include the Canary Current, South Central Atlantic and Gulf of Guinea because fewer than four birds were found in each area. To assess whether feather composition could be linked to specific oceanic areas, we used classificatory discriminant analyses (SPSS 2003) on the composition of both type of feathers in relation the breeding and wintering areas. Discriminant analyses were carried out separately for stable isotope signatures of C, N, S, H and O and for combined element concentrations of Se, Pb and Hg. We tested models by jackknife cross-validation. Models were built step by step including independent variables according to the Wilks' Lambda criterion, and breeding and wintering areas were weighted according to the sample size.

Supporting Information

Table S1 Biogeochemical composition of summer and winter feathers. Stable isotope signatures (‰) and log-transformed element concentrations (ng g-1) for primary feathers (P1) according to the breeding areas and for secondary feathers (S8) according to the wintering areas. Values are means±standard deviation and sample size is shown in brackets. Significant differences among breeding and among wintering populations are indicated by *** P<0.0001, ** P<0.05 and * P<0.1. ANOVA-test among wintering populations do not include Canary Current, South Central Atlantic and Gulf of Guinea. Standard coefficients from discriminant functions on original data (explained variance in brackets) are conducted separately for stable isotopes and element concentrations.

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Author Contributions

Conceived and designed the experiments: JGS XR. Performed the experiments: RR JGS DO. Analyzed the data: RR JGS. Contributed reagents/materials/analysis tools: JGS JPC DO XR. Wrote the paper: RR JGS JPC DO.

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Capítol 6:

Avaluant els nivells de contaminants en els ambients marins a través de migrants transoceànics

Presentem un estudi exhaustiu dels patrons espaciotemporals dels contaminants en els ambients marins, així com la influència de l'ecologia tròfica en els nivells de contaminants de les espècies d'aus marines migratòries. Presentem les concentracions de mercuri, seleni i plom, així com els valors d'isòtops estables de carboni i nitrogen en plomes mudades durant els períodes de cria i d'hivernada de dues espècies de baldrigues. Els resultats indicaren que mentre els isòtops estables es dipositen directament des de la dieta a les plomes, els metalls ho fan de manera gradual a partir de les reserves corporals. Per tant, aquest estudi proporciona la primera clara evidència que els nivells de contaminants acumulats durant un període poden ser transferits a plomes mudades en un altre període, destacant doncs, la necessitat de considerar el diferent marc temporal de les signatures isotòpiques i els nivells de contaminants.

ECOSYSTEM ECOLOGY - ORIGINAL PAPER

The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters

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Abstract Contamination in marine foodwebs is nowadays of great environmental concern owing to the increasing levels of pollution in marine ecosystems from different anthropogenic sources. Seabirds can be used as indicators of regional contaminant patterns across large temporal and spatial scales. We analysed Hg, Se and Pb levels as well as stable isotope ratios of C ($^{13}C/^{12}C$, $\delta^{13}C$) and N ($^{15}N/^{14}N$, $\delta^{15}N$) in breeding- and winter-season feathers on males and females of two related shearwater species, providing information on spatiotemporal patterns of contaminants as well as the influence of the trophic ecology of these seabirds on contaminant levels. During the breeding season, Se and Pb concentrations were highest at the Cape Verde archipelago, showing no differences among the other colonies or between the sexes. However, Hg levels varied among colonies, being highest in the Mediterranean, probably resulting from the larger emissions and fallout of this pollutant in Europe. Feathers grown during breeding also showed sexual differences in Hg concentrations and $\delta^{13}C$. Differences in Hg concentration between sexes are mainly due to egg-laying decontamination in females. In contrast, differences in Hg among colonies are probably related to differences in trophic ecology, as indicated by $\delta^{13}C$ and $\delta^{15}N$ measurements. Contaminant concentrations in winter-grown feathers did not show any relationship with stable isotope values but were affected by contaminant loads associated with the breeding season. These findings suggest that the interpretation of contaminant levels of migratory species from feathers moulted out of the breeding season should be made with caution because those values could reflect exposures to contaminants acquired during the breeding season. We conclude that factors other than feeding ecology may play an important role in the interpretation of contaminant levels and their annual dynamics at several spatial scales. Consideration of the relevant temporal context provided by isotopic signatures and contaminant concentrations is important in deciphering contaminant information based on various tissues.

Keywords Carbon-13 · Sealife contamination · Migratory connectivity · Nitrogen-15 · Marine foodweb pollutants

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Introduction

Oceans are increasingly becoming a repository for anthropogenic pollutants from aerial and aquatic sources and these are ultimately incorporated into the tissues of marine biota. Contamination discharges, however, are not spatially uniform and spatial differences in contaminant levels of marine organisms have been difficult to study

because species composition also changes across ocean regions (e.g. Cherel and Hobson 2007). Pelagic seabirds can help us to understand spatiotemporal dynamics of pollutants because many species have vast breeding distributions and undergo long-distance migrations. Thus, these traits provide opportunities to compare pollutant levels among remote populations as well as between breeding and wintering areas. In addition, since pelagic seabirds cover huge areas while foraging, they are relatively insensitive to local sources of pollutants and thus become excellent bioaccumulative integrators of baseline levels (Walsh 1990; González-Solís et al. 2002).

In seabirds, several factors can contribute to body burdens of heavy metals, such as foraging area, dietary preferences, breeding and moult schedules, migratory habits, body size, and taxonomic influences on metabolism (Walsh 1990; Monteiro and Furness 1995). Among them, differences in feeding ecology have been reported as some of the most important factors explaining differences in contaminant levels among individuals of the same species (González-Solís et al. 2002), among localities (Sanpera et al. 2000), and also among species (Monteiro et al. 1999; González-Solís et al. 2002). Indeed, many seabird species occur at high trophic levels in marine foodwebs, which make seabirds useful indicators of biomagnification processes of some pollutants, such as Hg (e.g. Honda et al. 1987). However, relationships among pollutants and feeding ecology are difficult to establish because conventional dietary studies suffer from several drawbacks including analytical biases and difficulty of access to birds during winter (González-Solís et al. 1997). In this respect, stable isotope ratios of N (^{15}N/^{14}N, δ^{15}N) and C (^{13}C/^{12}C, δ^{13}C) open new opportunities to explore relationships between feeding ecology and heavy metal burdens (Forero and Hobson 2003; Sanpera et al. 2007). Consumers are typically enriched in ^{15}N relative to their food and consequently δ^{15}N measurements are indicators of their diet and trophic position (e.g. Forero et al. 2004). By contrast, δ^{13}C values are used primarily to determine sources of primary production supporting foodweb components (Kelly 2000); indicating in the marine environment, inshore versus offshore, or pelagic versus benthic contribution to food intake (Hobson et al. 1994).

However, despite the growing number of studies describing heavy metal levels and stable isotope abundance in seabirds (Atwell et al. 1998; Bearhop et al. 2000), few papers combine analyses of both to tackle spatial and seasonal variation in metal burdens and its relationship with variability in feeding ecology (but see Nisbet et al. 2002; Sanpera et al. 2007). Analyses of both stable isotopes and contaminant levels in feathers are particularly appropriate for this objective in those species for which main moult pattern and time of feather formation are known.

Once formed, feathers become chemically inert, and thus their biogeochemical composition reflects metals and isotopes incorporated during growth. If moulting patterns are known, feathers can be sampled at any time of the year to examine feeding habits and heavy metal intake in specific time periods and colonies (Hobson 1999). At least for Hg, plumage has greater levels than other tissues (Thompson et al. 1990), feather growth being the major eliminatory pathway of that heavy metal in birds (Monteiro and Furness 1995). However, while dietary elements, and so their stable isotope signatures, are thought to be promptly routed to growing feathers, heavy metal dynamics seem to be more complex and feathers may not accurately reflect contaminant loads during the time of growth (Thompson et al. 1998a).

In this study we analysed Hg, Se and Pb concentrations and δ^{13}C and δ^{15}N values in feathers from three related taxa of shearwater, the Mediterranean Cory's shearwater *Calonectris diomedea diomedea*, the Atlantic Cory's shearwater *Calonectris diomedea borealis*, and Cape Verde shearwater *Calonectris edwardsii*, breeding in the Mediterranean, the northeast Atlantic and the Cape Verde archipelago, respectively. We sampled birds from the Chafarinas, Azores, Canary and Cape Verde archipelagos and analysed contaminants and stable isotopes in the first primary feathers (P1) and the eighth secondary feathers (S8), which are thought to be grown at the breeding and wintering grounds, respectively (Ramos et al. 2008). With this sampling strategy we aimed to: (1) explore the geographic variability in heavy metals of shearwater feathers from four remote archipelagos and relate them to the geographic differences in emissions and discharges of these elements; (2) relate interspecific, sexual and individual differences in heavy metal levels to the trophic ecology of the *Calonectris* shearwaters, as shown by δ^{15}N values; and (3) to study the dynamics of stable isotopes and heavy metals deposited in feathers by comparing feathers grown in breeding and wintering areas.

Materials and methods

Study species, study area and sampling strategy

Cory's shearwater *C. diomedea* is formed by two subspecies, *C. d. diomedea* breeding on islands in the Mediterranean, and *C. d. borealis* which breeds in the northeast Atlantic, from the Azores to the Canary archipelagos. The Cape Verde shearwater *C. edwardsii*, once considered a subspecies of Cory's shearwater, has recently been split off and it is currently considered as an endemic species of the Cape Verde Archipelago (Hazevoet 1995; Gómez-Díaz et al. 2006). This study included four different

archipelagos: Chafarinas, Azores, Canary and Cape Verde Islands (Is.) (Fig. 1). The Chafarinas Is. are located at 4.5 km off the Moroccan Mediterranean coast (35°11′N, 3°46′E); Azores Is. at the North-Mid Atlantic Ocean (36–39°N, 25–31°W), about 1,500 km west from the coast of Portugal; the Canary Is.. are about 120 km from the northwest African coast (27–29°N, 13–18°W); and the Cape Verde Is. are located 500 km off the western coast of Senegal (15–17°N, 23–25°W).

During the early breeding season of 2001, when adults were incubating eggs, we collected the P1 and S8 from 22 to 35 adult shearwaters at each locality (total $n = 118$). Cory's and Cape Verde shearwater moult P1 at the end of chick-rearing period, before departing from the breeding grounds to the wintering areas (personal observation; Monteiro and Furness 1996). Thus, since feathers were collected during the breeding period of 2001, the P1 was assumed to reflect dietary intake at the end of the 2000 breeding period. A recent study on moulting patterns of secondary feathers in Cory's shearwaters showed some birds may start moulting S8 around the breeding colony just before migration (Ramos et al. 2008). However, that study was based on specimens accidentally caught in longliners and probably include non-breeders, which are known to moult earlier than breeders (Edwards 2008). The present study only includes breeding birds, and therefore S8 is expected to be moulted on the wintering grounds. In addition to feathers, 0.5 ml blood was taken from the foot vein for further molecular sexing of individuals as described by Ellegren et al. (1996) (primers: 2550F and 2718R).

Sample preparation and laboratory analyses

At the laboratory, feathers were washed in a 0.25 M NaOH solution, rinsed thoroughly in distilled water to remove any
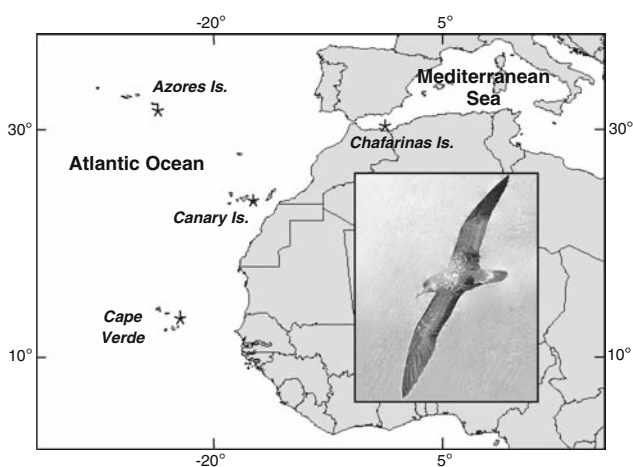

Fig. 1 Location of the studied area. *Asterisks* indicate archipelagos where samples were taken (original illustration from Ole Krogh)

surface contamination, dried in an oven at 60°C to constant mass, and ground to a fine powder in a freezer mill (Spex Certiprep 6750; Spex, Metuchen, N.J.) operating at liquid N temperature. For stable isotope analyses, a subsample of 0.4 mg feather powder was weighed to the nearest microgram, placed into tin capsules and crimped for combustion. Samples were oxidized in a Flash EA1112 coupled to a stable isotope mass spectrometer (Delta C) through a Conflo III interface (ThermoFinnigan, Bremen, Germany), where the $\delta^{15}N$ and $\delta^{13}C$ values were determined. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] \times 1000$$

where X (‰) is ^{13}C or ^{15}N and R is the corresponding ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), related to the standard values. $R_{standard}$ for ^{13}C is Pee Dee belemnite and for ^{15}N is atmospheric N (AIR). Isotopic ratio mass spectrometry facility at the Serveis Científico-Tècnics of University of Barcelona applies international standards (IAEA CH_7, IAEA CH_6 and USGS 24 for C and IAEA N1, IAEA N2 and IAEA NO_3 for N) inserted every 12 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of ±0.1 and ±0.2‰ for C and N, respectively, but these are likely underestimates of true measurement error for complex organics like feathers.

To determine concentrations of Pb, Hg and Se, 50 mg feather powder was digested in 1 ml HNO_3 (69–70%) and 0.5 ml H_2O_2 (30%) using Teflon bombs for 12 h at 60°C. The result of the digestion was diluted into 7 ml distilled water. Analyses were performed using an ICP-OES (Optima 3200 RL; Perkin Elmer, Norwalk, Conn.). Accuracy of analysis was checked by measuring certified reference material (human hair CRM 397; Community Bureau of Reference, Commission of the European Community). To check the reproducibility of the procedure, we included sample replicates as well as negative controls in each set of samples analysed.

Statistical analyses

Distributions of $\delta^{13}C$, $\delta^{15}N$, Se, Pb and Hg values partitioned by colony were inspected with a Q–Q plot analysis and tested for normality. Then, Se, Pb and Hg concentration values were log transformed to reach normality. Analyses of variability of contaminants and stable isotopes at breeding and wintering grounds were performed by applying separated generalized linear mixed models (GLMM; Littell et al. 1996). Species identity was treated as a random term in the GLMMs using SAS Macro program GLIMMIX (Littell et al. 1996). When each contaminant level during breeding (measured in P1) was the response

variable we tested the main effects and interactions of breeding colony, sex and stable isotopes (as an estimation of feeding ecology during breeding). When response variables were levels of contaminants during winter (measured in S8), we also considered the potential effect of breeding colony, sex, stable isotope signatures at wintering and levels of stable isotopes and contaminants at breeding grounds (measured in P1). Contaminant levels in P1 were fitted to control for the potential effects of the accumulation of heavy elements during the breeding season but being excreted at a later stage (i.e. throughout the winter moult period). All main effects of the explanatory variables and their interactions were also fitted to the observed data.

For a better understanding of the influence of feeding ecology on contaminant levels in our study species, the same statistical procedure was used to analyse variability in stable isotope values at breeding and wintering grounds. In addition to the random effect of species, breeding colony, sex and their interaction were fitted to the observed stable isotope values. Stable isotope values of P1 were also fitted in the models for $\delta^{15}N$ and $\delta^{13}C$ values in S8. In all cases, the final selected model was built following a forward stepwise procedure which includes only the significant effects retained.

Results

Breeding season

Considering levels of Hg in P1, and after controlling for species, the GLMM explained up to 64.2% of the initial variance and included three main explanatory variables: $\delta^{15}N$ in P1 ($F_{1,91} = 18.6$, $P < 0.0001$), sex ($F_{1,91} = 12.7$, $P = 0.0006$), and breeding colony ($F_{3,91} = 46.83$, $P < 0.0001$). Males exhibited higher levels of Hg than females (Fig. 2). Differences among colonies were mainly caused by the highest and lowest values of Hg at the Chafarinas and Cape Verde Is., respectively (Fig. 2a). Levels of Hg were positively related to $\delta^{15}N$ value (Fig. 3). The best-fit models for Se and Pb burdens during breeding explained 48.1 and 35.9% of the initial variation, respectively, and only retained the significant effect of breeding colony (Se, $F_{3,112} = 24.59$, $P < 0.0001$; Pb, $F_{3,112} = 20.92$,

Fig. 2 Mean and 95% intervals of confidence (*IC*) of pollutant concentrations (**a** Hg, **b** Se, **c** Pb) and stable C (**d**) and N isotope (**e**) in first primary (P1; *filled symbols*) and eighth secondary feathers (S8; *empty symbols*) of Cape Verde and Cory's shearwaters [Cape Verde (*CV*; *triangles*), Azores Is. (*A*; *diamonds*), Canary Is. (*C*; *squares*) and Chafarinas Is. (*Ch*; *circles*)]. Mean values of males (*asterisks*) and females (*dots*) are also shown for every colony when sexual differences were significant or marginally non-significant. Sample sizes are shown *in parentheses* (*n*)
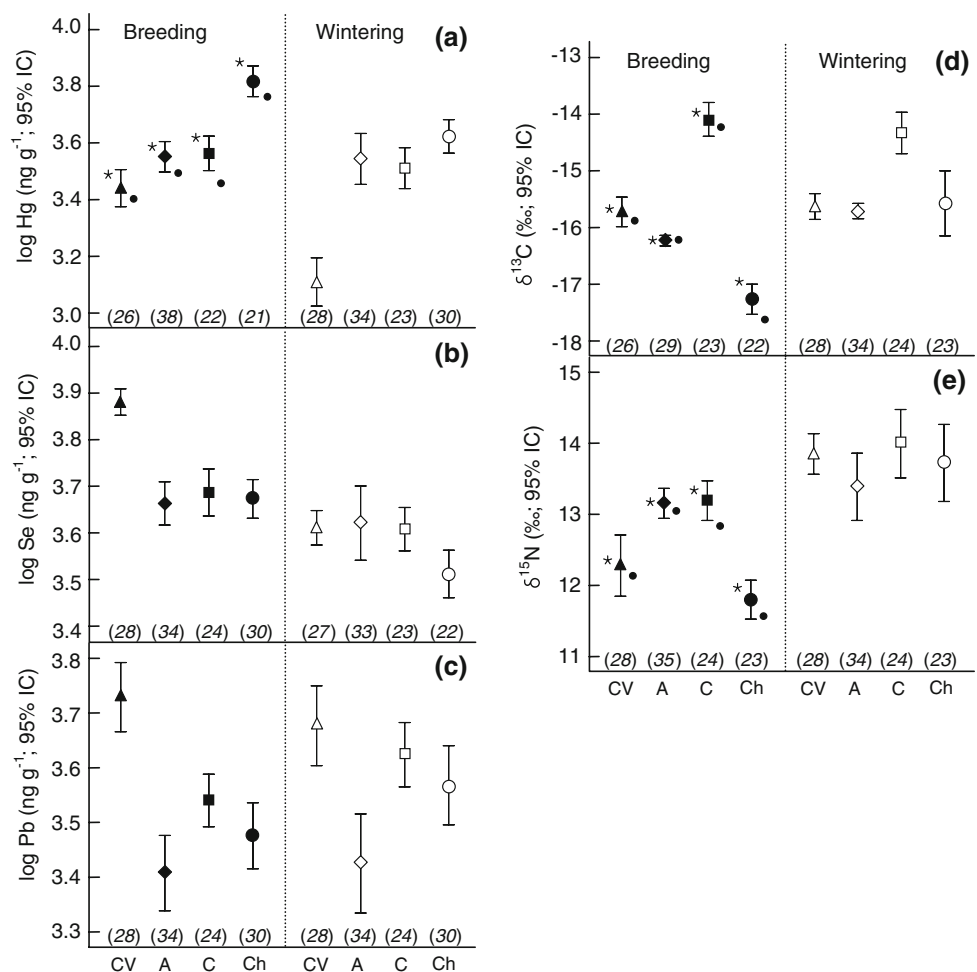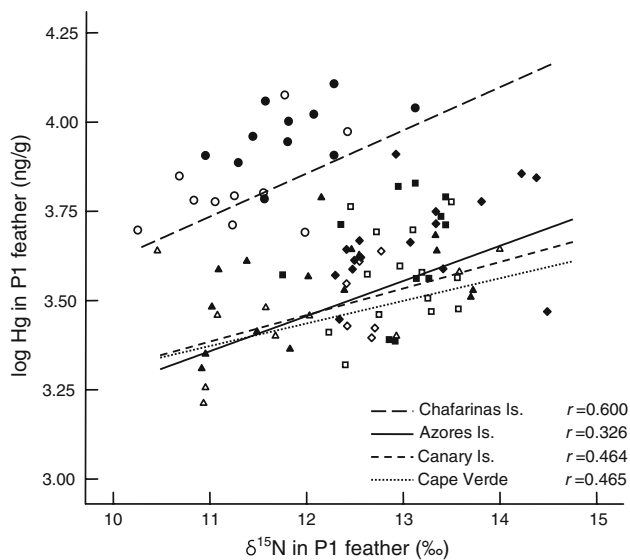
Fig. 3 Relationship between stable N isotope and Hg concentration in P1. Linear regressions are shown for each breeding locality separately: Cape Verde (*triangles*), Azores Is. (*diamonds*), Canary Is. (*squares*) and Chafarinas Is. (*circles*). Males represented by *filled symbols* and females by *empty symbols*

$P < 0.0001$). Effect of breeding colony was explained by the elevated values of Se and Pb in individuals from the Cape Verde archipelago, whereas values for the rest of the localities were similar (Fig. 2b, c).

Regarding stable isotopes during breeding, variability in $\delta^{13}C$ values (range: -18.5 to -12.4 ‰) was larger than in $\delta^{15}N$ values (range: 10.5–14.7 ‰). After controlling for species, models explained 80.1 and 45.0% of the original deviance in $\delta^{13}C$ and $\delta^{15}N$, respectively. Both models included the significant effect of breeding colony ($\delta^{13}C$, $F_{3,95} = 119.86$, $P < 0.0001$; $\delta^{15}N$, $F_{3,106} = 19.85$, $P < 0.0001$). All colonies differed in their $\delta^{13}C$ values: individuals from the Canary Is. showed the highest $\delta^{13}C$ values, whereas those from the Chafarinas showed the lowest (Fig. 2d). Birds from the Azores and Canary Is. presented higher $\delta^{15}N$ values than individuals from the Chafarinas and Cape Verde Is. (Fig. 2e). During breeding, males showed consistently higher values of stable $\delta^{15}N$ and $\delta^{13}C$ values than females. However, sex was only significantly retained in the model of $\delta^{13}C$ values ($F_{1,95} = 4.55$, $P = 0.03$; Fig. 2d), being marginally non-significant for $\delta^{15}N$ values ($F_{1,95} = 3.00$, $P = 0.09$).

Wintering season

Results of the GLMM showed different effects of the explanatory variables on levels of contaminants during winter (measured in S8), explaining 52.8, 21.9 and 37.2%, respectively, for Hg, Se and Pb. In the three models, and after controlling for species, levels of contaminants in S8

were significantly and positively affected by their respective values in P1 during breeding (Hg, $F_{1,110} = 12.92$, $P = 0.0005$; Se, $F_{1,99} = 8.19$, $P = 0.005$; Pb, $F_{1,111} = 42.85$, $P < 0.0001$). In addition, colony also influenced levels of Hg ($F_{3,110} = 19.12$, $P < 0.0001$) and Se ($F_{3,99} = 2.90$, $P = 0.04$) during winter: individuals that bred at Cape Verde and the Chafarinas showed the lowest levels of Hg and Se during winter, respectively (Fig. 2a, b). Finally, levels of Se were negatively affected by the $\delta^{15}N$ value in S8 ($F_{1,99} = 10.85$, $P = 0.00014$).

When analysing variability in feeding ecology during winter, we did not find any significant effect of the explanatory variables on feather $\delta^{15}N$ values, after controlling for species. The GLMM for feather $\delta^{13}C$ explained 36.4% of the original deviance and showed that its variability during winter was explained by $\delta^{13}C$ values of P1 ($F_{1,104} = 7.73$, $P = 0.0064$) and colony ($F_{3,104} = 5.09$, $P = 0.0025$). Effect of $\delta^{13}C$ values of P1 was only due to the positive correlation between $\delta^{13}C$ values of P1 and S8 at the Canary archipelago (Fig. 4). In addition, high $\delta^{13}C$ values of individuals that bred at this archipelago (Fig. 2d) explained the significance of breeding colony.

Discussion

Differences in Hg, Se and Pb among colonies and sexes

Although contaminant levels of the sampled colonies differed, they were generally similar to those previously reported for Cory's shearwater throughout the Mediterranean and Mid Atlantic Ocean (Renzoni et al. 1986; Monteiro et al. 1999). In particular, Monteiro et al. (1999) reported body feather Hg concentrations of Cory's shearwaters from several Mid-Atlantic colonies sampled in 1993–1995 ranging between 3.54 and 3.85 ng g^{-1}, which are close to the results we found for this area. Thus, in spite of the current environmental concern about the increasing oceanic pollution from anthropogenic sources, we found heavy metal and Se levels in seabird feathers not greater than those previously reported a decade ago (Thompson et al. 1992; Elliott et al. 1992; Sanpera et al. 2000; Arcos et al. 2002).

Our results corroborate the importance of understanding excretion routes to evaluate contaminant concentrations in marine organisms. Hg presented a more complex dynamic than Se and Pb, as shown by its additional association with stable isotope signatures and by the differences in levels between sexes. Dissociation between stable isotope signatures and Se or Pb levels could result from stable isotopes being deposited through dietary intake, whereas Se and Pb could have been deposited directly from the atmosphere onto the bird plumage (Rose and Parker 1982; Furness 1993). A number of studies with terrestrial birds reported

that Pb levels increase as feathers age or are more exposed than those lying under other plumage (e.g. Hahn 1991). Although Se may also deposit onto feather surfaces, it may originate from preen oils as well (Goede 1991). In contrast, Hg in feathers comes from diet because it occurs in the methyl-Hg form, whereas elemental and inorganic Hg are highly volatile and do not deposit onto feather surfaces (Thompson and Furness 1989). Alternatively, the lack of association of Se and Pb levels with stable isotopes could simply be due to different Se and Pb baseline levels among local foodwebs. A significant effect of breeding colony on Se and Pb concentrations in P1 was due to the high values of these elements in shearwaters from Cape Verde, which probably resulted from greater baseline levels of these two elements in this area. In fact, a much greater particulate Pb concentration in surface seawater around Cape Verde than around the Canary or Azores archipelagos was reported from a cruise in the Atlantic Ocean (Helmers 1996). Likewise, in a cruise transect between the Azores and Cape Verde, the total dissolved Se only varied slightly in surface waters but was significantly greater in deep waters around the Cape Verde archipelago (Cutter and Cutter 1995). Both studies concluded that concentrations of Pb and Se were mainly affected by local inputs from upwelling and atmospheric deposition, supporting the importance of baseline levels as a major factor influencing the dynamics of these two elements in local foodwebs. In the case of Hg, the highest levels were found in individuals from the Mediterranean colony (Chafarinas Is.; Fig. 2a). This result is probably related to the emissions and discharges of this pollutant in Europe, generating a relatively high Hg levels in the Mediterranean compared to the Atlantic, as previously reported in a number of studies on several top predators species (Renzoni et al. 1986; Andre et al. 1991; Lahaye et al. 2006). On the other hand, the greater levels of Hg and δ^{15}N values found in the Azores and Canary shearwaters compared to Cape Verde shearwaters suggest a geographic variation in baseline isotopic and contaminant levels. A differential use of fishery discards may also explain some differences, since discarded mesopelagic fish show greater Hg burden than the readily accessible epipelagic fish (Thompson et al. 1998b). However, in this case shearwaters from the Canary Is. should show greater Hg levels than those from the Azores due to their proximity to the trawler fleet operating on the western Africa continental shelf, but this was not the case.

In addition to the Hg variability associated with differences in δ^{15}N values among colonies, levels of Hg during breeding were also associated with individual δ^{15}N values within each locality (Fig. 3). This result indicated that biomagnification processes not only occur across species through the foodweb (e.g. Honda et al. 1987), but also among individuals at the intraspecific level.

The importance of excretion routes for the Hg concentration was further corroborated by sexual differences in isotopic signatures and Hg concentration. Male shearwaters showed slightly but significantly higher levels of Hg than females, consistent throughout the four studied colonies (Fig. 2a), even when accounting for potential differences in the trophic levels of the prey consumed by males and females, as indicated by the δ^{15}N values. This result agrees with some seabird studies, which also reported sexual differences in Hg levels in wing feathers (Braune and Gaskin 1987; Lewis et al. 1993), and probably reflects the different excretion opportunities of males and females. The main excretion route for both sexes is the deposition of Hg into feathers during moulting periods. Nevertheless, females have an additional route due to the potential to excrete Hg into the eggs between moulting periods (Becker 1992; Lewis et al. 1993; Monteiro and Furness 1995), which could further deplete their Hg levels relative to males (see Lewis and Furness 1993). Sexual differences in Hg levels could also be partly amplified by the slightly greater trophic level of males, as indicated by their greater δ^{15}N values, although differences in δ^{15}N values between sexes were not significant ($P = 0.09$, Fig. 2e). Sexual size dimorphism in these species is relatively small, with males being only 5–9% greater in bill and 9–10% greater in body mass than females (Thibault et al. 1997; Gómez-Díaz and González-Solís 2007; Navarro et al. 2008). Nevertheless, the slightly larger size of males may also confer access to slightly larger prey (see Bearhop et al. 2006) with both greater δ^{15}N values and greater Hg content (Braune 1987; Monteiro et al. 1992; Badalamenti et al. 2002; Cherel and Hobson 2005).

Feathers moulted in winter showed a general decrease in Hg and Se concentrations and similar levels of Pb coupled with a carry-over effect from concentrations accumulated during the breeding period (Fig. 2a–c). On one hand, the decrease in Hg and Se concentrations can be explained by the moult of flight feathers which is a continuous and steady process from the first moulted feather (i.e. P1) until all flight feathers are replaced. Consequently, birds excrete more metals in the first moulted feather compared with subsequent feathers (i.e. eighth secondary) (Braune and Gaskin 1987; Walsh 1990). On the other hand, the carry-over effect is interesting because it shows the complex dynamics of Hg, Se and Pb. That is, their concentrations in feathers grown at their winter quarters (eighth secondary) were partly explained by concentrations of feathers grown at the breeding grounds (P1). In consequence, contaminant levels of migratory species from feathers moulted out of the breeding season should be interpreted with caution because these values could reflect exposure to contaminants during the breeding season, and vice versa (see Thompson et al. 1992). Therefore, a fraction of contaminant burdens in

feathers has an endogenous origin, i.e. it is partially mobilized from various organs in which metals are stored (Goede 1991; Furness 1993). The deposition of contaminants acquired at breeding grounds on feathers grown out of the breeding period may be particularly important in procellariiform species, because they show long breeding seasons and relatively short wintering periods (Thompson et al. 1998a; Monteiro et al. 1999). For example, on average, Cory's shearwater spends 243 days at the breeding grounds, 80 days on the wintering grounds and 42 days travelling between the two areas (González-Solís et al. 2007). Alternatively to the carry-over effect, it is also possible that slight individual differences in physiology affect equally the efficiency with which birds excrete pollutants into the feathers throughout the moult sequence (Bearhop et al. 2000). However, such differences in individual physiology are generally considered irrelevant in influencing intraspecific variability in tissue pollutant concentrations (Becker et al. 2002; Nisbet et al. 2002). In addition, individual dietary specialisation could also explain an effect of contaminant levels during breeding on winter feathers. However, in that case it should be also observed in stable isotope levels, but in the present study $\delta^{15}N$ signatures did not show any correlation between P1 and S8. Therefore, it seems reasonable to rule out any possible trophic reason for this phenomenon. Another possible explanation was that concentrations of Hg, Se and Pb in the eighth secondary feather could also result from this feather being moulted at the breeding instead of the wintering grounds. It has been shown that some Cory's shearwaters moulting S8 before migration (Ramos et al. 2008). Although these birds are probably nonbreeders, this possibility cannot be completely discounted, but results from stable isotope analyses suggest otherwise. Whereas breeding colony was a significant factor explaining $\delta^{15}N$ values in P1, it was not significant for $\delta^{15}N$ in S8, suggesting that the signal of breeding colony vanishes because birds mix in several wintering areas where they grow the eighth secondary feathers. In the case of $\delta^{13}C$ values, breeding colony and P1 $\delta^{13}C$ values were significant factors for $\delta^{13}C$ in S8. However, this model explained much less variance (31.7%) than the model for $\delta^{13}C$ values of P1 (80.1%), suggesting rather weak effects of breeding colony on wintering $\delta^{13}C$ values. In fact, the influence of the breeding colony and the positive effect of $\delta^{13}C$ in P1 on $\delta^{13}C$ values of S8 mainly resulted from birds breeding at the Canary Is. (Fig. 4). This relationship could be explained because some birds from this breeding colony seem to winter on the Sahara shelf (González-Solís et al. 2007), the same area where they feed during the breeding season (see below). Thus, in contrast to the endogenous origin of Se, Pb and Hg burdens, stable isotope signatures of feathers reflect an exogenous origin, i.e. they are promptly routed to feathers when growing (Hobson 1999).
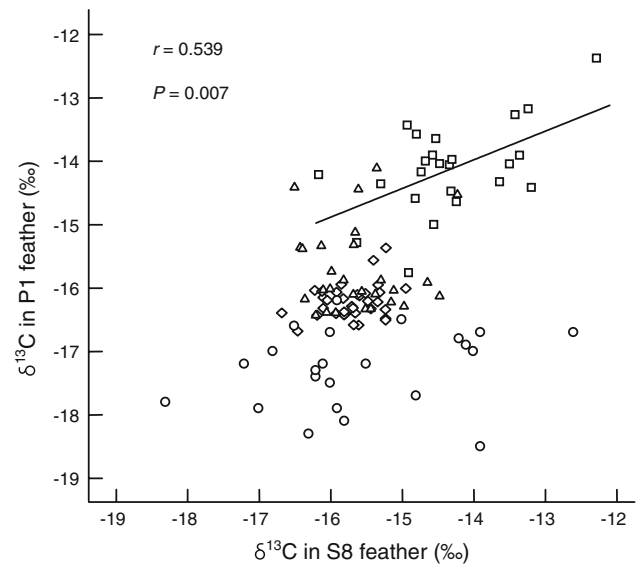

Fig. 4 Relationship between stable C isotopes for P1 and S8 are shown separately for each colony [Cape Verde (*triangles*), Azores Is. (*diamonds*), Canary Is. (*squares*) and Chafarinas Is. (*circles*)]. Only the regression line of the significant correlation for the colony on the Canary Is. is shown. For abbreviations, see Fig. 2

Differences in feeding ecology among colonies and sexes

Results from stable isotope analyses illustrated differences in the feeding ecology among different populations during breeding. Stable C isotope values of shearwaters breeding at the Canary Is. were higher than those of the other populations (Fig. 2d). Shearwaters breeding at the Canary Is. usually forage on the Sahara shelf at only 100–300 km from the islands (Navarro and González-Solís 2007), whereas those breeding at the Azores and Cape Verde Is. are expected to feed basically in offshore environments during the breeding season (Fig. 1; Magalhães et al. 2008). These results are therefore in accordance with most literature showing that offshore foodwebs have lower $\delta^{13}C$ values than those associated with inshore areas (France 1995; Hobson et al. 1995). Alternatively, such differences in $\delta^{13}C$ values could be due to a distinct use of fishery discards among colonies, since fisheries are mainly dominated by inshore or on-shelf species with greater $\delta^{13}C$ signatures. Differences in $\delta^{13}C$ values could also result from geographical variation in baseline values related to latitudinal gradients (Forero et al. 2005; Cherel and Hobson 2007). Indeed, analyses of feathers throughout most *Calonectris* populations in the Atlantic have shown some geographical isotope gradients in $\delta^{13}C$ values, with higher values in populations further south (Gómez-Díaz and González-Solís 2007). However, the Cape Verde archipelago is further south than the Canary Is. but showed more negative values, suggesting that the higher $\delta^{13}C$ values of

birds from the Canary Is. cannot be only explained by latitudinal gradients.

Small but consistent differences in δ^{13}C values between the sexes may result from a vertical or a horizontal gradient in foodweb δ^{13}C values, indicating respectively, a slightly different exploitation of resources along the water column or differences in the foraging areas used by males and females during the breeding season. Sexual differences in δ^{13}C values were consistent with both greater Hg concentrations and δ^{15}N values (although $P = 0.09$) in males during breeding, as previously found in some size-dimorphic Procellariiformes (González-Solís et al. 2000; Phillips et al. 2004; Forero et al. 2005; but see Navarro et al. 2008). In large- and medium-sized pelagic seabirds, larger body size has been related to diving longer and deeper (Watanuki and Burger 1999; Bearhop et al. 2006) as well as with greater wing-loading due to allometric relationships (Shaffer et al. 2003), which allows birds to cover longer distances in the presence of strong winds, probably leading to small differences in the areas exploited by males and females. Sexual differences found in both contaminant and isotopic levels could be imposed by differential reproductive tasks during breeding, and especially during the chick-rearing period (Granadeiro et al. 1998), when foraging areas and resources could be exploited differentially by males and females. In fact, a slightly larger use of fishery discards by males at this period could explain such higher values in both stable isotopes and contaminant concentrations (Hobson et al. 1994; Thompson et al. 1998b). The sex-specific reproductive task hypothesis is supported by the fact that sexual differences did not hold for feathers grown in winter as there are no reproductive constraints at that time. Moreover, the mixing of birds from distant breeding colonies with different contaminant burdens into different winter areas (González-Solís et al. 2007) with different baseline heavy metal loads could mask any potential sexual differences in feeding ecology occurring in winter.

This study emphasizes that combining contaminant with stable isotope analyses provides new insights into the dynamics of contaminants in relation to the feeding ecology of marine organisms. Whereas differences in background levels among localities and/or deposition directly from the atmosphere seem to mostly explain Pb and Se concentrations in feathers, the feeding ecology of shearwaters played a major role in explaining Hg concentrations. Furthermore, sexual segregation in feeding ecology and different reproductive constraints between males and females also seem to affect Hg concentrations. More studies exploring the relationships among stable isotope measurements and pollutants would help to elucidate the differential exposure of birds to pollutants in relation to their ecology. We also found Hg, Se and Pb

accumulated in one season could be transferred to feathers grown in another season. Future studies using feathers to assess the winter contaminant levels in birds with long breeding periods should consider the carry-over effects of contaminant loads that can occur between seasons. Thus, our results also highlight the need to consider the temporal context of isotopic signatures versus contaminant levels depending on the tissue chosen as well as their endogenous (bioaccumulated) or exogenous (dietary) origin. Nevertheless, for a better understanding of factors and processes explaining patterns of stable isotopes and contaminants in marine organisms, more detailed studies involving long-term monitoring of isotope ratios and contaminant loads or complementary approximations (i.e. tracked animals with some remote-sensing system) are needed.

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L'estudi de l'ecologia de les aus a través de les seves plomes

Aplicacions ecològiques dels biomarcadors intrínsecs

L'ESTUDI DELS PATRONS D'ESPACIOTEMPORALS EN ECOLOGIA TRÒFICA: EL CAS D'UNA ESPÈCIE PROBLEMÀTICA, EL GAVIÀ DE POTES GROGUES *Larus michahellis*

Definint les preferències alimentàries d'una espècie superabundant durant el període reproductor
Comprenent el component espaciotemporal de l'ecologia tròfica d'espècies oportunistes
Avaluant el paper dels hàbits d'alimentació dels ocells en la salut ambiental

L'ESTUDI DE DIFERENTS TRETS MIGRATORIS AL LLARG DELS OCEANS: EL CAS D'UNA AU MARINA PELÀGICA, LA BALDRIGA CENDROSA *Calonectris diomedea*

Esbrinant els patrons migratoris i de muda d'espècies discretes
Entenent les migracions oceàniques a través dels marcadors biogeoquímics intrínsecs
Avaluant els nivells de contaminants en els ambients marins a través de migrants transoceànics