Viabilitat demogràfica i efecte dels canvis ambientals antropogènics en la distribució, dieta i condició física d'una població d'aufrany *Neophron percnopterus* en expansió

Demographic viability and effect of anthropogenic environmental changes in the distribution, diet and body condition of an expanding population of Egyptian Vulture *Neophron percnopterus*

Helena Tauler Ametller

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2018

Helena Tauler Ametller

Tesi Doctoral

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Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
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Memòria presentada per HELENA TAULER AMETLLER

per optar al grau de doctora per la Universitat de Barcelona

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Índex

Capítols

Abstract

The Egyptian Vulture (*Neophron percnopterus*) is an endangered scavenger species that, in recent years, has experienced a population growth and an expansion of its range in Catalonia (NE of Iberian Peninsula). In this thesis the demographic and environmental factors that could have caused this increase are studied. Population models showed that, to explain the observed population growth, it has been needed a high adult survival and the arrival of immigrants from other populations. Also, it was assessed the influence of predictable anthropogenic food subsides, specifically landfills, to the distribution, diet and body condition of individuals of our study population. First, it was observed that occupied territories were located closer landfills than expected by chance, so the location of these installations are determining the distribution of breeding pairs. After, it was assessed the contribution of food from landfills to Egyptian Vulture diet using both conventional analyses and stable isotope analysis. Results showed that that both methods provide similar contributions of food from landfills in this species' overall diet, and also a high proportion of food derived from landfills (nearly 50%) was detected in some breeding pairs. The next step was to determine the influence of diet from landfills to the body condition of nestlings, using morphometrical and physiological approaches. It was found that the contribution of landfills to the diet was the main factor that explained body condition of individuals, especially for its effect to the antioxidant metabolism. Also, nestlings that fed in landfills seem to be better fed. Finally, concentrations of different families of Persistent Organic Pollutants and metals were analysed in nestlings of our population and it was found that levels of pollutants were in general low, so they not appear as susceptible to cause detrimental effects to nestlings' physiology. Overall, this thesis contributes to the knowledge of conservation status of Egyptian Vulture in Catalonia and it can be useful to assess the influence of landfills to vulture species.

INTRODUCCIÓ GENERAL

Impacte de l'ésser humà en les poblacions d'animals

Les activitats humanes han modificat els ecosistemes del planeta des de l'inici de la seva existència (Vitousek et al., 1997; Chapin et al., 2000; Foley, 2005). Actualment, la població humana està creixent exponencialment i gairebé la meitat d'aquesta viu en zones urbanes. Conseqüentment, la urbanització és una de les principals formes d'alteració dels ecosistemes (McKinney, 2006; Shochat et al., 2010). Durant tot aquest procés d'humanització del planeta, són molts els canvis que s'han produït sobre l'ambient, fins al punt que actualment es considera que hem entrat a l'Antropocè: l'era geològica dominada per l'impacte dels humans sobre els cicles biogeoquímics i sobre la biodiversitat (Steffen et al., 2011). En aquest sentit, els humans no només hem modificat els cicles de nutrients, que són fonamentals per a la vida a la terra, sinó que també hem transformat els paisatges naturals i la distribució dels recursos i estem provocant l'anomenada "sisena extinció massiva d'espècies", un fenomen que mai abans havia estat causat per la humanitat (Chapin et al., 2000; Barnosky et al., 2011; Dirzo et al., 2014).

A causa del gran declivi de moltes espècies provocat per les activitats humanes, la influència d'aquestes sobre les poblacions d'animals ha estat objecte de nombrosos estudis en els últims anys (Butchart et al., 2010). Així, s'ha pogut determinar que a més dels factors intrínsecs (com la fertilitat o la longevitat) i extrínsecs (com la disponibilitat d'aliment o la climatologia) que poden regular les poblacions d'animals (Begon, Harper, & Townsend, 1999), l'ésser humà també pot alterar la dinàmica poblacional de les espècies. Ho pot fer actuant directament sobre la supervivència dels individus d'una determinada població o espècie (Real et al., 2001; Green et al., 2004; Anderson et al., 2011), o bé modificant l'ambient i provocant, indirectament, canvis en les taxes vitals i en la seva dinàmica poblacional (Robb et al., 2008; Ripple et al., 2014). Així, la transformació de l'ambient cap a un nou paisatge antropitzat ha provocat grans canvis en les dinàmiques poblacionals i en les distribucions de les espècies a nivell mundial. D'una banda, hi ha espècies que cada vegada tenen una distribució més reduïda i es troben en estats preocupants de conservació (IUCN Red List; Stuart et al., 2004), però d'altra banda hi ha un seguit d'espècies que s'estan beneficiant d'aquests canvis ambientals, com és el cas de les espècies invasores o considerades plaga (Vidal, Medail, & Tatoni, 1998; Mack et al., 2000; Sakai et al., 2009). Tot i així, aquest escenari és molt més complex que això, ja que si bé algunes espècies es poden veure perjudicades per certes activitats humanes i estan catalogades com a amenaçades, a la vegada es poden estar beneficiant d'impactes provocats pels humans (Lunn & Stirling, 1985; García, Viñuela, & Sunyer, 1998a; Navarro et al., 2009). La forma com respondran les diferents espècies a aquests nous escenaris ambientals és encara desconeguda.

Nous escenaris de recursos tròfics

Una de les conseqüències de la transformació del paisatge derivada de les activitats humanes és el canvi en la distribució i la disponibilitat de recursos tròfics per a les espècies (Foley 2005). Així, entre les modificacions més importants de la distribució dels recursos hi ha l'aportament de recursos alimentaris d'origen antròpic, definits amb el nom de *Predictable Anthropogenic Food Subisdes* (PAFS) (Oro et al., 2013). Els PAFS tenen la característica comuna que es distribueixen de forma predictible en l'espai i/o en el temps, però poden provenir de moltes fonts diferents. Principalment són residus derivats de les activitats humanes; des de descarts pesquers fins a residus d'origen urbà, agrícola, ramader o de caça, però també poden ser punts d'alimentació suplementària dirigits a espècies amenaçades o d'interès (Oro et al., 2013).

Els residus urbans representen una part molt important dels PAFS globals en termes de quantitat d'aliment disponible per a les espècies silvestres. Aquests productes de rebuig es solen acumular en abocadors, instal·lacions que han augmentat molt en els últims 20 anys en gran part del planeta degut a l'augment de la població humana i a la forma de vida urbana. En aquest sentit, la quantitat de residus que s'aboquen diàriament al planeta augmenta anualment fins al punt que es preveu que es dupliqui en els pròxims 20 anys, especialment en països amb economies emergents. D'aquesta manera, s'espera que durant el pròxim segle s'arribi al màxim pic de residus urbans mai generats (Hoornweg, Bhada-Tata, & Kennedy, 2013).

En aquest context de sobreproducció de residus, és lògic pensar que aquesta concentració d'aliment està produint grans impactes en les poblacions d'animals i és previsible que aquests continuïn durant els pròxims anys (Newsome & van Eeden, 2017; Plaza & Lambertucci, 2017). D'una banda aquestes acumulacions de residus estan distribuïdes per tot el món, són abundants, predictibles en l'espai i en el temps i diàriament renovades. Aquestes característiques fan que moltes espècies de diferents nivells de la xarxa tròfica les puguin explotar per tal de satisfer els seus requeriments calòrics (Oro et al., 2013; Plaza & Lambertucci, 2017). D'altra banda, a més d'aquests residus orgànics, en els abocadors també hi ha vidres, metalls, plàstics, pintures i diferents tòxics i patògens que poden afectar a la salut dels animals que els utilitzen i, en conseqüència, la seva abundància (Flores-Tena et al., 2007; Houston, Mee, & McGrady, 2007; Matejczyk et al., 2011). A causa dels potencials impactes esmentats sobre la biodiversitat, diversos estudis s'han centrat en descriure la influència dels abocadors sobre diferents espècies que en fan ús, tan a nivell individual com demogràfic (Bino et al., 2010; Steigerwald et al., 2015; Plaza & Lambertucci, 2017). Aquesta influència pot ser tan negativa com positiva i, en alguns casos, pot provocar efectes contraris en funció de l'espècie.

Pel que fa als impactes descrits com a positius dels abocadors, trobem que a nivell individual poden fer augmentar la condició física dels individus, entesa com la massa corporal en alguns casos corregida pel pes. En aquest sentit, s'ha observat que els individus de diverses espècies que s'alimenten en abocadors solen ser més grossos i més pesants que els individus que utilitzen altres fonts d'aliment (Otali & Gilchrist, 2004; Cahill et al., 2012; Gould & Andelt, 2013). A banda d'aquests impactes descrits a nivell individual, el fet d'alimentar-se en abocadors ha tingut també conseqüències positives a nivell demogràfic en algunes espècies. Així, la majoria dels estudis coincideixen en què explotar aquestes fonts d'aliment predictible produeix una millora dels paràmetres reproductors, ja sigui incrementant la mida de la posta o l'èxit reproductor en algunes espècies d'ocells (Tortosa, Caballero, & Reyes-López, 2002; Tortosa, Pérez, & Hillström, 2003; Djerdali et al., 2008; Steigerwald et al., 2015). A més, també s'ha observat que l'aliment predictible pot provocar un augment de la supervivència de les espècies que en fan ús, possiblement a causa d'una menor mortalitat per inanició, així com també per una disminució de les interaccions agressives entre individus (Marzluff & Neatherlin, 2006; Bino et al., 2010).

Tot i així, no tots els estudis arriben a les mateixes conclusions, i hi ha un nombre important de treballs que descriuen els riscos que té el fet d'alimentar-se en abocadors. A nivell individual, els animals que s'alimenten en aquests punts estan exposats a la ingestió de tòxics i al contacte amb patògens que poden afectar la salut dels individus i, en casos extrems, poden acabar provocant-los la mort. Els amfibis són un dels grups més sensibles a aquest tipus de contaminació, ja que els pot provocar malformacions o fins i tot la mort (Bruner et al., 1998; Gibble & Baer, 2011). També per a algunes espècies d'ocells, els abocadors són una font d'ingestió de metalls pesants, que són tòxics per a ells (Pb, Hg, Cd, As) (de la Casa-Resino et al., 2014). D'altra banda, la ingesta de residus pot anar associada a la ingesta de plàstics o altres elements no degradables que poden causar obstrucció intestinal o intoxicació dels individus (Sazima, 2007; Rideout et al., 2012; Torres-Mura, Lemus, & Hertel, 2015). A més dels tòxics, també és molt comuna la presència de patògens en abocadors, i pot ser un risc important per als individus que s'hi alimenten (La Sala et al. 2013). Finalment, també s'ha descrit que els abocadors poden tenir conseqüències negatives indirectes en la supervivència d'altres espècies que no s'alimenten en aquests punts, sigui perquè en augmentar la població de les espècies que en fan ús, pot augmentar la pressió per depredació sobre les seves espècies presa (Yorio et al., 1998; Williams & Ward, 2006), sigui per la potencial transmissió de patògens per part d'espècies infectades que actuarien com a vectors (Coulson, Butterfield, & Thomas, 1983) (Figura 1).

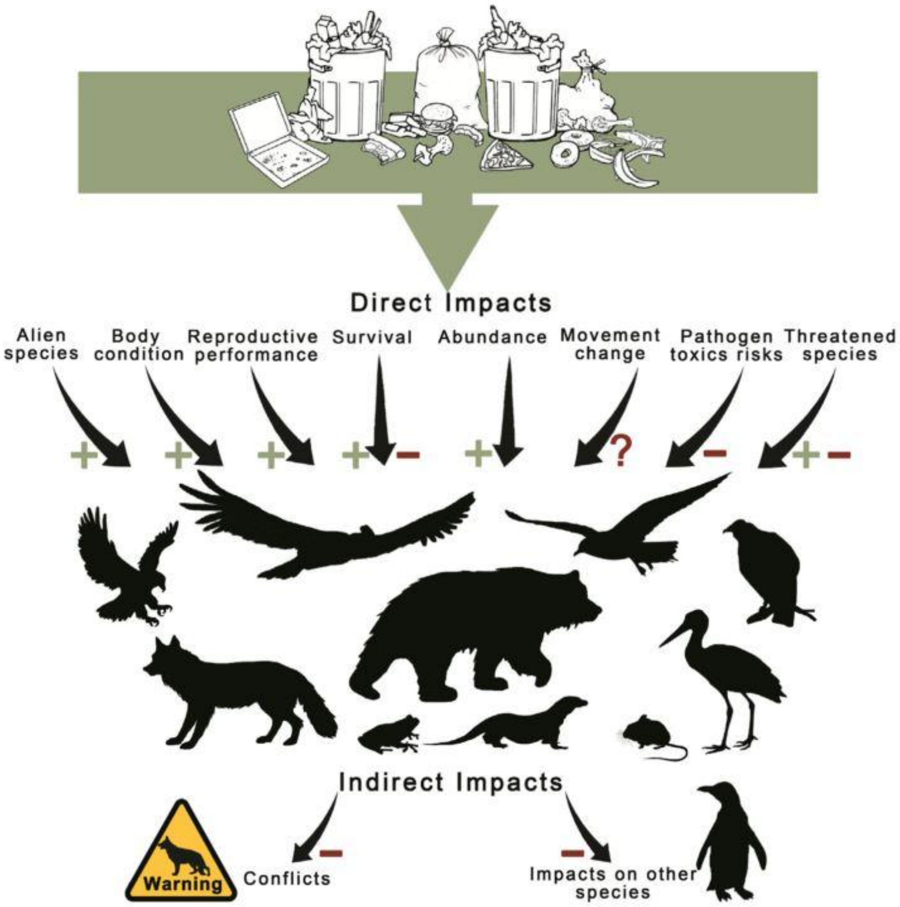

Figura 1. Impactes directes i indirectes dels abocadors en espècies de vertebrats. Font: Plaza and Lambertucci (2017).

Tot i la quantitat d'estudis dirigits a avaluar els efectes dels abocadors en les poblacions d'animals, encara queden molts buits de coneixement per a resoldre. Un dels més rellevants és saber exactament quines implicacions té el fet d'alimentar-se en abocadors sobre la condició física dels individus. Tal com s'ha comentat anteriorment, la majoria de treballs coneguts han estudiat la condició dels animals que s'alimenten en abocadors utilitzant índexs morfomètrics, és a dir, avaluant bàsicament la mida o el pes dels individus. Aquesta és un aproximació interessant però insuficient per explicar les conseqüències que té aquest tipus de dieta a nivell fisiològic per als individus. En aquest sentit, l'estudi dels paràmetres sanguinis és clau per a proporcionar informació sobre l'estat fisiològic dels animals (Stevenson & Woods, 2006; Norte et al., 2009; Resano-Mayor et al., 2016). D'una banda pot informar, per exemple, dels nivells de greixos (colesterol o triglicèrids) o del metabolisme proteic, paràmetres que estan relacionats amb l'estat nutritiu de l'individu (Sarasola, Negro, & Travaini, 2004; Amat et al., 2007; Rodríguez, Negro, & Figuerola, 2011). D'altra banda, l'anàlisi del metabolisme antioxidant ha rebut una atenció especial en els últims anys com a indicador de condició dels animals, ja que a més de tenir una relació estreta amb la dieta, pot tenir conseqüències en la reproducció i el sistema immunitari dels individus, entre d'altres (Monaghan, Metcalfe, & Torres, 2009; Herborn et al., 2016). Per tant, per a poder determinar com afecta el fet d'alimentar-se d'abocador en l'estat fisiològic dels individus és clau una aproximació que tingui en compte múltiples paràmetres indicadors, des de morfomètrics fins a fisiològics (Stevenson & Woods, 2006; Resano-Mayor et al., 2016).

Un altre buit de coneixement detectat és el fet que la major part dels estudis sobre els efectes dels abocadors en les poblacions d'animals s'han centrat principalment en descriure augments demogràfics d'espècies abundants o considerades plaga (Oro et al. 2013; Newsome et al. 2015), però hi ha poca informació sobre les possibles conseqüències dels abocadors en espècies amenaçades que també en fan ús (però veure García, Viñuela, & Sunyer, 1998; Gangoso et al., 2013; Katzenberger et al., 2017). Així, aquestes instal·lacions podrien tenir un paper clau per a la conservació d'aquestes darreres espècies, sigui perjudicant-les a causa, per exemple, de la ingestió de tòxics o de desajustos a nivell fisiològic, sigui afavorint-les proporcionant-los aliment predictible i en escreix i, per tant, millorant-ne els paràmetres demogràfics.

En aquest sentit, tot i que la tendència mundial és d'augment dels residus i, per tant, dels abocadors, a Europa s'estan aplicant normatives amb l'objectiu de reduir al màxim els residus exposats en abocadors a l'aire lliure. Les causes principals d'aquesta iniciativa són precisament l'augment d'espècies plaga i els impactes negatius sobre el medi que provoquen els abocadors (contaminació de sòls i aigües, entre d'altres), de manera que aquestes instal·lacions són vistes com a potencials problemes de salut pública. Per tal d'evitar les conseqüències negatives dels abocadors, la Comissió Europea va aprovar una Directiva l'any 2008 (Dir. 2008/98 del Parlament Europeu i del Consell, de 19 de novembre de 2008) que regula l'abocament de residus. Segons aquesta normativa, l'any 2020 s'han d'haver reduït els residus generats i els abocadors a l'aire lliure. Per tant, si bé d'una banda cal saber quines són les implicacions d'alimentar-se en aquestes instal·lacions, per tal d'avaluar fins a quin punt poden actuar com una amenaça per a les espècies que les utilitzin, sobretot i amb especial interès en les espècies amenaçades, d'altra banda també s'està preparant un nou escenari en què aquests residus es veuran reduïts en algunes parts del món com és el cas d'Europa. Aquest nou escenari és cert que podria ajudar a frenar l'augment d'espècies plaga, però també podria limitar l'accés als recursos a certes espècies amenaçades que utilitzen aquestes instal·lacions. Conèixer les implicacions dels abocadors sobre les espècies que en fan ús, tant a nivell individual com demogràfic, pot ser clau per a la conservació de les espècies amenaçades i la gestió de les espècies plaga en el futur.

Els voltors i la importància de l'aliment predictible

Els voltors representen una part important de les espècies que s'alimenten en els abocadors, instal·lacions on poden arribar a concentrar-s'hi grans quantitats d'individus (Donázar et al. 2010; Gangoso et al. 2013; Observació personal). A més, els voltors tenen un paper molt important en els ecosistemes, ja que són carronyaires i, per tant, s'encarreguen d'eliminar els cadàvers d'animals morts al camp, amb la qual cosa contribueixen en la reducció de potencials malalties i infeccions derivades de la putrefacció dels cadàvers. Per aquest motiu, es diu que ofereixen uns serveis ecosistèmics molt útils, tant per a la biodiversitat com per a l'espècie humana (Sekercioglu, 2006). Tot i així, estan exposats a una sèrie d'amenaces que han provocat grans declivis de les seves poblacions a nivell mundial (Ogada et al. 2012). Una de les amenaces més importants que afecta la majoria d'espècies de voltors del món és l'enverinament (Koenig, 2006). El fet d'alimentar-se a partir de restes d'animals morts provoca que els individus de les diferents espècies de voltors estiguin exposats a fàrmacs d'ús veterinari com el diclofenac, una substància molt tòxica que ha provocat un gran declivi de les poblacions de voltors a l'Àsia (Green et al., 2004; Oaks et al., 2004) i es tem que pugui també ocórrer a l'Àfrica i a Europa (Naidoo et al., 2009; Margalida et al., 2014). A banda, també estan exposats a altres substàncies com els pesticides, la ingestió dels quals també ha provocat disminucions dramàtiques de les poblacions de voltors al continent africà (Ogada et al., 2016). A nivell europeu, per això, la principal forma d'enverinament és deguda al consum de preses enverinades amb l'objectiu d'eliminar competència de depredadors per la caça (Hernández & Margalida, 2009; Demerdzhiev et al., 2014).

Una altra amenaça que afecta els voltors és la reducció de la disponibilitat d'aliment al medi que s'ha produït en les darreres dècades. En aquest sentit, a Europa l'abandonament de les pràctiques agrícoles i ramaderes tradicionals, lligades a la mecanització i a la intensificació ramadera, ha provocat una disminució de la disponibilitat de carronyes d'origen domèstic al camp. Sumat a aquest fet, l'any 2002 es va aprovar un Reglament Europeu (R (UE) 1774/2002 del Parlament Europeu i del Consell, de 3 d'octubre de 2002) que prohibia l'abandonament de cadàvers de ramaderia al camp, per tal d'evitar la propagació de la malaltia de l'encefalopatia espongiforme bovina. Des d'aleshores, els ramaders estan obligats a processar els cadàvers del seu bestiar i aquests deixen de quedar disponibles per a les espècies carronyaires (Donázar et al., 2009; Margalida et al., 2010).

Com que tant l'enverinament com la disminució dels recursos alimentaris han provocat davallades molt fortes de les poblacions de voltors arreu del món (Tella, 2001; Ogada, Keesing, & Virani, 2012), s'han aplicat mesures de conservació basades en l'alimentació suplementària amb la creació de canyets (o punts d'alimentació suplementària) en les àrees on les poblacions estan disminuint (Moreno-Opo et al., 2015; Cortés-Avizanda et al., 2016). Aquesta alimentació suplementària, sumada al nou recurs d'aliment disponible als abocadors, ha provocat que una part important de l'aliment que utilitzen els voltors es trobi actualment en punts d'alimentació predictible en l'espai i en el temps (Cortés-Avizanda et al., 2016). Aquest fet, ha modificat la forma d'alimentar-se dels voltors en moltes zones d'Europa, els quals han passat de dependre d'un aliment impredictible en l'espai i en el temps, la ramaderia extensiva, a un aliment proporcionat pels humans, concentrat en punts d'alimentació predictible: els canyets i els abocadors. A causa d’aquest canvi constant en la disponibilitat de recursos que utilitzen els voltors, el comportament d’aquestes espècies, així com les seves poblacions, també han patit modificacions importants. Conèixer com responen les poblacions d’aquestes espècies amenaçades als nous escenaris de disponibilitat d’aliment és un repte necessari per a la conservació dels voltors en el futur. Per aquest motiu, calen eines adequades per a conèixer els recursos que estan consumint els voltors i per a monitoritzar els possibles canvis en la seva dieta i els efectes que pot produir a nivell individual i poblacional.

Cas d’estudi: l’expansió de l’aufrany a Catalunya

L’aufrany *Neophron percnopterus* és una espècie de voltor de vida llarga, territorial, monògam i amb una maduresa sexual tardana. És carronyaire i generalista i s’alimenta bàsicament de preses de mida mitjana i petita, les quals pot obtenir de restes de ramaderia o també poden ser d’origen salvatge o d’abocadors (Donazar & Ceballos, 1988; Donazar et al., 1993; Hidalgo et al., 2005). Tot i tractar-se d’una espècie territorial, l’aufrany pot formar concentracions d’individus en punts d’aliment predictible com són els abocadors o els canyets, espais rellevants per la dinàmica social de l’espècie (Cortés-Avizanda et al., 2011). Les poblacions ibèriques d’aquesta espècie migren al Sahel de Mauritània i a zones limítrofes de Mail i Senegal al setembre i tornen a la Península Ibèrica a la primavera per criar (García-Ripollès, López-López, & Urios, 2010) (Figura 2).

L’aufrany està catalogat ‘en perill’ per la *International Union for Conservation of Nature* (IUCN), perquè les poblacions mundials d’aquesta espècie han patit un fort declivi en els últims anys, principalment a l’Índia, l’Àfrica i Europa (BirdLife International, 2016). La Península Ibèrica alberga la major part de la població del Paleàrtic Occidental d’aquesta espècie, que ha disminuït un 25% en els últims 20 anys (Donázar, 2004; Del Moral, 2009). Les causes principals d’aquesta tendència negativa són l’enverinament i la persecució il·legal, però també s’han vist afectats per l’abandonament de les pràctiques de l’agricultura i la ramaderia tradicionals (Liberatori & Penteriani, 2001; Donázar, 2004) així com per l’electrocució i la col·lisió amb línies elèctriques i parcs eòlics en algunes àrees de la Península (Carrete et al., 2007). Tot i que la tendència general a nivell mundial i a nivell de la Península Ibèrica és de declivi, hi ha algunes zones on les poblacions d’aufrany s’estan recuperant i estan augmentant en els últims anys gràcies a les mesures de conservació aplicades (Lieury et al., 2016) o per altres causes encara desconegudes (C. García-Ripollès & López-López, 2006; Del Moral, 2009).

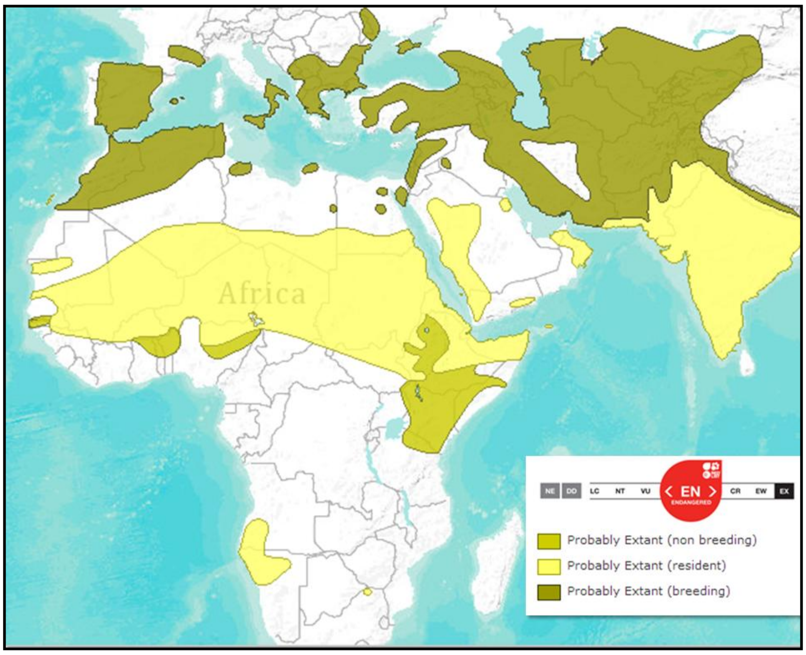

Figura 2. Distribució mundial de la població d'aufrany (*Neophron percnopterus*). S'indiquen les zones de reproducció (verd fosc), les zones d'hivernada (verd clar) i les poblacions residents (groc). Font: BirdLife International (2016).

En el cas de Catalunya, la població d'aufrany va disminuir durant els anys 60 i 70: es va extingir en la part més oriental i sud de la seva distribució, quedant reduïda a la zona occidental, amb 25 parelles censades l'any 1983 (Muntaner, Ferrer, & Martínez-Vilalta, 1983). A partir dels anys 80, però, aquesta tendència es va revertir i l'espècie va començar a recolonitzar alguns antics territoris i àrees de la Catalunya Central i de les Serres Prelitorals, on històricament no se n'havia conegut al seva presència com a reproductor, fins a arribar a unes 80 parelles censades l'any 2012 (Servei de Biodiversitat i Protecció dels Animals, Generalitat de Catalunya 2012). Paral·lelament a aquest augment demogràfic i a l'expansió territorial, a Catalunya, durant els anys 90, es van començar a construir abocadors comarcals que acumulen grans quantitats de residus d'origen urbà. Des d'aleshores, les observacions d'aufrany en aquestes instal·lacions han estat cada vegada més freqüents, fins al punt que s'han arribat a observar més de 40 individus en un mateix abocador (J. Baucells, comunicació personal 2016). A més a més, durant els anys 80 es van posar en marxa una sèrie de canyets destinats a millorar l'estat de conservació de les espècies necròfagues en la zona de distribució d'aquests carronyaires (Margalida, García, & Bertran, 2012).

L'espècie objectiu principal d'aquesta alimentació suplementària és el trencalòs, tot i així, al Pallars Jussà hi ha un canyet destinat exclusivament a l'aufrany, on també s'hi agrupen grans quantitats d'individus, sobretot a l'època de premigració, durant el mes d'agost (Castilló, 2012).

Així, l'expansió territorial de l'aufrany s'ha produït de forma paral·lela als canvis de disponibilitat de recursos en el medi els quals han passat d'una ramaderia extensiva (fins els anys 50) a l'augment dels abocadors i a la creació de punts d'alimentació suplementària. A més, aquests canvis continuaran en el futur, ja que es preveuen nous escenaris de disponibilitat d'aliment per als voltors. D'una banda, s'han aplicat normatives que preveuen el tancament dels abocadors a l'aire lliure (Dir. 2008/98 del Parlament Europeu i del Consell, de 19 de novembre de 2008), la qual cosa provocarà la disminució de l'aliment predictible disponible per a les espècies que en depenen. D'altra banda, també s'han aprovat normatives europees (R (UE) 1069/2009 del Parlament Europeu i del Consell, de 21 d'octubre de 2009 i R (UE) 142/2011 de la Comissió, de 25 de febrer de 2011) que permeten flexibilitzar les normatives d'eliminació dels cadàvers de ramaderia als estats que en formen part. Aquestes normatives ja van ser transposades a l'estat Espanyol (RD 132/2011, de 19 de març) i a Catalunya (O. AAM/387/2012 de 23 de novembre) amb l'objectiu de permetre l'abandonament de cadàvers de ramaderia en zones específiques. Tot i que actualment es poden abandonar les carronyes en zones limitades, la intenció és anar augmentant substancialment la disponibilitat d'aliment impredictible al camp (Donázar et al., 2009; Margalida et al., 2012).

I, encara, cal destacar que, sumat a aquests canvis en la disponibilitat d'aliment, en els darrers anys s'està produint una disminució de la persecució de les espècies rapinyaires, una regulació estricta de l'ús dels verins i un augment de la penalització de les persones que utilitzen aquestes substàncies. D'aquesta manera, els factors que han provocat l'elevada mortalitat de l'aufrany en les darreres dècades, tenen cada vegada menys impacte sobre la població d'aquesta espècie a Catalunya (Hernández, 2006) i s'espera que la tendència es mantingui. Per tant, en un context on l'aufrany està disminuint en gran part de la seva distribució mundial, l'estudi dels factors que han pogut determinar l'expansió d'aquesta espècie pot proporcionar informació molt útil per a ser aplicada en altres àrees on l'espècie està disminuint.

Aproximació interdisciplinària de la influència dels abocadors

En aquesta tesi s'analitza quins han estat els canvis ambientals provocats pels humans que han causat l'augment demogràfic i l'expansió de l'aufrany a Catalunya. Tenint en compte que una de les principals hipòtesis és que els abocadors hi han tingut un paper rellevant, aquest cas ofereix una oportunitat per a estudiar l'efecte d'aquestes instal·lacions a diferents nivells sobre un voltor amenaçat. La majoria d'estudis que han analitzat els efectes dels abocadors sobre les espècies d'animals ho han fet fixant-se en algun aspecte particular, com ara la condició física, la dieta, els paràmetres reproductors o els nivells de contaminants (Plaza & Lambertucci, 2017), però hi ha una manca d'estudis que analitzin quines implicacions poden tenir a nivell general per a una espècie. Així, el cas de l'aufrany a Catalunya i del seu ús dels abocadors, permet abordar la influència d'aquests punts d'aliment des d'un punt de vista interdisciplinari, combinant informació a nivell individual i demogràfic i intentant aportar informació als buits de coneixement detectats que pugui ser útil per a la conservació i gestió de les espècies que utilitzen els abocadors.

En el present treball s'utilitzen metodologies avançades que permeten modelitzar i estudiar diversos aspectes dels individus així com també la població d'aufrany a Catalunya, per intentar aproximar-nos al màxim possible a les causes que han provocat l'expansió de l'espècie. Aquestes metodologies són clau per a la seva conservació, ja que és la manera més fiable que tenim d'obtenir informació dels processos que actuen sobre les poblacions d'animals i, per tant, són necessàries per a la gestió de la biodiversitat.

Així, en el primer capítol d'aquesta tesi es realitza una anàlisi demogràfica de l'aufrany a Catalunya en els últims anys basada en models de viabilitat de la població. Aquests models tenen per objectiu predir l'estat futur d'una determinada població a partir de mètodes quantitatius, i han esdevingut eines bàsiques per a la conservació d'espècies amenaçades (Morris & Doak, 2002; Hernandez-Matias et al., 2013). En el nostre cas, s'han realitzat models demogràfics amb un objectiu doble: d'una banda, estudiar el creixement de la població d'aufrany en els últims 20 anys i quins han estat els factors demogràfics que l'han provocat i, de l'altra, projectar la població en el futur per veure quina serà la seva tendència en els pròxims anys si les condicions ambientals no canvien.

A continuació, en el segon capítol, s'analitzen les característiques ambientals dels territoris de cria, per descriure com són les zones que estan seleccionant les parelles territorials, i s'avalua especialment la contribució dels abocadors i els punts d'alimentació suplementària en la distribució dels territoris. L'objectiu d'aquest apartat és bàsicament determinar, utilitzant Sistemes d'Informació Geogràfica, si la distribució dels recursos d'origen humà al medi ha influït en la distribució dels territoris de cria.

El tercer capítol té per objectiu avaluar fins a quin punt la dieta de les parelles territorials depèn dels abocadors. L'estudi de la dieta d'aquesta espècie és un repte, ja que consumeix preses d'orígens molt diferents que sovint són difícils de distingir. Tradicionalment s'han utilitzat mètodes convencionals que consisteixen en l'anàlisi macroscòpica de les restes d'animals que es troben en el niu (principalment ossos, pèls i plomes). Però aquests mètodes poden presentar alguns biaixos, ja que no es pot saber la quantitat real d'una determinada presa ingerida pels individus, així com tampoc no tenim informació de les parts toves que han consumit sense deixar restes (Real, 1996). Per aquest motiu, en el tercer capítol es testa la utilitat de l'Anàlisi d'Isòtops Estables (AIE) com a eina per a caracteritzar la dieta de l'aufrany. Aquesta tècnica ha estat molt utilitzada en diverses espècies, ja que proporciona informació sobre l'aliment ingerit, però ha estat poc aplicada en voltors (però veure Chamberlain et al. 2005; Blázquez et al. 2016).

Una vegada coneguda la dieta de les parelles territorials, la qüestió que s'aborda en el quart capítol és la condició física dels polls, plantejant fins a quin punt el fet d'alimentar-se en abocadors pot provocar una millora de la condició dels individus o, contràriament, pot perjudicar-los d'alguna manera. Aquesta aproximació a la condició es fa mitjançant índexs morfomètrics, que s'han utilitzat en molts estudis (Peig & Green, 2009), però també s'ha dut a terme una anàlisi de paràmetres fisiològics que tenen en compte la bioquímica plasmàtica i els indicadors del metabolisme antioxidant dels individus.

Finalment, l'últim capítol està dedicat a l'estudi de la presència de diferents famílies de contaminants (metalls i compostos organoclorats, bromats i pefluorats) en els individus de la població i dels seus possibles efectes en la fisiologia dels polls. A més, també s'avalua la influència de les zones urbanes en els nivells de contaminants dels individus, per tal de veure si les zones més humanitzades són les que presenten valors més elevats d'aquestes substàncies.

Aquesta aproximació interdisciplinària proporciona informació clau per a la conservació de l'aufrany, així com per a conèixer quines són les potencials implicacions del fet d'alimentar-se en abocadors per a les espècies que en depenen. En un context on els recursos en el medi estan canviant, és clau saber quins seran els efectes sobre les espècies que els utilitzen, siguin espècies plaga o espècies amenaçades.

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OBJECTIUS

Objectiu general

Determinar els factors que han causat l'augment i expansió de la població d'aufrany (*Neophron percnopterus*) a Catalunya així com avaluar la influència dels canvis ambientals antropogènics, en especial els abocadors, sobre la dieta, la condició física i la presència de contaminants dels individus de la població.

Objectius específics

1) Caracteritzar l'augment demogràfic i els paràmetres vitals d'una població d'aufrany en expansió a la Catalunya Central i Oriental i analitzar-ne la seva viabilitat en el futur.

2) Determinar la influència dels abocadors i d'altres variables ambientals en la distribució dels territoris ocupats per parelles reproductores.

3) Avaluar la contribució dels abocadors en la dieta de les parelles territorials comparant l'anàlisi convencional de restes amb l'anàlisi d'isòtops estables.

4) Determinar les conseqüències d'alimentar-se en abocadors en la condició física dels polls d'aufrany utilitzant índexs morfomètrics i fisiològics.

5) Estudiar els nivells de contaminants (metalls pesants i compostos organoclorats, bromats i perfluorats) que presenten els polls de la població i la influència d'aquestes substàncies sobre la fisiologia dels individus.

Informe del director

Els directors de tesi Joan Real i Joan Ll. Pretus, acreditem que la doctoranda Helena Tauler Ametller ha dut a terme les investigacions de la tesi doctoral present titulada "Viabilitat demogràfica i efecte dels canvis ambientals antropogènics en la distribució, dieta i condició física d'una població d'aufrany *Neophron percnopterus* en expansió". La tesi consta de cinc treballs d'investigació en format d'article científic. Aquests es troben publicats, en revisió o preparats per enviar en revistes científiques internacionals reconegudes en el Science Citation Index (SCI). A continuació es detalla la referència de tots els articles, indicant-ne el factor d'impacte de la revista així com la contribució de la doctoranda en la elaboració de cada article.

Capítol 1

Tauler, H., Real, J., Hernandez-Matias, A., Aymerich, P., Baucells, J., Martorell, C., & Santandreu, J. (2015). Identifying key demographic parameters for the viability of a growing population of the endangered Egyptian Vulture Neophron percnopterus. *Bird Conservation International*, *25*(4), 426-439.
Factor d'impacte (2015): 1.59

H.T ha contribuït en el disseny de l'estudi, la obtenció de dades, l' anàlisi estadístic i la redacció científica.

Capítol 2

Tauler-Ametller, H., Hernández-Matías, A., Pretus, J. L. & Real, J. (2017). Landfills determine the distribution of an expanding breeding population of the endangered Egyptian Vulture Neophron percnopterus. *Ibis*, 159 (4), 757-768.
Factor d'impacte (2016): 2.279

H.T ha contribuït en el disseny de l'estudi, la obtenció de dades, l' anàlisi estadístic i la redacció científica.

Capítol 3

Tauler-Ametller, H., Hernández-Matías, A., Parés, F., Pretus, J. L., & Real, J. Assessing the applicability of stable isotope analysis to determine the contribution of landfills to vultures' diet. Acceptat a *PLOS ONE*.
Factor d'impacte (2016): 2.806

H.T ha contribuït en el disseny de l'estudi, la recollida de mostres, l'anàlisi experimental i estadístic de les dades i la redacció científica.

Capítol 4

Tauler-Ametller, H., Pretus, J. L., Hernández-Matías, A., Ortiz-Santaliestra, M., Mateo, R. & Real. Are landfills a good quality food source for vultures? En revisió a *Science of the Total Environment*
Factor d'impacte (2016): 4.900

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Capítol 5

Ortiz-Santaliestra, M., **Tauler-Ametller, H.**, Hernández-Matías, H., Lacorte, S., Real, J. & Mateo, R. Levels and physiological effects of persistent organic pollutants and metals in nestlings of Egyptian Vulture. En preparació per enviar.

H.T ha contribuït en el disseny de l'estudi, recollida de les mostres i anàlisi experimental i ha revisat la redacció científica.

A més a més, el director certifica que el coautors citats en els articles científics que conformen la present tesi doctoral, no han utilitzat, implícitament o explícitament, aquests treballs per a l'elaboració d'altres tesis doctorals.

Barcelona, 17 d' abril de 2018

Joan Real Orti Joan Lluís Pretus Real

CAPÍTOLS

CAPÍTOL 1

Identifying key demographic parameters for the viability of a growing population of the endangered Egyptian Vulture *Neophron percnopterus*

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Resum

L'aufrany (*Neophron percnopterus*) és una espècie de voltor que es troba amenaçat a nivell global. La Península Ibèrica alberga el 50% de la població mundial, però en aquesta regió el nombre d'individus ha disminuït un 25% en els últims 20 anys. Tot i aquesta tendència mundial negativa, a Catalunya, en els últims 25 anys, s'ha observat un augment del nombre d'individus i una expansió de la població cap a àrees on no es coneixia la presència històrica d'aquesta espècie. En el present capítol, es descriu l'evolució demogràfica d'una població en augment d'aufrany a la Catalunya Central i Oriental. Aplicant models poblacionals i procediments de màxima versemblança s'investiguen els processos demogràfics que expliquen les tendències observades i la viabilitat de la població en el futur. El nombre de parelles a la àrea d'estudi va augmentar d'1 a 22 durant el període 1988-2012. Els models més versemblants suggereixen que la supervivència adulta dels individus d'aquesta població ha estat superior a la d'altres poblacions ibèriques i, a més, hi ha hagut una entrada constant d'immigrants d'altres àrees. D'altra banda, els escenaris més versemblants de l'Anàlisi de Viabilitat de la Població, preveuen que la població continuarà augmentant en el futur. L'anàlisi de sensibilitat indica que la supervivència adulta és el paràmetre que té una major influència en la dinàmica poblacional, per tant els esforços de conservació seran més efectius si se centren en millorar aquesta taxa vital.

Abstract

The Egyptian vulture (*Neophron percnopterus*) is a threatened species throughout its worldwide range. The Iberian Peninsula holds 50% of its global population, which has declined by 25% over the last 20 years. Despite this negative global trend, an increase in the number of individuals over the last 25 years has been observed in Catalonia, where it has colonised areas in which it was previously unknown. In this study, we describe the demographic evolution of an increasing population of Egyptian Vulture in central and eastern Catalonia and we apply population models and maximum likelihood procedures to investigate both the main demographic processes driving the observed trends and the viability of this population. The number of pairs in this region increased from 1 to 22 in the period 1988–2012. The best-supported models suggest that adult survival in this population may be higher than in other Iberian populations and that, furthermore, there is a continuous influx of immigrants. Based on the most likely scenarios, Population Viability Analysis predicts that the population will continue to increase. Sensitivity analysis indicates that the adult survival rate has the greatest influence on population dynamics so conservation efforts will be more effective if concentrate on improving this rate.

Keywords: Egyptian vulture, population viability analysis, adult survival, immigration, vital rate uncertainty.

Introduction

Understanding the processes that shape population size and structure is one of the main objectives in ecology and is thus subject to extensive study (Begon et al., 1996; Levin et al., 2009). Population dynamics can be understood as the outcome of the addition (i.e. births and immigration) and loss (i.e. deaths and emigration) of individuals from a population. These demographic processes are regulated by both intrinsic and extrinsic factors: intrinsic factors consist of species' life-history traits such as age of first breeding, fertility, longevity and dispersal behaviour, whereas extrinsic factors are more connected to environmental conditions such as food resources or weather conditions, and are subject to marked variation over time and space (Levin et al., 2009). Currently, human activities have a severe impact on the environment (Loreau et al., 2001; Barnosky et al., 2012) and play an important part in the population declines and extinctions of species that are now occurring at unprecedented rates. Consequently, population theory plays a central role in modern conservation biology (Primack, 2012). Thus, population viability analysis (PVA) – the use of quantitative methods to predict the likely future status of a population – has become a basic tool in current conservation research and practice (Morris & Doak, 2002; Hernández-Matías et al., 2013). This type of analysis is based on a broad suite of population modelling and data-fitting methods of varying mathematical complexity whose aim is to estimate the expected values of the main descriptors of population dynamics that include the population growth rate and, particularly, the risk of extinction of a population over time (Beissinger & McCullough, 2002).

In a global context in which many species are decreasing and/or are threatened, most PVA studies focus on analysing the causes of and possible solutions to human-induced population declines (Caughley, 1994). Nevertheless, some species may in fact benefit from human activities (Duhem et al., 2003; Gangoso et al., 2012). The application of PVA methods to growing populations can provide useful quantitative information to understand demographic processes and to guide practitioners as it is commonly done for invasive species (Conroy & Senar, 2009) and other threatened raptor species (Ortega et al., 2009). In this sense, tendencies in a species may vary from one population to another and, therefore, the study of populations with positive trends can provide very relevant information for conservation of declining populations. Usually little demographic data is available for endangered species, but current statistical procedures may allow generating quantitative information useful to understand the demographic drivers of population dynamics and, consequently, provide evidence based prescriptions to be applied by practitioners (Doak et al., 2005; Hernández-Matías et al., 2013).

The present work examines the recent expansion of the Egyptian vulture population (*Neophron percnopterus*) in Catalonia (north-east Iberian Peninsula). To do so, we apply likelihood-based procedures on available field data in order to identify the demographic determinants of population dynamics and, then, we perform population viability analysis. The Egyptian vulture ranges from the Indian subcontinent, Middle East, south-east ex-USSR to the Mediterranean Basin, and, the Sahel and eastern and southern Africa (Donázar, 1993). This long-lived species lives in pairs in adult ages and nest in caves situated on cliffs defending the same territory year after year (Donázar, 1993). Immature individuals are not territorial and usually form groups near predictable food sources such as landfills or supplementary feeding stations (Donázar, 1993; Grande, 2006). European populations of this species spend the winter in Africa (Sahel) and return to Europe to breed during spring and summer (Benítez et al., 2004).

The Egyptian vulture is threatened worldwide and in recent decades its distribution has decreased significantly (Donázar, 2004). At present, the conservation status of the Egyptian vulture according to the IUCN is Endangered. Based on recent studies, the stronghold of this species' Palearctic population is in Spain (1320-1480 pairs) (Del Moral & Martí, 2002; Donázar, 2004). Even so, its Iberian population has declined by 25% over the last two decades (Donázar, 2004), mainly due to poisoning and illegal persecution, but also as a result of the loss of traditional agricultural practices (Liberatori & Penteriani, 2001; Donázar, 2004) as well as due electrocution and collision with power lines and wind farms in some areas of Spain (Donázar et al., 2002; Carrete et al., 2009).

In Catalonia (north-east Iberian Peninsula) this vulture declined in the 1960s and 1970s and became extinct in the most eastern part of its range (Muntaner et al., 1981; Muntaner et al., 1983). Nevertheless, in the late 1980s this tendency was reversed and some old abandoned territories were recolonised (Estrada et al., 2005) and areas with no historical records were colonised. This is a paradigmatic case of a species in worldwide decline whose populations are decreasing on a local scale – but with the exception of part of its European range (e.g. north-east Catalonia), in which they are increasing and even expanding their distributions (García-Ripollès & López-López, 2006; Mateo-Tomás et al., 2010).

The present study provides a demographic analysis of the colonisation by the Egyptian vulture of an extensive area of Catalonia from 1988 onwards. The general aim of the study was to identify the demographical determinants of the observed population trend and to provide a useful guide for the conservation of this population and of other European populations currently in decline. The specific aims of this work are: (1) to describe the population dynamics from colonisation to the present day in the study area, (2) to estimate the vital rates in the studied population, (3) to use population models and likelihood-based methods to evaluate the contribution of immigration and adult survival to the observed past population growth rate, (4) to perform a PVA to predict the expected trend of the population and its risk of extinction, and (5) to identify the conservation targets by analysing the sensitivity and elasticity of the population growth rate in relation to the main vital rates.

Materials and methods

Study area and data collection

The study area was located in central and eastern Catalonia (north-east Spain) (Figure 3) at altitudes in the range 200–1900 m a.s.l. throughout an area of cliffs running from the Prelitoral Mountains in the south to the pre-Pyrenean Mountains in the north. In between stretches a large lowland area covered mainly by farmland but with important extensions of forest and scrubland. The monitoring of this Egyptian vulture population was carried out from 1988 to 2012. In 1988 only one breeding pair was known in the study area (Muntaner et al., 1983; Aymerich & Santandreu, 1998). From this year up to 2012, regular cliff-nesting raptor censuses were performed in the area (Aymerich et al., 1991; Real & Mañosa, 1997; Aymerich & Santandreu, 1998; Baucells et al., 1998; Aymerich & Santandreu, 2002; Guixé, 2008; Hernández-Matías et al., 2013) and located the new colonising pairs. All new occupied territories were monitored regularly in order to determine the level of occupancy and a subset of territorial pairs was monitored to obtain data on breeding success.

To determine the occupancy of the territories, at least one visit was made with a spotting scope (20-60x) between March and mid-April. An area was considered unoccupied if no individuals were detected after four visits/days. At the end of the incubation period (42 days), nests were checked to detect the presence of nestlings. 70 days after hatching, the nests were visited again to check the number of fledglings, which was used to estimate fledgling rates.

Life-history traits and life cycle

According to current knowledge of the life-history traits of this species, we defined a life cycle to be used in the population models described below. All simulations were based on this life cycle and only took females into account. The life cycle was based on a post-reproductive census of six age classes (Figure 1). After each breeding cycle, surviving females move into the next age class; only adult birds produce new individuals. In the results, the number of adult females was considered to be equal to the number of territorial pairs in the population.

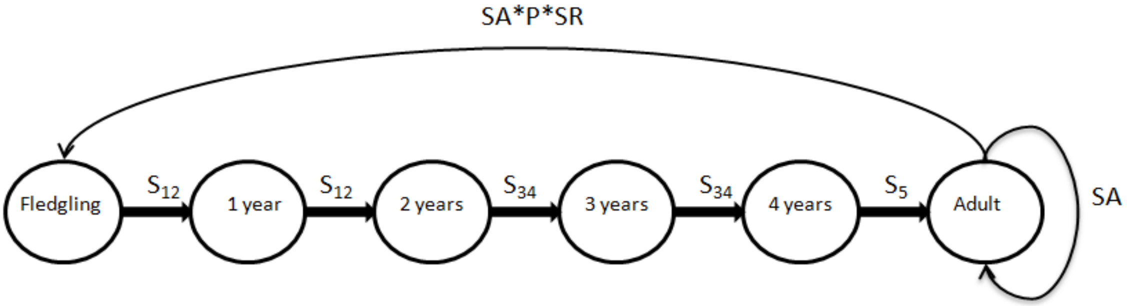

Figure 1. Diagram of the Egyptian Vulture life cycle. Nodes represent the different age classes considered in the model. S_{12}: yearly survival in first and second years of life, S_{34}: yearly survival in third and fourth years of life, S_5: yearly survival in fifth year of life when the recruitment of individual occurs, SA: yearly survival of adult breeders, P: productivity and SR: sex ratio which was assumed at 1:1.

Estimation of demographic parameters

Based on the observed number of pairs, the population growth rate was estimated for the whole study period as $\lambda = (N_t / N_0)^{1/t}$, where N_0 is the initial population size, N_t the final population size and t the number of years between the start and the end of the study period. We also calculated the population growth rate during the period 1994–

2012 given that this period was more appropriate for estimating the initial population size to be used in simulations. Productivity was calculated as the number of fledglings divided by the number of surveyed pairs. Other breeding parameters were calculated as breeding success (number of fledglings/number of laying pairs), fledgling rate (number of fledglings/number of pairs that have fledglings), percentage of laying pairs (number of laying pairs/number of territorial pairs), percentage of pairs with nestlings (number of pairs with nestlings/number of territorial pairs) and percentage of pairs with fledglings (number of pairs with fledglings/number of territorial pairs). Using the raw values of yearly productivity (and their associated sample variances), we applied White's method (White, 2000) to obtain an estimate of productivity (corrected by the sampling variation associated with small sample sizes) and the temporal variance that we then used to simulate the environmental stochasticity of the models.

Survival estimations could not be estimated in the study population since ringing schemes have only been initiated in recent years. Additionally, methods based on age ratios (e.g. Hernández-Matías et al., 2011) were not applicable for this species since only territorial individuals in full adult plumage could be monitored with ease. Therefore, we employed in the models estimates available for this species obtained from a large-scale ringing scheme in 1990–2005 in the Ebro Valley, part of the species' largest population in the Iberian Peninsula (Grande et al., 2008). Survival rates of non-adults used in our models were: $S_{12} = 0.73$, $S_{34} = 0.78$, $S_5 = 0.60$. Adult survival was estimated using a subsample of territorial individuals and considering a model with the time effect (model 24 in Table 5 from Grande et al., 2008). Based on yearly estimates and the corresponding standard errors, we applied White's method (White, 2000) to estimate the temporal variance of this parameter.

Population models and the evaluation of the model assumptions

To carry out the simulations, the life cycle described above and shown in Figure 1 was portrayed in the model. To calculate how many individuals of each age class will survive and will pass on to the next age we applied Monte Carlo simulations, which allowed us to account for demographic stochasticity in this rate. To introduce demographic stochasticity on productivity we determined the probability of having zero, one or two fledglings for each productivity and then applied a multinomial distribution to calculate the number of fledglings for each year. Environmental stochasticity for adult survival and for productivity were incorporated into the model using their temporal variance estimates and simulated with, respectively, the beta and stretched beta distribution functions in package POPBIO (Stubben & Milligan, 2007 based on the original code by Morris & Doak, 2002).

Population models were initially used to assess the most likely factors explaining the observed population growth rate. To do so, we applied likelihood-based methods. We evaluated a set of models considering several sources of uncertainty regarding the degree of connection of the population with other populations (i.e. whether the population was closed or received immigrants) and the values of adult survival for the population. Evaluated models considered all possible combinations of assumed adult survival from 0.7 to 0.975 at intervals of 0.025, and immigration from 0 to 10 immigrants per year. The number of immigrants considered is the net number of adult females that arrive to the population assuming that both emigration and immigration occurs. Essentially, model choice relied on how likely the observed lambda is given each model's set of predictions. For each model the simulated lambda values resulting from 5000 replicates were binned using fairly fine bins (0.05 bins). Then, the probability of the simulated lambdas being within each bin was considered as a means of estimating the probability of seeing the observed lambda for the population, P(λ). This probability was considered as a proxy of the likelihood of the observed lambda for each model. Models were chosen by comparing the P(λ) for each considered model (Hilborn & Mangel, 1997; Hernández-Matías et al., 2013). This analysis was restricted to the period 1994–2012 since before 1994 the population size was too small to be able to infer the initial population vector. By 1994 the territorial population consisted of three pairs and the initial population vector was approximated according to the stable distribution of ages obtained from the deterministic Leslie matrix model considering the life cycle and vital rates described above. The initial population vector considered was n0= (1, 1, 1, 1, 1, 3), each vector element corresponding to the number of females in each given age class. Model scripts were developed in R code (scripts are provided in the online Supporting Information).

Population viability analysis and the identification of conservation targets

We performed a PVA in which we considered four models. The first two corresponded to the two best-supported models in the previous analysis (scenarios 1 and 2 in the Results section). Additionally, we considered scenario 3 in which adult survival was assumed to be 0.891 (the most likely value provided by Grande et al., 2008) and the population was considered to be closed (0 immigrants per year). The last scenario (scenario 4) assumed the most likely adult survival estimated in the present study but considered a closed population. For each model, 5000 replicates were simulated with a time horizon of 50 years. Population growth rate and extinction probability were calculated under each scenario (Morris & Doak, 2002), whereby a population was considered to be extinct if the number of predicted breeding females was less than one. The probability of a fall by 50% in the number of pairs in 10 years was also calculated since this is one of the IUCN criteria used to classify a species as Endangered (IUCN, 2012).

To identify the vital rates that had the strongest effect on the population growth rate we applied a simulation-based method in which the value of the vital rate of interest was increased by 25% and the values of λ recalculated (except for the number of immigrants, which was increased from 1 to 2 immigrants). To do, so we considered the assumptions in scenario 1 in the Results section. Subsequently, we estimated the sensitivity and elasticity of λ to the main vital rates (S_{12}, S_{34}, S_5, SA, P) and to the number of immigrants (N_{imm}).

Results

Population trends and estimation of demographic parameters

The population of the Egyptian Vulture in the study area grew from 1 to 22 pairs during the period 1988–2012 and expanded eastwards (Figure 2). The population growth rate observed this period was estimated at 1.137 (1.117 in 1994–2012). Productivity corrected by White's method was estimated at 1.168 (temporal variance = 0.404). Adult survival obtained with the same method was 0.891 and its temporal variance corrected was 0.003.

Other breeding parameters calculated were: breeding success 1.11 ± 0.60, fledgling rate 1.17 ± 0.479, percentage of laying pairs 83.54 ± 33.90, percentage of pairs with nestlings 81.61 ± 32.47, percentage of pairs with fledglings 78.45 ± 31.54.

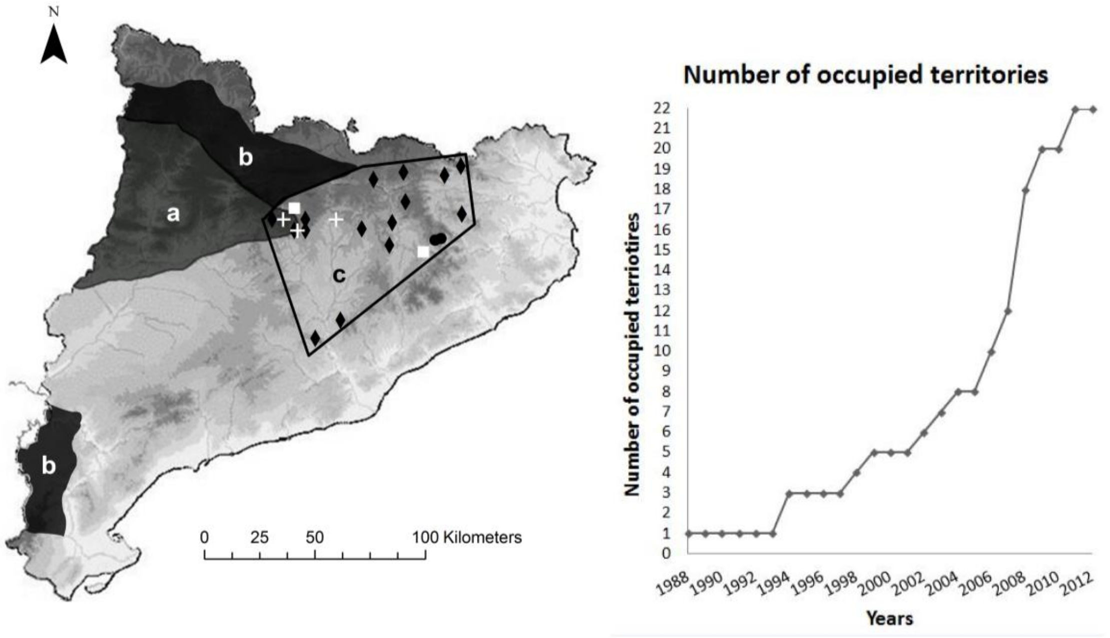

Figure 2. **Left:** Colonization of Egyptian Vulture in Catalonia*. (*a) Represents the range of the species according to *Atles dels Ocells nidificants de Catalunya* (1983). (Muntaner *et al*. 1983). (b) Represents the expansion of the species outside the study area, according to Servei de Biodiversitat i protecció dels Animals, Generalitat de Catalunya (2012) (c) Represents the study area. Symbols correspond to territorial pairs in the study area: white cross: 1988-1995, white square: 1996-2000, black circle: 2001-2005, black diamond: 2006-2012. **Right**: Number of occupied territories of Egyptian Vulture in the study area during the period 1988-2012.

Evaluation of the model assumptions

Our estimates of likelihood showed a crest of maximum values tracing a (non-linear) diagonal from high values of survival and low values of immigration to low values of survival and up to five immigrants per year (Figure 3). Based on available information (Grande et al., 2008), values of adult survival below 0.85 would seem to be unlikely. Thus, we selected two scenarios as the best supported, one of which (scenario 1) assumed the entry of one immigrant per year and adult survival of 0.950 (Likelihood estimated at 0.947), while the other (scenario 2) assumed the entry of two immigrants per year and adult survival of 0.875 (likelihood estimated at 0.962) (see white points in Figure 3).

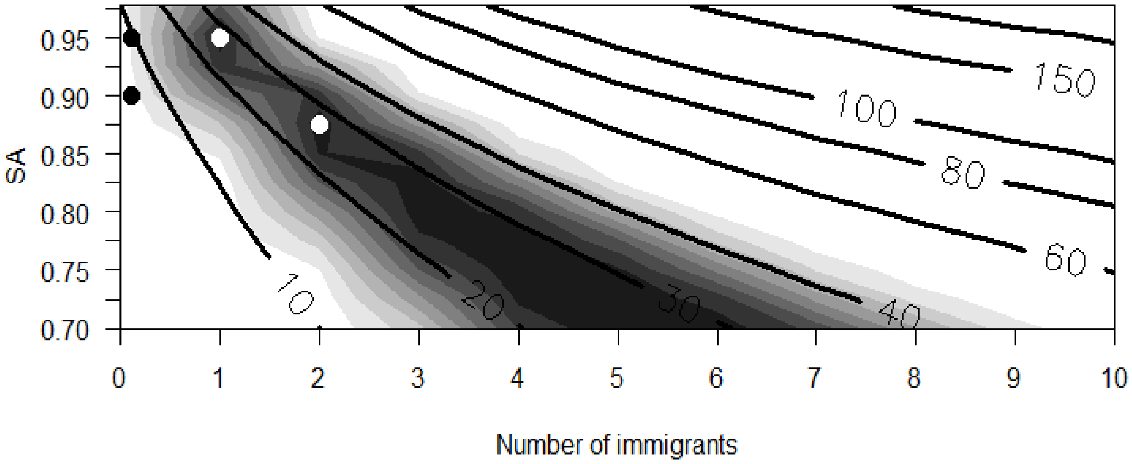

Figure 3. Likelihood estimated assuming different values of both adult survival (SA), from 0.7 to 0.975, and number of immigrants, from 0 to 10. Likelihood is represented by grey tones, from white (0) to dark grey (1). Black lines indicate the mean number of breeding pairs predicted under each scenario in 2012. White points indicate two most likely scenarios selected to perform the PVA (scenario 1 and 2). Black points indicate two closed population scenarios (3 and 4) selected to perform PVA.

Population Viability Analysis

Under scenario 1, the models predict that the population will continue to grow (λ = 1.051) and estimate an extinction probability of 0 in 50 years. Under scenario 2, the models predict a lower population growth rate (λ = 1.022) and an extinction probability also of 0. Under scenario 3, which assumes a closed population, the population growth rate was estimated at 0.994, implying that the population will remain at around 20 pairs, with an extinction probability of 0.011. Finally, under scenario 4, the population is predicted to increase with a growth rate of 1.039 and an extinction probability of 0 on a 50-year horizon (Figure 4). Under scenarios 1, 2 and 4, the probability of a 50% reduction in the number of pairs in 10 years was 0, but under scenario 3 was 0.049.

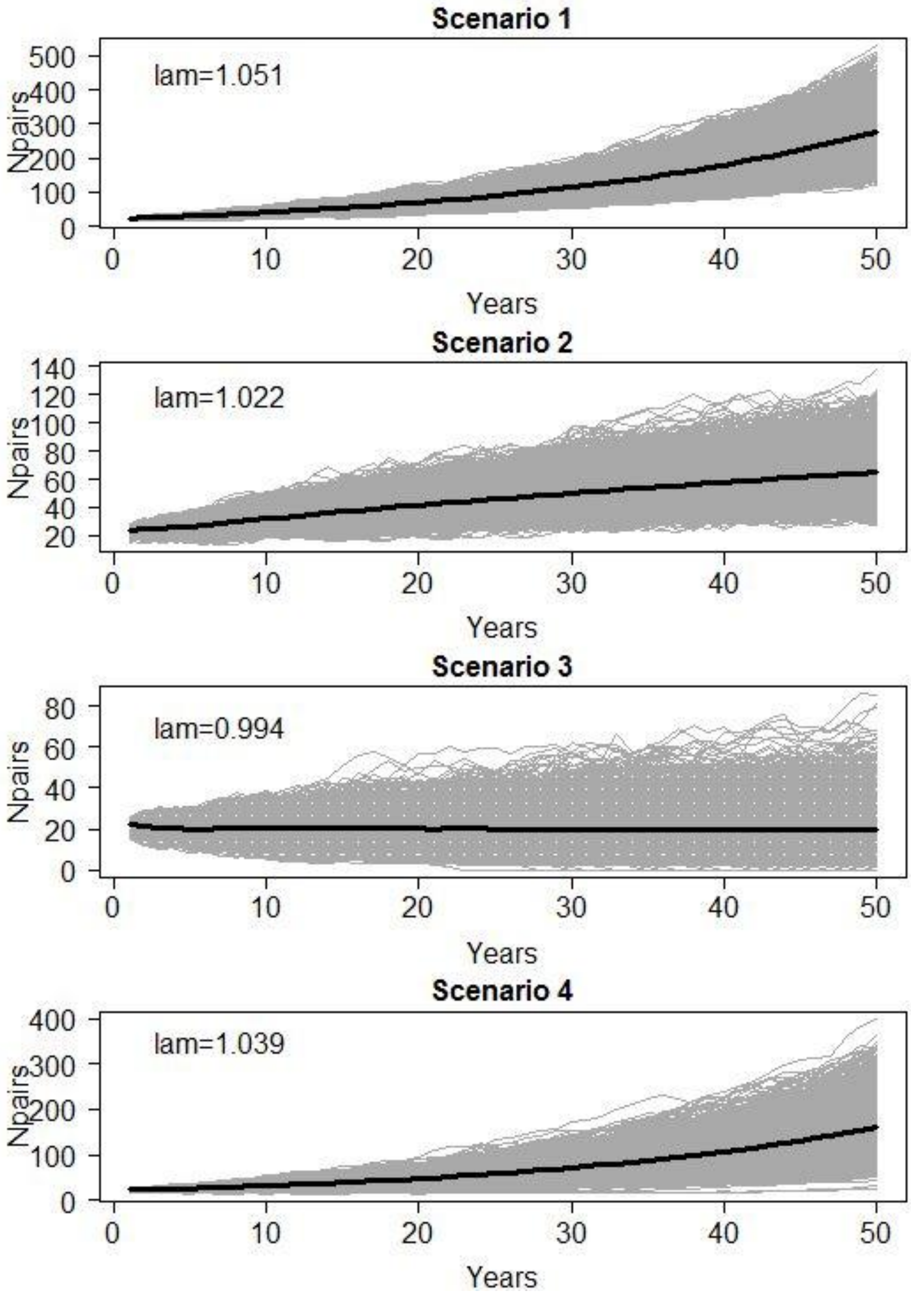

Figure 4. Projection of the population trend (in number of pairs) estimated for the next 50 years using models considered for PVA. All 5000 replicates are represented in grey as well as the average trend represented by the black line.

Sensitivity and elasticity of vital rates

Of all the parameters, adult survival has the greatest sensitivity (0.535) and elasticity (0.482); thus, relative increases in adult survival values would cause the greatest increase in the predicted population growth rate (Figure 5). The number of immigrants did not seem to have any important effect on the growth rate. This is because we used an increment of one immigrant (from one to two) to estimate the model's sensitivity and elasticity, thereby implying that the denominator of the expressions used to estimate these two metrics was much higher than for the other vital rates. As a result, the values of the metrics were much lower. However, the effect of immigration on the population growth rate was highly important, particularly between the scenarios of no immigration and of one immigrant (Figure 6).

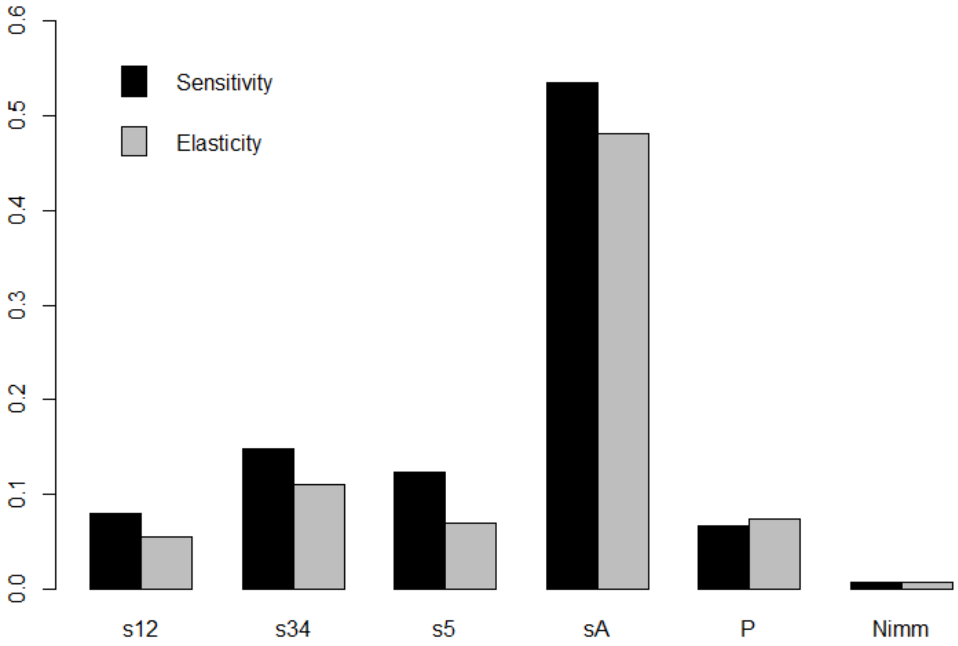

Figure 5. Sensitivity and elasticity of the population growth rate to main vital rates. S_{12}: yearly survival in the first two years of life, S_{34}: yearly survival in third and fourth years of life, S_5 survival in fifth year of life, SA survival of territorial individuals, P: productivity (number of fledglings per territorial pair), and Nimm: number of immigrants.

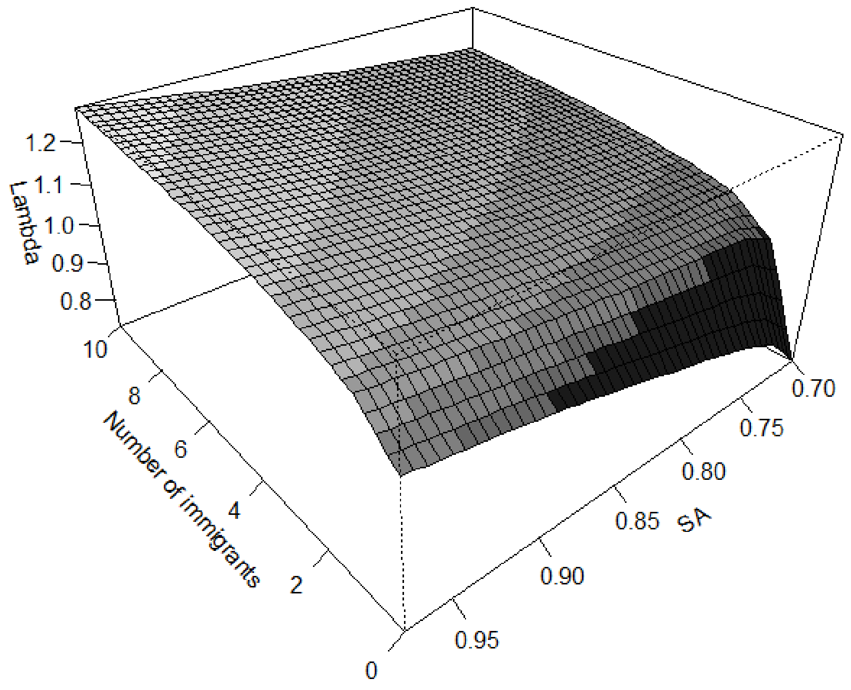

Figure 6. Contribution of Immigration and Adult survival to the population growth rate, assuming different values of both adult survival (SA), from 0.7 to 0.975, and number of immigrants, from 0 to 10. Values of lambda lower than 1 are represented in black, while values greater than one are represented in a grey scale, darker tones corresponding to lower values of lambda.

Discussion

The Egyptian Vulture is an endangered species that has dramatically declined throughout its range (BirdLife International, 2012), which includes the Iberian Peninsula, home to the bulk of the European population of this species. Despite this adverse scenario, a few local populations occupying small areas have recently grown (García-Ripollès & López-López, 2006; Mateo-Tomás et al, 2010). We focused on one of these increasing populations in eastern Catalonia to investigate whether the observed trend could be explained simply by the establishment of individuals born in the study population or whether the arrival of immigrants was a necessary factor. To do so, we applied likelihood-based methods using a comprehensive demographic analysis. While population viability analysis is frequently applied to declining populations (Carrete et al., 2009), it is used much more rarely to study endangered species that are increasing in number (Ortega et al., 2009). We argue that identifying the demographic determinants of population increases may provide relevant information for guiding conservation managers and practitioners whose task it is to preserve populations of target species.

The results of our models strongly suggest that the increase observed in recent years in the Egyptian Vulture population in Catalonia was probably caused by a combination of higher adult survival than in other Iberian populations and the entry into the population of adults from outside the study area. Both adult survival and immigration play a key role in determining the dynamics of the population, as is to be expected for long-lived species (Real & Mañosa, 1997; Saether & Bakker, 2000). Our analyses were based on limited field data, a common issue when studying endangered species (Doak et al., 2005). Even so, the framework we applied based on likelihood methods was useful to tackle this constraint (Hernández-Matías et al., 2013).

Instead of adult survival or immigration other demographic parameters could potentially affect the observed population trend. In the case of productivity, it was set at the values we estimated from the population and indeed, these values were higher than in other Spanish populations (C.R.P.R, 1984; Donázar & Ceballos, 1988; Fernández, 1994; Donázar et al., 2002). Nonetheless, sensitivity and elasticity analyses indicate that, of all factors, adult survival has the strongest effect on the population growth rate and that non-adult survival and, specially, productivity are much less decisive, suggesting that the positive trend and the expansion of our population was not due simply to high levels of productivity. Regarding non-adult survival, we assumed in all models it was the same as described in Ebro population. This assumption is reasonable if we consider that juvenile individuals from neighbouring populations share a similar life style; staying at the same wintering areas, aggregating in communal roosts and prospecting for potential breeding territories (Donázar, 1993). Another assumption we did in our models was that all individuals recruit at 5 years old. Again, this seem reasonable if we bear in mind that Egyptian vultures achieve maturity at this age (Donázar, 1993) and that we studied a growing population and, therefore, no denso-dependence is expected (Oro & Pradel, 2000).

Unlike other areas of the Iberian Peninsula, where poisoning is the main cause of non-natural mortality in Egyptian vultures (Hernández & Margalida, 2009), the probable higher survival rates in the Catalan population could be due to the less widespread use of poison in Catalonia, especially in the study area. Support for this affirmation comes from the fact that during the period 1995-2010 only three Egyptian vultures were found dead by poisoning in Catalonia, and all of them outside our study area (Hernandez, 2006; Programa Antídoto, unpubl. data). In other neighbouring areas like Aragon, 47 individuals were found dead during the period 1996-2006 (Hernandez, 2006) which highlights the different distribution and importance of poisoning events in the Iberian Peninsula. Electrocution and collision with power lines and wind farms are also important causes of non- natural mortality of Egyptian Vulture in Spain (Donázar et al., 2002; Carrete et al., 2009), but available data coming from recovery centres report that this is not a major cause of death of the species in Catalonia since only one case of electrocution has been reported up today (Servei de Biodiversitat, Generalitat de Catalunya, in litt., 2013). On the basis of our results, thus, management efforts in areas where the species' populations are decreasing should be directed to improving adult survival by eliminating the human related mortality the species suffers.

Our results also suggest that our study population is regularly receiving immigrating individuals from abroad. The Egyptian Vulture is a philopatric species with low natal dispersal distances (median natal dispersal 19.74 km, range = 0-150 km, n = 26; Grande et al., unpubl.). Our study area is placed adjacent to a nucleus in north-west Catalonia (Lleida Pyrenees), which has increased the number of pairs from 35 to 55 during the period 1983-2012. (Muntaner et al., 1983; Servei de Biodiversitat i Protecció dels Animals, Generalitat de Catalunya, 2012). The reasons of the growth of this adjacent population are not well understood although it has been argued that Egyptian Vulture benefit of some feeding points addressed to vultures (Margalida et al., 2012) but also the less use of poison and consequently high adult survival could be taken in account. Therefore, we hypothesize that this neighbouring population nucleus is the most likely origin of the new pairs being established in our area. Supporting this idea, the colonization pattern inside the study area shows that new colonized territories were always nearby another existing territory (less that 50 km), although long-distant natal dispersal events are also possible in this species (0-150 km) (Grande et al., unpubl.).

It is known that new sanitary policies that reduced the availability of carcass resources affected some species of vultures via reduction of their vital rates and/or dispersal of their individuals (Donázar et al., 2010; Margalida et al., 2010; Margalida et al., 2014). In the case of Egyptian vultures these policies seem not to be related with the increase of our population for two reasons, the neighbouring population of north-west Catalonia affected of the same regulations increased in the meantime and the breeding success seemed not affected by the possible consequent food shortages (Garcia & Margalida, 2009) that is in concordance with the trends predicted by Margalida & Colomer (2012). So probably the specific lifestyle of this species (Donázar et al., 2010) can be benefited by new environmental and human factors (landfills, extensive grazing) that occurred during the last decades in our area favouring their colonisation and increase (Kiff, 2000).

Assuming the most supported scenarios (1 and 2), both of which assume net immigration of adults, our population viability analysis predicts continued population increase at a lower rate than that observed in the study period. However, even in the event that immigration would stop (scenario 4) the population will grow if adult survival is high. In contrast, in scenario 3 where adult survival is assumed to be equal to that estimated at the Ebro population the population would stop growing although apparently it would remain stable. Even though the role of immigrants is commonly overlooked in population viability assessments, there is increasing evidence to suggest that immigration is a key determinant in population growth rates (Ward, 2005; Schaub et al., 2013). It is also worth mentioning that all our predictions were made under the assumption that ecological conditions will not change. However, if declining trends in most of the species range will continue, less potential immigrants will be available to support the Catalan population, so it is important that future research attempts to determine the ecological drivers of the vital rates of this population (e.g. Bakker et al., 2009). Conservation actions will thus have to take this question into account (Hernández-Matías et al., 2013).

Our results highlight that, despite major uncertainties, likelihood-based population analysis may provide relevant knowledge of the factors that regulate target populations. In the future, by reducing uncertainty, predictions can be refined so that advice for conservation managers can be more accurate. For example, it is essential to estimate survival, immigration and recruitment rates, to continue the long-term ringing scheme initiated in 2012 in this area and then to apply *ad hoc* statistical methods. Furthermore, the study of the ecological and/or behavioural factors driving the settlement of new territories must also be studied. It is known that conspecific attraction (Reed & Dobson, 1993; Grande, 2006) and environmental features, either for nesting or foraging, also play a very relevant role in colonisation processes (Webb et al., 2011). In our study area, most breeding pairs are located near landfills and farms, or in areas where extensive livestock farming is practised, and all these features are relevant sources of food for this species (Margalida et al., 2007; Gangoso et al., 2012, unpublished data). Therefore, it is crucial for the future effective management of this species to investigate the demographic relationships of the different local populations, the environmental determinants of its demographic characteristics and whether intensive human activities may have helped drive the increase in the studied Egyptian Vulture population.

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CAPÍTOL 2

Landfills determine the distribution of an expanding breeding population of the endangered Egyptian Vulture

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Resum

Les activitats humanes modifiquen la distribució de recursos a l'ambient creant aports alimentaris predictibles en l'espai i/o en el temps que poden ser aprofitats per diferents espècies. Aquests recursos, anomenats Predictable Anthropogenic Food Subisdes (PAFS), poden ser residus procedents de diferents activitats humanes o proporcionats intencionadament com a mesura de conservació per certes espècies. Alguns PAFS, incloent els abocadors, són utilitzats per espècies comunes, però existeix poca informació sobre els efectes que aquests punts d'aliment tenen sobre espècies rares o amenaçades. En aquest treball s'estudia la influència de la localització espacial dels PAFS en la distribució dels territoris de l'aufrany *Neophron percnopterus*. S'analitza la població del NE de la Península Ibèrica, que ha crescut en les últimes dècades i s'ha expandit cap a àrees on no es coneixia la seva presència. S'han utilitzat models nuls i models lineals per a determinar la influència dels PAFS i altres covariables en la ocupació territorial de l'espècie d'estudi. Els resultats apunten que els PAFS tenen un paper important en la selecció territorial de l'aufrany, ja que els territoris de cria estan situats més a prop dels abocadors del que s'esperaria a l'atzar. A més, la distància a PAFS (abocadors i punts d'alimentació suplementària per a voltors), té un paper rellevant en la probabilitat d'ocupació dels territoris. A part, altres variables ambientals com les zones rocoses orientades al sud, la urbanització i la proximitat a conespecífics també tenen importància en la selecció dels territoris. Tot i així, legislacions recents de la UE tenen per objectiu tancar els abocadors de residus a l'aire lliure per tal de reduir els impactes ambientals negatius associats a aquestes infraestructures. Aquest fet, podria tenir un impacte no desitjat per les espècies amenaçades que s'alimenten en abocadors. Caldria dur a terme mesures de gestió que per una banda puguin controlar l'abundància de espècies plaga, però també s'haurien de considerar altres mesures com l'alimentació suplementària per tal de compensar la potencial reducció d'aliment disponible dels abocadors per les espècies amenaçades.

Abstract

Human activities provide food resources for animals that are predictable in space and/or time (Predictable Anthropogenic Food Subsidies, PAFS). These resources are the result either of human-generated waste or are provided intentionally, sometimes as a conservation measure. Some PAFS, including landfills, are used by abundant species. However, little information exists about the effects that these feeding points have on non- abundant species that feed there, some of which are of conservation interest. This study focuses on the influence of PAFS and their spatial location on the distribution of territories of the endangered Egyptian Vulture *Neophron percnopterus*. We analysed a population in the NE Iberian Peninsula that has in recent decades expanded its range. We used both null model and linear model analyses to ascertain the effect of PAFS and other covariables on the occupancy of territories by the study species. Results suggested that PAFS played an important role in territory selection by Egyptian Vultures since occupied territories were nearer landfills than expected by chance. In addition, the distance from PAFS (landfills and vulture feeding stations or 'restaurants') played an important role in the probability of territory occupancy by Egyptian Vultures, even though other environmental variables such as surface areas of rocky south-facing slopes, human settlement and the proximity of conspecifics were also important. However, recent EU legislation aims to phase out open-air landfills to reduce the negative environmental effects of these facilities, which could have an undesired impact on the endangered species that use these feeding points. We recommend management measures that can control abundant pest species but, in the long term, other measures as supplementary feeding should be considered to counteract the probable negative effect of disappearance of landfills on endangered species.

Keywords: null model, Predictable Anthropogenic Food Subsidies, rubbish dumps, scavengers, threatened species, vulture restaurants.

Introduction

Human activities are provoking environmental changes in natural systems that may seriously affect ecosystem components such as the dynamics of plant and animal communities over time (Burney & Flannery, 2005). Thus, understanding the processes underlying the responses of species to non-natural perturbations has become a key topic in applied ecology and conservation biology (Nilsson & Grelsson, 1995; Gill et al., 1996; Primack, 2012).

One of the alterations that causes a serious direct impact on animal populations is change in the distribution and availability of food resources. This type of alteration may take the form of a drastic reduction in food supplies (Rodenhouse & Holmes, 1992; Mateo-Tomás & Olea, 2010) or an improvement in food availability due to the provision of food subsidies that were previously unavailable in the environment (Oro et al., 2013). The term 'Predictable Anthropogenic Food Subsidies' (PAFS) was coined to refer to resources that humans waste or intentionally offer to animals, but whose appearance is predictable in space and/or time (Oro et al., 2013). When referring to 'artificial' food availability, landfills are one of the most important types of PAFS and have an effect on terrestrial ecosystems, since the resources in the form of waste they provide are present in most modern societies (Olea & Baglione, 2008). By contrast, resources intentionally offered to animals (e.g. supplementary feeding stations) usually form part of specific conservation actions aimed at enhancing populations of a target (usually endangered) species (Donázar et al., 2009; Lieury et al., 2015; Cortés-Avizanda et al., 2016). Both landfills and resources intentionally offered to animals can have both positive and negative effects on wildlife that have to be analysed if PAFS are to be managed effectively within a conservation framework (Robb et al., 2008; Cortés-Avizanda et al., 2016).

Intentionally offered resources may improve the breeding parameters of some avian species (Robb et al., 2008) and increase survival rates in a part of their populations, thereby aiding their recovery (Oro et al., 2008; Lieury et al., 2015). Nevertheless, a number of studies (Carrete, Donázar & Margalida, 2006; Robb et al., 2008; García-Heras et al., 2013) have highlighted the unintended impact of supplementary feeding on individuals, populations and communities of avian scavengers. The predictability of food availability causes an increase in the number of individuals of the target species in areas around feeding points and may cause density-dependence depression of demographic parameters (Carrete, Donázar & Margalida, 2006). These studies indicate that this conservation strategy must be used with caution as it may potentially affect the viability of target species (Robb et al., 2008; Cortés-Avizanda, Carrete & Donázar, 2010). In addition, in some areas supplementary feeding in isolation has been seen to be insufficient to offset the loss of adult birds due to, for example, poisoning (Oppel et al., 2016). By contrast, less attention has been paid to non-target species that also use these feeding points intensively and so little information exists on how the presence of these artificial food resources affects the distribution and dynamics of the scavenger species for which these resources are not initially designed.

Landfills typically generate a series of negative environmental effects, including soil contamination, disease and/or the presence of invasive species (Ahel et al., 1998; Pyšek et al., 2003). Nevertheless, most research on the impact of landfills on animal populations has to date focused on generalist species such as gulls, ravens, foxes and rats. These species benefit from predictable resources and have increased locally in areas near landfills, thereby causing problems due to their abundance and the fact that they are regarded as pests (Bosch, Oro & Ruiz, 1994; Boarman et al., 2006; Duhem et al., 2008; Bino et al., 2010). Consequently, recent modifications in legislation have aimed to close open-air landfill sites (EU Commission 2008), thereby leading to a progressive reduction of predictable food resources that is expected to help control the populations of some abundant species (Payo-Payo et al., 2015). However, resources from landfills are also used by other species that are not considered as pests, some of which are even threatened (Viada, 1994; Martina & Gallarati, 1997; Newsome et al., 2015). The implementation of current landfill legislation may negatively affect some of these species, although there is still little available information about the effects of artificial food resources on the distribution and population dynamics of the species of conservation concern that rely on landfills. Further knowledge of this topic is a prerequisite if management decisions are to be taken that will not have negative effects on the populations of these threatened species.

In this study, we address the question whether PAFS have influenced the distribution of a territorial vulture species, the Egyptian Vulture *Neophron* *percnopterus*, a globally endangered species that is able to exploit a wide range of food resources including landfills and supplementary feeding points (Donázar, 2004). Vultures are useful for evaluating the effects of PAFS on spatial distribution since all vulture species are scavengers and most benefit from PAFS. Under this scenario, we focused on an Egyptian Vulture population that is currently expanding its range, an exceptional situation for this species in Europe. Interestingly, our study population has colonized new areas, which are not thought to have been previously occupied by this species (Tauler et al., 2015). These newly colonized locations are found in (i) a mountainous area with livestock where there is a network of feeding stations, also termed 'vulture restaurants', that was set up primarily to help preserve the endangered Bearded Vulture Gypaetus barbatus, and in (ii) a more humanized contiguous area that possesses a network of landfill sites.

The aim of this study was to assess whether there is an association between the spatial distribution of PAFS and the spatial distribution of Egyptian Vulture territories in central Catalonia (NE Spain), an area that has been recolonized by this species over the past two decades. To do so, we performed a dual approach that combined a null model analysis (taking into account relevant environmental constraints on the locations of vulture territories) and linear models (controlling for the most relevant environmental variables known to affect habitat selection in the study species). Our main predictions were that (i) Egyptian Vulture territories would be located closer to PAFS than expected by chance, (ii) breeding territories would more probably be found in remote areas with a low degree of humanization, and (iii) occupied territories would be situated in areas with a high availability of livestock-derived resources.

Materials and methods

Study species

The Egyptian Vulture is classified as Endangered on the IUCN Red List (BirdLife International, 2015). Its Iberian population has declined by 25% over the last two decades, mainly due to poisoning and illegal persecution, but also as a result of a decline in traditional agricultural practices (Liberatori & Penteriani, 2001; Donázar 2004), and in certain areas of Spain collision with power lines and wind farms (Donázar et al., 2002; Carrete et al., 2009). Paradoxically, there are at least two populations of Egyptian Vulture in the Iberian Peninsula that are increasing and expanding their ranges (Garcia-Ripollès & Lopez-Lopez, 2006; Tauler et al., 2015).

Study area and data collection

The study area (10500 km^2) lies in central eastern Catalonia (NE Iberian Peninsula) at altitudes of 200–1900 m a.s.l., and encompasses an area of cliffs running from the Prelitoral Mountains in the south to the pre-Pyrenean Mountains in the north. The study population was monitored from 1988 to 2014 (Fig. 1).

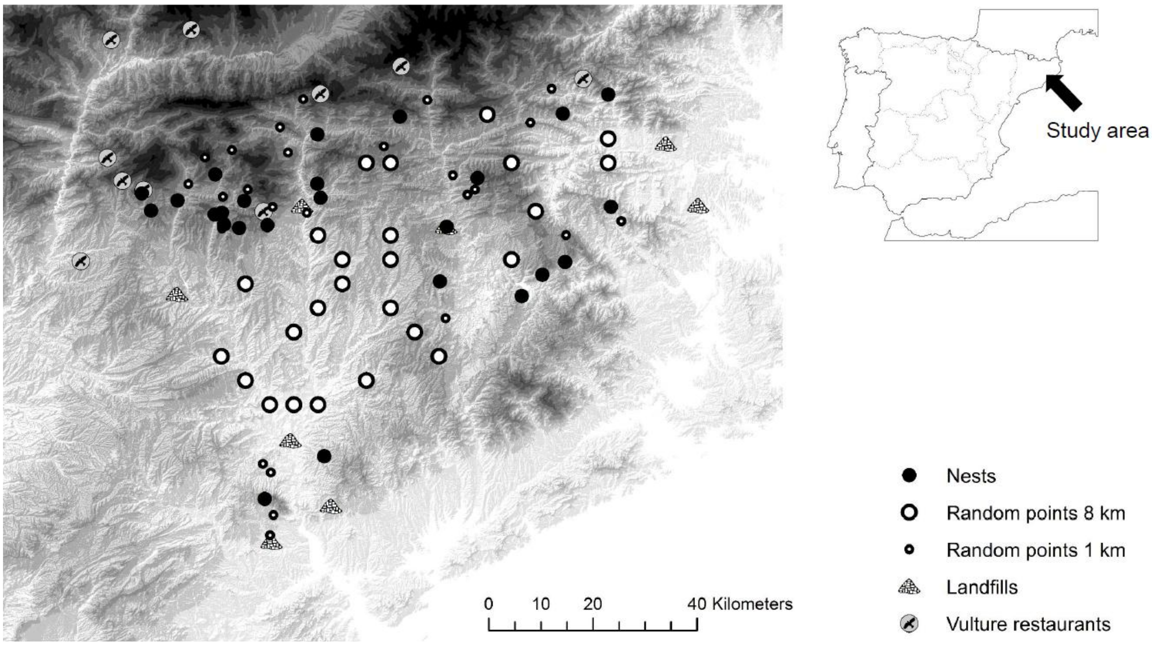

Figure 1. Distribution of occupied territories of Egyptian Vulture, landfills and vulture restaurants used in the GLM and null model analysis. Random points used in 1 km and 8 km GLM analyses are also presented.

During this period, regular cliff-nesting raptor censuses were performed in the whole area and potential suitable habitats where new colonizing pairs were recorded were prospected. To determine territory occupancy, each territory was visited during the breeding season (March–August) by observers equipped with a spotting scope (20–60x). An area was considered unoccupied if no individuals were detected in the course of four visits in different days. The population grew from 1 to 22 breeding pairs between 1988 and 2012 (λ=1.13) (Tauler et al., 2015). To perform the statistical analysis, we selected the period 2010 - 2014 since the monitoring effort during this period was more intensive, and information regarding nest sites was more accurate than for the remaining part of the period. Breeding territories included in the analyses were occupied almost all of the period (a mean of 4.2 ± 1.35 years) and if a territorial pair used more than one nest, the nest that was used for most years was selected.

Effect of PAFS on territory distribution – the null model analysis

To study the influence of PAFS on the distribution of occupied territories, we first used a null-model approach (Nitecki & Hoffman, 1987; Gotelli & Graves, 1996; Colwell & Lees, 2000). A null model is 'a pattern-generating model that is based on randomization of ecological data or random sampling from a known or imagined distribution' (Gotelli & Graves, 1996). In contrast to most other modelling approaches (Caswell, 1988), the null-model strategy involves the construction of a model that deliberately excludes the mechanism being tested. The aim is to see how well the available data fits this model (Hilborn & Mangel, 1997) — in other words, can the patterns in the data be reproduced by a simple model that does not incorporate biologically important mechanisms? Does the data have a non-random distribution with respect to the null hypothesis? If so, the analysis provides evidence in support of the mechanism under study (Gotelli, 2001).

In our case, the mechanism being tested was whether or not occupied territories were located nearer PAFS than expected under the assumption that territories were not related to the presence of PAFS. We thus defined a statistic – the Nearest PAFS Distance (NPD) – to measure this relationship. Consequently, to describe the observed pattern, we calculated the NPD for all occupied territories in the study area using data on landfills and vulture restaurants from the Agencia de Residus de Catalunya and the Servei de Biodiversitat, Departament de Territori i Sostenibilitat, respectively (Fig. 1).

To perform the null model, the NPD of occupied nests was compared with the NPD of 1000 randomly simulated nest sites located inside the study area on cliffs identified on a very precise land cover map (resolution of 2 m, Mapa de Cobertes del Sòl de Catalunya, Generalitat de Catalunya). By selecting only rocky areas, we imposed an ecological restriction on the null distribution that preserves some features of the real data and thus gives greater biological authenticity to the random locations of the simulated nests. The model operated in the following way: first, a sample of 25 points (i.e. the same number as the number of occupied territories) was selected randomly from the simulated set. Two calculations were then made: (i) the distance between each point and the nearest PAFS (NPD) in the study area and (ii) the mean of all the obtained NPD (MNDP). This process was repeated 999 times to generate a frequency histogram of expected MNDP values in a random distribution of nests. The position of the observed value of MNPD in occupied territories in the null distribution of the randomly generated values was then used to assign a probability value (P), just as in a conventional statistical analysis (Dolédec et al., 2000; Raes & ter Steege, 2007). Finally, the 95% confidence interval of the obtained null distribution was calculated.

This model was initially performed using data from all PAFS in the study area (PAFS model), but was then also simulated separately for the distance from points to the eight landfills in the study area (MNLD; landfill model) and to the 10 vulture restaurants (MNVD; vulture restaurant model) to test for possible differential effects of these two types of predictable feeding points.

Effect of PAFS on territory selection – Generalized Linear Model analysis

We performed a Generalized Linear Model (GLM) to test the effect of PAFS on Egyptian Vulture territory selection by taking into account other environmental variables known to affect the distribution of this species (see below). Environmental variables were measured at two spatial scales to account for the fact that nest sites may be selected by vultures on the basis of the habitat characteristics of both the immediate area around the nest and the overall home-range that vultures need to exploit to find resources. For the two spatial scales considered, we conducted a GLM using variables measured within a 1-km and 8-km radius around nests, which correspond, respectively, to the core area and the home range of breeding Egyptian Vultures according to radio-tracking data from southern Spain (Carrete et al., 2007).

To perform GLMs, occupied territories were compared with a set of 25 randomly generated points located inside the Minimum Convex Polygon (MCP) delimited by the 25 occupied territories. The response variable in this case was the occupation of the territories, which were either occupied or unoccupied (i.e. random points). To carry out the analysis at the breeding-area scale (1-km radius around the nest), random points were modelled that had to be inside the 8-km radius of the area of influence of occupied territories in order to highlight the characteristics of the breeding area, given that the larger area had already been selected. The points used at the home-range scale (8-km radius) were simulated randomly, but with the limitation that their areas of influence overlapped the occupied territories by a maximum of 50% to avoid replication and the overlapping of buffers of occupied and non-occupied territories.

The choice of explanatory variables to be considered in the analyses was made on the basis of previous knowledge of Egyptian Vultures (Liberatori & Penteriani, 2001; Sarà & Di Vittorio, 2003; Carrete et al., 2007; Mateo-Tomás & Olea, 2010), and specific hypotheses regarding their possible effects on territorial selection were formulated (Supporting information, Table S1). To describe food availability, data on livestock from the national cattle census was obtained (Instituto Nacional de Estadística, 2009), and the distances between occupied territories and PAFS existing inside the study area were calculated. Variables regarding food availability were only collected for the 8-km analysis because data for livestock were not available at a 1-km scale. Topographical variables (altitude, roughness and aspect) were obtained from a Digital Elevation Model (DEM) with a spatial resolution of 15 m. Landscape structure and land-use variables were obtained from a land-cover map (Mapa de Cobertes del Sòl de Catalunya, Generalitat de Catalunya), and the Simpson diversity index was calculated based on the information for each land-cover type. The effect of human presence and disturbance was assessed by calculating the distance to the nearest paved road, as well as by determining the total length of unpaved tracks inside the areas of influence around the nest sites. These variables were obtained from the Topographical Map of Catalonia (1:25000, Institut Cartogràfic de Catalunya). Finally, as the Egyptian Vulture is a territorial breeder, the effect of the presence of conspecific pairs was analysed by calculating the distance to the nearest neighbouring breeding pair. All variables were obtained using Arcgis 10.2 (ESRI). Correlation analyses were performed to detect co-linearity and non-independence between variables, and, if detected, those variables that were considered to be less biologically meaningful were not subsequently used. At the 1 km scale, we selected 5 variables: roughness (correlated with altitude), rocky surface, urban surface (correlated with other landscape and human presence variables), habitat diversity and aspect. At the 8 km scale, we retained 7 variables: distance to PAFS, extensive and intensive livestock, urban surface (correlated with other landscape and human presence variables), roughness (correlated with aspect and rocky) and habitat diversity.

We used the logit link function to model this binomial variable (presence/pseudo-absence) and assumed a binomial error distribution. Model selection was performed by a backward-forward stepwise procedure considering the two models to be statistically different when there was a difference of two AICc units (AICc: Akaike information criterion corrected for small samples). All models that did not differ by more than two AICc points from the best model were selected if they had a lower AICc than the null model (i.e. an intercept-only model). If AICc values were nearly equal (i.e. within 2), we used full model averaging over the whole model set to calculate the parameter estimates and standard errors using Akaike weights. Parameter estimates or predictions obtained by model averaging are robust in the sense that they reduce model selection bias and account for model selection uncertainty (Johnson & Omland, 2004). For the best selected models, the relative variable importance was calculated as the sum of the Akaike weights for all models in which the variable w+(i) appeared to quantify the evidence of the importance of each variable in the set (Burnham & Anderson, 2002).

All variables were standardized and models were performed using the R.3.2.2 (R Development Core Team, 2015) program and the package MuMIn was used to perform GLM model selection and the relative variable importance.

Results

Effects of PAFS on territory distribution –the null model analysis

The Mean Nearest PAFS Distance (MNPD) observed between occupied nests and PAFS was 8.75 km. This figure is not significantly different from the expected frequency distribution under the null model (mean MNPD = 9.31 km; 95% CI: 7.17–11.77), and so in this case the null hypothesis was accepted (P = 0.330). In the case of the landfill model, the MNLD between occupied nests and landfills (14.06 km), was significantly lower than the MNLD predicted by the null model (mean =17.76 km, 95% CI: 14.7–21.30) and so the null hypothesis was rejected, thereby supporting a non-random relationship between the locations of occupied territories and landfills (P = 0.0013). Finally, the MNVD between occupied territories and vulture restaurants (18.21 km) was not found to be significantly different from the expected MNVD from the null model (mean = 21.67 km, 95% CI: 14.42–28.90) and so this null hypothesis was also accepted (P = 0.178). In summary, the landfill model was the only model that was significant when explaining the relationship between feeding points and the distribution of occupied nests (Fig. 2).

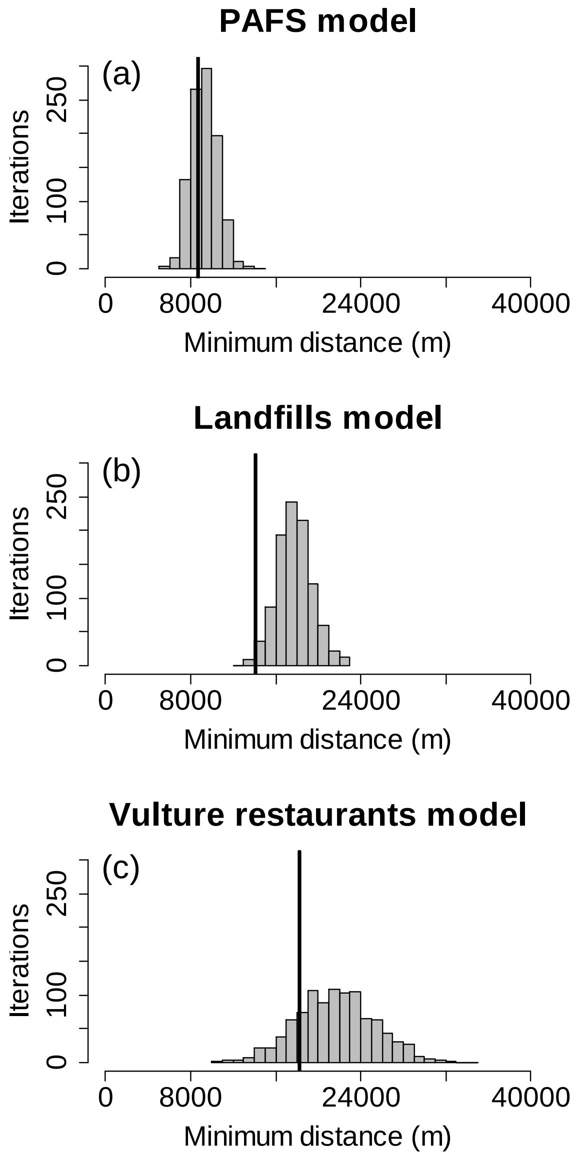

Figure 2. Frequencies of distances from simulated nests to (a) PAFS (landfills and vulture restaurants), (b) landfills and (c) vulture restaurants obtained in each null model. N iterations = 999. The black line indicates the distance from real nests to PAFS, landfills and vulture restaurants.

Effects of PAFS on territory selection– Generalized Linear Model analysis

At the breeding-area scale (1-km radius), two models were selected that did not differ by more than two AICc units from the model with the lowest AICc. All selected models at this scale included the variables ‘rocky’, ‘aspect’, ‘urban’ and ‘roughness’. The first three variables had the same relative importance (*w+(i)*= 1), but the relative importance of roughness was 0.83 (Table 1).

Table 1. Variables included in the best models (1 if included, 0 otherwise) selected at the breeding-area scale (1-km radius around the nest) and the home-range scale (8-km radius around the nest). For each variable, relative importance weights w + (i) and parameter estimates and standard errors obtained from full model averaging are shown. For the best models (within 2 AICc units of the best model), the number of parameters (k), AICc, Δ AICc, AICc weight and R^2 are also presented. More information on the other models is given in supporting information (Tables S3 and S4).

	w + (*i*)	1	2	3	4	5	Estimate	se
AICc top models (1-km)								
Rocky	1	1	1				1.98	0.74
Aspect	1	1	1				1.43	0.55
Urban	1	1	1				−1.77	0.75
Roughness	0.83	1	1				1.03	0.71
Habitat diversity	0.68	1	0				−1.14	1.13
k		6	5					
AICc		46.79	48.14					
ΔAICc		0	1.35					
AICc weight		0.55	0.28					
R^2		0.53	0.47					
AICc top models (8-km)								
PAFS	0.93	1	1	1	1	1	−1.11	0.57
Conspecifics	0.81	1	1	1	1	0	−0.96	0.71
Roughness	0.61	1	0	1	0	1	0.48	0.52
Intensive livestock	0.61	1	1	0	1	0	−0.57	0.62
Urban	0.43	0	0	1	0	1	−0.34	0.63
Extensive livestock	0.27	0	0	0	1	0	−0.09	0.29
Habitat diversity	0.23	0	0	0	0	0	0.04	0.25
k		5	4	5	5	4		
AICc		48.67	48.69	48.95	50.35	50.37		
ΔAICc		0	0.02	0.28	1.68	1.7		
AICc weight		0.1	0.1	0.08	0.04	0.04		
R^2		0.46	0.42	0.45	0.44	0.40		

Occupied territories tended to have a greater proportion of rocky surface areas, were generally south facing and had a lower proportion of built-up areas (Fig. 3). At the home-range scale (8-km radius), five models were selected. All selected models included the variable PAFS distance with a relative importance of 0.93, but the presence of conspecifics was also present in four models with a high relative importance (0.81, Table 1). Occupied territories were situated nearer PAFS and conspecifics than were random points (Fig. 3). Roughness and intensive livestock had a lower variable importance (0.61), and were present only in three of the best models. All selected models in the two sets of analyses, at both breeding-area and home-range scales, improved the AICc relative to the null model (AICc null model = 71.39 in the two analyses).

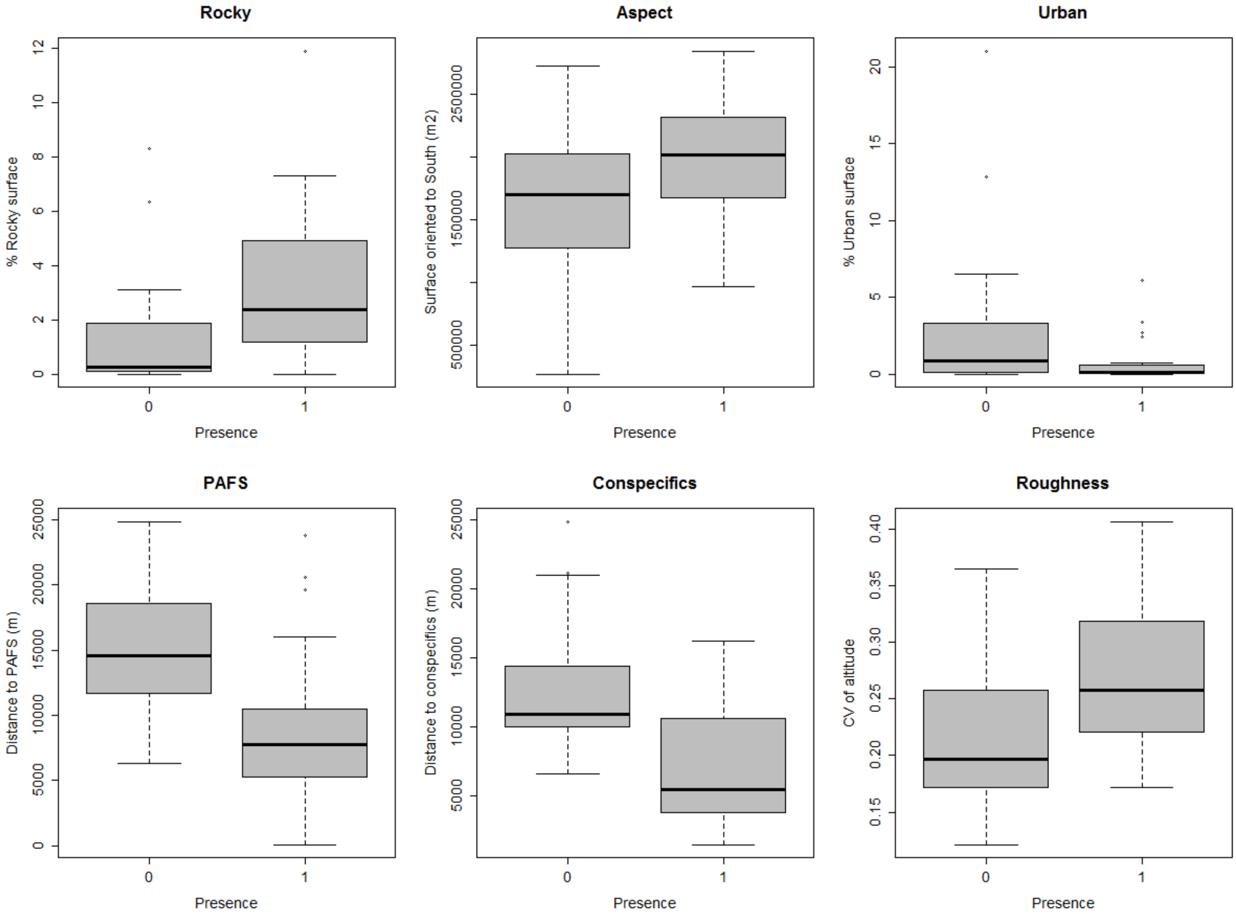

Figure 3. Boxplots of variables with the highest relative importance included in all best sets of models obtained with GLM analysis at breeding-area (1-km radius, top) and home-range (8-km radius, bottom) scales. The original data distribution of random points (occupied = 0) and occupied territories (occupied = 1) are also shown.

Discussion

Our study focused on an expanding population of Egyptian Vultures that has recently occupied areas situated outside its historical range. We thus assumed that territorial locations reflect preferred environmental characteristics in the study species and that other factors including historical ones, have not confounded our main findings. Our overall results indicated that PAFS played an important role in determining the current distribution of this species in the study area, which has been shaped by the presence of these artificial food resources (at least in the case of landfills). However, unlike in the linear model analysis, in the null model analysis only landfills were selected to explain territorial distribution, a finding that, as we discuss below, has serious implications for management and conservation.

The linear models allowed us to control for the effects of other environmental variables that, if not taken into account, may affect territorial choice and potentially confound the results. Model uncertainty led to large errors for some variables. Nevertheless, models including these variables appeared well ranked under the AICc and they explained a relevant fraction of variance of the response variable. Additionally, they showed relatively high Akaike weights, suggesting that they also played a relevant role in nest-site selection. We found that the PAFS weight was important and was present in all five best models at the 8-km scale, thus, all territories were located close to a PAFS, be it a landfill or a vulture restaurant (Table 1). However, landfills and vulture restaurants do not appear as very important variables if they are considered separately in the linear models, as individual territories may also be situated far from either a landfill or a vulture restaurant (Supporting information, Table S2).

Home-range-scale analysis (8-km radius) also showed that the presence of conspecifics is important when selecting a new territory, as individuals have occupied areas close to other breeding pairs. Distance to PAFS and conspecifics presence were the variables that presented lower standard errors, so drawn conclusions of these two variables are stronger than of the others (Table 1). Additionally, the location of nests was linked to very rough terrain and the presence of intensive livestock. Thus, our results match those of other habitat analyses that indicate that cattle are one of the main sources of food for Egyptian Vultures (our pers. obs., Donázar, 1993; Mateo-Tomás & Olea, 2009). At the breeding-area scale (i.e. 1-km radius), nests were located preferentially on south-facing cliffs far from built-up areas (Fig. 3), a result that agrees with other studies of this vulture (Liberatori & Penteriani, 2001; Sarà & Vittorio, 2003; Carrete et al., 2007; Mateo-Tomás & Olea, 2009).

In our linear model approach, real nest sites were compared with a single sample of randomly selected non-nest points. Therefore, to address this potential constraint on our approach we also performed a null model analysis. The assumption that randomized territories could only exist in rocky areas (Estrada et al., 2005, this study) prevented type-I errors in all tests, as well as type-II errors in the test for landfills, since these infrastructures are usually placed in humanized areas far from rocky outcrops. Nevertheless, vulture restaurants are usually placed in rocky areas corresponding to the distribution of the Bearded Vulture (Fig. 1) and so the possibility of type-II errors increases in the vulture restaurants model. We did not detect any effect of vulture restaurants on the observed distribution of Egyptian Vultures (Fig. 2), although it is known that these sites have acted as attractors for high densities of pairs of Egyptian Vultures and other scavenger species (Carrete, Donázar & Margalida, 2006; Lieury et al., 2015). Thus, although our approach probably does not completely disentangle the effect of vulture restaurants, it does strongly support the idea that landfills have played an important role in the observed distribution of Egyptian Vulture territories in the study area. In this regard, our results are relevant since landfills are important sources of food for Egyptian Vultures, as has been described in other areas (Liberatori & Penteriani, 2001; Gangoso et al., 2013, our pers. obs.), but the implications of the presence of landfills for the territorial distribution of Egyptian Vultures had never been demonstrated before.

Implications for conservation

Feeding in landfills may have certain implications at individual and population levels that may potentially affect the conservation status of scavenging species. At an individual level, abundant and predictable food should improve body condition and breeding performance of the individuals that feed in these areas (Oro et al., 2013 and references therein). On the other hand, landfills can also act as a threat as they represent a source of low-quality food with high levels of different pollutants such as heavy metals (de la Casa-Resino et al., 2014, Carneiro et al., 2015), rodenticides (Quarles, 2011) and organochlorines (Martinez-Lopez et al., 2015). The presence of these pollutants can have negative effects on body condition (Ortiz-Santaliestra et al., 2015) or even cause mortality (Sanchez-Barbudo et al., 2012). Moreover, the presence of the species in highly humanized areas where landfills are found could also increase the risk of fatal casualties such as collisions with power lines and other infrastructures (Martínez-Abraín et al., 2012, Sanz-Aguilar et al., 2015).

PAFS could also have positive effects at a population level by promoting the immigration of individuals attracted by the availability of food and increasing the number of Egyptian Vulture breeding pairs in Catalonia (Tauler et al., 2015). In this sense, the presence of breeding pairs near PAFS could also have acted as an additional attracting factor of non-breeding individuals (Carrete et al., 2007). Immature individuals favoured by the presence of predictable food and conspecifics could have formed new pairs that could have colonized other unoccupied territories even further from PAFS, thus continuing the population expansion. On the other hand, PAFS can act as a drivers of population change, improving survival rates of both adult and immature individuals, and hence recruitment (Oro & Pradel, 2000, Oro et al., 2008), but also could improve productivity of pairs that feed on PAFS. In this sense, the high predictability and availability of food associated with landfills have also led to steep population increases in gull, Raven Corvus corax and rat populations. Several studies have focused on the ecological and social consequences of this overabundance (Hario, 1994; Vidal et al., 1998; Bosch et al., 2000) and for instance, the growth of gull populations has caused social disturbance and public health issues (Hatch, 1996), thereby triggering population control measures worldwide (Bosch et al., 2000; Brooks & Lebreton, 2001).

As a response to the negative impact of open-air landfills and to the above mentioned concern regarding abundant problematic species, the European Commission approved a Landfill Waste Council Directive (EU Commission 2008) that aims to halt the building of open-air landfill sites. This reduction in the predictable resources available to scavenger species could have a strong negative impact on populations of vultures if compensatory measures are not implemented. Our study shows that the presence of landfills may have promoted the colonization of new areas by the Egyptian Vulture and, furthermore, that landfills provide an important proportion of the diet of some pairs in the study area (our pers. obs.). Thus, if the food resources provided by landfills disappear, some breeding failures and territory abandonment of certain breeding pairs may occur in our study area (Liberatori & Penteriani, 2001). Current European legislation thus needs to promote research addressed at evaluating the potential effects on wildlife of the closure of these infrastructures. Such research can assist in the design of suitable conservation measures by considering how the predictability of food sources affects the species that feed on them and by assessing their suitability — in other words, solutions that take into account both pest and non-pest species must be found. To mitigate the potential negative effects for endangered populations of species due to the closure of landfills, other conservation measures such as supplementary feeding should be considered. However, it is worth mentioning that supplementary feeding has also had certain negative impacts on the population dynamics of some endangered species in the form of a decrease in productivity due to a rise in the number of pairs (Carrete, Donázar & Margalida, 2006; Lieury et al., 2015) and to an increase in intraspecific competition (Oro et al., 2008). This conservation tool must thus be used with caution and its effects should be constantly assessed. In our study area, the above mentioned EU legislation has recently been put into practice in at least one landfill and will also be implemented in other landfills in the area in the future. Thus, well designed monitoring and conservation actions are urgently required.

Overall, this study provides highly relevant information for conservation practices given that it shows that human-induced alterations in natural food availability can have meaningful consequences that are not immediately apparent or are difficult to calculate. Such modifications may affect pest species – typically generalists in the wild – whose abundances can cause problems. However, they may also have an impact on certain threatened non-pest species and even determine their distributions and influence other possible factors that have not yet been assessed.

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Supporting information

Table S1 (next page). Explanatory variables used in the environmental analysis. Also given are the name of the variable, its description, the radius at which the analysis was considered (1-km or 8-km radius around nests) and the source of data (INE: Instituto Nacional de Estadística, ARC: Agència de Residus de Catalunya, SB: Servei de Biodiversitat, Departament de Territori i Sostenibilitat, Generalitat de Catalunya, ICGC: Institut Cartografic i Geologic de Catalunya, MCSC: Mapa de Cobertes del Sòl de Catalunya, DEM: Digital Elevation Model). Finally, a hypothesis for the contribution of each variable to the selection of breeding areas (according to Mateo-Tomás & Olea, 2009) is given.

Table S1

	Description	Radius	Source	Hypotheses
Food availability				
Extensive livestock	Number of cows, sheeps and goats in extensive (non-housing cattle) regarding livestock census corrected by the surface of the municipality inside the buffer	8	INE	Livestock is a food resource
Intensive livestock	Number of cows, sheeps, goats, horses, pigs, rabbits and poultry in intensive (housing cattle) regarding livestock census corrected by the surface of the municipality inside the buffer	8	INE	Livestock is a food resource
PAFS distance	Distance to the nearest landfill or supplementary feeding station (m)	8	ARC+SB	Predictable food resource
Topography				
Altitude	Average altitudes of the pixels of DEM (m)	1,8	ICC	Altitude facilitates the presence of potential breeding areas
Roughness	Coefficient of variation of the altitudes of the pixels of DEM (m)	1,8	ICC	Irregularity of the terrain can provide habitat for breeding
Aspect	Surface oriented to the south (between 45-225º)	1,8	ICC	South exposures can provide protection against inclement weather
Landscape and land uses				
Forest	% of land covered by forest	1, 8	MCSC	Dense vegetation makes foraging more difficult
Shrub	% of land covered by shrub	1, 8	MCSC	Dense vegetation makes foraging more difficult
Pasture	% of land covered by pasture	1, 8	MCSC	Open land facilitates foraging
Rocky	% of land covered by rocky	1, 8	MCSC	Rocky terrain can provide habitat for breeding
Water	% of land covered by water	1, 8	MCSC	Water reduces natural habitat for foraging
Urban	% of surface urbanized	1, 8	MCSC	Urbanization reduces natural habitat and can disturb breeding territories
Diversity	Habitat diversity according to Gini-Simpson index: $1-\sum p_i^2$	1,8	MCSC	More diverse areas can provide more diverse sources of food
Human presence				
Paved road	Distance to the nearest paved road (m)	1,8	MCSC	Presence of roads can increase disturbance of breeding territories
Unpaved roads	Length of unpaved roads inside the influence areas (m)	1,8	MCSC	Presence of unpaved roads can increase disturbance of breeding territories
Conspecifics				
Conspecifics presence	Distance to the nearest territory of Egyptian vulture considering all known territories in 2014 (m)	8	own data	Presence of conspecifics can facilitate establishment of other breeding pairs but can also increase interespecific competition for resources

Table S2: Results of an additional GLM analysis performed to test the influence of PAFS, landfills and vulture restaurants separately on territory selection by Egyptian vultures. Model S1 include PAFS, landfills and vulture restaurants along with all other variables included in the 8-km analysis; Models S2 and S3 include landfills and vulture restaurants respectively as well as also environmental variables. In each case there are indicated variables included in the best five models (1 if included, 0 otherwise), variable relative importance weights w + (i) and parameter estimate and standard error obtained from full model averaging. For the best 5 models there are also indicated the number of parameters (k), AICc, Δ AICc and AICc weight.

AICc top models (Model S1)								
	w+(i)	**1**	**2**	**3**	**4**	**5**	**Estimate**	**Std.error**
PAFS	1	1	1	1	1	1	-9.48	4.01
Landfill	1	1	1	1	1	1	5.44	2.68
Vulture Restaurant	1	1	1	1	1	1	5.36	2.50
Intensive Livestock	0.72	1	1	1	0	1	-2.02	1.84
Urban	0.65	0	1	0	1	0	-1.72	1.83
Conspecifics	0.64	1	0	1	1	0	-0.99	1.08
Habitat	0.49	0	1	1	1	1	0.72	1.03
Roughness	0.33	0	0	0	0	0	0.33	0.72
Extensive Livestock	0.32	0	0	0	1	0	-0.27	0.71
k		6	7	7	8	6		
AICc		34.16	34.89	34.93	34.95	34.96		
ΔAICc		0	0.72	0.77	0.78	0.79		
AICc weight		0.08	0.05	0.05	0.05	0.05		

AICc top models (Model S2)								
	w+(i)	**1**	**2**	**3**	**4**	**5**	**Estimate**	**Std.error**
Conspecifics	1	1	1	1	1	1	-1.33	0.52
Intensive Livestock	0.67	1	1	0	1	1	-0.60	0.59
Roughness	0.66	1	0	1	1	1	0.48	0.48
Extensive Livestock	0.30	0	0	0	0	0	-0.10	0.30
Urban	0.28	0	0	0	0	0	-0.03	0.37
Landfill	0.25	0	0	0	1	0	-0.07	0.25
Habitat	0.22	0	0	0	0	1	0.00	0.20
k		4	3	3	5	5		
AICc		52.13	53.40	53.62	54.15	54.50		
ΔAICc		0	1.27	1.49	2.03	2.38		
AICc weight		0.13	0.07	0.06	0.05	0.04		

AICc top models (Model S3)								
	w+(i)	**1**	**2**	**3**	**4**	**5**	**Estimate**	**Std.error**
Conspecifics	1	1	1	1	1	1	-1.36	0.53
Intensive Livestock	0.68	1	1	0	1	1	-0.62	0.60
Roughness	0.65	1	0	1	1	1	0.48	0.48
Extensive Livestock	0.29	0	0	0	0	0	-0.10	0.29
Urban	0.26	0	0	0	0	0	-0.01	0.34
Vulture restaurant	0.23	0	0	0	1	0	0.04	0.26
Habitat	0.22	0	0	0	0	1	0.00	0.19
k		4	3	3	5	5		
AICc		52.13	53.40	53.62	54.31	54.50		
ΔAICc		0	1.27	1.49	2.18	2.38		
AICc weight		0.14	0.07	0.06	0.05	0.04		

Table S3: Additional results obtained with GLM 1km analyses. There are indicated the variables included in each model (1: Roughness, 2: Aspect, 3: Rock, 4: Habitat, 5: Urban), the number of parameters (k), AICc, Δ AICc and AICc weight obtained with the confidence model set (cumulative weights above 95%).

Variables	k	AICc	Δ AICc	AICc weight
12345	6	46.79	0.00	0.55
1235	5	48.14	1.35	0.28
2345	5	49.67	2.89	0.13
235	4	51.91	5.12	0.04

Table S4: Additional results obtained with GLM 8 km analyses. There are indicated the variables included in each model (1: Roughness, 2: PAFS, 3: Conspecifics, 4: Extensive, 5: Intensive, 6: Habitat, 7: Urban), the number of parameters (k), AICc, Δ AICc and AICc weight obtained with the confidence model set (cumulative weights above 95%).

Variables	k	AICc	Δ AICc	AICc weight
1235	5	48.67	0.00	0.10
235	4	48.69	0.02	0.10
1237	5	48.95	0.28	0.08
2345	5	50.35	1.68	0.04
127	4	50.37	1.70	0.04
12357	6	50.84	2.16	0.03
123	4	50.95	2.27	0.03
2356	5	50.95	2.27	0.03
12356	6	50.95	2.28	0.03
12347	6	51.15	2.48	0.03
2357	5	51.16	2.48	0.03
12345	6	51.24	2.57	0.03
12367	6	51.51	2.84	0.02
125	4	51.56	2.89	0.02
2347	5	51.61	2.93	0.02
25	3	51.98	3.30	0.02
135	4	52.13	3.46	0.02
1267	5	52.33	3.66	0.02
237	4	52.34	3.67	0.02
1257	5	52.35	3.68	0.02
23456	6	52.46	3.79	0.01
234	4	52.73	4.06	0.01
1247	5	52.74	4.07	0.01
1236	5	52.78	4.11	0.01
23457	6	52.81	4.14	0.01
1234	5	52.99	4.32	0.01
23	3	53.08	4.41	0.01
1256	5	53.09	4.42	0.01
123567	7	53.15	4.48	0.01
35	3	53.40	4.73	0.01
123457	7	53.41	4.74	0.01
23567	6	53.54	4.86	0.01
123456	7	53.59	4.91	0.01
13	3	53.62	4.95	0.01
123467	7	53.70	5.03	0.01
256	4	53.72	5.05	0.01
12567	6	53.75	5.08	0.01
257	4	53.93	5.26	0.01
1245	5	54.01	5.34	0.01
23467	6	54.01	5.34	0.01
245	4	54.10	5.42	0.01
236	4	54.22	5.55	0.01
1356	5	54.51	5.83	0.01
137	4	54.52	5.85	0.01
1345	5	54.54	5.87	0.01
345	4	54.56	5.89	0.01
1357	5	54.57	5.89	0.01
12467	6	54.60	5.92	0.01
27	3	54.60	5.93	0.01
2367	5	54.69	6.02	0.00
247	4	54.77	6.09	0.00
234567	7	54.88	6.20	0.00
357	4	54.92	6.24	0.00
12457	6	54.92	6.24	0.00
2346	5	54.95	6.28	0.00

CAPÍTOL 3

Assessing the applicability of stable isotope analysis to determine the contribution of landfills to vultures' diet

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Resum

Les activitats humanes provoquen canvis a l'ambient que afecten als recursos disponibles per les diferents espècies. L'augment de població humana en ciutats ha comportat grans acumulacions de residus que són dipositats en abocadors, instal·lacions que han esdevingut una nova font de recursos per espècies plaga o amenaçades com els voltors. En aquest estudi, vam utilitzar l'anàlisi d'isòtops estables (AIE) i la identificació convencional de restes d'alimentació de l'aufrany (*Neophron percnopterus*) per a avaluar la aplicabilitat de l'anàlisi d'isòtops com una eina per a determinar la composició de la dieta dels voltors. Ens vam centrar en una població en expansió d'aufrany situada al NE de la Península Ibèrica per tal de determinar la contribució dels abocadors i la ramaderia en la dieta de l'espècie, amb l'objectiu de reduir els biaixos associats a la identificació convencional de restes. Vam comparar les proporcions de dieta obtingudes amb els models isotòpics mixtos i l'anàlisi convencional de cinc preses principals. El major acord entre els dos mètodes es va donar en les categories 'abocadors' i 'ocells' i les diferències més grans entre els resultats dels dos mètodes van ser en les categories 'ramaderia', carnívors' i 'herbívors salvatges'. Tot i la incertesa associada als models de l'AIE, els resultats mostraren que aquest mètode pot ajudar a distingir entre animals que s'alimenten de residus i per tant presenten nivells més elevats de $\delta^{13}C$, d'aquells que s'alimenten al camp. De fet, en algunes parelles reproductores es va detectar una elevada proporció d'aliment procedent d'abocadors (prop d'un 50%). A més, vam dur a terme anàlisis GLMM que mostraren que valors elevats de $\delta^{13}C$ a les plomes d'aufrany (indicador d'alimentar-se en abocadors) estan relacionats amb nivells alts d'urbanització dels territoris. Aquest mètode té el potencial de ser aplicat a altres espècies de voltors amenaçats dels quals existeix poca informació sobre els recursos que estan consumint, essent especialment important degut a que les principals causes del declivi dels voltors a nivell mundial estan relacionades amb el consum i la disponibilitat d'aliment.

Abstract

Human activities cause changes to occur in the environment that affect resource availability for wildlife. The increase in the human population of cities has led to a rise in the amount of waste deposited in landfills, installations that have become a new food resource for both pest and threatened species such as vultures. In this study we used stable isotope analysis (SIA) and conventional identification of food remains from Egyptian Vultures (*Neophron percnopterus*) to assess the applicability of SIA as a new tool for determining the composition of the diets of vultures, a group of avian scavengers that is threatened worldwide. We focused on an expanding Egyptian Vulture population in NE Iberian Peninsula to determine the part played by landfills and livestock in the diet of these species, and aimed to reduce the biases associated with conventional ways of identifying food remains. We compared proportions of diet composition obtained with isotope mixing models and conventional analysis for five main prey. The greatest agreement between the two methods was in the categories 'landfills' and 'birds' and the greatest differences between the results from the two methods were in the categories 'livestock', 'carnivores' and 'wild herbivores'. Despite uncertainty associated to SIA, our results showed that stable isotope analysis can help to distinguish between animals that rely on waste and so present enriched levels of C than those that feed on the countryside. Indeed, a high proportion of food derived from landfills (nearly 50%) was detected in some breeding pairs. Furthermore we performed GLMM analyses that showed that high values of δ 13C in Egyptian Vulture feathers (a proxy of feeding in landfills) are related with high levels of humanization of territories. This method has the potential to be applied to other threatened vulture species for which there is a lack of information regarding resources they are consuming, being especially important as the main causes of vultures decline worldwide are related to the consumption and availability of food resources.

Keywords: conventional diet analysis, Egyptian Vulture, Neophron percnopterus, rubbish dumps, scavengers and threatened species.

Introduction

Human activities have greatly transformed the Earth's land surface. Natural landscapes have been altered irremediably by human use and management practices in many human-dominated lands are constantly changing (Foley et al., 2005). During this transformation of the landscape, humans have modified the natural resources available to wild species with serious implications for their population dynamics and distributions (Donazar et al., 1993; Blanco, Tella, & Torre, 1998; Laiolo et al., 2004).

The increase in the human population in cities has led to an accumulation of large quantities of waste, which is deposited in open-air landfills scattered throughout the countryside (Oro et al., 2013). These sites provide plentiful and predictable food resources for many generalist or scavenger species such as gulls, raven, fox and rats that are able to feed there. In some cases, these species have undergone a rapid population increase as a result (Bosch, Oro, & Ruiz, 1994; Boarman et al., 2006; Duhem et al., 2008; Bino et al., 2010; Navarro et al., 2016). However, resources derived from landfills are also used by other species that are not regarded as pests, some of which are classified as threatened (Viada, 1994; Martina & Gallarati, 1997). Although it is known that certain species of conservation concern use landfills as feeding sites (Ciucci et al., 1997), few studies have quantified this use (Ceballos & Donázar, 1990).

Over the last 20 years in the Iberian Peninsula the sight of large groups of vultures feeding in landfills has become commonplace (Martínez-Abraín et al., 2012) and it has been suggested that these installations are today a key source of food for certain vulture populations. Moreover, vultures also feed in other resources that could act as a threat, as poisoned animals. The consumption of these resources has caused in recent decades the crash of vulture populations in Asia and, more recently, in many parts of Africa (Oaks et al., 2004; Koenig, 2006). The generalized use of veterinary drugs on livestock, pesticides, or intentional poisoning by poachers have pushed some raptor species that just two decades ago were common worldwide to the brink of extinction (Margalida et al., 2014; Ogada et al., 2016). Indeed, vultures are considered today to be one of the most threatened groups of birds worldwide (IUCN Red List).

Despite these human-driven changes in resource distribution are carrying serious behavioural and demographic responses of vultures populations, our knowledge of the effect of these novel scenarios on vulture diets is still limited (but see

Mundy et al., 1992; Donázar, 1993; Margalida, Bertran, & Heredia, 2009; Milchev, Spassov, & Popov, 2012). This is partly due to complexity to quantify the net contribution in terms of ingested or assimilated biomass in this group of birds. In raptors, most conventional diet studies are based in the analysis of food remains or pellets sampled at nests or resting sites (Real, 1996; Donázar, Cortés-Avizanda, & Carrete, 2010; Margalida et al., 2012). These method allows the identification of prey at a high taxonomic accuracy (Hidalgo et al., 2005; Milchev, Spassov, & Popov, 2012). However, many vulture species such as those of *Gyps* genus may ingest large amounts of meat from corpses from big sized animals that may contribute scarcely to food sampled remains. In addition, species that use to feed on smaller species, such as Egyptian vultures, commonly carry to nests pieces from large prey while only a small fraction of it was feed; all these making challenging to stablish a correspondence between sampled food remains and ingested biomass. Consequently, the potential biases that have been recognized for conventional diet analysis, such as those linked to prey size or digestibility (Real, 1996; Margalida et al., 2007), might be present in available quantitative assessments of diet composition in vultures.

As an alternative, stable isotope analysis (SIA) can provide a representation of the food digested and absorbed by an animal (Hobson & Clark, 1992). In this field, recent Bayesian isotopic mixing models have been developed to generate potential dietary solutions for multiple dietary sources and to account for uncertainty and variation in model estimates (Parnell et al., 2010), and has been widely used to infer the diet composition of a large range of organisms (Ramos et al., 2009; Resano-Mayor et al., 2014; Payo-Payo et al., 2015). This method has been proven useful for determining the part played in animals' diets of important food resources such as wild herbivores or food from landfills (Weiser & Powell, 2011; Resano-Mayor et al., 2014). In spite of this, SIA has been very little used to study the diet of vultures (but see Chamberlain et al., 2005; Blázquez et al., 2016). Thus, it would appear to be a promising tool for investigating vulture diets under the changing scenarios in which vultures live and may be useful as an indicator of on-going environmental changes.

Nevertheless, SIA is not exempt of limitations, as it requires accurate prior information regarding the trophic ecology of the studied species and results usually present a wide range of uncertainty (Phillips et al., 2014; Robinson, Franke, & Derocher, 2018). Incorporating prior information obtained from the analysis of stomach in SIA has been shown to provide very reliable results (Polito et al., 2011). However, this method requires the regurgitation of ingested food or the death of the animal, so its applicability is very limited when working with species whose individuals are difficult to capture and cannot be sampled repeatedly in time, an issue particularly concerning in endangered species. In addition, despite the great popularity of SIA in ecological studies, formal comparisons between SIA and conventional methods of diet analysis are still limited (but see Weiser & Powell, 2011; Resano-Mayor et al., 2014). In that context where more available and feasible methodologies present some limitations, providing quantitative assessments of diet composition of vultures based on the most easily applicable methods is crucial to better understand their potentialities and the possible differences between these methods.

This study focused on the diet of the Egyptian Vulture *Neophron percnopterus*, a species located at the top of the food web and therefore a good indicator of mature ecosystems. This particularity, together with the fact that it is sensitive to human activities, makes it a good indicator of environmental changes (Margalida, García, & Avizanda, 2007; Zuberogoitia et al., 2008; Tauler-Ametller et al., 2017). We used both conventional diet analysis and SIA to infer the diet of this endangered vulture, we assumed that if both methodologies agree, then both methods would be providing a good approximation of resources consumed. Unlike the negative global trend in the species (BirdLife International, 2016), the study population in Catalonia (NE Iberian Peninsula) has grown over the past 20 years, and has expanded and colonized new highly humanized areas from where it was not known before (Tauler et al., 2015). This process has happened in parallel with the increase in open-air landfills that has contributed to a higher probability of recruitment of breeding pairs in the area as well as to a lesser probability of territory disappearance (Carrete et al., 2007; Tauler-Ametller et al., 2017). The main objective of our study was to determine the part played by landfills in the diet of the Egyptian Vulture breeding pairs, using conventional identification of food remains and stable isotope analysis. We thus hypothesized that breeding pairs found in humanized areas close to landfills are feeding on these facilities and therefore that the contribution of resources from waste tips in their diet will be high.

Materials and methods

Study area and data collection

The study area lies in central eastern Catalonia (NE Iberian Peninsula, Spain) at altitudes of 200–1900 m a.s.l., and encompasses an area of cliffs running from the Prelitoral Mountains in the south to the pre-Pyrenean Mountains in the north (Fig. 1). It includes humanized zones with high human population density (more than 150 hab/km^2), as well as less populated rural areas (13 hab/km^2). Due to this dense human population there are nine landfills within the study area that receive municipal waste, which, before being processed, is exposed and available for different opportunistic species.

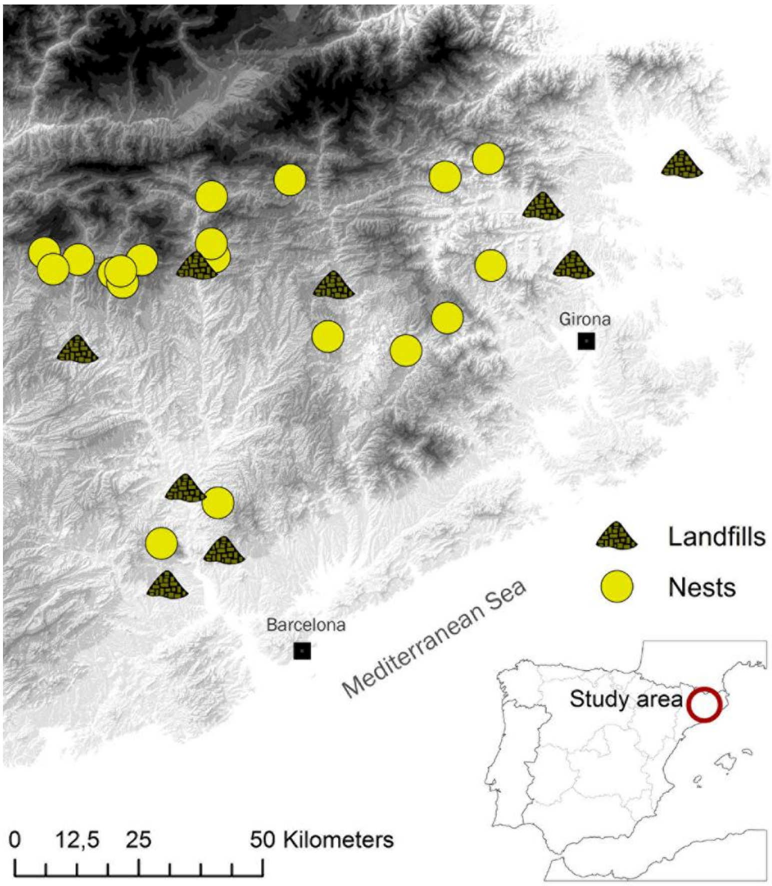

Figure 1. Distribution of breeding pairs (yellow circles) and landfills (green symbols) located inside our study area and considered for diet analysis. Grey gradient represent altitudes from 0 m (white) to 3000 m (black). Two important cities are also represented.

In 2012–2015, all occupied Egyptian Vulture territories were visited during the breeding season (March–August) by observers equipped with a spotting scope (20–60x). Once nestlings were approximately 45–55 days old, they were caught with the assistance of experienced climbers and three/four mantle feathers from each individual were taken for sampling by SIA (2012–2015; n=60 chicks from 19 territories). Food remains for conventional diet analysis were collected in two moments during reproduction: (i) from the nests when the nestlings' feathers were sampled and (ii) after the breeding season at the same nests (2012–2014; n=32 samples from 18 territories). Both two samples from each nest and year were considered as one single statistical observation for diet analysis. Only data of successful breeding pairs was considered for analysis in order to results were comparable.

Conventional analysis

Food components were identified to the lowest possible taxonomic level by consulting collections in the Barcelona Museum of Natural Sciences, the Centre de Recursos de Biodiversitat Animal (Biology Faculty, Barcelona University), the collection of one of the authors (J. Real) and a reference guide (Brown et al., 2003). Counts of items were carried out following (Milchev, Spassov, & Popov, 2012). Numbers of invertebrates and small- and medium-sized vertebrates were recorded as a minimum number of individuals. Carnivores and ungulates were counted as the number of bones or skeletal fragments as it was impossible to determine with precision the number of whole individual specimens for most species. The three distal parts of the limbs, i.e. the tarsus/carpus, metatarsus/metacarpus and digits associated with other smaller bones, were recorded as one skeletal fragment. The same applied to skull fragments, which usually consisted of several connected bones.

Food components were categorized into six groups: landfill, livestock, wild herbivores, carnivores, birds and others (mainly micro-mammals, reptiles, amphibians and fish). To differentiate between extensive livestock remains and landfill remains, cut bones or bones with traces of butchery or cooking were assumed to have been obtained from landfills (Milchev, Spassov, & Popov, 2012), while the rest of the livestock remains were assumed to have been found as extensive livestock carcasses in the field. We believe this assumption is plausible, first, because due to the strict legislation and controls of intensive farms existing in Central Catalonia, it is very unlikely to find carcasses of intensive livestock abandoned in the field or near farms. Second, because despite there are some supplementary feeding points in our study area, most of them were no longer active during our study period. Also, they were mostly intended to the Bearded Vulture, so the remains that were provided were goat and sheep limbs. This type of remains are difficult to carry to the nests by Egyptian vultures as they use the beak and, consequently, the size of remains is not the optimal for this species. In addition, the period when remains were provided in these feeding points for the Bearded Vulture was from November to April or beginning of May (Catalan Government data, personal communication). In our study population chicks are born at the end of May or June, so it is very unlikely that these remains could be found in sampled nests.

In the case of remains of *Oryctolagus cuniculus* and *Sus scrofa* we classified them as landfill origin when they had signs of butchery, while the rest were considered wild individuals. Regarding hair remains, white rabbits were considered domestic as there are not white rabbits in the wild and hair samples of brown rabbits were classified using a collection that allowed us to identify their origin according to their length (wild rabbits present longer hair than domestic). In the case of pigs, in our study area there is not black Iberian pig in farms so we assumed that black hair was from wild boar. When we had doubts about its origin, we classified it as undetermined.

Stable isotope analysis

Nestling feathers were first cleaned in a solution of NaOH (0.25 M) and oven-dried at 40°C for 24h. Feathers were then ground into a fine powder and subsamples of 0.35 mg were loaded into tin receptacles for combustion. Isotopic measurements were performed at the Scientific and Technological Centres of the University of Barcelona using the methods described in Resano et al., (2011). Stable isotope ratios are reported as δ values and expressed in ‰, according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Rstandard is the ratio of the international standards: Pee Dee Belemnite (PDB) for ^{13}C and atmospheric nitrogen (AIR) for ^{15}N. Measurement precisions for $\delta^{13}C$ and $\delta^{15}N$ were ≤ 0.15 ‰ and ≤ 0.25‰.

Isotope analysis can differentiate sources of food given that organisms reliant on plants that use a C4 photosynthesis pathway have higher (more enriched) stable carbon isotope ratios than those from food webs reliant on plants that use a C3 pathway (Wooller et al., 2007). This relationship is useful in studies of livestock species since it can differentiate between cattle raised on intensive farms that are fed with C4 plants (mainly corn) and cattle raised extensively feeding on pastures (Schmidt et al., 2005). In our study area (Mediterranean and pre-Pyrenean mountains), the species that live in extensive are those aimed to meat production and, as the climate allows it, they graze in pastures during all the year without any supplement based in corn (personal communication from farmers of our study area). More, most plant species that cover pasture lands (*Brachypodium, Festuca, Lolium, Agropyron, Koeleria, Oryzopsis, Molinia, Anthoxanthum, Holcus, Dactylis*) use a C3 pathway (Osborne et al., 2014; De Bolòs & Vigo, 2015) so we assumed that C4-type isotopic signature (reflected in more enriched stable carbon isotope ratios) detected in Egyptian Vulture tissues will be due to food obtained from the landfills where humans dispose of waste from cattle bred intensively.

The isotopic signatures of muscle of prey items were obtained from the bibliography, considering main prey determined by conventional analysis and collecting values of our study area or from other regions with similar climatic conditions when possible. Landfill isotopic values were obtained from isotopic analysis of regurgitates composed of meat (mainly chicken and pork) of *Larus michaellis* feeding on a landfill inside our study area where Egyptian Vultures have been observed foraging (Ramos et al., 2009; Institut Català Ornitologia, 2016). These values were coherent with isotopic values of livestock animals raised in intensive and fed with corn in other areas (González-Martin et al., 1999; Heaton et al., 2008; Osorio et al., 2011). Extensive livestock isotopic values were obtained from beef and lamb fed with C3 plants (Schmidt et al., 2005; Perini et al., 2010); the wild herbivores analysed were *Oryctolagus cuniculus* (Resano-Mayor et al., 2014) and *Sus scrofa* (Nadjafzadeh, Voigt, & Krone, 2015). To account for the diet fraction containing carnivores, isotopic signatures of *Vulpes vulpes* were used (Nadjafzadeh, Voigt, & Krone, 2015). Finally, the isotopic signatures of *Columba palumbus* and other passerines (Corvidae, Sturnidae and Turdidae) were taken from Resano-Mayor et al., (2014). To obtain the isotopic value of categories that included more than one prey item (livestock, wild herbivores and birds), the mean and variances of $^{13}\delta C$ and $^{15}\delta N$ were estimated by assuming stratified random sampling and giving equal weight to each prey type in a given category (Williams, Nichols, & Conroy, 2002) (Table 1). The mixing model considered that all food sources were taken into account, which means that there was no 'others' category.

Table 1: Isotopic values of each source of food considered in the Bayesian isotopic mixing models. For each source, it is indicated the mean of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ and the standard deviation. The area and bibliographic reference of the study of data collection are also specified.

Source	$\delta\ ^{13}C$	SD $\delta\ ^{13}C$	$\delta\ ^{15}N$	SD $\delta\ ^{15}N$	Area	Reference
Landfills	-21.67	1.44	5.5	1.74	Catalonia	Ramos et al., 2009
Livestock	-25.68	0.19	6.07	0.40	Ireland and Italy	Schmidt et al., 2005; Perini et al., 2010
Wild herbivores	-24.22	1.17	2.54	1.63	Germany Catalonia	Resano-Mayor et al., 2014; Nadjafzadeh, Voigt, & Krone, 2015
Carnivores	-24.60	0.70	9.00	2.30	Germany	Nadjafzadeh, Voigt, & Krone, 2015
Birds	-23.67	0.57	6.40	1.07	Catalonia	Resano-Mayor et al., 2014

The Trophic Enrichment Factor (TEF) is necessary to perform stable isotope analysis, as the isotope ratios of a consumer tissue are usually different from their diet due to biochemical processes when proteins are digested, absorbed, and then re-arranged for new tissue synthesis. This is the first-ever analysis of the diet of Egyptian Vultures using isotope analysis and also the first of any vulture from the Accipitridae family. Thus, there are no reference values available in the literature since Egyptian Vultures consume different resources or are taxonomically distant from other species. To solve this shortcoming, we used the SIDER package from R software, which estimates the TEF of species with unknown enrichment factors by taking into account phylogeny, tissue type sampled, general type diet (in this case we treated our study species as a carnivore), isotopic signature of the food source and measurement error (Healy et al., 2017). To perform the SIDER model, the values of $\delta\ ^{13}C$ and $^{15}\ \delta\ N$ for the food sources obtained from the literature (see above) were used. TEF values obtained with SIDER model and used in mixing models were 1.11 ‰ ±1.12 for $\delta\ ^{13}C$ and 3.33 ‰ ±1.18 for $\delta\ ^{15}N$.

Once we had calculated the isotopic mean values and the standard deviations of the nestlings' feathers, prey sources and TEF, we performed Bayesian isotopic mixing models using the SIMMR package from R software (Parnell, 2016) to estimate the relative contribution of each prey type to the diet of the Egyptian Vulture. Models were built over four Markov chains with 10000 steps per chain with a burn in of 1000 iterations. Each nest and year was considered as a single statistical observation by estimating the mean isotopic values of sampled siblings.

Statistical analyses

To assess the agreement between the conventional and stable isotope analyses we performed three different test. First, the weighted Kappa statistic (Kw) was used to compare methods on an ordinal scale by ranking prey categories from higher to lower levels of consumption. Secondly, an intra-class correlation coefficient (ICC) was used to test for the agreement of the two methods in the quantitative prey consumption estimates. We performed a third analysis that took into account uncertainty of prey estimations obtained with mixing models. We used simulated values obtained with mixing models (selecting 1000 random vales for each nest) and calculated the difference between each simulated value and the proportion of each prey obtained with conventional methods for each territory and year. After, we calculated the proportion of these difference values that were below 0 in order to study the distribution of the probability that the estimates of prey consumption by mixing models would be greater than the estimates by conventional methods. We assumed that if there were no differences between methods, the distribution of this probability for each prey category would be symmetrical and would have median values close to 0.5. As much as the distribution would be skewed towards 1 or 0 the methods would be providing different estimates of consumption for the food resource in question.

Also, we reported mean differences between methods by subtracting the consumption estimates obtained with conventional analyses from the predicted consumption obtained with the SIA (Weiser & Powell, 2011). Only data from 2012–2014 was used to perform the comparisons as there was no information for the conventional analysis from 2015.

Finally, as $\delta^{13}C$ on animal tissues reflects the consumption of C3 or C4 plants and thus if they had fed on intensive (ie landfills) or extensive livestock (Schmidt et al., 2005), we assessed whether isotopic values of $\delta^{13}C$ of Egyptian vulture feathers were correlated with the availability of food and the level of humanization in our study area.

We performed Generalized Linear Mixed Models (GLMM) to account for the potential non-independence of clustered observations from the same territories and years. We performed four different models, all of them with $\delta^{13}C$ as a response variable. The first two models considered food availability as explanatory variables, using distance to landfill as a proxy of the access of vultures to landfills resources (Model 1) and the number of cattle in extensive farming present in the territory (taken from the municipal census) and potentially available to vultures (Model 2). The other two models used urban surface (Model 3) and forest surface (Model 4) with an 8-km-radius buffer area around the nest also as explanatory variables and as a proxy of the level of humanization of territories (Table 2). We chose these variables as they can simply describe if territories are more urban or rural and so, they could provide information of the typology and distribution of food in the environment. For more details of the explanatory variables, see Tauler-Ametller et al., (2017). Territory and year were included in all four models as random factors.

All models were compared with the null model that did not consider the explanatory variable using a Likelihood Ratio Test and we report the level of significance but also AICc of each model. GLMM were performed with the lmer function from the lme4 package of R software.

Results

Diet estimates

Using conventional analyses we identified 1483 prey items corresponding to at least 62 different species. According to this methodology, the diet of the study population was composed of (mean ± SD) 30.46% (± 18.96) livestock, 19.85% (± 11.09) wild herbivores, 18.12% (± 11.64) resources from landfills, 17.45% (± 10.70) birds, 9.33% (± 13.71) carnivores and 4.79% (± 3.66) others.

According to stable isotope mixing models the diet was composed of 15.52% (± 5.86) livestock, 15.82% (± 11.87) wild herbivores, 26.04% (± 5.03) resources from landfills, 19.79% (± 3.52) birds and 22.84 % (± 11.88) carnivores (Fig. 2). Isotope mixing models for diet inference converged.

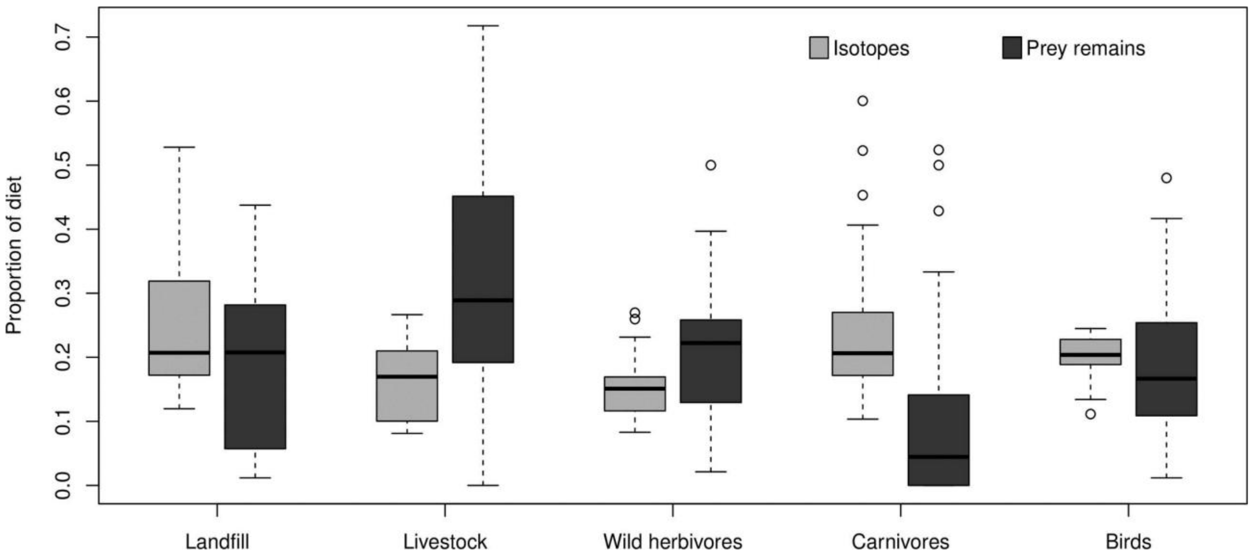

Figure 2. Proportion of diet of Egyptian Vulture represented by main prey categories obtained by stable isotope analysis and conventional identification of food remains. Medians, quartiles and outliers of each prey category are represented in the boxplot.

The arithmetic mean isotopic values (±SD) for 60 Egyptian Vulture nestlings were -21.97 (± 1.088) for δ^{13}C and 10.08 (± 1.653) for δ^{15}N. Isotope biplots (δ^{13}C, δ^{15}N) showed that Egyptian Vulture nestlings lay within the space delineated by the main prey categories previously corrected by TEFs (Fig. 3).

Individual estimates for diet according to isotope mixing models predicted a high degree of individual variability in regard to resources derived from landfills (Fig. 4). Although there was a large degree of overlap in confidence intervals, the results indicate that a number of breeding pairs obtain an important proportion of their diet from landfills and that these pairs are likely to consume fewer livestock resources. Livestock in general is represented in lower proportions than landfills but still plays a significant role, especially in territories where the consumption of prey from landfills is lower (Supporting Information, Fig. S1).

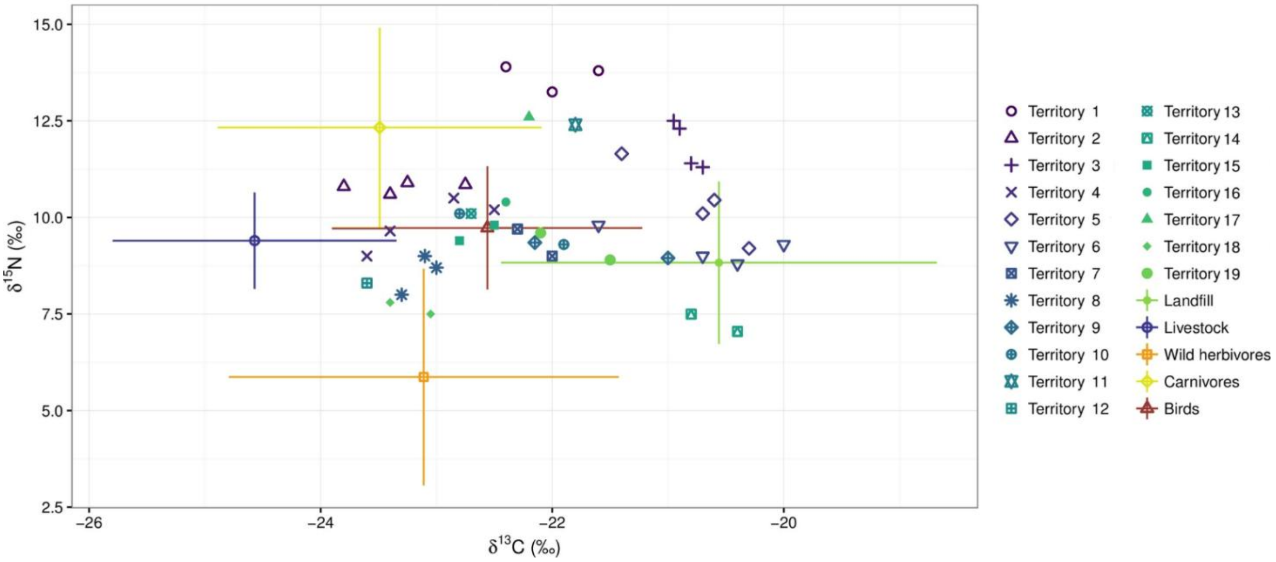

Figure 3. Isotopic values (δ ^{13}C and δ ^{15}N) of Egyptian Vulture nestlings in Catalonia (n=60) and main sources of food (mean ± SD). Same symbols correspond to the isotopic values of nestlings from the same territory but different years.

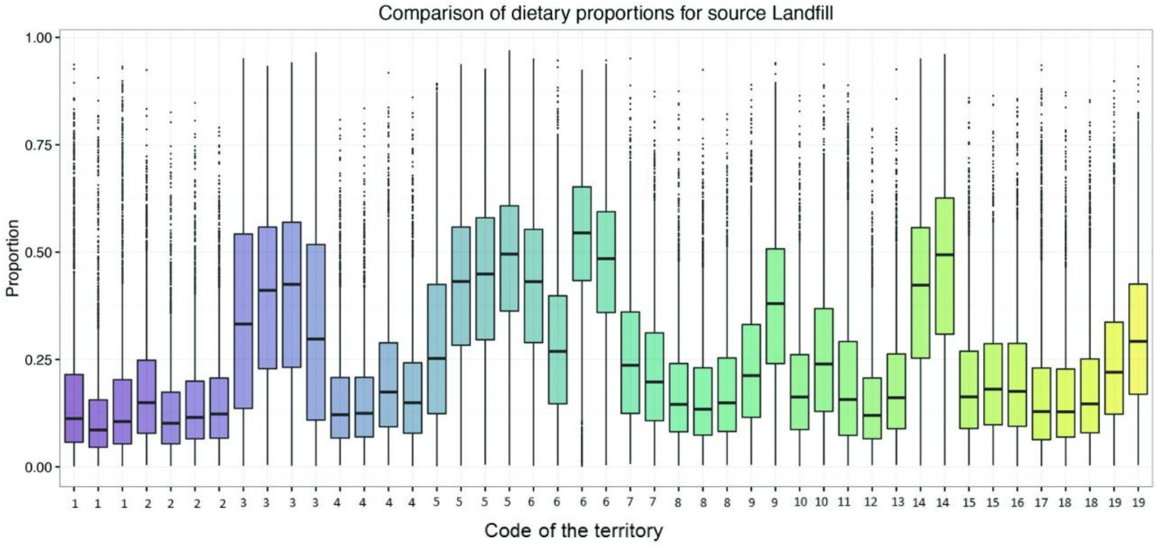

Figure 4. Percentage of the contribution of landfills in the diet of Egyptian vulture nestlings estimated with Bayesian mixing models (SIMMR). Data of each territorial pair but different year is represented with the same number. Boxes represent the credible interval of 50% and error bars the credible interval of 95% obtained with SIMMR.

Comparison of conventional methods and isotope mixing models

When ordering prey categories from higher to lower levels of consumption, the Kappa test found good agreement for 'landfills' (Kw=0.564, P<0.001) and 'birds' (Kw= 0.255, P=0.0129) but disagreement for the rest of prey sources. The ICC test only found agreement between prey remains counts and SIAR estimates for landfills (ICC=0.565, P<0.001) and no significant agreement between methods for all other categories. According to the test that incorporated uncertainties of mixing models, we obtained symmetrical distribution of probabilities of differences below 0 and median values close to 0.5 for landfills (0.65) and birds (0.48) categories, but asymmetrical distribution of probabilities and median values far from 0.5 for livestock (0.07), wild herbivores (0.21) and carnivores (0.89) (Fig.5a). This results supported findings obtained with Kw and ICC test, showing that landfills and birds were the less biased categories and livestock, wild herbivores and carnivores presented more disagreement between methods. Finally, regarding the mean differences obtained for each prey using the different methods (Fig. 5b) in accordance with all performed tests, there were fewest differences for the categories 'landfill' and 'bird', followed by the 'wild herbivores'. Most disagreement was observed in the categories 'livestock' and 'carnivores'.

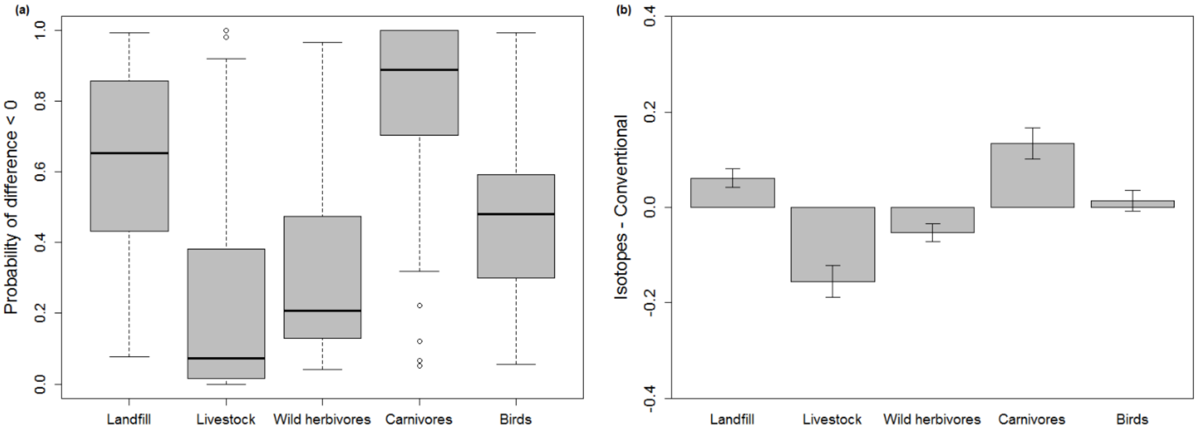

Figure 5. Results of comparison of conventional methods and isotope analysis for the diet of Egyptian Vulture in Catalonia during the period 2012-2014. (a) Probability of difference below 0 between stable isotope analysis and conventional analysis for main prey of Egyptian Vulture. (b) Mean differences between stable isotope analysis and conventional methods in estimates of the main prey of Egyptian Vulture. Standard errors of the differences are represented.

δ ^{13}C, food availability and level of humanization

According to the GLMM analyses, there was no significant relationship between availability of food expressed as distance to landfill and extensive livestock availability and δ ^{13}C of feathers of Egyptian Vulture chicks (Model 1 P = 0.0928 and Model 2 P = 0.3308). However, our results revealed a positive relationship between δ ^{13}C and the level of humanization reported with urban surfaces (Model 3, P = 0.0009) being most urbanized areas those with higher levels of δ ^{13}C in Egyptian vulture feathers. Consequently, δ ^{13}C was negative correlated with rural areas reported with forest surface (Model 4, P = 0.0002) (Table 2).

Table 2. Design and results of the four Generalized Linear Mixed Models performed to relate δ ^{13}C (Response variables) with food availability and the level of humanization (Explanatory variables). There are indicated parameter estimates and standard errors (SE), AICc of each model, AICc of null model and also P Value obtained with the ANOVA performed between null and full model.

Model	Response variable	Explanatory variables	Estimate	SE	AICc	AICc null	P Value
1	δ ^{13}C	Landfill proximity	-6.31E-05	3.57E-05	104.31	104.60	0.0928
2	δ ^{13}C	Extensive livestock	-4.45E-05	7.10E-05	106.74	104.60	0.5336
3	δ ^{13}C	Urban areas	0.25	0.06	96.10	104.60	0.0009
4	δ ^{13}C	Rural areas	-0.06	0.01	93.13	104.60	0.0002

Discussion

In this study we assessed the role played by landfills in the diet of Egyptian Vultures, as well as the usefulness of SIA as a tool for determining vulture diets. Both conventional analysis of food remains and SIA are the most easily applicable methods to quantify diet composition of vultures, though both of them are known to show some limitations (Real, 1996; Votier et al., 2003; Phillips et al., 2014; Robinson, Franke, & Derocher, 2018). So far, no comparisons have ever been made between isotope and conventional analyses of a scavenger species at the top of the food web. Here, we assumed that the agreement between methods for certain food resources consumption would support the idea that the methods are reliably approximating to the real consumption of those resources and, in this sense, we found that both types of analyses provide similar contributions of food from landfills in this species' overall diet. Importantly, our results also suggest that landfills are a major food resource for the Egyptian Vulture, thus highlighting that human waste, besides its strong impact on populations of generalist abundant species, may be also of great importance for populations of endangered species able to exploit this type of resources.

Comparison of conventional methods and SIA

By comparing the most easily applicable methods to describe the diet of scavenger bird species we provide valuable information to understand its potential biases and constraints, an issue particularly concerning in our study group for which most available diet assessments where done by conventional diet analysis (Donazar & Ceballos, 1988; Hidalgo et al., 2005). We grouped food resources into five categories and we considered three approaches to evaluate the agreement between methods.

Our results revealed that the two methods showed good agreement for two of the considered categories: ‘landfills’ and ‘birds’. Consequently, we suggest that that isotope analysis can be used as an approximation of the consumption of food from landfills in vultures, a question of major interest in our study. In contrast, the categories that were least in agreement were ‘livestock’, ‘carnivores’ and ‘wild herbivores’. It is likely that conventional methods overestimate ‘livestock’ importance since cattle bones are usually large, easy-to-identify and remain in the nest for long periods of time (Margalida et al., 2007). In the case of wild herbivores, despite there is less disagreement between methods, it is possible that there were also overestimated by conventional methods due to the easy identification and long permanence of bones in the nest. On the other hand, it is possible that carnivores contribute substantially to the diet of Egyptian vulture as they are frequent roadkill (Hidalgo et al., 2005; Dobrev et al., 2015). However, these high levels of N detected in some chicks could be due to the use of fertilizers or animal waste products that can increase the levels of N in the environment and so arise N levels in Egyptian Vulture’s prey (Hebert & Wassenaar, 2001).

In spite of the observed mismatches between methods, uncertainty in parameters estimates provided by SIA were quite wide and, consequently, the percentages of diet provided by conventional methods fell within the credible intervals provided by SIA for all categories (see Figure 5).

Thus, our results show that both methods present limitations to describe de diet of the Egyptian Vulture mainly due to its wide range of prey consumed from different sources (Robinson, Franke, & Derocher, 2018). Nevertheless, the combination of both methods seems to provide complementary results. On the one hand conventional methods allow the identification of prey at a high taxonomic accuracy and this information of prey consumed is necessary to be incorporated in SIA. However, the relationship between the type and amount of a given prey that is consumed and the type and amount of a given prey that is quantified from nest remains has not been yet performed, meaning that the magnitude and direction of potential biases in conventional analysis for vulture species is unknown yet.

On the other hand, and as is usual in stable isotope studies of animal diets, our mixing models were based on several assumptions. First, we used bibliographical isotopic values of prey sources. A major constraint in isotopic analysis is to obtain representative samples of consumed prey at the time that the food resource is used, since spatial and temporal variation in isotopic values have been detected for some species (Dalerum & Angerbjörn, 2005; Nadjafzadeh, Voigt, & Krone, 2015). Our study species shows a very wide dietary range and we studied it for a fairly large area and time period, so it would have been impossible to sample all potential food sources. Therefore, and according to our aims, we considered the five main sources represented by dominant species according to conventional analysis and selected those values in the literature with the highest geographic, ecological and climatic similarities. In addition, the isotopic values of food resources obtained from other areas corresponded to species or prey types that show low variation in isotopic values across large geographic ranges (Piasentier et al., 2003; Boner & Förstel, 2004; Jose M. Moreno-Rojas et al., 2010; Russo et al., 2017), which suggest that the effect of having chosen those values was small in our main results.

Second, we estimated TEF values using SIDER models as we had no values of a species phylogenetically close and ecologically similar to the one we are interested in. As a result, uncertainty of TEF values was high. However, it is more correct to use TEF values with large uncertainty than use values from other species with less uncertainty but probably wrong (Healy et al., 2017). Overall, any potential biases in our stable isotope models were incorporated as much as possible and, furthermore the models provide us the level of uncertainty in parameter estimates. In this sense, mixing models provided large credible intervals around dietary proportions that should be taken into account when interpreting results.

Despite the difficulty of describing the diet of vultures, it is showed that SIA can help to distinguish between animals that rely on waste and so present enriched levels of C than those that feed on the countryside. However it is worth to mention that in our study area although there are few supplementary feeding points, most of them are intended to Bearded Vulture so its influence to Egyptian Vulture population is predicted to be low (Tauler-Ametller et al., 2017; Catalan Government data, personal communication). In this sense, in other areas where supplementary feeding points are intended to Egyptian Vulture where the remains provided could come from intensive farms, it would be difficult to distinguish between landfill and feeding points origin. Therefore, our study provides an initial step towards further research on vultures' diet that should be refined in the future to disentangle meaningful ecological and conservation questions.

Contribution of landfills to Egyptian Vulture diet

Diet studies have been described as powerful tools for monitoring food resources obtained by raptors on large spatio-temporal scales since they are able to detect changes in resource availability caused by new environmental scenarios (Real, 1996). Thus, our results are consistent with these studies of other species as our diet analysis shows that an important part of the Egyptian Vulture's diet is obtained from landfills, a novel food resource that has recently become available in the environment.

The diet of Egyptian Vulture has been described to be very variable between different areas. In our case, pairs that feed most on landfills could obtain nearly a 50% of their diet from these sources, but those pairs that consume most livestock this resource represents less than 25% of their whole diet (Supporting information, Fig. S1). Despite the contribution of landfills to diet of this species has been little quantified because most of the studies have focused on taxonomic groups and not on the origin of these prey (Donázar, Cortés-Avizanda, & Carrete, 2010; Milchev, Spassov, & Popov, 2012), it is known that waste can be important to the diet of Egyptian Vulture in some areas (Gangoso et al., 2013).

In addition, the proportion of livestock in our population is less than the livestock consumption described in other areas where cattle has been defined as one of the main sources of food in the Egyptian Vulture (Donazar & Ceballos, 1988; Milchev, Spassov, & Popov, 2012), however it is not the only exception described (Margalida et al., 2012; Dobrev et al., 2015).

This result is relevant for conservation as vultures are among the most endangered groups of birds at a global scale and that one of their major threats comes from the type of food resources that they consume (Hernández & Margalida, 2009; Donázar et al., 2009; Ogada et al., 2016). The main cause of their decline is poisoning due to the consumption of dead animals that contain high levels of pesticides or veterinary drugs such as diclofenac, which is causing a sharp reduction in vulture populations throughout the world, especially in *Gyps* genus (Oaks et al., 2004; Swan et al., 2006; Ogada et al., 2016). Despite it is a first approximation, our results are important as they show that isotope analysis can provide information about the use of livestock resources and the potential change to consuming novel resources derived from landfills, which therefore allows us to assess the new threats to which vultures are currently exposed.

Feeding on landfills can have several conservation implications at both individual and population levels. At individual level, abundant and predictable food supplies should improve the body condition and breeding performance of the individuals that feed at these sites (Oro et al., (2013) and references therein). In this sense, it is worth to mention that we only sampled chicks of successful breeding pairs, so it could be possible that pairs that feed on landfills might be those with higher breeding success. Further analysis of diet of pairs that fail would be necessary to study if feeding of landfills could favour reproduction in our area.

However, landfills can also act as a threat as they represent a source of food that potentially contains pollutants and poisons (Quarles, 2013; de la Casa-Resino et al., 2014; Carneiro et al., 2015; Casas-Díaz et al., 2016). Moreover, the presence of the species in highly humanized areas where landfills are found could also entail an increase in the risk of fatal casualties due to collisions with power lines and other infrastructures (Carrete et al., 2009). Many of these threats are emerging as major causes of mortality in large avian scavengers and may turn out to be catastrophic if they operate in tandem with other threats such as poisoning (Martínez-Abraín et al., 2012; Sanz-Aguilar et al., 2015).

At a population level, landfills can promote the settlement of individuals attracted by the availability of food (Tauler-Ametller et al., 2017) and improve survival rates and recruitment (Oro et al., 2008), but may also reduce fecundity (Lieury et al., 2015). Nevertheless, the great predictability and availability of food associated with landfills has also led to rapid population increase of some species and several studies have focused on the ecological and social consequences of this overabundance (Vidal, Medail, & Tatoni, 1998; Bosch et al., 2000).

Moreover, results of GLMM analysis pointed out that the level of $\delta\ ^{13}C$ is linked to the level of humanization of territories. High levels of $\delta\ ^{13}C$ are associated with intensive livestock and so landfill consumption (Schmidt et al., 2005; Weiser & Powell, 2011), therefore territories that present more $\delta\ ^{13}C$ are more likely to be located in humanized areas. However, we didn't find any relation with $\delta\ ^{13}C$ and our measurement of food availability, estimated as distance to landfill and number of cattle present in the territory, possibly due to the limitations of having an accurate metric for food availability. Obtaining this metrics is particularly challenging in our study species, since breeding pairs of Egyptian Vultures make long daily movements to feed at predictable feeding sources located over 100 km away (López-López, García-Ripollés, & Urios, 2014) and so, despite having territories located far from landfills, these pairs could be feeding on these installations.

In our study case, feeding on landfills in addition to the reduced mortality of individuals from our study area (Tauler et al., 2015) could have prompted the Egyptian Vulture population increase that has taken place over the last 30 years in Catalonia. This is a paradoxical situation in which an endangered species has been favoured by human waste when, typically, modern human activities are in fact detrimental to biodiversity. Similar processes could be occurring in other threatened species but could hitherto have been overlooked. However, a new scenario is expected to occur when landfills are closed in compliance with European legislation (EU Comission, 2008), together with a resolution by the European Parliament (European Parliament resolution of 9 July 2015 on resource efficiency: moving towards a circular economy), urges the European Commission to reduce levels of residual waste to close to zero by 2020. In this context it is thus expected that the amount of waste available to vultures in coming years will fall and the effect of a landfill closure to vulture populations has been little studied (Katzenberger et al., 2017). Thus, this study provides a first approximation of a new tool that should be refined in order to monitor the potential diet change caused by this new resource scenario that will help design conservation measures for endangered vultures in the future.

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Supporting information

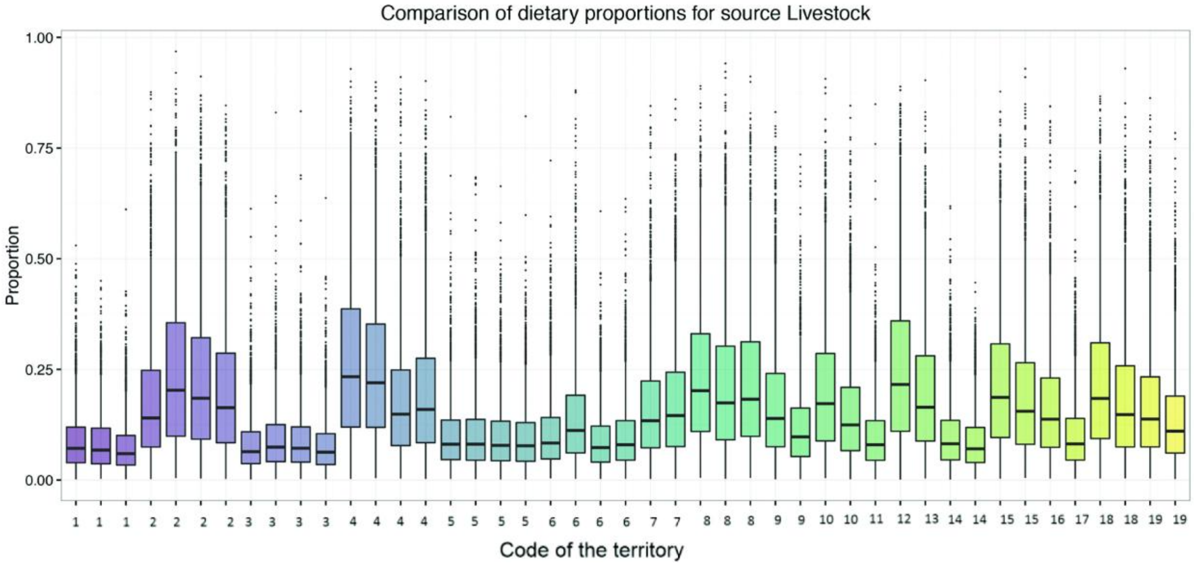

Figure S1. Percentage of the contribution of livestock in the diet of Egyptian vulture nestlings estimated with Bayesian mixing models (SIMMR). Data of each territorial pair but different year is represented with the same number.

CAPÍTOL 4

Are landfills a good quality food source for vultures?

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Resum

Els humans llencem una gran quantitat de restes de menjar que acaba en abocadors. Aquests recursos alimentaris són explotats per moltes espècies, que conseqüentment, poden alterar la seva dinàmica poblacional. Tot i així, els efectes d'alimentar-se en abocadors en l'estat de salut dels individus i la seva condició física encara són poc coneguts. Tradicionalment, la condició física dels animals s'ha estudiat utilitzant índexs morfològics, però la combinació d'aquests índexs amb indicadors fisiològics pot proporcionar informació molt més completa de l'estat de salut d'un determinat individu. En aquest treball, vam estudiar com els diferents nivells d'ingestió de residus (avaluats utilitzant l'anàlisi d'isòtops estables), poden afectar a la condició física dels polls d'una població d'aufrany (*Neophron percnopterus*) en expansió. Vam utilitzar dues aproximacions: la morfomètrica i la fisiològica, que incloïa l'anàlisi de la bioquímica plasmàtica i del metabolisme antioxidant dels individus. A més, també vam tenir en compte la influència d'altres factors individuals i ambientals que poden afectar a la condició física dels polls. Els resultats de l'anàlisi de redundància i de la partició de la variància indiquen que la proporció de la dieta que prové d'abocadors és el factor principal que afecta els indicadors fisiològics dels individus. Els polls alimentats a partir de residus humans presenten nivells més baixos d'algunes vitamines (α-tocopherol) i carotens (zeaxantina i luteína), els quals tenen funcions importants com a defenses antioxidants, propietats immunoestimulants i finalitats ornamentals. Els resultats també apunten que aquests nivells baixos de vitamines i carotens, estan correlacionats amb un augment dels nivells d'enzims antioxidants superòxid dismutasa i glutatió peroxidasa. A més a més, els polls alimentats en abocadors, semblen estar més ben alimentats i experimentar menys períodes de dejú que els individus que no utilitzen aquests recursos, que presenten nivells més baixos de colesterol. Aquesta aproximació integral ha sigut clau per a descriure la condició física dels polls d'aufrany en funció del consum d'aliment procedent d'ambients humans alterats. Són necessaris més estudis per a descriure com aquests canvis en la condició física dels individus podrien causar efectes a llarg termini en la supervivència i productivitat dels individus així com en la seva dinàmica poblacional.

Abstract

Humans discard an enormous amount of food that ends up in landfills. This food resource is exploited by many species whose population dynamics have as a result been altered. Nevertheless, the effects of feeding on human waste on individual health parameters such as body condition are still unknown. Traditionally, body condition in wildlife has been assessed using morphological indices, completing these indices with the analysis of indicators at the physiological level can provide a fuller picture of the animals' health status. In this work, we studied how different amounts of waste consumption (assessed using stable isotope analysis) affects nestling body condition in an expanding population of the endangered Egyptian Vulture (*Neophron percnopterus*). We used morphometric and physiological approaches, including plasma biochemistry, antioxidant defences and oxidative stress biomarkers, but also took into account other individual and environmental factors that influence nestlings' body condition. The results of the multivariate redundancy analysis and variance partitioning showed that the proportion of diet originating from landfills is the main factor explaining physiological indicators in the studied vulture population. Nestlings that were fed on food from landfills had lower levels of vitamins (e.g. α-tocopherol) and carotenoids (zeaxanthin and lutein), which have important functions as antioxidant defences, immunostimulant properties and ornamental purposes. Our results also showed that lower vitamin and carotenoid levels correlate with an increase in the levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase. Nevertheless, nestlings that were fed on food from landfills seemed to be better nourished and experience fewer food shortages than individuals not fed using this resource, which had lower levels of cholesterol. Our integrative approach using diverse components was crucial for a reliable assessment of body condition in Egyptian Vulture nestlings and its relationship to the consumption of food derived from human-altered environments. Further studies are necessary to describe how these changes in individual body condition could have long-term effects on individual survival and reproduction and, ultimately, on population dynamics.

Keywords: null model, Predictable Anthropogenic Food Subsidies, rubbish dumps, scavengers, threatened species, vulture restaurants.

Introduction

Human activities are drastically transforming a large proportion of the Earth's land surface (Foley, 2005) and changes in food availability have become one of the most notable changes in the environment (Oro et al., 2013). While habitat destruction and species' declines generally imply a drastic reduction in natural food resources for most species, humans also waste an enormous amount of food that becomes available to and is exploited by many species that inhabit human-altered ecosystems (Oro et al., 2013; Newsome & van Eeden, 2017; Plaza & Lambertucci, 2017). This waste is usually deposited in landfills, which have proliferated in our urbanized world.

The impact of landfills on animal species has been reported at many levels ranging from individual performance to population dynamics. At population level, the most frequently reported response in animal species is an increase in abundance, especially in birds but also in mammals and reptiles (reviewed in Plaza & Lambertucci 2017). These groups of species have been shown to respond to waste consumption by improving reproductive (Bosch, Oro & Ruiz, 1994; Tortosa, Caballero & Reyes-López, 2002; Steigerwald et al., 2015) or survival rates (Bino et al., 2010; Payo-Payo et al., 2015; Rotics et al., 2017). Nevertheless, certain studies have highlighted how feeding on rubbish can negatively affect reproductive performance (Pierotti & Annett, 2011) and individual survival due to the ingestion of waste and/or metal (Rideout et al., 2012).

However, these impacts have been traditionally reported at population level and little is known about the individual effects of relying on food resources obtained from landfills. Although some studies have detected improvements in morphometric body condition of individuals feeding on landfills (Auman, Meathrel & Richardson, 2009; Cahill et al., 2012; Gould & Andelt, 2013), very few studies have ever comprehensively tackled the consequences of differing degrees of waste consumption on individual body condition parameters at physiological level (but see Jessop et al. 2012) or, to our knowledge, the consequences for the antioxidant system.

Body condition is a key concept used in studies of animal biology to quantify the health and physiological state of individuals (Brown, 1996; Stevenson & Woods, 2006) traditionally evaluated using morphological indices (Stevenson & Woods, 2006; Peig & Green, 2009). These indices are usually based on the premise that, once corrected for the structural size of an individual, body mass is indicative of the amount of non-structural energy reserves (e.g. fat and proteins) and, ultimately, individual body condition (Stevenson & Woods, 2006). However, these methods suffer from limitations (Peig & Green, 2009) and it is now thought that the most accurate way of describing the body condition of an individual is to use multiple approaches combining morphological and physiological information (Norte et al., 2009; Jacobs et al., 2012; Resano-Mayor et al., 2016).

Blood parameters are useful for estimating animal body condition and individual fitness (Milner et al., 2003) since, for instance, certain plasma components are informative of fat levels (cholesterol and triglycerides) or protein metabolism (urea and uric acid). These parameters have been shown to be related to the physiological and nutritional state of animals (Sarasola, Negro & Travaini, 2004; Amat et al., 2007; Rodríguez, Negro & Figuerola, 2011).

Antioxidant defences (AO) and oxidative stress (OS) have received considerable attention as indicators of an animal's fitness (Monaghan, Metcalfe, & Torres, 2009; Herborn et al., 2016). As a by-product of normal metabolic processes, aerobic organisms generate reactive oxygen species (ROS) that can damage key biomolecules due to their high reactivity (Balaban, Nemoto & Finkel, 2005). OS occurs as a result of an imbalance between the production of ROS and the body's ability to mitigate their harmful effects through AO defences (Monaghan, Metcalfe & Torres, 2009). Antioxidants include exogenous molecules obtained from diet (e.g. vitamins and carotenoids) and endogenous peptides (e.g. glutathione) or enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) that act as defences and prevent or minimize oxidative damage through interrelated mechanisms. Given that animals obtain some of their AO defences from their diet (which also determines energy and nutrient intake), an individual's nutritional state can thus potentially affect the balance that generally exists between ROS production and AO defences (Catoni, Peters, & Martin Schaefer, 2008; Monaghan, Metcalfe & Torres, 2009). Overall, although AO and OS biomarkers offer a powerful tool for assessing individual body condition or stress, their interpretation requires the integration of the presence of AO defences with biomarkers of oxidative stress.

In this study, we used the Egyptian Vulture (*Neophron percnopterus*) as a model species with which to explore the potential implication of feeding on landfills on the body condition of individual vultures. We performed morphometric and physiological analyses that included a broad spectrum of blood biochemical parameters such as certain OS and AO system biomarkers. This vulture is globally endangered and its populations are declining across most of its range (BirdLife International, 2016). By contrast, in our study area in the NE Iberian Peninsula the population of this vulture has increased in recent years (Tauler et al., 2015) and it has expanded its range and colonized territories close to landfill sites filled with urban waste (Tauler-Ametller et al., 2017) on which they feed (see chapter 3 of present thesis). Egyptian vultures are territorial and our study population is spread over a broad area in which individuals hold territories at unequal distances from landfills and so differ in their degree of consumption of this food resource. Thus, Stable Isotope Analysis (SIA) is a good potential tool for providing dietary estimations of the prey consumed by the individuals in this population (Hobson & Clark, 1992; Inger & Bearhop, 2008). SIA can assess how much food from different sources is assimilated by individuals (Votier et al., 2010). Thus, when the consumption of a certain resource such as waste varies between individuals in a population, SIA enables us to study the consequences of these different degrees of consumption at individual level.

This system offers us an opportunity to study the consequences of urban waste consumption on individuals' body condition. It has been hypothesized that feeding on landfills could have contributed to the population expansion of the Egyptian Vulture (Tauler-Ametller et al., 2017). However, to fully understand the effects of landfills, be it positive or negative, it is crucial to investigate to what extent this predictable and anthropic food resource has consequences for this vulture's body condition. For example, the predictability of resources from landfills could facilitate food provisioning but the quality of this food could be poor and could modify certain physiological parameters. The aims of this study were (1) to provide reference values for body condition measures (morphometric body condition, plasmatic biochemistry and antioxidant metabolism) in free-living Egyptian Vulture nestlings; (2) to analyse using morphometric and physiological indices whether the contribution of landfills to diet affects body condition; and (3) to assess the potential influence of individual and environmental factors other than diet on body condition estimates.

Materials and methods

Study area and data collection

The study area (10 500 km^2) lies in central eastern Catalonia (NE Iberian Peninsula) at altitudes of 200–1900 m a.s.l., and encompasses an area of cliffs running from the Prelitoral Mountains in the south to the pre-Pyrenean Mountains in the north. It includes humanized zones with high human population density, as well as less populated rural areas. Due to this dense human population there are as many as nine landfills within the study area (Fig. 1 from chapter 3 of present thesis).

In 2012–2016, Egyptian Vulture territories were visited during the breeding season (March–August) by observers equipped with a spotting scope (20–60x). Once nestlings were around 45–55 days old, experienced climbers accessed the nests to capture the chicks, which were then carefully transported to a secure place for morphometric measurements and sample collection. Body mass was then recorded to the nearest 25 g and tarsus length measured to the nearest 0.01 mm using an electronic calliper; these measurements were subsequently used to estimate nestling body condition based on morphometrics (see below). We also measured the length of the seventh primary to accurately estimate nestling age in days (Donazar & Ceballos, 1989). Three mantle feathers were then sampled from each chick for individual diet estimates via isotopic analyses (see below) as isotopic composition in feathers is an indicator of nestling diet at the time of tissue development.

Finally, before the nestlings were returned to the nest, we sampled 2.2 ml of blood from the brachial vein with a 20-gauge needle. Blood was collected in two heparinized tubes of 1 ml and was used to fill two haematocrit capillaries. The remaining blood was collected in an Eppendorf tube and mixed with 0.5 ml of absolute ethanol for molecular sex determination, which was performed following the method described in Fridolfsson & Ellegren (1999). All blood samples were immediately stored at 4°C in a portable fridge until processed in the laboratory within 12 h. Once in the laboratory, haematocrit capillaries were centrifuged at 9794 rcf for 5 min and the haematocrit was measured as the percentage of packed red cell volume in relation to the total column height (plasma plus packed cell volume). Heparinized tubes were centrifuged at 9794 rcf for 5 min to separate the plasma (supernatant) from the red blood cell (RBC) fraction. Then, RBC samples were washed with a cold saline solution followed by centrifugation at 9794 rcf for 5 min to remove the supernatant. Finally, the four vials (two with plasma and two with RBC; an aliquot for each fraction) were frozen in liquid nitrogen and then stored a few days later at −80°C until analysis. The blood samples in ethanol were directly frozen at −20°C until sex determination. During the study period blood samples were collected from 77 nestlings in 20 different territories.

Diet analysis

Nestlings' main prey consumption was estimated by analysing the isotopic ratios of carbon (^{13}C:^{12}C; $\delta^{13}C$) and nitrogen (^{15}N:^{14}N; $\delta^{15}N$) in feathers. Isotopic measurements were performed at the Centres Científics i Tecnològics of the University of Barcelona using the methods described in Resano et al. (2011). The Bayesian mixing model SIMMR (Parnell et al., 2010; Parnell & Inger, 2016) was used to estimate the relative contribution of the main prey categories in the chicks' diets. The main prey categories included in the SIA were determined using conventional identification of food remains and classified into 5 groups: landfills, livestock, wild herbivores, carnivores and birds. Prey isotopic values were obtained from different published studies (see Table 1 form chapter 3 of present thesis). The trophic discrimination factors included in the SIMMR were 1.11‰ ± 1.21 for δ13C and 3.33‰ ± 1.18 for δ15N. These values were calculated using the SIDER package from R software (Healy et al., 2017). Mean prey consumption estimates from SIMMR were selected for subsequent analyses. These prey consumption percentages were used with the Shannon-Wiener index (1949) to estimate diet.

Morphometric body condition index

When traditional morphological approaches are used to estimate body condition, the covariation between energy reserves (e.g. fats and proteins) and body size can result in the overestimation of body condition in large animals. To correct this, we calculated a morphometric body condition index that controlled for the growth effect on body size. Firstly, we log-transformed all recorded length measures of the chicks and then standardized the data to give a mean of 0 and a SD of 1. Body size was estimated from the scores on the first axis (PC1, hereafter 'body size') of a principal components analysis (PCA) of the covariance matrix built using tarsus, claw, beak, seventh primary feather and central tail feather length. These traits were selected because they gave the highest loadings in the PCA and therefore are the traits that best contribute to explaining size variation.

Furthermore, we performed a linear model with body weight as a dependent variable and body size as an independent variable. Initially, we included the effect of sex and its interaction with body size to exclude the possibility that there were any allometric differences between sexes; however, after checking that these effects were not relevant we only considered body size as an independent variable. The residuals from the fitted model were taken as a measure of the morphometric body condition of each nestling. We validated these values by checking that they did not show any strong correlation with the chosen size variables, which meant that the size effect had been successfully removed.

Plasma biochemistry

The plasma fraction stored at −80°C was used to measure calcium, magnesium, phosphorus, glucose, cholesterol, triglycerides, creatinine, urea, uric acid, total proteins and the activities of alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase and creatine kinase, as described in Martinez-Haro et al. (2011). Plasma biochemistry was measured spectrophotometrically using an A25 autoanalyser and commercial kits from BioSystems S.A. (Barcelona, Spain). As well, levels of exogenous antioxidant defences such as vitamin A (free retinol in alcoholic form), vitamin E (α-tocopherol) and carotenoids (lutein and zeaxanthin) were determined in plasma by high-pressure liquid chromatography (HPLC, Agilent Technologies 1100 Series), as described in Rodríguez-Estival et al. (2011).

Oxidative stress biomarkers in red blood cell analysis

Endogenous antioxidant defences and oxidative stress biomarkers were analysed in RBC after homogenization (1:10 w/v) in a stock buffer (1.15% KCl in 0.01 M PBS – pH 7.4 – with 0.02 M EDTA), as previously described in Reglero et al. (2009). Firstly, the activities of two antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD), were determined spectrophotometrically with an A25 autoanalyser using Ransel and Ransod kits, respectively (Randox Laboratories, Crumlin, UK). Homogenized samples were diluted by 1:20 and 1:25 (v:v) with Ransel diluting agent and Ransod sample diluents for GPx and SOD determinations, respectively. Enzyme activities were expressed in terms of milligrams of protein in the homogenates, quantified spectrophotometrically as per Bradford (1976). Membrane lipid peroxidation in erythrocytes was estimated by the quantification of malondialdehyde (MDA) in the homogenates. Determination of MDA was performed by HPLC following the method described by Agarwal and Chase (2002) but incorporating the modifications described by Romero-Haro and Alonso-Alvarez (2014). Levels of total glutathione (GSHT) and GSH in oxidized form (GSHO; 2 GSHO = 1 GSSG) were quantified through a reaction coupled to GSH reductase, as described by Reglero et al. (2009) with an A25 autoanalyzer. The GSHO was expressed as a molar concentration and as a percentage of the GSHT (GSHOT).

Data analysis

Descriptive parameters of all body condition measures (i.e. morphometric body condition index, haematocrit, plasma biochemistry, antioxidant defences and oxidative stress biomarkers) were calculated as the mean and standard deviation (±SD) of all individuals sampled in our population. Before performing the statistical analysis, we plotted the data to detect outliers, which we replaced with the mean value for that variable obtained for all individuals. We applied the centred log-ratio transformation to the mean compositional item diet estimates, and rescaled those variables that included negative values (as morphometric body condition index).

To assess the effect of diet and that of other individual and environmental parameters on nestling body condition we conducted a redundancy analysis (RDA) (Ter Braak, 1994) run on a correlation matrix. The response variables considered were morphometric body condition index, haematocrit, plasma biochemistry parameters, antioxidant defences and biomarkers of oxidative stress. Explanatory variables were consumption of each prey category, diversity of diet, number of chicks per nest, nestlings' age and sex, and altitude of nesting site. As recent food ingestion and the time of the day may influence plasma biochemistry and thus hinder parameter interpretation (Resano-Mayor et al., 2016), we considered (i) whether nestlings had a full crop or not as a proxy of recent ingestion of food and (ii) whether nestlings were sampled before or after midday. These considerations were then included as categorical covariates in the RDA. In addition, we included the year of sampling as a third covariate to tease out this potential effect (see Supporting information, Table

S1). The most significant explanatory variables were chosen using forward selection and a Monte Carlo Permutation Test (9999 permutations) constrained by blocks defined by the covariate year.

The significant variables selected were retained and used for the variance partitioning calculations to quantify the relative importance of the selected diet components and other individual and environmental factors for each condition and physiological variable. By doing so, we derived the variance explained separately by the three main components: (A) diet, (B) individual and environmental factors other than diet, and (C) the temporal trend. Also, we determined the variance shared by these three main components. The specific variables included in each component are specified in the results section.

The significance of the different fractions (diet, individual and environmental factors, and temporal trend) was evaluated using 9999 permutations of the original data. RDA and variance partitioning were conducted with CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA).

Results

The descriptive values of the body condition measures (i.e. morphometric condition index, haematocrit, plasma biochemistry parameters, and biomarkers of oxidative stress) for nestlings of Egyptian vulture (38 males and 39 females) are given in Table 1.

Table 1. Descriptive values of Egyptian Vulture nestling body condition in Catalonia 2012-2016 (n = 77). The parameters shown are the morphometric body condition index, haematocrit, plasma biochemistry components, plasma exogenous antioxidants, and oxidative stress biomarkers analyzed in red blood cells. There are shown the units of measurement, the abbreviated names of measures, the mean, standard deviation (±SD) and the minimum and maximum for all individuals.

Body condition measures	**Name**	**Mean**	**SD**	**Min**	**Max**
Morphometric body condition index	COND	0.00	0.08	-0.19	0.15
Haematocrit (%)	HEM	30.32	3.06	21.49	43.56
Plasma biochemistry					
Calcium (mg/dL)	CA	12.32	2.40	5.64	18.92
Magnesium (mg/dL)	MG	2.01	0.92	0.64	3.09
Phosphorus (mg/dL)	P	9.03	10.26	0.35	69.63
Glucose (mg/dL)	GLU	310.87	97.06	10.00	566.00
Cholesterol (mg/dL)	COL	252.17	65.17	52.00	397.00
Tryglicerides (mg/dL)	TRY	153.83	69.23	64.00	587.00
Creatinine (mg/dL)	CRE	0.51	0.20	0.16	1.34
Urea (mg/dL)	UREA	10.23	4.09	1.00	21.00
Uric acid (mg/dL)	AURI	11.30	5.16	1.99	26.65
Total proteins (g/L)	PROT	34.44	5.66	10.00	48.00
Alkaline phosphatase (U/L)	ALK	903.56	188.25	251.00	1472.00
Aspartate aminotransferase (U/L)	ASP	144.75	29.39	92.00	258.00
Lactate dehydrogenase (U/L)	LAC	1824.10	510.64	832.00	3448.00
Creatine phosphokinase (U/L)	CREP	4954.18	1208.62	2456.00	7980.00
Plasma exogenous antioxidants					
Retinol (uM)	RET	52.13	13.91	20.77	108.03
Tocopherol (uM)	TOC	79.95	22.96	30.70	127.17
Lutein (uM)	LUT	30.12	17.30	2.25	73.99
Zeaxanthin (uM)	ZEA	7.84	4.28	0.81	16.95
Oxidative stress biomarkers					
Glutathione peroxidase (U/mg protein)	GPX	0.35	0.12	0.12	0.73
Superoxide dismutase (U/mg protein)	SOD	1.14	0.34	0.42	2.42
Malondialdehyde (uM/g pellet)	MDA	0.07	0.02	0.03	0.13
Total glutathione (umol/g pellet)	GSHT	3.96	1.41	0.42	6.82
Oxidized glutathione (umol/g pellet)	GSHO	1.30	0.55	0.16	2.33
% Oxidized glutathione (% of total)	GSHOT	35.59	16.62	5.53	117.00

The significant explanatory variables selected according to RDA and after Monte Carlo permutations were landfill (P=0.0001), altitude (P= 0.0012) and the number of chicks per nest (P=0.012) (Fig. 2); the remaining variables were non-significant. The first axis was characterized mainly by the landfill variable and explained up to 12% of the response variables variance. The second axis was best explained by the number of chicks and the third by altitude. Although the overall variance explained by the three axes was only 17.7%, there was a strong relationship between the explanatory and responses variables, with a response-predictor correlation of 0.85 on the first axis, 0.63 on the second and 0.60 on the third (Table 2).

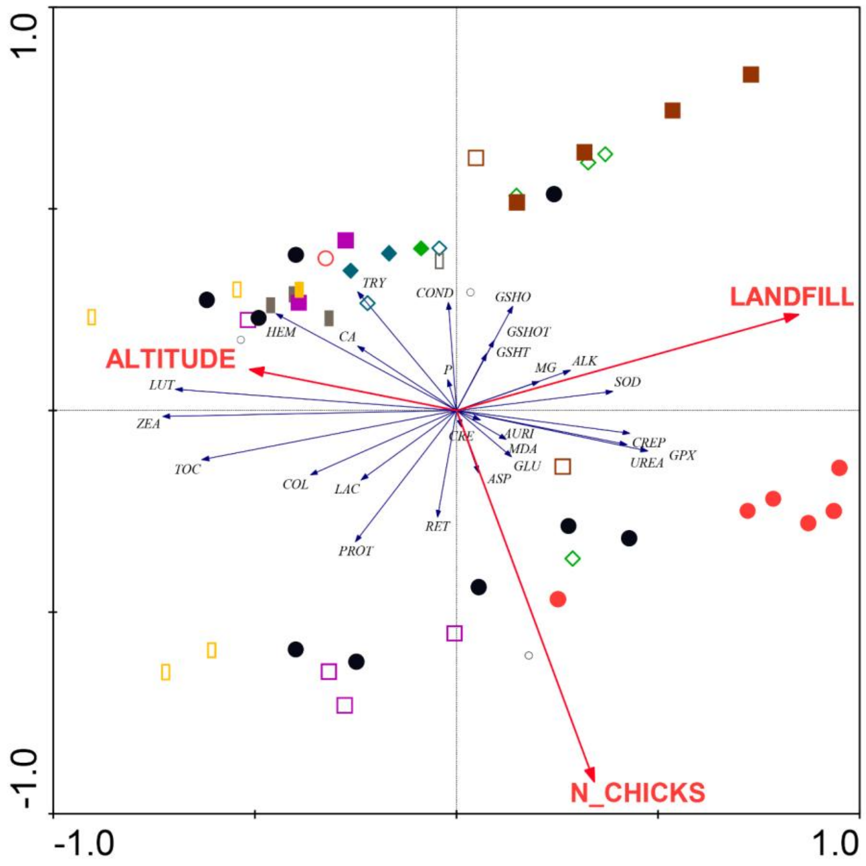

Figure 2. Graphic representation of the results of the redundancy analysis (RDA) and Monte Carlo permutation test to analyse the effect of diet and individual and environmental variables (red arrows, see text for a description), on condition variables (blue arrows). Only explanatory variables selected by RDA analysis are represented. Nestlings of same territory are represented by same symbol and color (n=77).

Table 2. Main results of the redundancy analysis (RDA). Eigenvalues obtained, correlations between explanatory and response variables and proportion of variance of response variables explained by explanatory variables are shown. Also the contribution of selected significant explanatory variables to each axis is shown.

		Axis 1	**Axis 2**	**Axis 3**
Eigenvalues		0.112	0.027	0.017
Predictors- responses correlation		0.854	0.635	0.603
Cumulative percentage variance of response variables		12.0	15.7	17.7
Cumulative percentage variance of responses-predictors correlation		71.8	88.9	100
Regression coefficients for selected explanatory variables	*Landfill*	0.872	0.404	0.504
	Number of chicks	0.182	-0.979	0.241
	Altitude	-0.386	0.016	0.995

To perform variance partitioning between A, B and C components, we selected both ‘landfill’ and ‘altitude’ as a proxy of diet (component A). We considered these two variables together because they were highly correlated on the first axis given that the pairs that consume most food from landfills breed at lower altitudes. Component B accounted for individual and environmental factors and was represented by the variable ‘number of chicks’, while the component C temporal trend was represented by the variable ‘year’. The total variance explained by these three components was 22.52 %, explained mainly by component A (landfill + altitude, 53.23%), component C (temporal trend, 25.18%) and component B (number of chicks, 15.76%). The variance shared by components AB and AC was low, and contribution of the other interactions was negligible (Fig. 3).

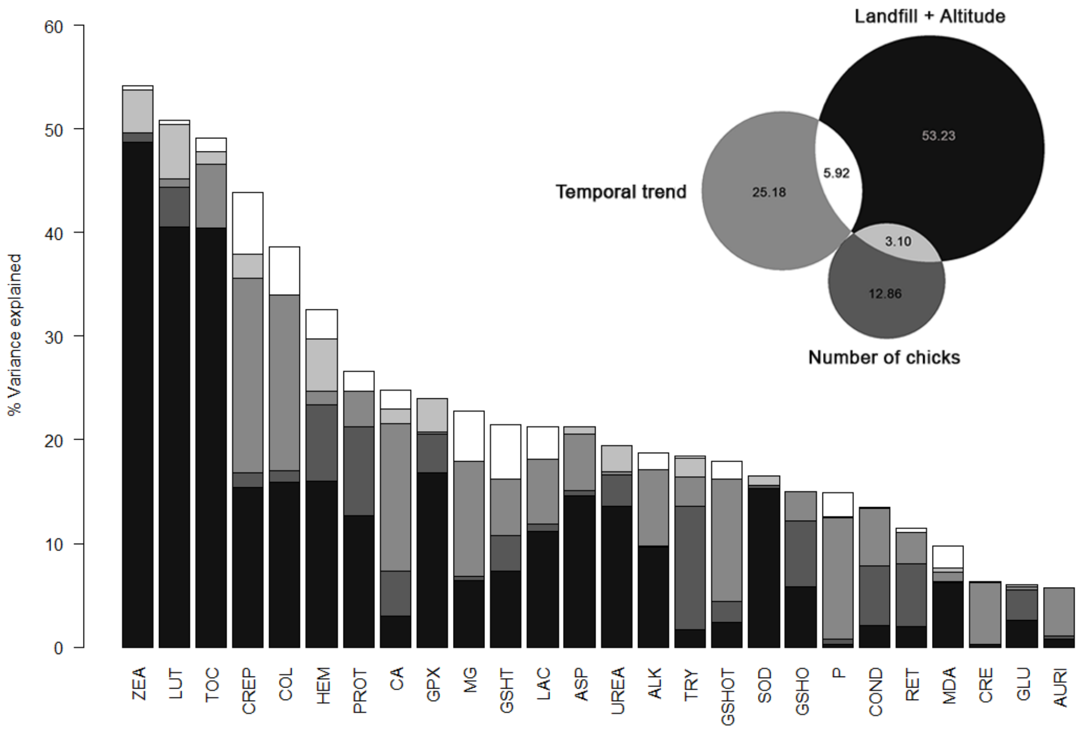

Figure 3. Percentage of variance of condition variables (barplot, x axis) explained by component A (landfill + altitude, black), component B (number of chicks, dark grey) and component C (temporal trend, light grey) according with variance partitioning analysis. Also it is represented the variance shared between diet and temporal trend (AC, white) and diet and number of chicks (AB, light grey). Right top side: Venn diagram representing the contribution of the three main components to the 22.52 % of total variance according to variance partitioning analysis.

Effects of feeding on landfills on physiological parameters

Antioxidant metabolism

According to the RDA analysis, territories with a high consumption of resources from landfills had less antioxidant exogenous defences represented by plasma lutein, zeaxanthin and α-tocopherol levels (Fig. 4). As well, the consumption of resources from landfills was correlated with an increase in the activities of the two analysed antioxidant enzymes (GPx and SOD). However, landfill consumption did not influence the appearance of oxidative stress since MDA, GSHT, GSHO and GSHOT only had a weak relationship with diet (Fig. 2).

Variance partitioning also provided evidence for the fact that the contribution of landfills to diet explained a high proportion of the carotenoid variance (48.69% for

ZEA and 40.55% for LUT) and vitamin E levels (40.46% for TOC) in plasma. Regarding endogenous antioxidant defences, diet explained 16.82 % of GPX and 15.35 % of SOD variance, but less than 10% of the variance of the other oxidative stress biomarkers (MDA, GSHT, GSHO and GSHOT) (Fig. 3).

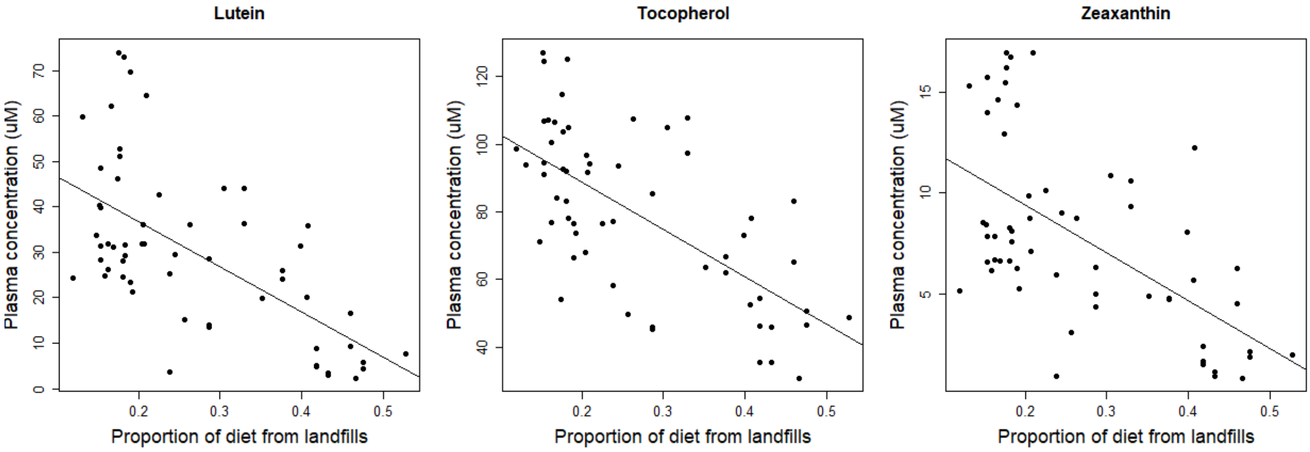

Figure 4. Relationship between plasma concentration of lutein, tocopherol and zeaxanthin (µM) in Egyptian Vulture nestlings and the proportion of diet from landfills obtained with stable isotope analysis. The trend line of the relationship between the variables is shown.

Plasma biochemistry and haematocrit

Cholesterol and proteins were negatively correlated with the proportion of the diet obtained from landfills. The percentage of variance of these two variables explained by diet was 15.93% for COL and 12.72% for PROT. Conversely, CREP and UREA were positively correlated with the frequency of feeding on landfills and the variance in these two parameters explained by diet was 15.45% in the case of CREP and 13.59% for UREA.

Haematocrit was positively correlated with the altitude of nesting areas, as territories located in higher areas presented a greater percentage of haematocrit than those located in more lower-lying areas (Fig. 5). This correlation (R^2= 0.18, P= 0.0007) suggests that altitude was the main contributor to the 16.02% of variance in haematocrit explained by component A (landfill + altitude).

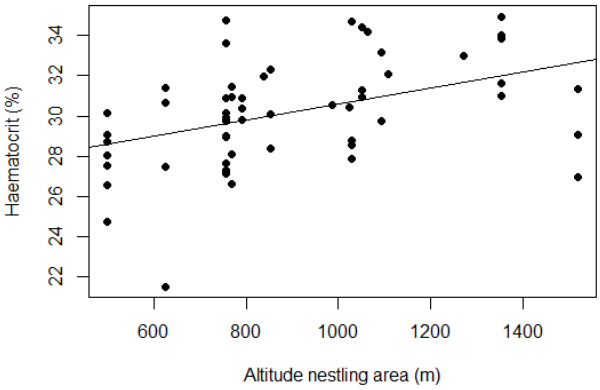

Figure 5. Relationship between haematocrit values (%) in Egyptian Vulture nestlings and the altitude of nesting areas (m a.s.l.). The trend line of the relationship between the variables is shown.

Number of chicks and temporal trend

The number of chicks per nest also had a significant effect on body condition parameters, despite being the selected variable with the lowest percentage of total variance explained. Nestlings from territories with only one chick per nest had better morphometric body condition than nestlings raised in two-chick nests (Fig. 2). Despite this correlation, the percentage of variance in the morphometric body condition explained by the number of chicks was only 5.71%. In fact, there were other parameters with a higher percentage of variance explained by the number of chicks. Triglycerides plasma levels were negatively correlated with the number of chicks, the variance explained by the number of chicks being 11.88%. Total protein and retinol levels, were positively correlated with the number of chicks, which explained 8.53% of the variance of PROT and 6.04% of that of RET. Moreover, breeding pairs that fed on landfills had a more constant number of chicks per year (either one or two chicks) than those that were less dependent on this resource, which raised one or two chicks per nest in alternate years (Fig. 2).

The temporal trend also had a relevant contribution in explaining the variance of CREP (18.73%), COL (16.96%), CA (14.26%), MG (11.11%), GSHOT (11.75%) and P (11.79) (Fig. 3).

Discussion

This study of an expanding population of the endangered Egyptian vulture in the NE Iberian Peninsula revealed strong correlations between the consumption of food from landfills and antioxidant metabolism. Our approach, based on the simultaneous analysis of nestlings from territories spread over a large area with an unequal consumption of waste, enabled us to undertake a fine-scale individual level analysis of the relationship between landfill usage and body condition parameters. Interestingly, those individuals that fed on waste were to a large extent those that had the lowest concentrations of plasma carotenoids and vitamins, which are a crucial part of the antioxidant defence system and, in addition, are thought to play a relevant role in the coloration and behaviour of this species. In turn, several parameters suggested that individuals feeding on landfills have easier access to food since the proxies for food shortages had lower values and more constant reproductive rates than the individuals with lower frequencies of waste consumption.

Effects of feeding on waste on the antioxidant metabolism

Our results showed that a landfill-based diet is the best explanatory parameter for the total variance in the pool of biochemical and antioxidant metabolism indicators analysed. We found that lower levels of carotenoids (lutein and zeaxanthin) and vitamin E (α-tocopherol) were related to increased frequency of diet acquisition from landfills. Vitamins and carotenoids are important plasmatic antioxidants that have to be obtained from diets as vertebrates are unable to synthesize them (Armstrong & Hearstt, 1996; Møller et al., 2000; Monaghan, Metcalfe & Torres, 2009).

A possible explanation of these lower levels is that nestlings fed on waste from landfills could be incorporating fewer antioxidant defences from their diets than those fed with other food resources. However, the ingestion of poorer quality food from landfills could increase the level of ROS and individual birds may use carotenoids and vitamins to compensate for this increase, thereby leading to lower levels of these substances. Another possibility is that the presence of pathogens in rubbish dumps – an obvious danger to the animals that feed there (Ortiz & Smith, 1994; La Sala et al., 2013) – activated their immune systems and birds employed carotenoids and vitamins as immune defences (Olson & Owens, 1998).Regardless of the origin of the reduced levels of circulating exogenous antioxidant defences, this effect was associated with an increase in the levels of endogenous antioxidant enzymes (i.e. glutathione peroxidase and superoxide dismutase), which could be a compensatory response for dealing with the ROS generated as part of the physiological processes of the organism. This hypothesis regarding the compensating for the lower levels of exogenous antioxidants with increased endogenous defences is also consistent with the lack of differences in the oxidative stress indicators (i.e. increased MDA levels or percentage of oxidized glutathione) between nestlings fed from landfills and those using other food sources.

The relationship between carotenoid ingestion and landfill-based diets is important in the case of the Egyptian Vulture. This species feeds on excrements of ungulates to obtain the pigments necessary for its yellow facial coloration (Negro et al., 2002; Blanco et al., 2014). The faeces of ungulates contain large amounts of carotenoids, which, besides being valuable micronutrients due to their antioxidant and immunostimulant properties, have a function as pigments (Brush, 2017). The ornamental purpose of these molecules in Egyptian Vultures is supported by the fact that the amount of these pigments that they obtain from faeces is more than needed for maintaining a healthy physiological function and also more than expected according to body mass (Blanco et al., 2014). Yellow faeces coloration could be associated with high-quality individuals given the potential cost of eating excrements: excrements may expose consumers to gastrointestinal parasites (Hutchings et al., 2001) and individuals may spend time and effort searching for food with negligible nutritional content (Negro et al., 2002).

Individuals from the studied population had a wider range of concentrations of carotenoids than individuals from other parts of Spain (Fig. 6). On one hand, nestlings from our population that were fed on landfills had low concentrations of lutein, in some cases similar to captive individuals fed only with flesh or to free-living nestlings from areas with little availability of livestock resources. On the other hand, individuals from areas with more extensive livestock have been found to have levels of carotenoids in their blood plasma that are as high as those from areas where free-grazing cattle are very abundant (Negro et al., 2002).

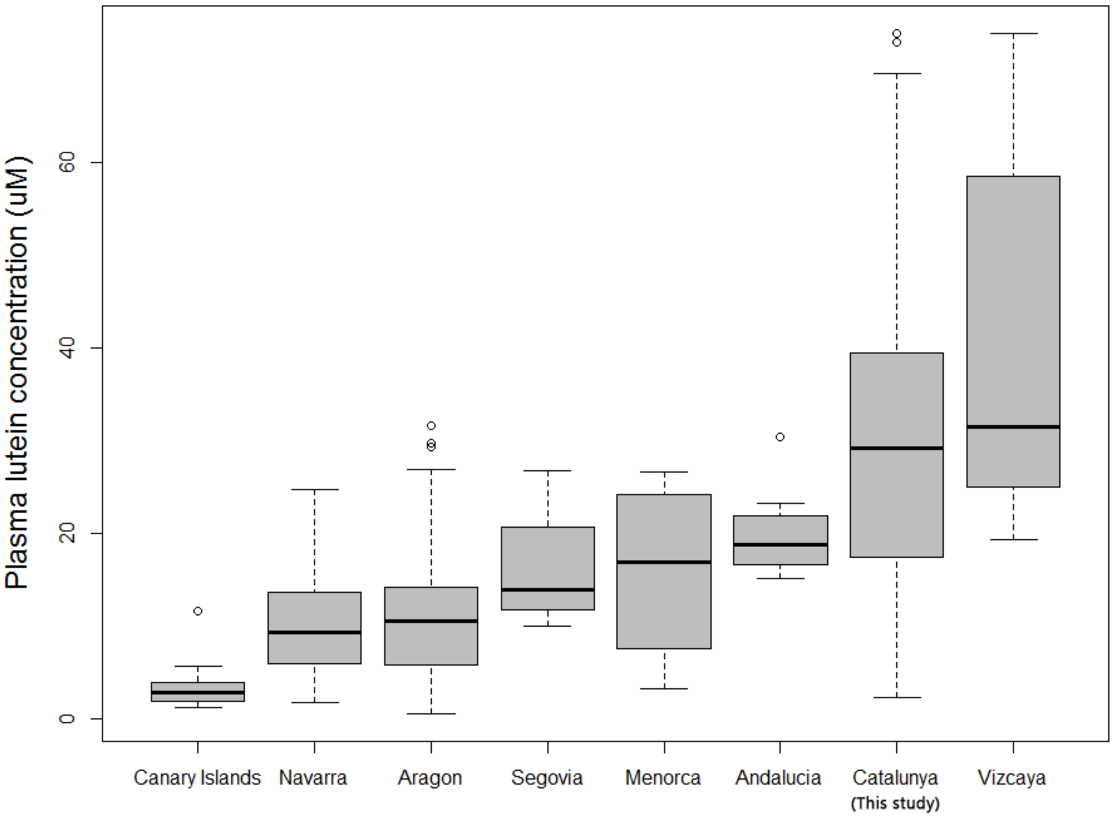

Figure 6. Plasma lutein concentration (μM) of Egyptian Vulture in different areas of Spain obtained from Negro et al. 2002 compared with lutein concentration of nestlings from Catalonia (this study).

In adult individuals of other species, the alteration of ornamental traits can influence sexual selection as coloration provides information on general health, resistance to parasites or the ability to avoid predators (Hill, 1991; Olson & Owens, 1998 and references therein). Therefore, lower levels of carotenoids could be a disadvantage for sexual selection in individuals feeding on landfills. In addition, despite little being known about the consequences of this lower level of carotenoids in Egyptian Vulture nestlings (which are not yet subject to sexual selection), Romero-Haro et al. (2016) report that early oxidative stress during development in some bird species could influence the future cost of reproduction. In summary, the antioxidant role of carotenoids in individuals with lower levels of these substances might be compensated by increased endogenous defences; however, the consequences of these lower levels of carotenoids in individuals of different ages associated with landfills appear to be complex and therefore should be studied further.

Morphometric condition index and plasma biochemistry

Despite the existence of a number of studies describing a positive relation between feeding on rubbish dumps and the morphometric body condition of individuals (Otali & Gilchrist, 2004; Auman, Meathrel & Richardson, 2009; Steigerwald et al., 2015), our morphometric body condition index corrected for growth did not identify any relationship between landfill consumption and morphometric body condition. However, we found that the morphometric body condition index was mainly explained by the number of chicks per nest, that is, the nestlings raised without siblings had better body condition. We also found that nestlings raised alone had higher levels of triglycerides than those raised with a sibling. In birds, an increase in plasma levels of triglycerides has been related to food shortages due to the mobilization of endogenous fats during shortages (García-Rodríguez et al., 1987; Rubio et al., 2014); even so, the opposite could be true as higher levels of plasma triglycerides have been found in well-fed birds (Castellini & Rea, 1992). Our case seems to resemble the latter situation –– nestlings raised without siblings are better fed, face fewer periods of food shortage and so can store triglycerides as lipid reserves.

Moreover, individuals that feed on landfills also have lower levels of cholesterol. As in triglycerides, cholesterol levels could increase during food shortages (Jeffrey et al., 1985; García-Rodríguez et al., 1987), which suggests that individuals that feed on landfills have fewer food restrictions and therefore do not mobilize these lipid reserves. Breeding pairs that use landfills have more regular brood sizes, a finding that is possibly related to the constant availability of food; by contrast, pairs that depend on other food resources alternate between one or two chicks according to the environmental conditions in a given year. This result suggests that pairs that feed on rubbish dumps could be less dependent on the availability of natural environmental food resources and so their brood size may be related to the characteristics and reproductive capacity of individuals.

We also found that breeding pairs that feed on landfills had territories in more lower-lying areas, thereby implying that landfill diet is indirectly related to altitude. In this sense, we found that altitude was correlated with the haematocrit, as has been observed in other species (Fair, Whitaker & Pearson, 2007; Resano-Mayor et al., 2016). Finally, we detected an important role of the temporal trend when explaining the variance of some biochemical and oxidative stress parameters. Even so, our data set is small and it is possible that the importance of this effect may be much greater if longer time spans are monitored. Further studies are needed to assess potential changes in demographic parameters of individuals over the years.

Integrative approach and conservation implications

Overall, our results highlight the importance of performing integrative approaches combining multiple biological components when attempting to describe the implications of the consumption of food from landfills on the body condition of individuals. In our study system, this multi-parameter approach turned out to be key in understanding the consequences of changes in food availability in human-altered environments at individual level. Our results also showed that landfills are an easy source of food that allow animals to be better fed but that the consumption of this type of food could modify antioxidant metabolism. In the same way, it would be well-worth studying in the future which other factors could be acting on body condition since toxic substances present in landfills can also affect physiology and/or cause intoxication in individual birds (Espín et al., 2014; Ortiz-Santaliestra et al., 2015). There are still many gaps in our knowledge regarding how these metabolic changes cause long-term effects on individuals and populations. Thus, in a context in which human-altered environments are expanding, an understanding of the consequences of feeding on new resources offered by humans will be crucial for the suitable design of future conservation and management strategies for both endangered and abundant species.

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Supporting information

Table S1. Response variables, explanatory variables and covariates considered in redundancy analysis (RDA).

Response variables	Explanatory variables
Morphometric body condition index	Landfill consumption
Haematocrit	Wild herbivores consumption
Calcium	Livestock consumption
Magnesium	Carnivores consumption
Phosphorus	Birds consumption
Glucose	Diet diversity
Cholesterol	Number of chicks
Tryglicerides	Age of individual
Creatinine	Sex of individual
Urea	Territory
Uric acid	Altitude
Total proteins	
Alkaline phosphatase	
Aspartate aminotransferase	
Lactate dehydrogenase	**Covariates**
Creatine phosphokinase	Recent ingestion of food
Retinol	Time of the day
Retinyl Palmitate	Year
Tocopherol	
Lutein	
Zeaxanthin	
Glutathione peroxidase	
Superoxide dismutase	
Malondialdehyde	
GSHT	
GSHO	
GSHOT	

CAPÍTOL 5

Levels and physiological effects of persistent organic pollutants and metals in nestlings of Egyptian Vulture

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Resum

Els voltors són susceptibles a acumular i concentrar contaminants orgànics persistents (COPs) i metalls en els seus teixits degut a la seva posició al capdamunt de la xarxa tròfica. Així, les espècies carronyaires estan exposades als potencials efectes tòxics causats per aquests contaminants. En el present estudi, es van analitzar les concentracions de diferents famílies de COPs: compostos organoclorats (Ocs), èters difenílics polibromats (PBDEs), substàncies perfluorades (PFAS), així com diferents metalls: plom (Pb), cadmi (Cd), arsenic (As), coure (Cu) i zinc (Zn) en els polls d'una població d'aufrany (*Neophron percnopterus*) a Catalunya (NE de la Península Ibèrica). A més, es va dur a terme una anàlisi de redundància (RDA) per tal d'estudiar els efectes fisiològics de l'acumulació de contaminants, a partir de diferents paràmetres sanguinis indicadors de la bioquímica plasmàtica i metabolisme antioxidant dels individus. Finalment, es va utilitzar la proporció de superfície urbana al voltant de cada niu com una aproximació del nivell d'humanització dels territoris de cria per tal d'explorar la relació entre aquesta variable i l'acumulació de contaminants a la sang dels polls d'aufrany. Els resultats apunten que la major part dels nivells detectats de contaminants es troben per sota el límit de detecció o són en general baixos, per tant, l'acumulació de contaminants en polls d'aufrany a l'àrea d'estudi no sembla estar causant greus efectes negatius sobre la seva fisiologia. La principal correlació de l'exposició de contaminants va ser l'associació entre PFAS i alguns paràmetres del sistema anitoxidant (α-tocoferol, luteïna i zeaxantina). Tot i així, aquesta relació sembla ser conseqüència de l'explotació de fonts d'aliment antropogèniques més que d'un efecte directe dels contaminants sobre la fisiologia dels individus.

Abstract

Vultures are at risk of accumulating and concentrating persistent organic pollutants (POPs) and metals in their tissues due to their position at the top of the food web, so they may be more predisposed to the toxic effects of these substances. In this study, we analysed the concentrations of different families of POPs: organochlorine compounds (OCs), ploybrominated diphenyl ethers (PBDEs) and perfluoroalkyl substances (PFAS) as well as different metals: lead (Pb), cadmium (Cd), arsenic (As), copper (Cu) and zinc (Zn) in nestlings of the population of Egyptian Vulture *Neophron Percnopterus* in Catalonia (NE Iberian Peninsula). Also, we performed a redundancy analysis (RDA) to study the physiological effects of pollutant accumulation assessing several nestling blood parameters indicative of health status including plasma biochemistry, antioxidant defenses and oxidative stress biomarkers. Finally, we used the proportion of urban surface around each nest as a proxy of the humanization of breeding territories to explore the relationship between this variable and accumulation of the different pollutants in vulture nestlings. As most detected levels of pollutants were below the detection limit or were, in general low, pollutant accumulation in nestlings of Egyptian Vulture from Catalonia does not appear as susceptible to cause detrimental effects to their physiology. The main physiological correlation of pollutant exposure was the association between PFAS exposure and parameters like α-tocopherol, lutein and zeaxanthin, which seems to be consequence of the exploitation of artificial food sources rather than of a direct effect of PFAS on the organism physiology.

Keywords: scavengers, threatened species, biomonitoring, physiology

Introduction

Accumulation of persistent organic pollutants (POPs) and metals is one of the main environmental threats to raptor birds (Drouillard et al., 2001), which, because of their high position in trophic webs, tend to accumulate large amounts of these chemicals and are considered as sentinels for pollutant biomonitoring (Gómez-Ramírez et al., 2014). Under Stockholm Convention, most POPs are currently discontinued from industrial and domestic use, although their massive utilization in the past together with their low degradation rate make their environmental levels still high enough to affect human and wildlife health.

Organochlorine compounds (OCs) were used as insecticides since the 1940s, being replaced by less persistent alternatives from the 1970s until their complete ban in developed countries (Turusov et al., 2002). OCs have also been used for decades as part of several industrial activities and materials (e.g. PCBs as components of electrical transformers). Brominated compounds, and particularly polybrominated diphenyl ethers (PBDEs) were added to a wide variety of materials due to their properties as flame retardants (Hites, 2004), being the most toxic congeners banned at early 21st century. Perfluoroalkyl substances (PFAS) have been also widely used since mid-20th century as impermeable coatings, thermal stabilizers or surfactants (Lindstrom et al., 2011), and some actions have also been taken to reduce their environmental release, although their complete ban has not been implemented so far (Gómez-Ramírez et al., 2017). All these POPs are commonly released to the atmosphere and transported until deposition in remote cold-climate areas (Daly and Wania, 2005; Lau et al., 2007), thus showing potential for global scale pollution.

Metals are substances naturally occurring in the environment, being the Earth's crust their main reservoir. Some human activities like mining and groundwater extraction have contributed to mobilize metals from the lithosphere, increasing their bioavailability and potential for causing toxic effects (Maiz et al., 2000). In addition, extracted metals are also used in many products and materials, some of which can reach high environmental concentrations and become a relevant source of exposure for wildlife (e.g. ammunition as a source of environmentally available lead, Mateo et al., 2014).

Among the studies of biomonitoring pollutant accumulation in raptors, only a few focus on scavengers. Due to their special feeding regime among birds of prey, obligate avian scavengers can be of special interest in the study of pollutant accumulation and of the relationship of this accumulation with responses at the physiological level. A review of the available literature on pollutant accumulation in diurnal raptors does not reflect any particular trend pointing to a higher or lower susceptibility of avian scavengers relative to other species (Van Wyk et al., 1993; van Wyk et al., 2001; Garcia-Fernandez et al., 2005; Shlosberg et al., 2012). However, there is hardly no data on how accumulated pollutants associate with biochemical indicators of general health or with biomarkers of oxidative stress that are commonly studied in response to pollutant exposure in wildlife.

The aim of this study was to analyse the relationship between pollutant accumulation and biochemical responses in an obligate avian scavenger, the Egyptian Vulture (*Neophron percnopterus*). This is a globally endangered species known to exploit a wide range of food resources (Donázar, 2004). The studied population, located in Catalonia (NE Iberian Peninsula), is known to be expanding its range and colonizing new territories (Tauler et al., 2015). This expansion is presumably related to the exploitation of novel food resources such as landfills (Tauler-Ametller et al., 2017; chapter 3 of present thesis), whose influence in pollutant accumulation patterns remains unknown for raptor birds in general. In this context, we used the proportion of urban surface around each nest as a proxy to estimate potential use of novel food sources in this expanding population, and explore the relationships between this variable and accumulation of the different pollutants in vulture nestlings being presumably fed from nearby sources.

Materials and methods

Sample collection and estimation of human influence

Samples (N=77 individuals) were obtained between 2012 and 2016 from several Egyptian Vulture territories located across central eastern Catalonia (NE Iberian Peninsula) (Fig. 1 from chapter 3 of present thesis). Nests were identified and monitored during the breeding season (March–August) with the help of a spotting scope (60x). Once nestlings were around 45-55 days old, experienced climbers accessed the nests to capture the chicks, which were then carefully transported to the top of the cliff for blood collection. We extracted 2.2 ml of blood from the brachial vein with a 20 gauge needle; for the analyses described in the present study, we used 2 ml, which were centrifuged at 10000 rpm for 5 min to separate plasma from the cellular fraction (red blood cells, RBCs). Both samples were frozen in liquid nitrogen and stored at −80°C until analysis.

The percentage of surface occupied by urban areas within a circle of 8 km around each nest was estimated from the information contained in the Topographical Map of Catalonia (1:25 000, Institut Cartogràfic de Catalunya) using ARCGIS 10.2 (ESRI). This area is consistent with the home range of breeding Egyptian Vultures from southern Spain (Carrete et al., 2007). The proportion of urban surface in the study area is correlated with other variables indicative of human presence (Tauler-Ametller et al., 2017) and is indirectly related with waste consumption (chapter 3 of present thesis).

Analysis of metals and trace elements

RBCs were freeze-dried (Christ Alpha 1e2, Braun Biotech) and analysed for lead (Pb), cadmium (Cd), arsenic (As), copper (Cu) and zinc (Zn) as detailed in Ortiz-Santaliestra et al. (2015). All concentrations are given relative to dry weight (d.w.). Briefly, Pb, Cd and As were analysed using graphite-furnace atomic absorption spectroscopy (GF-AAS; AAnalyst800 with autosampler AS800, Perkin-Elmer), while Cu and Zn were analysed by flame-AAS. Dried samples were digested with nitric acid and hydrogen peroxide and taken to a final volume of 15 ml with Milli-Q water. Calibration standards were prepared in Milli-Q water from 1 g/l commercial solutions (Panreac). A reference material (Lobster Hepatopancreas; TORT-2, National Research Council, Canada) with certified levels (µg/g; mean±95% CI) of Pb (0.35±0.13), Cd (26.7±0.6), As (21.6±1.8), Cu (106±10), Zn (180±6), was analysed (N=4) to ensure the quality of the methodology. Mean±SD recovery values were 83.0±15.0% for Pb, 119.2±24.4% for Cd, 116.8±12.2% for As, 104.7±16.5% for Cu, and 80.8±5.7% for Zn. The detection limits were 0.080 µg/g Pb, 0.007 µg/g Cd, 0.056 µg/g As, 0.001 mg/g Cu, and 0.004 mg/g Zn.

Analysis of organohalogen compounds

OCs, PBDEs and PFAS were determined from plasma samples. Solvents and reagents used were analytical grade or equivalent high purity grade and purchased form Merck (Darmstad, Germany) or Panreac (Montcada i Reixac, Spain). Target compounds were extracted with n-hexane from plasma samples previously spiked with a known amount of PCB 209 (Dr. Ehrenstorfer, Augsburg, Germany) as internal standard, followed by at least three clean-up repetitions with sulfuric acid as described in Mateo et al., (2012).

OC quantification was performed by high-pressure liquid chromatography (HPLC) in an Agilent Technologies 6890 N series equipped with a 30 m fused silica capillary column of 0.32 mm ID and 0.25 µm of film thickness (HP-5 from J&W Scientific, USA), coupled to an electron capture detector (ECD), in conditions optimized for the analytes and specified in Ortiz-Santaliestra et al. (2015). Quantification of OC was done with calibration curves prepared with Pesticide-Mix 13 and PCB-Mix 20 (Dr. Ehrenstorfer, Augsburg, Germany). The recovery of the method, calculated with plasma samples spiked with Pesticide-Mix 13 at four different concentrations (n=3 per concentration level) ranged from 79.1% to 107.5%. Corrections based on recovery data were not taken into account for quantification.

The same extracts used for OC analysis were reinjected for PBDEs determination. PBDEs were analysed following the method described in Zapata et al. (2018) and their quantification was performed by external standard quantification.

Mixture of native PFAS containing PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTriDA, PFTeDA, PFHxDA, PFODA, PFBS, PFHS, PFOS and PFDS were supplied by Wellington Laboratories (Ontario, Canada). Stock standard solutions were prepared in acetonitrile at a concentration of 5 ng/µL. Perfluoro-n-(1, 2, 3, 4-13C4) octanoic acid (MPFOA) and sodium perfluoro-1-(1, 2, 3, 4-13C4) octane sulfonic acid (MPFOS), also from Wellington Laboratories, were used as surrogate standards. HPLC grade water and acetonitrile were supplied by Merck (Darmstadt, Germany) and glacial acetic acid from Panreac (Barcelona, Spain). The extraction of plasma samples and instrumental analysis was performed following Vicente et al. (2012). The recoveries of surrogate standards were of 88±18% for MPFOS and 92±19% for MPFOA. The calibration was performed over a concentration range of 0.005 to 0.3

µg/mL and good reproducibility based on the calculation of the coefficient of variation was obtained for all test compounds (<10%).

Detection limits of OCs, PBDEs and PFAS were all <0.01 ng/ml.

Plasma biochemical parameters

Plasma samples were used to determine circulating levels of calcium, cholesterol, creatinine, glucose, magnesium, phosphorus, total proteins, triglycerides, urea and uric acid, and the enzymatic activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine phosphokinase (CK) and lactate dehydrogenase (LDH). These parameters were determined spectrophotometrically in an automatic analyzer A25 using the reaction kits available for each parameter (BioSystems, Barcelona, Spain). We determined the levels of free retinol, α-tocopherol and carotenoids (zeaxanthin and lutein) in plasma using HPLC (Agilent Technologies 1100 Series) coupled to a photodiode detector (DAD) and a fluorescence detector (FLD), following the extraction and chromatographic methods described in Rodríguez-Estival et al. (2010).

Oxidative stress biomarkers

We measured oxidative stress biomarkers by spectrophotometry in homogenates of RBCs following the methods described in Reglero et al. (2009). We quantified total and oxidized glutathione (GSH) concentrations, and calculated the molar proportion of total GSH present as oxidized form. We used Ransel and Ransod kits (Randox Laboratories, Crumlin, UK) to measure the activities of glutathione peroxidase (GPX, EC 1.11.1.9) and superoxide dismutase (SOD, EC 1.15.1.1), respectively. Enzyme activities were calculated relative to mg of protein, which were determined in RBC homogenates according to Bradford (1976). As a measure of oxidative damage caused by ROS to membrane phospholipids, we quantified malondialdehyde (MDA) in the RBC homogenates by HPLC in an Agilent Technologies 1100 Series coupled to a photodiode detector (DAD) and a fluorescence detector (FLD). Determination of MDA was performed following the method by Agarwal and Chase (2002) with the modifications of Romero-Haro and Alonso-Alvarez (2014).

Statistical analysis

In order to minimize variance heterogeneity, all variables were log transformed prior to the analysis. Those analysed chemicals that, because of a high proportion of measures below detection limits, had a null interquartile range, were excluded from further statistical analyses. Consequently, the substances considered for analysis included all tested PFAS and PBDEs, as well as p,p'-DDE, δ-HCH, PCBs 28, 52 and 180, and Zn.

To analyze the influence of the set of pollutants on blood parameters we run a redundancy analysis (RDA) using the correlation matrix (ter Braak, 1994). In order to reduce the redundancy among variables, we run a principal component analysis (PCA) with the independent variables (i.e. chemicals, N=22) and another PCA with the response variables (i.e. blood parameters, N=23). We used the PC scores to create the variables that were used in the RDA; the first four PCs of the PCA run on chemicals (PCchem) were used as environmental variables, whereas the first four PCs of the PCA run on blood parameters (PCparam) were used as response variables in the RDA.

The most significant PCchem influencing the pool of PCparam in the RDA were determined using a forward selection according to a Monte Carlo permutation test (999 permutations). In order to identify which specific responses were influenced by the selected chemical components, we run bivariate correlations of every PCparam with the specific PCchem selected by the Monte Carlo test. We further explored the associations between chemicals and blood parameters represented in the significantly correlated PCs. With this purpose, we run a multiple linear regression for each relevant blood parameter as dependent variable, including as independent variables those chemicals represented in the PCchem. We used the regressions instead of correlations between specific pairs of variables to minimize the influence of zero-like (i.e. values below limit of detection) inflation existing in the majority of analysed pollutants.

In order to analyse how human presence influenced the accumulation of pollutants by Egyptian Vulture nestlings, we correlated the proportion of urban area (log-transformed) around the nest with the PCchem. When significant correlations were found, the specific contaminants whose measurements in nestling were associated with the proportion of nearby urban areas were determined through regressions with each pollutant associated with the significant PCchem as dependent variables and the proportion of urban areas as explanatory variable.

Results

Chemical analysis

For the majority of analysed chemicals, we found a high frequency of values below the corresponding limits of detection (Table 1). Geometric means were in the range of quantifiable levels for the two DDE isomers, the δ and ε isomers of HCH, isomer A of heptachlor epoxide, PCBs 28, 52, 101 and 180, all PFAS, all PBDEs but congener 183, and Zn. As explained in the methods, only compounds showing some variability among samples, as indicated by an inter-quartile range different from zero, were considered for further statistical analyses.

The four first axes (PCs) of the PCA grouping chemical substances explained 56.6 % of the system variance. The first PCchem (PCchem1) was indicative of increasing plasma concentrations of PFAS. PCchem2 reflected increased levels of most PBDEs (i.e. congeners 100, 153, 154 and 183) and δ-HCH. PCchem3 correlated with increased blood levels of PCB28, PFBA, and PBDEs 183 and 209, and with decreased levels of p,p'-DDE and PCB180. Finally, PCchem4 showed a positive association with p,p'-DDE and a negative one with PBDE99.

Table 1. Summary of results (geometric means maximum values) obtained from the analysis of pollutants in blood of Egyptian Vulture chicken (N=77) from Catalonia. Percentage of samples with detectable values (% > LoD) is shown for each substance. Substances marked with an asterisk (*) had an inter-quartile range different from zero and were included in further statistical analyses.

Substance	% > LoD	Geometric mean	Maximum
Metals			
Pb (μg/g w.w.)	18.18	< LoD	0.215
Cd (μg/g w.w.)	2.60	< LoD	0.013
As (μg/g w.w.)	15.58	< LoD	0.779
Cu (mg/g w.w.)	0.00	< LoD	-

Substance	% > LoD	Geometric mean	Maximum
Organochlorine compounds (OCs)			
p,p’-DDT (ng/ml)	3.90	< LoD	1.190
o,p’-DDT (ng/ml)	1.30	< LoD	1.222
p,p’-DDE (ng/ml)*	18.18	0.014	3.522
o,p’-DDE (ng/ml)	12.99	0.010	3.291
o,p’-DDD (ng/ml)	3.90	< LoD	3.202
p,p’-DDD (ng/ml)	2.60	< LoD	1.259
Aldrin (ng/ml)	1.30	< LoD	1.005
Dieldrin (ng/ml)	12.99	< LoD	1.709
Endrin (ng/ml)	1.30	< LoD	0.692
Isodrin (ng/ml)	1.30	< LoD	0.946
α-HCH (ng/ml)	2.60	< LoD	0.608
β-HCH (ng/ml)	3.90	< LoD	13.825
γ-HCH (ng/ml)	5.19	< LoD	3.786
δ-HCH (ng/ml)*	41.56	0.044	3.521
ε-HCH (ng/ml)	15.58	0.011	1.855
HCB (ng/ml)	1.30	< LoD	0.619
cis-Chlordane (ng/ml)	11.69	< LoD	4.085
trans-Chlordane (ng/ml)	2.60	< LoD	1.900
Metoxichlor (ng/ml)	0.00	< LoD	-
Heptachlor (ng/ml)	2.60	< LoD	2.383
Heptachlor epoxide (isomer B) (ng/ml)	2.60	< LoD	1.408
Heptachlor epoxide (isomer A) (ng/ml)	14.29	0.010	2.496
β-Endosulfan (ng/ml)	3.90	< LoD	2.327
Mirex (ng/ml)	1.30	< LoD	0.686
PCB28 (ng/ml)*	97.40	6.322	38.822
PCB52 (ng/ml)*	19.48	0.018	15.746
PCB101 (ng/ml)	14.29	0.012	6.301
PCB138 (ng/ml)	9.09	< LoD	2.733
PCB153 (ng/ml)	7.79	< LoD	1.399
PCB180 (ng/ml)*	58.44	0.084	5.126
Polybrominated diphenil ethers (PBDEs)			
PBDE47 (ng/ml)*	86.49	0.026	0.103
PBDE99 (ng/ml)*	82.43	0.025	0.140
PBDE100 (ng/ml)*	44.59	0.011	0.113
PBDE153 (ng/ml)*	55.41	0.014	0.103
PBDE154 (ng/ml)*	51.35	0.011	0.089
PBDE183 (ng/ml)*	33.78	< LoD	0.039
PBDE209 (ng/ml)*	83.78	0.023	1.100
Perfluoroalkyl substances (PFAS)			
PFBA (ng/ml)*	60.81	0.133	3.850
PFHxS (ng/ml)*	56.76	0.075	4.534
PFOA (ng/ml)*	24.32	0.027	818.628
PFNA (ng/ml)*	48.65	0.165	62.545
PFOS (ng/ml)*	93.24	2.273	55.899
PFDA (ng/ml)*	77.03	0.253	8.267
PFUnA (ng/ml)*	86.49	0.380	3.151
PFDoA (ng/ml)*	64.86	0.141	6.659
PFTriDA (ng/ml)*	89.19	0.175	6.447

Relationship between chemicals and blood parameters

Summarized results of the parameters measured in plasma (biochemical indicators) and red blood cells (oxidative stress biomarkers) of the analysed nestlings are shown in Table 1 from chapter 4 of present thesis. The PCA run to group all these parameters explained a 46.2% of the total variance in its four first axes. The first PC (PCparam1) seemed to reflect a good nutritional status, as it was positively associated with plasma levels of Ca, cholesterol, α-tocopherol and carotenoids (lutein and zeaxanthin) and negatively associated with the activity of CK. PCparam2 was mostly associated with increased nutritional metabolism, as correlated positively with cholesterol, glucose, urea and total proteins in plasma; it was also associated with increased levels of MDA in erythrocytes. The third and fourth PCs looked like indicative of the antioxidant status; PCparam3 reflected an increased activity of the antioxidant system as revealed by its positive association with plasma lactate dehydrogenase activity and negative association with the activity of superoxide dismutase in RBCs. PCparam3 was also positively associated with AST activity in plasma. On the other hand, PCparam4 was an indirect indicator of oxidative stress, as it correlated positively with an increased proportion of oxidized GSH and negatively with increased total levels of GSH in RBCs.

The RDA selected the PCchem 1, 2 and 3 as significantly influencing the full set of PCparam obtained from the blood parameters measured in nestlings (Fig. 1). The three canonical axes considered in the RDA explained a low cumulative percentage of the variance of the responses (PCparam) system (12.3%) (Table 2), but there was a strong relationship between the PCchem and PCparam, with a responses–predictors correlation of 0.589 in the first axis.

Figure 1. Graphic representation of the results of the RDA and Monte Carlo permutation test to analyse the effect of pollutant-extracted principal components (PCchem, continuous arrows) on principal components extracted from physiological parameters (PCparam, dotted arrows).

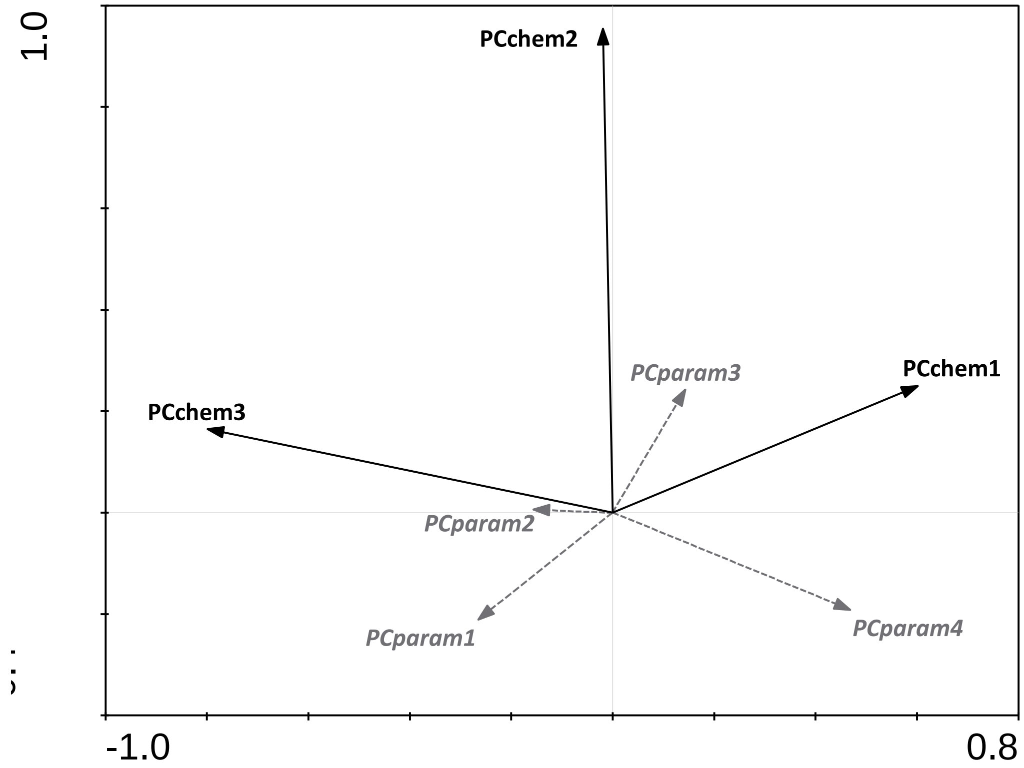


Table 2. Eigenvalues and percentage of variance explained by the redundancy analysis (RDA), with Pearson's correlations between the canonical axes and the predictor variables (i.e. PCchem) selected as significant, and results of the Monte Carlo permutations tests to check the significance of canonical axes.

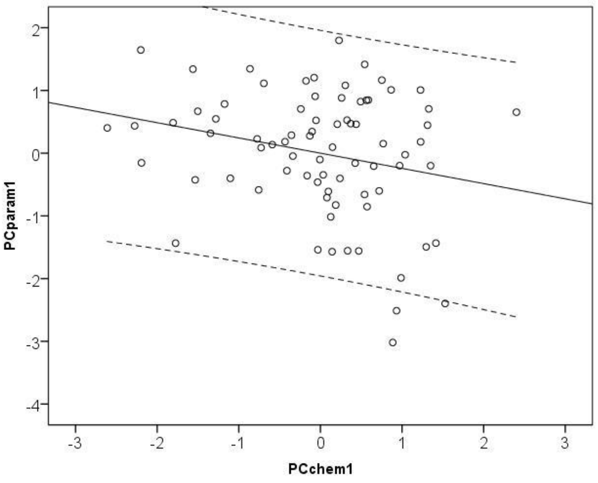
		Axis 1	**Axis 2**	**Axis 3**
Eigenvalues		0.087	0.035	0.001
Chemicals - responses correlation		0.589	0.375	0.064
Cumulative percentage variance of response variables		8.7	12.2	12.3
Cumulative percentage variance of responses-predictors correlation		70.5	99.1	99.9
Regression coefficients for selected chemical variables	*PCchem 1*	0.347	0.098	-0.048
	PCchem 2	-0.016	0.357	0.019
	PCchem 3	-0.111	0.056	-0.038
Monte Carlo test		**F**	**P**	
Significance of first canonical axis		4.34	0.002	
Significance of all canonical axes		1.514	0.006	

PCchem1 was negatively correlated with PCparam1 (R=-0.243, p=0.033, Fig. 2a). Among parameters represented by PCparam1, significant regression models were obtained for Ca (negatively associated with PFTriDA, Fig. 3a), α-tocopherol (negatively associated with PFDoA, Fig. 3b), lutein (positively associated with PFNA and negatively with PFDA, Fig. 3c), zeaxanthin (positively associated with PFNA and negatively with PFDoA, Fig. 3d), and CK (positively associated with PFHxS and negatively with PFNA, Fig. 3e).

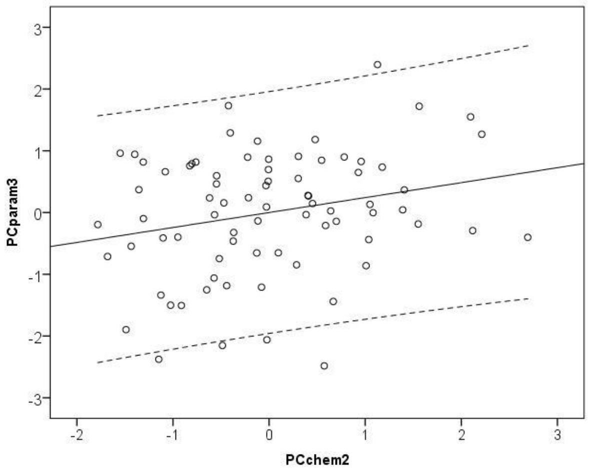
PCchem2 was positively correlated with PCparam3 (R=-0.242, p=0.034, Fig. 2b). Considering the parameters more associated with PCparam2, significant regression models were found for AST (negatively associated with PBDE100 and positively with PBDE154, Fig. 4a) and for LDH (positively associated with PBDE153, Fig. 4b).

PCchem3 was negatively correlated with PCparam4 (R=-0.414, p<0.001, Fig. 2c). The only significant model explaining the variability in the parameters associated with PCparam4 was obtained for total GSH levels, which were positively related to plasma concentrations of PCB180 (Fig. 5).

2a



2b



2c

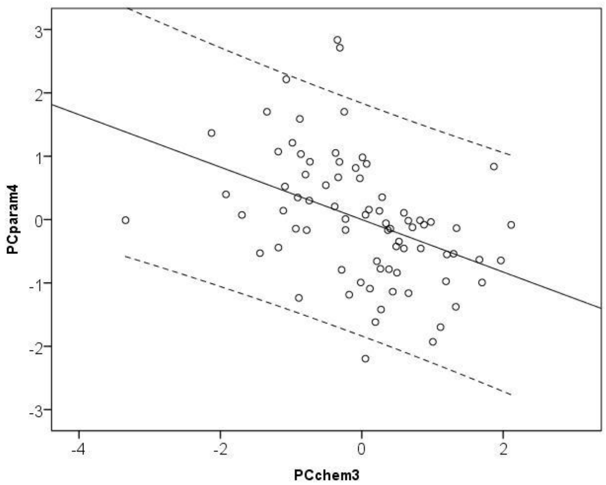
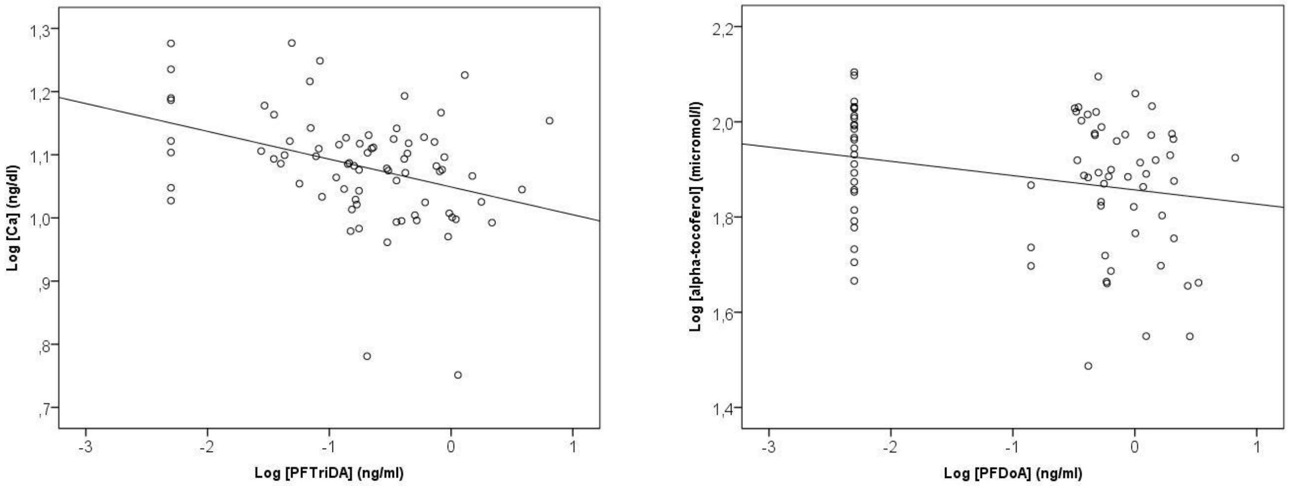
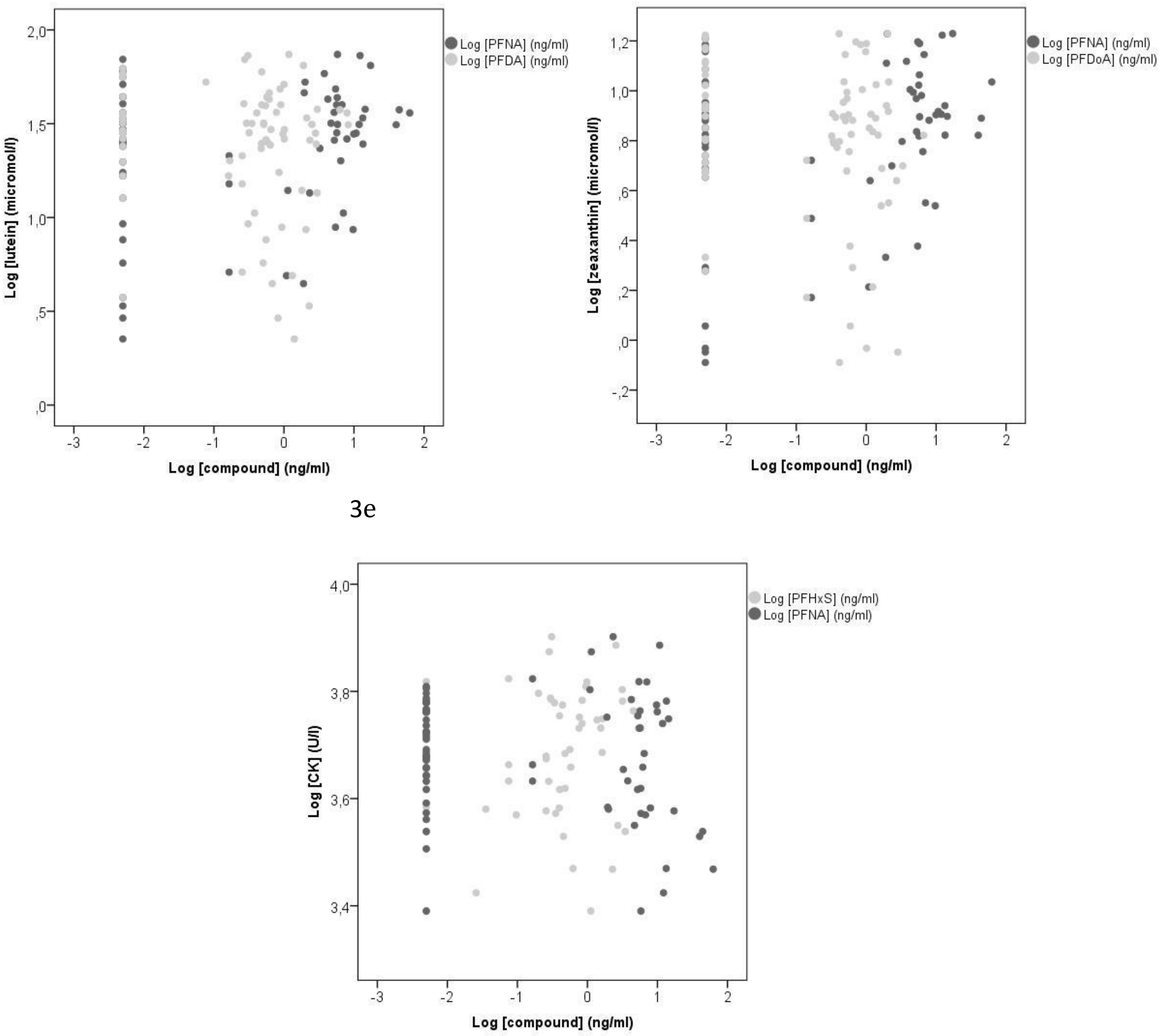


Figure 2. Scatter plots of significantly correlated components considering all possible relationships between those principal components grouping chemical concentrations (PCchem) selected as significant in the RDA and principal components grouping physiological parameters (PCparam) in blood of nestlings Egyptian Vultures. Significant correlations were found between (a) PCchem1 and PCparam1 (R=-0.243, p=0.033), (b) PCchem2 and PCparam3 (R=0.242, p=0.034), and (c) PCchem3 and PCparam4 (R=-0.414, p<0.001).

3a

3b



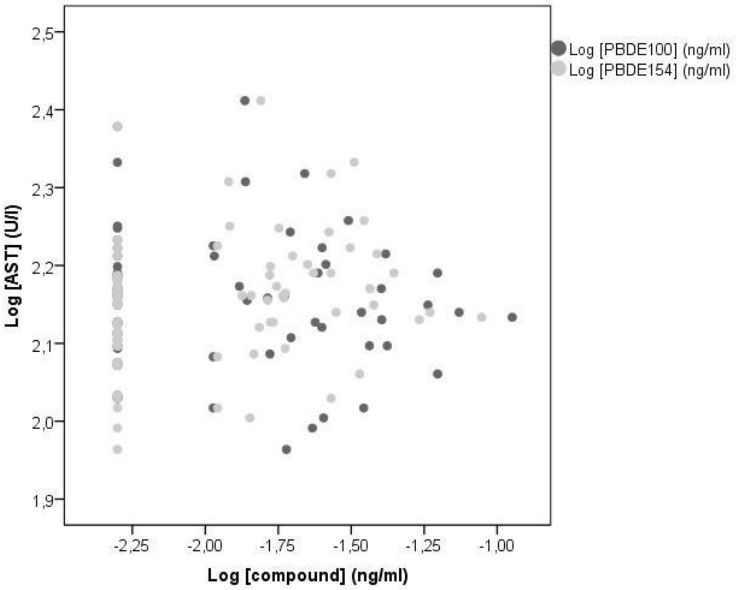

3c

3d

3e

Figure 3. Scatter plots of variables related to PCchem1 and PCparam1, measured in nestling Egyptian Vultures, for which significant associations were identified from multiple regression models: (a) plasma levels of calcium (Ca) vs. plasma levels of perfluorotridecanoic acid (PFTriDA). (b) plasma levels of α-tocopherol vs. plasma levels of perfluorododecanoic acid (PFDoA). (c) plasma levels of lutein vs. plasma levels of perfluorononanoic (PFNA) and perfluorodecanoic (PFDA) acids. (d) plasma levels of zeaxanthin vs. plasma levels of PFNA and PFDoA. (e) plasma levels of creatine phosphokinase (CK) vs. plasma levels of perfluorohexane sulfonate (PFHxS) and PFNA. Adjustment lines extracted from the regression models selecting a single response variable are shown. All variables are depicted in logarithmic scale.

4a



4b

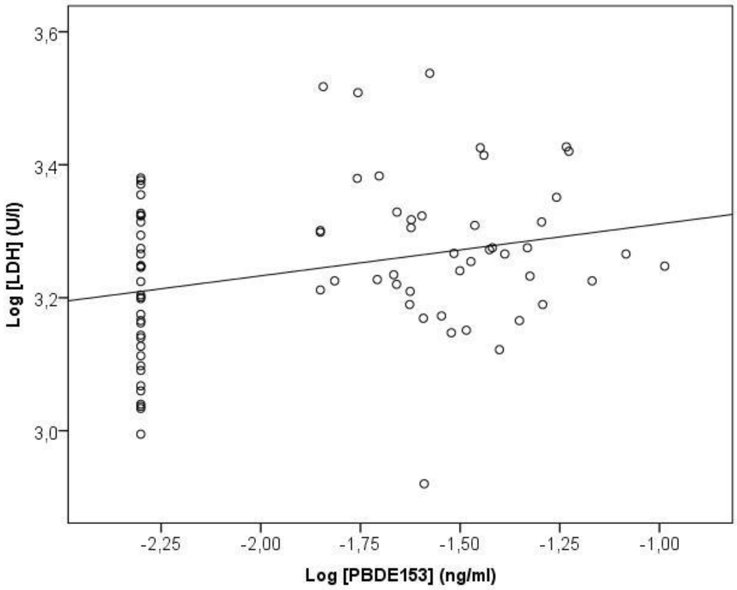


Figure 4. Scatter plots corresponding to the significant regression models between dependent variables represented in PCparam3 (a: AST, b: LDH) and chemicals represented in the correlated PCchem2 selected in each regression model. Adjustment line extracted from the regression model selecting a single response variable is shown. All variables are depicted in logarithmic scale.

5

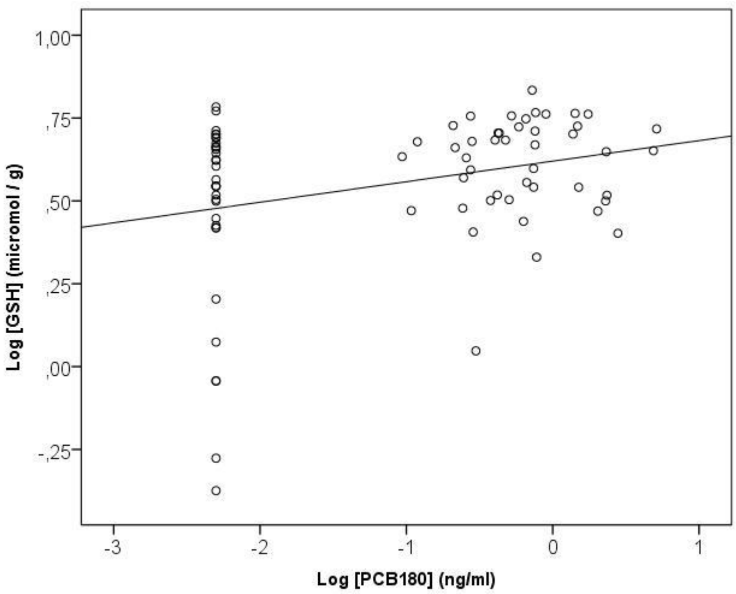
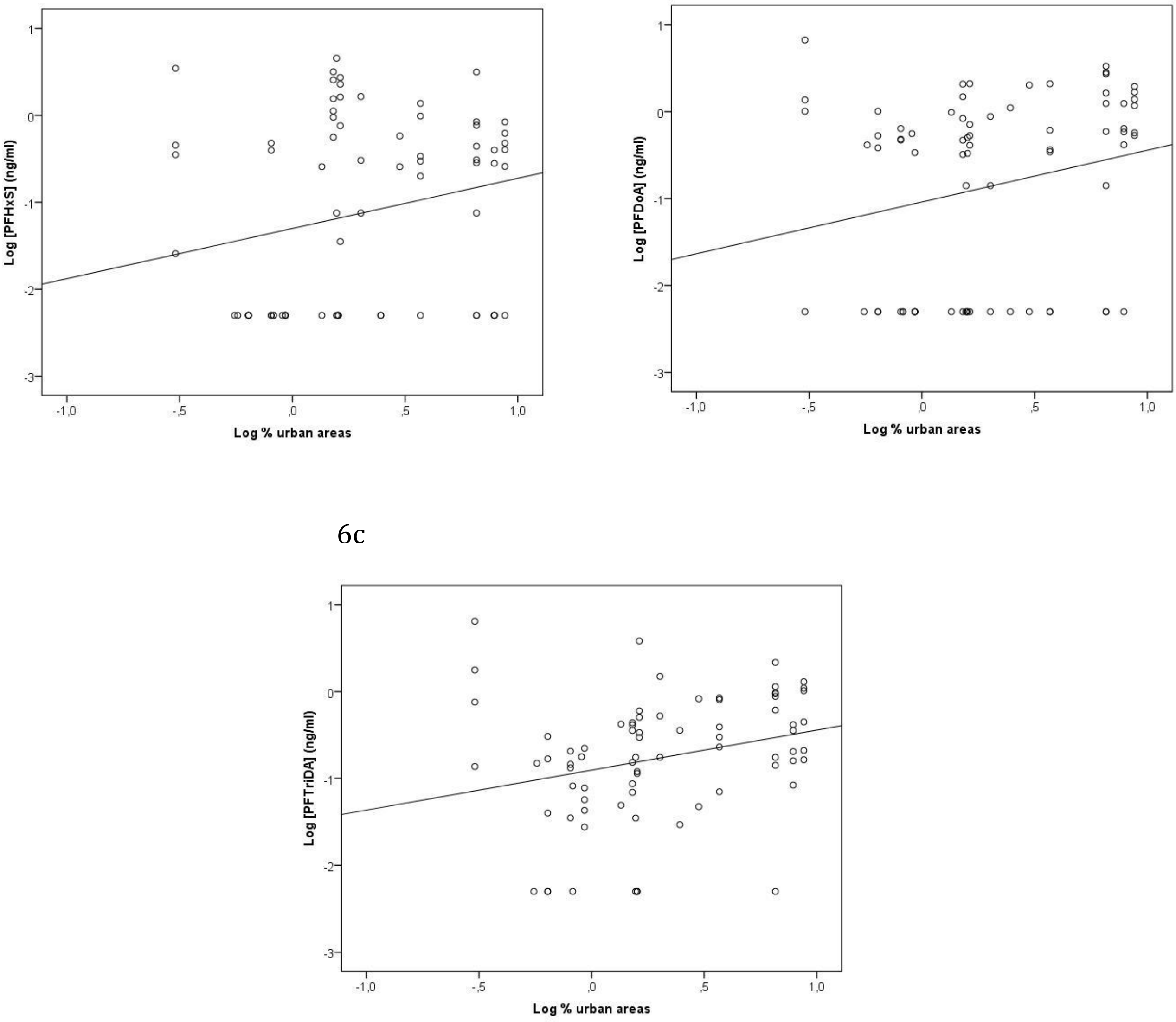


Figure 5. Scatter plot of the relationship between plasma levels of total glutathione (GSH) and PCB180 measured (and log-transformed) in plasma of Egyptian Vulture nestlings. Adjustment line extracted from the regression model is shown.

Influence of human presence on pollutant accumulation

The proportion of urban areas around the nests was significantly correlated with the PCchem1 (R=0.228, p=0.047, N=76). Among the chemical substances mostly associated with this PCchem, we found significant regressions of the percentage of urban area with plasma levels of three specific PFAS: PFHxS (B=0.577±0.289, p=0.049), PFDoA (B=0.595±0.289, p=0.043) and PFTriDA (B=0.460±0.192, p=0.019). In all cases, a positive association of human presence with accumulation of these PFAS in plasma of Egyptian Vulture nestlings was observed (Fig. 6).


6a

6b

6c

Figure 6. Scatter plots corresponding to the significant regression models between proportion of urban areas around the nests and chemicals represented in the correlated PCchem1 (a: PFHxS, b: PFDoA, c: PFTriDA). Adjustment lines extracted from the regression models are shown. All variables are depicted in logarithmic scale.

Discussion

The results of the present study constitute the first published report of accumulation of brominated and perfluoroalkyl POPs in Egyptian Vultures. Considering other species, PFAS levels measured in Egyptian Vultures from Catalonia seem relatively low. Shlosberg et al. (2012) measured mean blood values of 5.8-6.0 ng/ml PFOS and

1.3-2.4 ng/ml PFOA in Griffon Vultures (*Gyps fulvus*) from Israel, with maximum values of 9.4 ng/ml of PFOS in an adult individual and 3.5 ng/ml of PFOA in a juvenile bird. These data suggest that exposure of Egyptian Vulture nestlings monitored in the present study to PFOS and PFOA was lower than that of Griffon vultures from Israel, with the exception of some individual who showed outlying values relative to the entire pool of samples. Route et al. (2014) analysed PFAS in plasma of Bald Eagle (*Haliaeetus leucocephalus*) nestlings from six areas in Minnesota and Wisconsin, and found in all sampled populations higher mean levels than in our study (mean levels per population in ng/ml: PFBA 0.31-0.55, PFHxS 0.77-2.73, PFOA 0.34-1.01, PFNA 4.15-8.13, PFOS 77.53-800.41, PFDA 8.49-12.55, PFUnA 9.46-17.45, PFDoA 3.18-7.03, PFTriDA 2.18-3.87). Bustnes et al. (2013) quantified PFOS in plasma of nestlings of two raptor species, Northern Goshawk (*Accipiter gentilis*) and Marine White-Tailed Eagle (*Haliaeetus albicilla*), from northern Norway, and found significant differences among values obtained from repeated measures with a few weeks of time lapse. Mean values were 12.1 and 22.0 ng/g plasma for Northern Goshawks and 25.4 and 38.7 ng/g for White-Tailed Eagles, which, assuming a plasma density ~1, reflects much higher concentrations than those found in Egyptian Vulture nestlings from Catalonia. Age of nestlings monitored in the present study would be close to that of nestlings at the second sampling by Bustnes et al. (2013), which corresponds to the highest reported value. Maximum PFOS levels found at that sampling time were 90.4 ng/g in Northern Goshawks and 144.2 ng/g in White-Tailed Eagles, approximately two and three times higher than the maximum PFOS level measured in our study (55.9 ng/ml). For PFAS other than PFOS or PFOA, there are hardly no data relative to blood levels in raptors.

In general, data on PFAS accumulation in birds reflect a strong dependence on the species, geographical origin, feeding regime or sampling time for the majority of compounds (Bustnes et al., 2013; Bustnes et al., 2015; Munoz et al., 2017). Therefore, without additional data specifically obtained from Egyptian Vultures is difficult to estimate the reasons for the comparatively low values of PFOS and PFOA found in the present study. Likewise, it is difficult to interpret the potential toxicity associated with the measured levels. Reference values for PFOS toxicity have been proposed for fish-eating birds at levels much higher than those found in the present study (i.e. 5900 and 360 ng/mL for adult males and females, respectively; Newsted et al., 2005). Toxicity tests conducted on non-raptor species revealed a significant increase of mortality rate in Bobwhite Quails (*Colinus virgnianus*) showing 8700 ng/ml of PFOS in plasma, and no lethal effects in Mallard Ducks (*Anas platyrhynchos*) showing plasma PFOS levels up to 16600 ng/ml (Newsted et al., 2007). Jiang et al. (2012) experimentally exposed chick embryos to PFOA and found that treatments leading to a serum concentration of 1230.8 ng/ml had some effects in the cardiac system but did not affect individual survivorship.

Guo et al. (2018) and Venier et al. (2010) found PBDE plasma levels in Bald Eagle nestlings from Michigan higher than those measured in the preset study for all congeners. Only in the case of BDE209, the concentrations measured by Guo et al. (2018) (0.02 ng/g) resembled those observed in our Egyptian Vultures. Fernie et al. (2017) also found higher concentrations of all analysed PBDE congeners than those found in our study when monitoring three Peregrine Falcon (*Falco peregrinus*) populations in Canada, one of them from a remote area in the Arctic, and the same findings were reported by Eulaers et al. (2014) in White-Tailed Eagles from central Norway. BDE209 and the low brominated BDEs 47 and 99 were the most frequently found congeners in our study. BDE209 has been one of the most commercially used congeners (Birnbaum & Staskal, 2004), and therefore is one of the most abundant in the environment. However, because of its low bioavailability and of the potential for environmental biodegradation (Stapleton et al., 2006), concentrations of BDE209 accumulated in wildlife specimens relative to other congeners are very variable. Some studies are in line with ours reporting BDE209 as one of the most abundant PBDEs (e.g. Venier et al., 2010), whereas others found it among the less abundant congeners (e.g. Fernie & Letcher, 2010; Guo et al., 2018). The appearance of BDEs 47 and 99 as highly abundant PBDEs in wildlife monitoring is also frequent (e.g. Fernie et al., 2017), and is surely related to the high bioavailibity of these congeners. The comparatively low PBDE values measured in plasma point to a lack of risk associated with the exposure to these compounds. Toxicity reference values of PBDEs in raptor blood are scarce. Henny et al. (2009) and Nordlöf et al. (2010) reported no reproductive effects of PBDEs in Osprey (*Pandion haliaetus*) and White-Tailed Eagles, respectively, at egg concentrations that would correspond, according to correlations established by Jaspers et al. (2013), to blood levels lower than those measured in Egyptian Vultures sampled in the present study.

OC levels measured in blood of Egyptian Vulture nestlings from Catalonia were generally low compared to what has been measured in other populations of this species. Gómara et al. (2004) monitored OC accumulation in serum of five Egyptian Vulture populations across Spain and reported mean levels of DDE ranging from 0.82 to 6 ng/ml and mean DDT levels ranging from 0.22 to 1.9 ng/ml. Maximum levels per population varied from 1 to 38 ng/ml of DDE and from 0.46 to 5 ng/ml of DDT. Dhananjayan et al. (2011) reported average plasma levels of p,p'-DDT and p,p'-DDE of 5.4 and 8.8 ng/ml, respectively, for Egyptian Vultures captured in India. Maximum concentrations measured in individuals from that population were 11.3 ng/ml of p,p'-DDT and 25.6 ng/ml of p,p'-DDE. The lower concentrations of DDT and DDE found in Catalonian Vultures relative to Indian ones could be explained by the fact that DDT is still in use for certain purposes in India (Kunisue et al., 2003). However, this reason cannot explain the observed low levels relative to findings by Gómara et al. (2004), as DDT was banned in the entire territory of Spain more than 40 years ago. Therefore, the differences between our results and those of Gómara et al. (2004) could be attributable to: i) differences in the degree of environmental pollution among regions related to eventual differences in the intensity of pre-ban use of the insecticide; ii) differences among populations in the potential exposure in their overwintering areas; or iii) a time-related reduction in the accumulation of DDTs because of their progressive environmental degradation, considering that there were between 15 and 17 years of difference between samplings performed by Gómara et al. (2004) and ours. The comparison between populations of plasma levels of PCBs reflects a more comparable pattern than that found for DDT. Actually, the most abundant congener measured in our study, PCB28, showed considerably higher values than those found by Gómara et al. (2004) in the other Spanish populations, with average values per population in that study of 0.06-0.61 ng/ml and maximum values up to 5.4 ng/ml. For PCBs 52, 101 or 180, levels shown by Catalonian vultures were more or less consistent with those reported by Gómara et al. (2004), who reported mean values ranging from 0.46 to 3.3 ng/ml (maximum 0.49 to 6.3 ng/ml) for PCB52, from 0.29 to 1.3 ng/ml for PCB 101, and from 0.40 and 2.80 ng/ml (maximum 0.78-18 ng/ml) for PCB 180. This similarity in some PCB congener values with other Spanish populations in spite of the time lapse between our samplings and those conducted by Gómara et al. (2004) could be expected as Catalonia is one of the most industrialized and densely populated regions in Spain, and the density of PCB-containing apparatus is probably higher in Catalonia than in any of the regions sampled by Gómara et al. (2004). Dhananjayan et al. (2011) did not report concentrations per specific congeners, but measured a mean total PCB level in plasma of Egyptian Vultures from India (226 ng/ml) much higher than what we could calculate from the sum of individual congener levels measured in our study.

Blood levels of analysed metals and metalloids were below detection limits in all cases but for Zn. Donázar et al. (2002) reported mean Pb, As and Cd concentrations in blood of Egyptian Vultures from the Canary Islands of 146, 18.8 and 1.1 ng/ml, respectively, and maximum levels of 1780 ng/ml of Pb, 42.30 ng/ml of As and 3.489 ng/ml of Cd. Although we did not measure metal levels referred to whole blood volumes, an estimation using the average water content in the pellet (68.8%), the average hematocrit value of the analysed samples (30.32%; range 21.49-43.56), and an approximate density of 1.1 g/ml of the blood pellet, would result in maximum levels of 22.4 ng/ml of Pb, 81.2 ng/ml of As, and 1.4 ng/ml of Cd in our samples. The relatively high levels of As could be related to the proximity of sampled territories to industrialized areas; a similar trend was observed by Ortiz-Santaliestra et al. (2015) when comparing As levels in populations of Bonelli's eagles (*Aquila fasciata*) from Catalonia with those of other less industrialized regions in Spain. Donázar et al. (2002) proposed that normal As blood levels in raptors should be below 20 ng/ml, a concentration that was surpassed in four out of the 77 blood samples analysed in the present study.

In the case of Zn, blood concentrations measured in Egyptian Vultures were low compared to those reported by Donázar et al. (2002) for Canary individuals (mean 3.615 µg/ml and maximum 4.467 µg/ml vs. estimated mean and maximum levels in our samples of 0.834 µg/ml and 1.147 µg/ml, respectively). These authors mentioned that the levels they found would be well below those shown by birds living in contaminated areas. Compared to other species, Zn levels measured in our samples were higher than those found in Black Kite (*Milvus migrans*) nestlings from central Spain (mean 5.37, maximum 8.63 ppm; Blanco et al., 2003) or in adult Great Horned Owl (*Bubo virginianus*) from Pennsylvania (mean 1.8, maximum 2.3 ppm; Stout et al. 2010), and lower than levels reported for Peregrine Falcon nestlings from SW Sweden (mean ~15 ppm; Ek et al. 2004) or for Osprey nestlings from several sites along US

Atlantic shore (mean 22.4-30.5, maximum 54 ppm; Rattner et al. 2008). Fluctuations in Zn blood levels are normal in raptors as a function of individual and environmental parameters (Rattner et al., 2008; Ortiz-Santaliestra et al., 2015).

PFAS were the main determinants in the variability of blood parameters measured in Egyptian Vulture nestlings. In general, increased concentrations of different PFAS were associated with decreased levels of the blood parameters indicative of the nutritional status represented in PCparam1 (i.e. calcium, α-tocopherol and carotenoids). Only PFNA showed a positive association with carotenoid levels, and at the same time was related to decreased levels of CK. Decreased levels of vitamins or carotenoids are common in organisms exposed to pollutants generating oxidative stress, which occurs as a consequence of an excessive generation of reactive oxygen substances (ROS) from activation of metabolizing CYP 450 enzymes (e.g. Fernie et al., 2005). Both carotenoids and some free vitamin forms like α-tocopherol counteract the excess of ROS by scavenging electrons from these substances (Di Mascio et al., 1991). However, the relationship between exposure to PFAS and induction of oxidative stress in birds does not seem as clear as for other organohalogen compounds, at least under realistic environmental exposure concentrations. Miljeteig et al. (2012) found a negative association of α-tocopherol levels in Ivory Gull (*Pagophila eburnea*) eggs from three colonies in the Russian Arctic with several OC and brominated compounds, but not with PFAS. Sletten et al. (2016) reported a decrease in the activity of the first-line antioxidant enzyme, SOD, in White-Tailed Eagles from the Norwegian Sub-Arctic showing increased OC levels, but no relationship involving PFAS was detected.

The only direct evidence of effects on the endogenous antioxidant system found in the present study is the positive association between PCB180 and total GSH levels, which was also observed in Bonelli's eagles relative to total PCB levels (Ortiz-Santaliestra et al., 2015). GSH is the major intracellular antioxidant molecule that helps neutralizing free radicals (Apel & Hirt, 2004), and therefore it is common that its circulating levels drop at the first instance as a response to pollutant exposure. However, upon a sustained exposure to ROS generated by pollutant metabolism, organisms may react by synthesizing GSH *de novo* as a compensatory response (DeLeve & Kaplowitz 1991). Therefore, our results follow the line observed by Miljeteig et al. (2012) or Sletten et al. (2016) indicating that capacity of OC to induce oxidative stress in birds is higher than that of PFAS.

Egyptian Vultures, as any other vertebrate, obtain tocopherols and carotenoids from diet, and therefore the relationship with PFAS could be consequence of an impoverished diet leading to increased intake of contaminants in parallel to a reduced ingestion of vitamins and carotenoids. We have recently observed that Egyptian Vultures from the studied populations who feed on landfills, which could constitute a source of PFAS present in human waste materials, show reduced circulating levels of vitamins and carotenoids compared to individuals not exploiting this food source (Chapter 4 of present thesis.). This hypothesis would be supported by the correlation between accumulation of three different PFAS in nestlings and the proportion of urban surface around nests, which could be indicating a comparatively high use of human waste as food source by those couples with nestlings showing the highest levels of PFHxS, PFDoA and PFTriDA. Positive associations of degree of urbanization with pollutant accumulation in raptor birds have been reported for some POPs (Brogan et al., 2017). However, although increased environmental levels of most PFAS are normally related to urbanization (Gewurtz et al., 2013), the present study reports, to the best of our knowledge, the first quantitative evidence of an association between the degree of habitat urbanization and PFAS accumulation in birds. Gewurtz et al. (2016) monitored PFAS accumulation in gull eggs across Canada and found that, whereas some substances like PFOS were more abundant in sampling sites from the densely populated zone of southern Ontario, other compounds like PFNA, PFDA, and PFUnDA were more abundant in colonies from the Arctic regions, probably because of the atmospheric transport and deposition of these compounds. Interestingly, compounds that, according to observations of Gewurtz et al. (2016), are apparently susceptible to pass to the atmosphere and be carried to remote areas were not among those that we found positively correlated to the percentage of urban surface.

Hexa-BDEs appeared positively associated with either AST or LDH. AST is present in a variety of tissues in the organism, and thereby its altered circulating levels may reflect damage to various organs. Likewise, the ubiquitous presence of LDH in almost all cell types makes it a poorly specific indicator of physiological damage, being potentially related to hormonal distress and/or injury of several organs (see review in Maceda-Veiga et al., 2015). One of the effects that could be associated with increased AST or LDH levels is liver injury, as damage to this organ has been related to PBDE exposure in birds. For instance, Sonne et al. (2010, 2012) found positive correlations between PBDE exposure in White-Tailed Eagles, Northern Goshawks and Golden Eagles (*Aquila chrysaetos*) from northern Norway and some biochemical indicators of liver damage, although neither AST nor LDH were analysed in those studies. In the present study, however, we did not find any association between plasma levels of PBDEs and indicators such as ALP that are more specific of liver damage than AST or LDH. With regards of other systems potentially affected by PBDE exposure, Sonne et al. (2013) found signs, in one of the colonies of North Atlantic Great skua (*Stercorarius skua*) that they studied, of altered kidney function associated with increased PBDE concentrations. However, such signs consisted of altered levels of urea in blood, whereas in the Egyptian Vultures analysed in the present study urea showed no correlation with PBDE levels. Thus, increased levels of AST and LDH associated with hexa-BDE accumulation in the sampled individuals could be reflecting damage at the respiratory, cardiac or endocrine levels, but further studies analyzing additional biomarkers will be necessary to determine the reasons for these correlations.

Pollutant accumulation in nestlings of Egyptian Vulture from Catalonia does not appear, in general, as susceptible to cause detrimental effects, although the establishment of reference levels for some compounds like PFAS needs further research. The main physiological correlate of pollutant exposure was the association between PFAS exposure and parameters like α-tocopherol, lutein and zeaxanthin, which seems to be consequence of the exploitation of artificial food sources rather than of a direct effect of PFAS on the organism physiology. Therefore, other ecological factors such as food availability and/or diet composition must be explored in order to improve our understanding of the relationships between pollutants and biochemical responses in Egyptian Vultures.

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DISCUSSIÓ GENERAL

En la present tesi, s'estudien les causes potencials de l'augment demogràfic i expansió geogràfica de la població d'aufrany (*Neophron percnopterus*) a Catalunya, incidint especialment en el paper que han tingut els abocadors en tot aquest procés. Aquest estudi aporta informació rellevant en dos sentits. En primer lloc, perquè descriu el cas particular de l'augment demogràfic d'una espècie amenaçada que es troba en declivi en gran part de la seva distribució mundial; el coneixement de les causes que han provocat aquesta tendència positiva podria ser molt útil per a la conservació de l'aufrany en d'altres àrees on està disminuint. En segon lloc, perquè ofereix l'oportunitat d'avaluar quines són les conseqüències del fet d'alimentar-se en abocadors des d'un punt de vista interdisciplinari sobre una espècie, és a dir, tenint en compte els efectes a nivell individual i poblacional. En un món antropitzat on els escenaris de la distribució dels recursos estan canviant constantment, aportar coneixement sobre els efectes de l'alimentació en abocadors pot ser clau per a dissenyar mesures de gestió futures de les espècies que utilitzen aquests punts, siguin amenaçades o plaga.

Viabilitat demogràfica

L'aufrany és una espècie que ha patit una disminució poblacional molt important al llarg de la seva distribució mundial (BirdLife International, 2016). Malgrat aquesta tendència mundial negativa, existeixen algunes poblacions d'aquesta espècie que no es troben en declivi, sinó que estan augmentant en els últims anys (García-Ripollès & López-López, 2006; Mateo-Tomás & Olea, 2010). Una d'aquestes excepcions és la població d'estudi de la Catalunya Central i Oriental, on el nombre de parelles reproductores ha augmentat d'1 a 22 entre els anys 1988 i 2012.

Per tal de poder avaluar els mecanismes implicats en la dinàmica poblacional de les espècies, una de les eines clau que existeixen en la conservació de la biodiversitat és l'Anàlisi de Viabilitat de Poblacions (AVP). Usualment, aquestes anàlisis demogràfiques han estat aplicades a poblacions en declivi (Carrete et al., 2009), però poques vegades s'han aplicat a espècies amenaçades en augment (Ortega et al., 2009). En el nostre cas, aquests models ens han permès identificar quins són els factors demogràfics implicats en l'augment poblacional de l'aufrany a l'àrea d'estudi. Els resultats dels models indiquen que el creixement poblacional observat en els últims anys es pot explicar per la combinació de dos factors demogràfics principals. El primer, una supervivència dels individus adults de la població més elevada que en d'altres àrees de la Península Ibèrica. El segon, la immigració d'individus procedents d'altres àrees, ja que, segons aquests models, només amb un augment de la supervivència adulta la població no hagués experimentat el creixement observat.

Així mateix, les anàlisis de sensibilitat i elasticitat de les taxes vitals confirmen que la supervivència adulta és el paràmetre que té una influència més gran en la taxa de creixement de la població, tal com sol succeir en les espècies de vida llarga (Hernandez-Matias et al., 2013). Per tant, tenint en compte que la supervivència adulta és un paràmetre clau per a la dinàmica demogràfica de l'espècie, es deriva que qualsevol amenaça que l'afecti implicarà grans canvis en la seva mida poblacional.

En aquest sentit, la principal hipòtesi que explicaria perquè en la nostra àrea d'estudi la supervivència adulta és més elevada que en altres zones, és que existeix una mortalitat més baixa per enverinament que en d'altres àrees de la Península Ibèrica. Tal i com s'ha comentat, l'enverinament és una de les principals amenaces que afecten a l'espècie (Hernández & Margalida, 2009) i sembla que en la població d'estudi se n'ha reduït l'impacte en els últims anys (Hernández, 2006). Així, durant el període 1995-2010 només es van registrar tres individus morts per enverinament a Catalunya, i tots ells fora de la nostra àrea d'estudi. Aquestes xifres són molt més baixes que les registrades en d'altres zones properes com a l'Aragó, on es van registrar fins a 47 individus morts durant el període 1996-2006 (Hernández, 2006). D'aquesta manera, la població estudiada podria servir com a model de l'impacte potencial de la disminució de l'enverinament sobre les taxes vitals i la dinàmica poblacional de l'aufrany, mostrant que si en altres àrees aquesta activitat disminueix l'espècie podria veure's afavorida en uns anys.

D'altra banda, els models demogràfics també apunten que ha estat necessària l'entrada d'individus d'altres poblacions per explicar el creixement observat a la Catalunya Central i Oriental. L'aufrany és una espècie filopàtrica, és a dir, els individus es reprodueixen en zones properes al seu lloc de naixement (dispersió natal mitjana 19.74 km, rang = 0–150 km, n = 26; Grande et al., dades no publicades). Per tant, la hipòtesi més plausible per explicar aquesta entrada d'immigrants seria l'arribada d'individus del nucli contigu situat a la zona occidental de Catalunya. Aquesta població occidental, va ser el nucli que es va mantenir quan la població d'aufrany va disminuir durant els anys 60. Així, es podria considerar que la nostra població d'estudi és la continuació de la de la zona occidental de Catalunya, ja que aquesta ha anat creixent i densificant-se en els últims anys (Servei de Biodiversitat i Protecció dels Animals, 2012), i per tant, el més probable és que s'hagi expandit cap a l'est.

A més, l'anàlisi demogràfica també ens ha permès projectar la població en els pròxims 50 anys per tal d'estudiar-ne la seva tendència sota diferents escenaris. Els més versemblants, que preveuen l'entrada d'immigrants i una supervivència adulta elevada, apunten que la població continuaria creixent en el futur. En el cas que l'entrada d'immigrants s'aturés, la població continuaria creixent només si la supervivència dels individus adults de la població es mantingués elevada (per sobre 0.95), però si aquesta es reduís (per sota 0.9), la població tendiria a estancar-se. Per tant, podríem dir que si les condicions ambientals i antròpiques no canvien, és previsible que la població segueixi augmentant en el futur. És per aquest motiu que avaluar quines són aquestes condicions ambientals, ecològiques i antròpiques que afecten els individus i la dinàmica poblacional de l'espècie en tot aquest procés, podria ser clau per a la conservació de l'aufrany en el futur.

Influència dels canvis ambientals antropogènics

a) Distribució de la població

Els canvis en la distribució de recursos al medi derivats de les activitats humanes tenen una gran influència sobre les espècies que en depenen (Blanco, Tella, & Torre, 1998; Laiolo et al., 2004; Foley, 2005). Una de les modificacions més destacades de la distribució dels recursos a l'ambient és l'aportació d'aliment d'origen antròpic (*Predictable Anthropogenic Foof Subsides*, PAFS), essent els abocadors una font molt important d'aquests nous recursos disponibles (Oro et al., 2013). Així, el primer pas per a conèixer quins efectes han pogut tenir aquests nous escenaris de canvis ambientals i de disponibilitat d'aliment sobre l'expansió de la població d'aufrany, va ser l'estudi de les característiques ambientals que han causat la distribució de les parelles territorials.

D'una banda, i per tal d'estudiar la influència de la disponibilitat d'aliment predictible en la distribució dels territoris, es van dur a terme una sèrie de models que consideraven tant els canyets com els abocadors. D'aquesta manera, es va poder determinar que els territoris ocupats estan situats a una distància aproximada d'uns 8 km de mitjana d'algun canyet o abocador. Tot i així, l'estudi de l'efecte dels canyets va presentar algunes limitacions a l'hora d'avaluar quina contribució real han tingut aquests punts d'aliment en l'ocupació territorial de les parelles. En primer lloc, l'espècie objectiu de l'alimentació suplementària proporcionada en la majoria de canyets propers a l'àrea d'estudi és el trencalòs, per tant el tipus d'aliment que s'hi aporta no és l'òptim per a l'aufrany. A més, molts dels canyets van començar a funcionar fa uns anys però actualment alguns han deixat de fer-ho o ho fan de forma intermitent (Diego Garcia, Generalitat de Catalunya, comunicació personal). Per tant, tot i que els canyets potser van tenir algun paper rellevant en el moment de la selecció del territori d'algunes parelles, possiblement actualment no són importants per la dieta de les parelles de l'àrea d'estudi. Un altre factor que en limita l'anàlisi, és que la distribució dels canyets enfocats al trencalòs es solapa amb la distribució de roquissars, l'hàbitat de cria de l'aufrany. Aquest fet provoca que els models estadístics realitzats no poguessin distingir entre l'efecte canyet i l'efecte roquissar. Justament, per tal d'eliminar aquest efecte confusiu, es va dur a terme una aproximació basada en una sèrie de models nuls que restringien els punts potencials de nidificació als roquissars. Els resultats apunten que el factor canyet no és significatiu i els abocadors són els únics punts d'aliment que determinen significativament la distribució de les parelles territorials d'aufrany en la zona d'estudi.

Així, la presència d'abocadors esdevé la principal explicació del perquè la població s'hagi expandit cap a l'est de Catalunya i hagi colonitzat àrees com les serres prelitorals on, abans de la construcció d'aquestes infraestructures cap als anys 90, segurament la disponibilitat d'aliment era massa baixa per a ser una zona atractiva per la colonització de les parelles d'aufrany. Així doncs, aquest resultat és rellevant ja que estaria indicant que la distribució de l'aliment en abocadors estaria alterant la distribució clàssica de les parelles d'aufrany, que històricament estava lligada a les àrees ramaderes de Catalunya, on aquestes podien trobar cadàvers per alimentar-se (Muntaner, Ferrer, & Martínez-Vilalta, 1983; Margalida, García, & Bertran, 2012). Tot i així, cal esmentar que tot i que a la nostra àrea el paper dels canyets sembla ser poc rellevant, probablement pel fet que aquests no han estat dissenyats per a l'aufrany, si que és conegut que els canyets dissenyats específicament per a l'espècie (Moreno-Opo et al., 2015), poden ser freqüentats pels aufranys i tenir un paper molt important en els nuclis i poblacions objecte de les mesures (López-López, García-Ripollés, & Urios, 2014; Lieury et al., 2015).

D'altra banda, a part dels abocadors, altres factors ambientals també han condicionat la distribució de les parelles. Els models realitzats a nivell d'àrea de cria (radi 1 km al voltant del niu) van seleccionar els roquissars orientats al sud com a factors preferents, ja que les parelles crien en coves situades en cingleres i l'orientació sud proporciona unes millors condicions microclimàtiques. La influència humana també pot condicionar negativament l'ocupació territorial a nivell d'àrea de cria, ja que els resultats indiquen que els territoris ocupats estan situats en zones amb poca presència de construccions urbanes a prop del niu, evidenciant que les parelles prefereixen zones tranquil·les i sense molèsties humanes. Finalment, un altre dels factors que és rellevant a nivell d'àrea territorial (radi de 8 km al voltant del niu) és la presència de conespecífics, de manera que la presència de parelles reproductores podria atraure altres parelles colonitzadores a criar en zones properes. A més, la presència de conespecífics alimentant-se en abocadors, així com les primeres parelles colonitzadores al costat d'aquestes infraestructures, pot haver causat l'atracció de més parelles i sumar així l'efecte abocador i l'efecte conespecífic. Tots aquests resultats són coherents amb les preferències de l'espècie descrites en altres àrees de la Península Ibèrica i Europa (Ceballos & Donázar, 1989; Liberatori & Penteriani, 2001; Sarà & Vittorio, 2003; Carrete et al., 2007; Mateo-Tomás & Olea, 2010).

En definitiva, l'expansió de l'aufrany de la Catalunya Central i Oriental, així com d'altres zones on no hi havia registre d'ocupació previ com les Serres Prelitorals (Muntaner, Ferrer, & Martínez-Vilalta, 1983), apunta que les característiques ambientals d'aquestes noves àrees d'ocupació han sigut adequades per a permetre la colonització de les parelles. Així, segons els resultats dels models, la disponibilitat d'aliment present en abocadors seria un dels principals factors que ha afavorit la colonització de la zona oriental de Catalunya. D'aquesta manera, la colonització de zones properes a abocadors hauria provocat l'expansió de l'aufrany cap a zones més humanitzades que la distribució històrica de les parelles que estava vinculada a les zones ramaderes.

b) Dieta

Els voltors representen una part important de les espècies que s'alimenten en els abocadors, instal·lacions on poden arribar a concentrar-s'hi grans quantitats d'individus (Donázar et al. 2010; Gangoso et al. 2013; Observació personal). Tot i així, degut a les dificultats que presenta l'estudi de la seva dieta (veure més avall), els efectes que han tingut els nous escenaris de recursos tròfics i que aquests tindran en el futur sobre les seves poblacions són encara poc coneguts. En aquest sentit, una altra de les aproximacions dutes a terme per tal d'avaluar la influència dels nous escenaris de disponibilitat de recursos en la població d'aufrany va ser estudiar la dieta dels polls de les parelles territorials i intentar quantificar quina part d'aquesta procedia de l'abocador.

Tradicionalment, per tal d'estudiar la dieta de les diferents espècies de voltors s'han utilitzat metodologies convencionals que consisteixen en la identificació de les restes de plomes, pèls o ossos residuals de l'aliment que les parelles porten al niu (Mundy et al., 1992; Donázar, 1993; Margalida, Bertran, & Heredia, 2009; Milchev, Spassov, & Popov, 2012). Aquesta aproximació, si bé pot donar bons resultats a nivell qualitatiu, és a dir, de la representació taxonòmica de les diferents preses en la dieta, també pot presentar biaixos a nivell quantitatiu. Un dels més importants és que les preses de major mida o que presenten més parts no digestibles són aquelles que deixen més restes i, contràriament, les preses petites o que presenten més parts toves són ingerides totalment i deixen molt poques restes. Per tant, a més de les dificultats per estimar la quantitat que s'ha ingerit d'una determinada presa, les preses que deixen més restes són sovint sobreeestimades en el total de la dieta (Real 1996; Margalida et al. 2007).

D'altra banda, l'Anàlisi d'Isòtops Estables és un mètode més innovador que estima la proporció de les diferents preses ingerides a partir de models bayesians (Hobson & Clark, 1992). Aquesta metodologia s'ha utilitzat per a determinar la dieta de moltes espècies (Ramos et al., 2009; Resano-Mayor et al. 2014b; Payo-Payo et al., 2015), però poques vegades s'ha aplicat en voltors (Chamberlain et al., 2005; Blázquez et al., 2016) i, fins ara, no s'havia realitzat cap estudi comparatiu de les dues metodologies per a avaluar els potencials biaixos de cadascuna en l'anàlisi de dieta dels voltors.

En el nostre cas, vam utilitzar les dues metodologies per tal de comprovar si l'anàlisi d'isòtops estables pot ser una bona eina per a quantificar la proporció de la dieta que prové dels abocadors. Vam seguir el criteri pel qual si els dos mètodes coincidien en el consum estimat per una determinada categoria de presa, assumíem que aquesta podia ser una aproximació acceptable del seu consum real. En aquest sentit, els resultats del present estudi demostren que, tot i les limitacions existents en els dos mètodes, ambdós proporcionen valors similars de la proporció de la dieta de l'aufrany que prové d'abocadors, per tant considerem que les dues metodologies són vàlides per a quantificar l'ús d'aquest recurs.

A més, les anàlisis convencionals i isotòpiques estableixen que els residus d'origen humà contribueixen substancialment en la dieta d'algunes parelles, ja que, en alguns casos, al voltant d'un 50% de l'aliment prové de l'abocador. Aquest fet és rellevant ja que confirma els abocadors són una font d'aliment força utilitzada per a alimentar els polls de les parelles d'aufrany. Tot i que és conegut que els residus urbans poden contribuir substancialment en la dieta d'aquesta espècie (Gangoso et al., 2013; Fazari & Mcgrady, 2016), fins ara no havia estat quantificat l'ús que en feien. En aquest sentit, la majoria d'estudis s'havien centrat principalment en classificar les preses per grups taxonòmics i no pel seu origen o bé en determinar la presència de plàstics o altres materials, però no de l'aliment ingerit procedent d'abocador (Donázar, Cortés-Avizanda, & Carrete, 2010; Milchev, Spassov, & Popov, 2012).

D'altra banda, no totes les parelles fan el mateix ús dels abocadors. Tot i que algunes de les que estan criant prop d'aquests punts són les que consumeixen majors proporcions de residus antròpics, no totes s'adeqüen a aquesta norma i no trobem una relació lineal entre consum d'abocador i distància a aquestes infraestructures. Segurament, això és degut al fet que l'aufrany pot realitzar llargs desplaçaments per a alimentar-se (López-López, García-Ripollés, & Urios, 2014), i podria ser que parelles que estan lluny d'abocadors consumeixin aquest recurs igualment. Tot i així, el que sí que es compleix és que els individus que presenten una major concentració de $^{13}\delta C$, associat al consum de preses alimentades amb pinso que poden trobar als abocadors, estan localitzats en ambients més humanitzats que els que presenten concentracions més baixes.

A banda del consum d'abocador, el mètode convencional d'estimació de la dieta i l'anàlisi d'isòtops estables també coincideixen en l'estimació del consum d'ocells. Per tant, els dos mètodes utilitzats serien igualment útils per estimar el consum d'aquest grup de preses. Tot i així, hi ha desacord entre els mètodes a l'hora d'estimar la proporció consumida de ramaderia, herbívors salvatges i carnívors. En el cas de les categories ramaderia i herbívors salvatges, aquestes solen presentar ossos grossos i identificables que poden romandre en el niu durant llargs períodes de temps i, per tant, és bastant possible que l'anàlisi convencional estigui sobreestimant aquestes preses. En el cas dels carnívors, l'anàlisi d'isòtops preveu un consum més elevat que el convencional. Tot i que aquests animals són sovint atropellats i poden ser una font important d'aliment per a l'aufrany (Hidalgo et al., 2005; Dobrev et al., 2015), també és possible que els elevats nivells de $^{15}\delta N$ detectats provinguin de l'ús de fertilitzants orgànics que en faci augmentar els seus nivells a l'ambient a la vegada que els de les preses de les que s'alimenta l'aufrany (Hebert & Wassenaar, 2001).

De manera global, tot i que l'anàlisi d'isòtops estables té limitacions ja que el consum de les preses estimat presenta uns nivells d'incertesa amplis, aquesta eina ofereix l'oportunitat de ser un bon mètode per tal de monitoritzar la contribució dels abocadors en la dieta dels voltors. El punt més fort de l'ús d'aquesta tècnica és que permet distingir entre els individus que consumeixen animals alimentats amb pinso i, per tant, obtinguts en abocadors, dels individus que consumeixen preses de ramaderia extensiva o animals salvatges. A més, aquesta metodologia també pot estalviar un esforç considerable en la identificació i el recompte de preses associada a la metodologia convencional, així com també ens ha servit per a detectar-ne els seus potencials biaixos. Tot i així, aquest estudi és una primera aproximació a l'ús de l'anàlisi d'isòtops per a voltors, però la tècnica es podria millorar en el futur. Per tal de fer-ho, es podrien utilitzar els valors isotòpics de les preses de la població considerada així com incorporar les proporcions de dieta com a informació prèvia als models bayesians (Franco-Trecu et al., 2013). D'aquesta manera, la combinació dels resultats de tots dos mètodes podria proporcionar unes estimacons encara més acurades de la dieta de l'aufrany. En un escenari on la disponibilitat de recursos al medi està canviant constantment és clau disposar de les eines que permetin fer un seguiment detallat de la dieta dels animals, per així poder estudiar quins efectes estan tenint aquests nous escenaris sobre els individus.

c) Condició física

Malgrat que els abocadors són utilitzats com a font d'aliment per a moltes espècies (Plaza & Lambertucci, 2017), existeix poca informació sobre els efectes d'alimentar-se de residus sobre l'estat de salut dels individus. En aquest sentit, per tal de determinar la relació entre la dieta i la condició física d'un determinat individu, la majoria d'estudis solen utilitzar índexs morfomètrics (Auman, Meathrel, & Richardson, 2009; Gould & Andelt, 2013). Aquests índexs, poden basar-se en determinar el pes i la talla dels animals (Peig & Green, 2009), però recentment alguns estudis han combinat l'ús d'aquests índexs morfomètrics amb els paràmetres sanguinis dels individus, que aporten informació sobre el seu estat fisiològic a partir d'indicadors de la bioquímica plasmàtica així com del seu metabolisme antioxidant (Meillère et al., 2015; Resano-Mayor et al., 2016).

En la present tesi es combinen l'ús de mètodes morfomètrics i fisiològics per tal de veure quina influència té el fet d'alimentar-se en abocadors en els individus de la població d'aufrany. D'aquesta manera, s'ha pogut determinar que el consum de recursos d'abocador és el factor que té una influència més gran en la condició física dels polls, principalment per les seves conseqüències a nivell de metabolisme antioxidant. El que es detecta és que els individus que consumeixen més residus d'origen urbà presenten nivells més baixos de carotens i vitamines. Aquestes substàncies són molt importants per a l'organisme, ja que actuen com a defenses antioxidants, són claus en el sistema immunitari i poden tenir un paper important en la coloració dels individus i, per tant, durant la selecció sexual (Olson & Owens, 1998; Møller et al., 2000; Blount, 2004; Monaghan, Metcalfe, & Torres, 2009). Les causes d'aquests nivells més baixos de vitamines i carotens en els polls que s'alimenten de residus d'abocadors podrien ser diverses.

D'una banda, degut al fet que aquestes defenses antioxidants s'obtenen de la dieta (Olson & Owens, 1998), podria ser que l'aliment procedent dels abocadors fos pobre en aquest tipus de substàncies. Tot i així, tenint en compte que una de les fonts principals d'aquestes substàncies antioxidants per l'aufrany són els excrements d'espècies ramaderes (Negro et al., 2002), podria ser que el fet de consumir en abocador provoqués un menor consum d'aquests excrements i, per tant de vitamines i carotens. D'altra banda, també podria ser que l'aliment ingerit dels abocadors fos de poca qualitat i generés un estrès oxidatiu que provoqués el consum de vitamines i carotens com a defenses antioxidants per tal de restablir l'equilibri homeostàtic (van de Crommenacker et al., 2011). Una altra possibilitat seria que la presència de patògens en abocadors (Ortiz & Smith, 1994; Giusti, 2009; Camacho et al., 2016) causés que els individus haguessin d'activar el seu sistema immunitari, consumint així aquestes defenses antioxidants (Blount, 2004). Independentment de l'origen d'aquest nivell baix de vitamines i carotens, els resultats apunten que els individus que s'alimenten d'abocador també presenten nivells més alts d'enzims antioxidants (SOD i GPx). Aquests nivells més alts d'enzims possiblement servirien per compensar la disminució de les defenses antioxidants exògenes, les vitamines i carotens (Monaghan, Metcalfe, & Torres, 2009).

Malgrat les diferències detectades en les defenses antioxidants entre els individus de la població en funció del seu consum d'abocador, no hem observat que aquests presentin un dany oxidatiu sever. En aquest sentit, les conseqüències dels nivells observats de defenses antioxidants són incertes. En el cas de l'aufrany, els carotens tenen un paper ornamental important en la coloració de la seva cara i s'ha proposat que aquests podrien ser rellevants per a la selecció sexual dels individus reproductors (Negro et al., 2002). Si això es complís, una menor ingestió d'aquestes substàncies podria comprometre els individus en el moment de trobar parella. Tot i així, val a dir que els individus analitzats no són adults i, per tant, encara no estan subjectes a la selecció sexual. Caldria veure com podrien influir a curt i a llarg termini aquests nivells més baixos de vitamines i carotens detectats durant l'etapa de desenvolupament del poll.

A més a més, l'anàlisi de la bioquímica plasmàtica ens ha permès conèixer que els individus que s'alimenten més en abocadors presenten nivells més baixos de colesterol que els altres. Com que en ocells els nivells de colesterol poden augmentar en el cas que es produeixi un període de dejú (García-Rodríguez et al., 1987), aquest resultat apuntaria que els individus que consumeixen residus d'origen humà tindrien menys limitacions de menjar, passarien menys períodes d'inanició i per tant no haurien de mobilitzar aquestes reserves lipídiques. A més, el fet d'alimentar-se en abocador també ha provocat que les parelles siguin més constants pel que fa al nombre de polls que tiren endavant anualment. Així, semblaria que les parelles que s'alimenten d'abocador podrien ser més independents de la disponibilitat de recursos que hi ha al medi i el fet de tirar endavant un o dos polls podria dependre més de les característiques dels individus.

De tota manera, existeixen altres factors que també poden afectar a la condició dels individus. D'una banda, el nombre de polls que neixen per parella, ja que l'índex morfomètric apunta que els polls que neixen sols en un niu presenten millor condició que els que es troben en nius de dos polls. D'altra banda, també hem observat que els polls que estan sols al niu tenen nivells més alts de triglicèrids, possiblement perquè passen menys períodes de dejú (Castellini & Rea, 1992).

En resum, l'ús d'un índex morfomètric combinat amb diversos indicadors sanguinis ha proporcionat informació molt útil per a entendre de forma global quina és la condició física dels polls d'aufrany de la població que ha colonitzat la Catalunya Central i Oriental i la seva relació amb el consum de recursos tròfics provinents d'abocador. D'aquesta manera hem observat que els abocadors proporcionen aliment fix per als individus, que poden passar menys dejú gràcies a la predictibilitat d'aquest, així com també poden ser més constants en la reproducció. Tot i així, aquest tipus d'alimentació té conseqüències a nivell de metabolisme antioxidant, malgrat que les implicacions d'aquestes variacions metabòliques a llarg termini sobre els individus encara són desconegudes.

d) Contaminants

A causa de la seva posició al capdamunt de la xarxa tròfica, els voltors són susceptibles d'acumular i concentrar contaminants orgànics persistents (COPs) i metalls en els seus teixits (Gómara et al., 2004; Ogada, 2014; Carneiro et al., 2015). Així, les espècies carronyaires estan exposades als potencials efectes tòxics causats per aquests contaminants. En el present estudi es van analitzar les concentracions de diferents famílies de COPs: compostos organoclorats (Ocs), èters difenílics polibromats (PBDEs), substàncies perfluorades (PFAS), així com diferents metalls: plom (Pb), cadmi (Cd), arsènic (As), coure (Cu) i zinc (Zn) en els polls de la població d'aufrany així com la seva influència en els diferents paràmetres sanguinis dels individus. Els resultats apunten que, en general, els nivells detectats de totes les famílies de contaminants analitzades són més baixos que els descrits en altres espècies i àrees.

Pel que fa als PFAS, els motius d'aquests nivells més baixos detectats són difícils de determinar, ja que l'acumulació de PFAS en ocells pot dependre tant del seu origen geogràfic com del tipus d'alimentació de l'animal o del moment del mostreig (Bustnes et al., 2013, 2015; Munoz et al., 2017). A més, també és difícil d'interpretar quina és la toxicitat potencial associada als nivells observats, però els valors detectats en la nostra població són, en general, més baixos que els nivells de referència de toxicitat proposats en altres espècies (Bustnes et al., 2013; Route et al., 2014). Pel que fa als PBDES, la majoria d'estudis també descriuen nivells més alts d'aquests compostos (Venier et al., 2010; Fernie et al., 2017), per tant, semblaria que els individus de la nostra població estan poc exposats a la toxicitat causada per aquests contaminants. En la mateixa línia, els valors dels diferents compostos de la família dels organoclorats són, en general, baixos comparats amb els descrits per a l'aufrany en altres àrees (Gómara et al., 2004). Així, en el cas del DDT i DDE, les diferències entre els nostres valors i els descrits per Gómara et al. (2004) podrien ser degudes a i) diferències en el grau de contaminació entre les diferents regions influïdes pel grau d'utilització d'insecticides rics en aquests compostos abans a la seva prohibició, ii) diferències entre les poblacions relacionades amb l'exposició a aquestes substàncies en les zones d'hivernada o iii), una reducció de l'acumulació de DDT al medi entre quan es va realitzar l'estudi de Gomara et al. (2004) i l'actualitat (han passat uns 15-17 anys). Els valors de PCBs, en canvi, van ser més comparables als descrits en el mateix estudi. Aquest fet pot ser degut a que malgrat la diferència temporal entre els dos estudis que comportaria una degradació dels compostos, el fet que Catalunya sigui una àrea industrial on la presència d'aquests compostos és més elevada (Ortiz-Santaliestra et al., 2015) podria compensar la seva degradació temporal. Finalment en el cas dels metalls, tots ells estaven per sota el límit de detecció excepte en Zn. En aquest cas, les concentracions també són més baixes que les reportades per Donázar et al., (2002) de la població d'aufrany de Canàries.

A més, els PFAS són els principals determinants de la variabilitat dels paràmetres sanguinis dels individus, ja que alts nivells d'aquests compostos s'associen a nivells baixos d'alguns indicadors de l'estat nutritiu, principalment les vitamines i els carotens. Aquestes substàncies s'obtenen de la dieta, per tant, els nivells més baixos observats en alguns individus podrien estar relacionats amb un pitjor estat nutritiu d'aquests. Aquesta relació negativa entre la presència de PFAS i els nivells de vitamines i carotens podria explicar-se per dues possibles causes. La primera, podria ser que la ingestió de PFAS causés estrés oxidatiu i les vitamines i carotens serien utilitzades per a compensar l'efecte de l'estrés oxidatiu (Di Marcio, Murphy, & Sies, 1991). Tot i així no hi ha descrita en la bibliografia una clara relació entre PFAS i estrés oxidatiu en ocells. L'altra explicació, coherent amb els resultats del capítol 4, seria que els individus que s'alimenten més en abocadors ingerissin directament menys quantitat de vitamines i carotens i fossin aquests individus els que justament presenten uns nivells de PFAS més elevats. Aquesta hipòtesi concordaria també amb el fet que s'hagin detectat nivells més elevats de PFAS en zones urbanes, ja que tal i com es demostra al capítol 4, els individus de zones urbanes són els que s'alimenten més d'abocador.

Finalment, també s'ha detectat una la relació positiva entre l'organoclorat PCB180 i els nivells totals de GSH, també observada per Ortiz-Santaliestra et al., (2015). Aquest fet pot explicar-se amb el fet que els organismes sintetitzin GSH (principal mol·lècula antioxidant intracel·lular), per tal de contrarestar l'estrés oxidatiu generat pels contaminants. Així els resultats són coherents amb altres estudis que descriuen que la capacitat dels organoclorats de generar estrés oxidatiu és més elevada que la dels PAFS (Miljeteig et al., 2012; Sletten et al., 2016).

En resum, els nivells observats de les diferents famílies de contaminants no semblen susceptibles de causar efectes molt greus sobre la fisiologia dels polls de la nostra població, tot i que cal més recerca sobre quins serien els valors de referència de toxicitat dels PFAS sobre els individus. La principal correlació observada entre contaminants i indicadors fisiològics és entre els PFAS i les vitamines i carotens. Tot i així, els nivells d'aquests compostos semblen més relacionats amb l'explotació d'abocadors que no pas amb els efectes dels PFAS sobre la seva fisiologia.

Nous escenaris de recursos tròfics: aplicacions en la conservació de l'espècie

Els resultats exposats en aquesta tesi tenen aplicacions importants en la conservació de l'aufrany, ja que aporten informació que permet entendre el creixement demogràfic de la població objecte d'estudi, així com també expliquen la contribució que han tingut els abocadors en l'expansió, dieta i condició física de l'espècie. A més, conèixer com estan afectant els canvis en la disponibilitat de recursos al medi derivats de l'activitat humana en aquesta espècie amenaçada, pot ser molt útil tan per a predir possibles conseqüències de nous canvis ambientals, així com per a enfocar mesures de conservació en àrees on l'aufrany es troba en declivi. En aquest sentit, en els propers anys, s'esperen canvis en la disponibilitat de recursos al medi que podrien afectar tan a l'aufrany com a la resta d'espècies carronyaires que utilitzen recursos similars.

En primer lloc, l'any 2008 es va aprovar una normativa europea destinada a reduir els residus exposats a l'aire lliure en abocadors (Dir. 2008/98 del Parlament Europeu i del Consell, de 19 de novembre de 2008). Un dels objectius d'aquesta legislació és disminuir els impactes negatius dels abocadors sobretot a nivell de riscos per a la salut humana, però que indirectament afectarà a totes les espècies que se n'alimenten, tan les abundants com les amenaçades. Per tant, una de les conseqüències esperables del tancament dels abocadors és el canvi en la dieta d'aquells individus que s'alimenten de residus humans. Tot i així, és complicat predir quin impacte tindrà aquest canvi brusc de disponibilitat de recursos en els individus de la població d'aufrany. D'una banda és cert que l'aufrany és una espècie capaç d'utilitzar un ventall de recursos molt ampli, de manera podria ser capaç de trobar altres fonts de recursos per alimentar-se, tal i com ha fet en altres zones on s'ha produït un canvi d'aliment disponible (Donázar, Cortés-Avizanda, & Carrete, 2010; Katzenberger et al., 2017). De tota manera, no s'hauria de confiar en aquest fet ja que també que podria passar que les parelles territorials trobessin dificultats per habituar-se al canvi brusc d'aliment disponible.

A més a més, els canvis en la dieta podrien comportar conseqüències importants en els paràmetres demogràfics dels individus (Resano-Mayor et al., 2014a). Per aquesta raó, un dels següents aspectes que caldria abordar és la influència dels abocadors en la supervivència i la productivitat dels individus de la nostra població d'estudi. En aquest sentit, cal tenir en compte que tal i com s'ha demostrat en el primer capítol, un dels factors més important que ha condicionat l'augment de la població d'aufrany a Catalunya ha estat la supervivència dels individus adults. Així, si bé la principal explicació és que aquesta no està tan castigada pels enverinaments com en altres àrees, també caldria estudiar si la predicabilitat de l'aliment causada pels abocadors, podria a més, haver afavorit la supervivència dels individus en els últims anys, tal i com ha passat en altres àrees (Lieury et al., 2015).

Altrament, també cal tenir en compte que el fet d'alimentar-se en abocadors podria provocar l'efecte contrari sobre la supervivència dels individus: augmentant-ne la mortalitat a causa de les amenaces derivades d'aquestes instal·lacions. Així, un potencial risc sobre la supervivència dels individus podria ser el d'intoxicació per ingesta de substàncies tòxiques com ha passat en altres espècies (Rideout et al., 2012; de la Casa-Resino et al., 2014). A més, segons els resultats observats les parelles que s'alimenten més d'abocador es troben en hàbitats més humanitzats. La presència d'infraestructures humanes en aquests ambients podria suposar una altra amenaça que augmentés el risc de mortalitat pels individus, ja que aquests podrien col·lisionar o electrocutar-se amb torres elèctriques o altres construccions. Aquestes amenaces s'han detectat com a importants per l'espècie i en altres àrees els seus efectes causen una mortalitat important en els individus de població (Donázar et al., 2002; Carrete et al., 2009). De moment, però, no sembla que la toxicitat ni la presència d'infraestructures humanes siguin una amenaça alarmant per a l'espècie a l'àrea d'estudi ja que d'una banda, els nivells detectats de contaminants són baixos, i de l'altra no tenim constància d' una mortalitat important dels individus adults.

Un altre factor que també caldria estudiar amb deteniment seria la influència d'alimentar-se en abocadors sobre la productivitat de les parelles. Tot i que l'efecte dels abocadors sobre les taxes reproductores de les parelles no s'ha estudiat directament, el que sí s'ha calculat és la productivitat mitjana de la població. Aquesta, és lleugerament superior a la observada en algunes altres àrees (Donazar & Ceballos, 1988; Donázar et al., 2002; Dobrev et al., 2015; Katzenberger et al., 2017), però les causes d'aquests valors més elevats són encara desconegudes. A banda, també s'ha detectat que les parelles que s'alimenten en abocadors solen ser més regulars pel que fa al nombre de polls que porten a l'envol anualment, però no s'ha estudiat si la productivitat mitjana d'aquestes parelles és més elevada que les que s'alimenten més de restes de ramaderia i animals salvatges. Tenint en compte que en altres espècies el fet d'alimentar-se en abocadors pot tenir implicacions importants en els paràmetres reproductors dels individus (Tortosa, Caballero, & Reyes-López, 2002; Steigerwald et al., 2015), no és descartable que en la nostra població també hi pugui tenir una influència positiva.

Per tant, atès que els abocadors tenen rellevància en la distribució i la dieta de l'espècie i podrien afectar també la supervivència i productivitat dels individus, com ja s'ha detectat en altres espècies (Tortosa, Caballero, & Reyes-López, 2002; Marzluff & Neatherlin, 2006), s'haurien de considerar els efectes potencials del tancament d'aquestes instal·lacions sobre l'aufrany en el moment de l'aplicació de la legislació. Una eina molt útil per a avaluar els possibles riscos del tancament seria dur a terme models demogràfics que prediguessin l'estat futur de la població sota diferents escenaris de disponibilitat d'aliment. A més, un cop es tanquessin els abocadors, també caldria seguir monitoritzant la població i continuar estudiant-ne la supervivència i productivitat per tal de poder actualitzar els models demogràfics periòdicament. Aquests models podrien esdevenir una eina de gestió clau per a la conservació de l'espècie tant de l'àrea d'estudi com en d'altres àrees que depenen de recursos similars.

A banda, si els models prediguessin que el tancament d'abocadors podria causar la disminució del nombre de parelles, també es podria considerar l'alimentació suplementària de les parelles més dependents d'aquest recurs, és a dir, les amenaçades per la seva desaparició. Les mesures basades en l'alimentació suplementària s'han mostrat útils per a la conservació de l'aufrany en zones on es volia recuperar la seva població (Lieury et al., 2015). Tot i així s'han d'utilitzar adequadament ja que sinó poden no ser efectives (Moreno-Opo et al., 2015; Oppel et al., 2016) o fins i tot poden comportar problemes poblacionals (García-Heras, Cortés-Avizanda, & Donázar, 2013).

En segon lloc, un altre canvi ambiental que ja s'ha iniciat és la flexibilització de les normatives ramaderes de l'abandonament de cadàvers al camp. Aquest procés ja ha començat en els últims anys a partir de l'aprovació de reglaments europeus (R (UE) 1069/2009 del Parlament Europeu i del Consell, de 21 d'octubre de 2009 i R (UE)

142/2011 de la Comissió, de 25 de febrer de 2011) i de la seva transposició a l'estat espanyol (RD 132/2011, de 19 de març) i a Catalunya (O. AAM/387/2012 de 23 de novembre. De moment, la legislació vigent permet "l'abandonament de cadàvers en zones de protecció per a l'alimentació d'espècies d'ocells necròfags d'interès comunitari en forests públics, comunals o en altres terrenys d'altitud superior a 1400 m" en municipis especificats en la mateixa legislació, tots ells de la zona prepirinenca i pirinenca. Si aquesta normativa es continua flexibilitzant, i s'ampliés l'àrea permesa per a l'abandonament de cadàvers, podria provocar que l'aliment predictible actualment acumulat en abocadors tornés a ser impredictible al medi. Tot i que aquest escenari és encara incert, tal i com està descrit en la bibliografia, aquest tipus d'aliment distribuït de forma impredictible podria afavorir tan l'aufrany com altres espècies necròfagues amenaçades (Cortés-Avizanda et al., 2012).

En resum, aquesta tesi demostra que la població d'aufrany ha augmentat a la Catalunya Central i Oriental degut a una elevada supervivència dels individus adults lligada a l'entrada d'immigrants d'altres zones. A més, la presència d'abocadors ha afavorit que les parelles colonitzessin noves àrees que no havien estat ocupades històricament per l'espècie. D'altra banda també es confirma que algunes de les parelles territorials utilitzen els abocadors per alimentar-se, i que aquests poden contribuir de forma important en la seva dieta. Tot i que les implicacions d'alimentar-se en aquestes instal·lacions a llarg termini són desconegudes, els nostres resultats apunten que alimentar-se de residus humans té implicacions rellevantsa nivell de condició física dels individus, en especial en el seu metabolisme antioxidant. A més, també s'ha confirmat que els polls dels territoris més urbanitzats, que són els que s'alimenten més en abocadors, presenten nivells de contaminants més elevats. Tot i així, els valors detectats d'aquestes substàncies són baixos en general i els seus efectes sobre la fisiologia dels individus requereixen més recerca. En definitiva, els resultats d'aquesta tesi tenen implicacions rellevants per a la conservació de l'aufrany en l'àrea d'estudi i aporten informació que podrà ser útil per a la conservació de l'espècie en àrees on aquesta es troba en regressió.

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CONCLUSIONS FINALS

- La població d'aufrany ha augmentat a la Catalunya Central i Oriental d'1 a 22 parelles durant el període 1988-2012. Els models demogràfics han permès determinar que aquest augment poblacional ha estat causat per dos factors principals: una supervivència elevada dels individus adults de la població segurament provocada per una baixa mortalitat per enverinaments, així com per una entrada d'immigrants possiblement de zones properes (Catalunya Occidental). L'Anàlisi de Viabilitat de la Població estableix que, si les condicions ambientals no canvien, la població seguirà augmentant en el futur.

- L'augment demogràfic ha anat lligat a una expansió de la població cap a la zona est de Catalunya, on les parelles han colonitzat àrees que no havien estat ocupades per l'espècie històricament. El factor ambiental que determina la ocupació territorial és, principalment, la localització dels punts d'alimentació predictible, en concret els abocadors. Tot i així, altres factors com l'existència de roquissars orientats al sud, la distància a infraestructures humanes i la proximitat a conespecífics també influeixen en la selecció dels territoris.

- L'ús de l'anàlisi convencional d'identificació de restes i l'anàlisi d'isòtops estables ha permès caracteritzar la dieta de l'espècie a l'àrea d'estudi. Les dues metodologies presenten estimes similars pel que fa al consum de restes obtingudes en abocadors i de diferents espècies d'ocells, però desacord en l'estima del consum de ramaderia, herbívors salvatges i carnívors. Els resultats apunten que algunes parelles territorials podrien consumir prop d'un 50% de la dieta en abocadors, per tant aquestes instal·lacions són importants per a l'alimentació dels polls.

- El consum de residus urbans és, d'entre els factors estudiats, el que té majors conseqüències a nivell de condició física dels individus. Utilitzant un índex morfomètric i diversos indicadors fisiològics hem observat que els polls que s'alimenten més en abocadors tenen nivells més baixos de defenses antioxidants (vitamines i carotens), que estarien compensant amb uns nivells més alts d'enzims antioxidants. A més, aquests polls també presenten menys colesterol, indicant que passen menys períodes de dejú.

- Els nivells de contaminants detectats són en general més baixos que en altres espècies i àrees i no sembla que estiguin causant efectes greus sobre la fisiologia dels individus. La principal correlació observada entre contaminants i indicadors fisiològics és entre els PFAS i les vitamines i carotens. Tot i així, els nivells d'aquests compostos antioxidants semblen més relacionats amb l'explotació d'abocadors que amb els efectes dels PFAS sobre la fisiologia dels individus.

- En els propers anys, s'esperen una sèrie de canvis en la disponibilitat de recursos que podrien afectar a la conservació de l'espècie. D'una banda es preveu una disminució de la disponibilitat de residus urbans a causa de l'aplicació de normatives europees que regulen el tancament dels abocadors, d'altra banda es preveu una flexibilització de les normatives d'abandonament de cadàvers al camp. Disposar de les eines per a poder monitoritzar els possibles canvis ambientals és clau per a la conservació d'aquesta espècie amenaçada en el futur.

APÈNDIX

Identifying key demographic parameters for the viability of a growing population of the endangered Egyptian Vulture *Neophron percnopterus*

HELENA TAULER, JOAN REAL, ANTONIO HERNÁNDEZ-MATÍAS, PERE AYMERICH, JORDI BAUCELLS, CARLES MARTORELL and JOAN SANTANDREU

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Identifying key demographic parameters for the viability of a growing population of the endangered Egyptian Vulture *Neophron percnopterus*

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Summary

The Egyptian Vulture *Neophron percnopterus* is a threatened species throughout its worldwide range. The Iberian Peninsula holds 50% of its global population, which has declined by 25% over the last 20 years. Despite this negative global trend, an increase in the number of individuals over the last 25 years has been observed in Catalonia, where it has colonised areas in which it was previously unknown. In this study, we describe the demographic evolution of an increasing population of Egyptian Vultures in central and eastern Catalonia and we apply population models and maximum likelihood procedures to investigate both the main demographic processes driving the observed trends and the viability of the population. The number of pairs in this region increased from one to 22 in the period 1988–2012. The best-supported models suggest that adult survival in this population may be higher than in other Iberian populations and that furthermore, there is a continuous influx of immigrants. Based on the most likely scenarios, Population Viability Analysis predicts that the population will continue to increase. Sensitivity analysis indicates that the adult survival rate has the greatest influence on population dynamics so conservation efforts will be more effective if concentrated on improving this rate.

Introduction

Understanding the processes that shape population size and structure is one of the main objectives in ecology and is thus subject to extensive study (Begon *et al.* 1996, Levin *et al.* 2009). Population dynamics can be understood as the outcome of the addition (births and immigration) and loss (deaths and emigration) of individuals from a population. These demographic processes are regulated by both intrinsic and extrinsic factors: intrinsic factors consist of species' life-history traits such as age of first breeding, fertility, longevity and dispersal behaviour, whereas extrinsic factors are connected to environmental conditions such as food resources or weather, and are subject to marked variation over time and space (Levin *et al.* 2009). Currently, human activities have a severe impact on the environment (Loreau *et al.* 2001, Barnosky *et al.* 2012) and play an important part in the population declines and extinctions of species that are now occurring at unprecedented rates. Consequently, population theory plays a central role in modern conservation biology (Primack 2012). Population viability analysis (PVA) – the use of quantitative methods to predict the likely future status of a population – has become a basic tool in current conservation research and practice (Morris and Doak 2002, Hernández-Matías *et al.* 2013). This type of analysis is based on a broad suite of population modelling and data-fitting methods of varying mathematical complexity whose aim is to estimate the expected values of the main descriptors of population dynamics that include the population growth rate and, particularly, the risk of extinction of a population over time (Beissinger and McCullough 2002).

In a global context in which many species are decreasing and/or are threatened, most PVA studies focus on analysing the causes of and possible solutions to human-induced population declines (Caughley 1994). Nevertheless, some species may in fact benefit from human activities (Duhem *et al.* 2003, Gangoso *et al.* 2012). The application of PVA methods to growing populations can provide useful quantitative information to understand demographic processes and to guide practitioners as it is commonly done for invasive species (Conroy and Senar 2009) and other threatened raptor species (Ortega *et al.* 2009). In this sense, tendencies in a species may vary from one population to another and, therefore, the study of populations with positive trends can provide very relevant information for conservation of declining populations. Usually, little demographic data are available for endangered species, but current statistical procedures may generate quantitative information useful to understand the demographic drivers of population dynamics and, consequently, provide evidence- based prescriptions to be applied by practitioners (Doak *et al.* 2005, Hernández-Matías *et al.* 2013).

The present work examines the recent expansion of the Egyptian Vulture population *Neophron percnopterus* in Catalonia (north-east Iberian Peninsula). To do so, we apply likelihood-based procedures on available field data in order to identify the demographic determinants of population dynamics and then we perform population viability analysis. The Egyptian Vulture ranges from the Indian subcontinent, Middle East, south-east of the former USSR to the Mediterranean Basin and the Sahel and eastern and southern Africa (Donázar 1993). This long-lived species lives in adult pairs that nest in caves situated on cliffs, defending the same territory year after year (Donázar 1993). Immature individuals are not territorial and usually form groups near predictable food sources such as landfills or supplementary feeding stations (Donázar 1993, Grande 2006). European populations of this species spend the winter in Africa (Sahel) and return to Europe to breed during spring and summer (Benítez *et al.* 2004).

The Egyptian Vulture is threatened worldwide and in recent decades its distribution has decreased significantly (Donázar 2004). At present, the conservation status of the Egyptian Vulture according to the IUCN Red List is 'Endangered'. Based on recent studies, the stronghold of this species' Palearctic population is in Spain (1,320–1,480 pairs) (Del Moral and Martí 2002, Donázar 2004). Even so, its Iberian population has declined by 25% over the last two decades (Donázar 2004), mainly due to poisoning and illegal persecution, but also as a result of the loss of traditional agricultural practices (Liberatori and Penteriani 2001, Donázar 2004) as well as electrocution and collision with power lines and wind farms in some areas of Spain (Donázar *et al.* 2002, Carrete *et al.* 2009).

In Catalonia this vulture declined in the 1960s and 1970s and became extinct in the most eastern part of its range (Muntaner *et al.* 1981, 1983). Nevertheless, in the late 1980s this tendency was reversed and some abandoned territories were recolonised (Estrada *et al.* 2005) and areas with no historical records were colonised. This is a paradigmatic case of a species in worldwide decline whose populations are decreasing at a local scale, except for part of its European range (north-east Catalonia), in which they are increasing and even expanding their distribution (García-Ripollès and López-López 2006, Mateo-Tomás *et al.* 2010).

The present study provides a demographic analysis of the colonisation by the Egyptian Vulture of an extensive area of Catalonia from 1988 onwards. The general aim of the study was to identify the demographical determinants of the observed population trend and to provide a useful guide for the conservation of this population and of other European populations currently in decline. The specific aims of this work are: (1) to describe the population dynamics from colonisation to the present day in the study area, (2) to estimate the vital rates in the study population, (3) to use population models and likelihood-based methods to evaluate the contribution of immigration and adult survival to the observed past population growth rate, (4) to perform a PVA to predict the expected trend of the population and its risk of extinction, and (5) to identify conservation targets by analysing the sensitivity and elasticity of the population growth rate in relation to the main vital rates.

Materials and methods

Study area and data collection

The study area was located in central and eastern Catalonia (Figure 3) at altitudes in the range of 200–1,900 m asl throughout an area of cliffs running from the Prelittoral Mountains in the south to the pre-Pyrenean Mountains in the north. In between stretches a large lowland area covered mainly by farmland but with important extensions of forest and scrubland. The monitoring of this Egyptian Vulture population was carried out from 1988 to 2012. In 1988 only one breeding pair was known in the study area (Muntaner *et al.* 1983, Aymerich and Santandreu 1998). From this year up to 2012, regular cliff-nesting raptor censuses were performed in the area (Aymerich *et al.* 1991, Real and Mañosa 1997, Aymerich and Santandreu 1998, Baucells *et al.* 1998, Aymerich and Santandreu 2002, Guixé 2008, Hernández-Matías *et al.* 2013) and new colonising pairs located. All new occupied territories were monitored regularly in order to determine the level of occupancy and a subset of territorial pairs was monitored to obtain data on breeding success.

To determine the occupancy of territories, at least one visit was made with a spotting scope (20–60x) between March and mid-April. An area was considered unoccupied if no individuals were detected after four visits/days. At the end of the incubation period (42 days), nests were checked to detect the presence of nestlings. Seventy days after hatching, the nests were visited again to check the number of fledglings, which was used to estimate fledgling rates.

Life-history traits and life cycle

According to current knowledge of the life-history traits of this species, we defined a life cycle to be used in the population models described below. All simulations were based on this life cycle and only took females into account. The life cycle was based on a post-reproductive census of six age classes (Figure 1). After each breeding cycle, surviving females move into the next age class; only adult birds produce new individuals. In the results, the number of adult females was considered to be equal to the number of territorial pairs in the population.

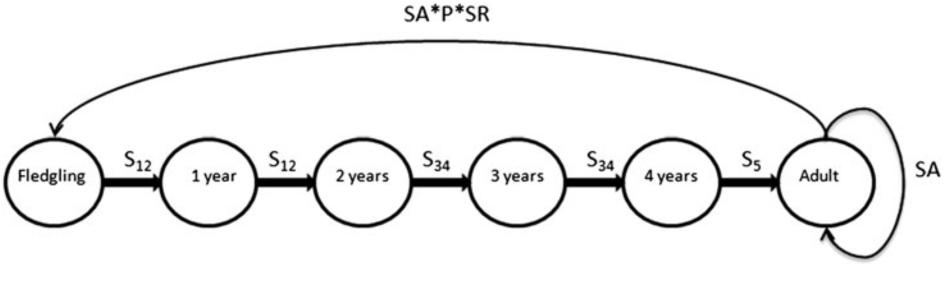

Figure 1. Diagram of the Egyptian Vulture life cycle. Nodes represent the different age classes considered in the model. $S_{12:}$ yearly survival in first and second years of life, $S_{34:}$ yearly survival in third and fourth years of life, $S_{5:}$ yearly survival in fifth year of life when the recruitment of individual occurs, SA: yearly survival of adult breeders, P: productivity and SR: sex ratio which was assumed at 1:1.

Estimation of demographic parameters

Based on the observed number of pairs, the population growth rate was estimated for the whole study period as $\lambda = (N_t / N_0)^{1/t}$, where N_0 is the initial population size, N_t the final population size and t the number of years between the start and the end of the study period. We also calculated the population growth rate during the period 1994–2012 given that this period was more appropriate for estimating the initial population size to be used in simulations. Productivity was calculated as the number of fledglings divided by the number of surveyed pairs. Other breeding parameters were calculated as breeding success (number of fledglings/number of laying pairs), fledgling rate (number of fledglings/number of pairs that have fledglings), percentage of laying pairs (number of laying pairs/number of territorial pairs), percentage of pairs with nestlings (number of pairs with nestlings/number of territorial pairs) and percentage of pairs with fledglings (number of pairs with fledglings/number of territorial pairs). Using the raw values of yearly productivity (and their associated sample variances), we applied White's method (White 2000) to obtain an estimate of productivity (corrected by the sampling variation associated with small sample sizes) and the temporal variance that we then used to simulate the environmental stochasticity of the models.

Survival estimations could not be made in the study population since ringing schemes have only been initiated in recent years. Additionally, methods based on age ratios (e.g. Hernández-Matías *et al.* 2011) were not applicable for this species since only territorial individuals in full adult plumage could be monitored with ease. Therefore, we employed in the models estimates available for this species obtained from a large-scale ringing scheme in 1990–2005 in the Ebro Valley, part of the species' largest population in the Iberian Peninsula (Grande *et al.* 2008). Survival rates of non-adults used in our models were: $S_{12} = 0.73$, $S_{34} = 0.78$, $S_5 = 0.60$. Adult survival was estimated using a subsample of territorial individuals and considering a model with the time effect (model 24 in Table 5 from Grande *et al.* 2008). Based on yearly estimates and the corresponding standard errors, we applied the method of White (2000) to estimate the temporal variance of this parameter.

Population models and the evaluation of the model assumptions

To carry out the simulations, the life cycle described above and shown in Figure 1 was portrayed in the model. To calculate how many individuals of each age class will survive and will pass on to the next age we applied Monte Carlo simulations, which allowed us to account for demographic stochasticity. To introduce demographic stochasticity on productivity we determined the probability of having zero, one or two fledglings for each productivity and then applied a multinomial distribution to calculate the number of fledglings for each year. Environmental stochasticity for adult survival and for productivity were incorporated into the model using their temporal variance estimates and simulated with, respectively, the 'beta' and 'stretched beta' distribution functions in package POPBIO (Stubben and Milligan 2007 based on the original code by Morris and Doak 2002).

Population models were initially used to assess the most likely factors explaining the observed population growth rate. To do so, we applied likelihood-based methods. We evaluated a set of models considering several sources of uncertainty regarding the degree of connection of the population with other populations (i.e. whether the population was closed or received immigrants) and the values of adult survival for the population. Evaluated models considered all possible combinations of assumed adult survival from 0.7 to 0.975 at intervals of 0.025, and immigration from 0 to 10 immigrants per year. The number of immigrants considered is the net number of adult females that arrive in the population assuming that both emigration and immigration occurs. Essentially, model choice relied on how likely the observed lambda is given each model's set of predictions. For each model the simulated lambda values resulting from 5,000 replicates were binned using fairly fine bins (0.05 bins). Then, the probability of the simulated lambdas being within each bin was considered as a means of estimating the probability of seeing the observed lambda for the population, P(λ). This probability was considered as a proxy of the likelihood of the observed lambda for each model. Models were chosen by comparing the P(λ) for each considered model (Hilborn and Mangel 1997, Hernández-Matías *et al.* 2013). This analysis was restricted to the period 1994–2012 since before 1994 the population size was too small to be able to infer the initial population vector. By 1994 the territorial population consisted of three pairs and the initial population vector was approximated according to the stable distribution of ages obtained from the deterministic Leslie matrix model considering the life cycle and vital rates described above. The initial population vector considered was n0 = (1, 1, 1, 1, 1, 3), each vector element corresponding to the number of females in each given age class. Model scripts were developed in R code (scripts are provided in the online supplementary material).

PVA and the identification of conservation targets

We performed a PVA in which we considered four models. The first two corresponded to the two best-supported models in the previous analysis (scenarios 1 and 2 in the Results section). Additionally, we considered scenario 3 in which adult survival was assumed to be 0.891 (the most likely value provided by Grande *et al.* 2008) and the population was considered to be closed (0 immigrants per year). The last scenario (scenario 4) assumed the most likely adult survival estimated in the present study but considered a closed population. For each model, 5,000 replicates were simulated with a time horizon of 50 years. Population growth rate and extinction probability were calculated under each scenario (Morris and Doak 2002), whereby a population was considered to be extinct if the number of predicted breeding females was less than one. The probability of a fall by 50% in the number of pairs in 10 years was also calculated since this is one of the IUCN criteria used to classify a species as Endangered (IUCN 2012).

To identify the vital rates that had the strongest effect on the population growth rate we applied a simulation-based method in which the value of the vital rate of interest was increased by 25% and the values of λ recalculated (except for the number of immigrants, which was increased from one to two immigrants). To do, so we considered the assumptions in scenario 1 in the Results section. Subsequently, we estimated the sensitivity and elasticity of λ to the main vital rates (S_{12}, S_{34}, S_5, SA, P) and to the number of immigrants (N_{imm}).

Results

Population trends and estimation of demographic parameters

The population of the Egyptian Vulture in the study area grew from one to 22 pairs during the period 1988–2012 and expanded eastwards (Figure 2). The population growth rate observed this period was estimated at 1.137 (1.117 in 1994–2012). Productivity corrected by White's method was estimated at 1.168 (temporal variance = 0.404). Adult survival obtained with the same method was 0.891 and its temporal variance corrected was 0.003.

Other breeding parameters calculated were: breeding success 1.11 ± 0.60, fledgling rate 1.17 ± 0.479, percentage of laying pairs 83.54 ± 33.90, percentage of pairs with nestlings 81.61 ± 32.47, percentage of pairs with fledglings 78.45 ± 31.54.

Evaluation of the model assumptions

Our estimates of likelihood showed a crest of maximum values tracing a (non-linear) diagonal from high values of survival and low values of immigration to low values of survival and up to five immigrants per year (Figure 3). Based on available information (Grande *et al.* 2008), values of adult survival below 0.85 would seem to be unlikely. Thus, we selected two scenarios as the best supported, one of which (scenario 1) assumed the entry of one immigrant per year and

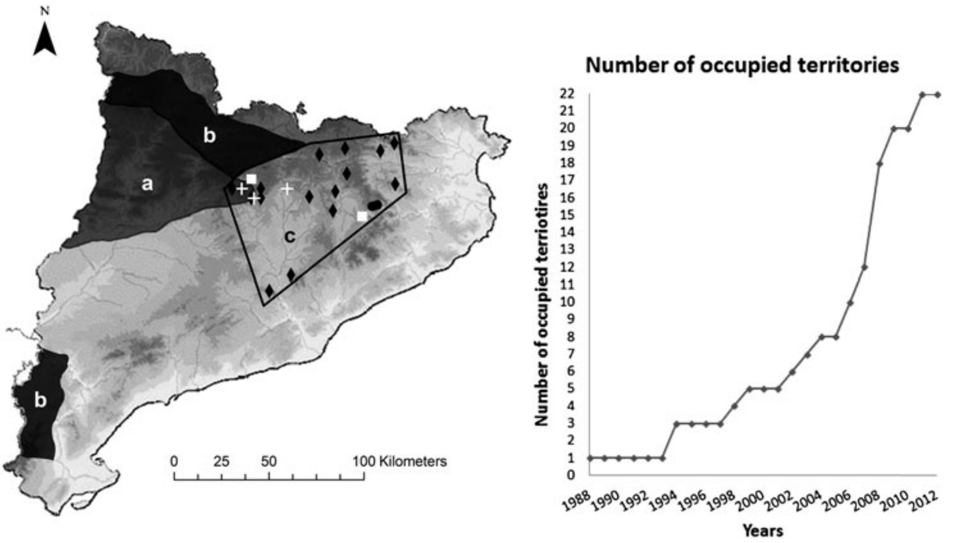

Figure 2. Left: Colonisation of Egyptian Vulture in Catalonia. (a) Represents the range of the species according to *Atles dels Ocells nidificants de Catalunya* (Muntaner *et al.* 1983). (b) Represents the expansion of the species outside the study area, according to Servei de Biodiversitat i protecció dels Animals, Generalitat de Catalunya (2012). (c) Represents the study area. Symbols correspond to territorial pairs in the study area: white cross: 1988–1995, white square: 1996–2000, black circle: 2001–2005, black diamond: 2006–2012. Right: Number of occupied territories of Egyptian Vulture in the study area during the period 1988–2012.

adult survival of 0.950 (likelihood estimated at 0.947), while the other (scenario 2) assumed the entry of two immigrants per year and adult survival of 0.875 (likelihood estimated at 0.962) (see Figure 3).

Population Viability Analysis

Under scenario 1, the models predict that the population will continue to grow ($\lambda = 1.051$) and estimate an extinction probability of 0 in 50 years. Under scenario 2, the models predict a lower population growth rate ($\lambda = 1.022$) and an extinction probability also of 0. Under scenario 3, which assumes a closed population, the population growth rate was estimated at 0.994, implying that the population will remain at around 20 pairs, with an extinction probability of 0.011. Finally, under scenario 4, the population is predicted to increase with a growth rate of 1.039 and an extinction probability of 0 on a 50-year horizon (Figure 4). Under scenarios 1, 2 and 4, the probability of a 50% reduction in the number of pairs in 10 years was 0, but under scenario 3 was 0.049.

Sensitivity and elasticity of vital rates

Of all the parameters, adult survival had the greatest sensitivity (0.535) and elasticity (0.482); thus, relative increases in adult survival values would cause the greatest increase in the predicted population growth rate (Figure 5). The number of immigrants did not seem to have any important effect on the growth rate. This is because we used an increment of one immigrant (from one to two) to estimate the model's sensitivity and elasticity, thereby implying that the denominator of the expressions used to estimate these two metrics was much higher than for the other vital rates.

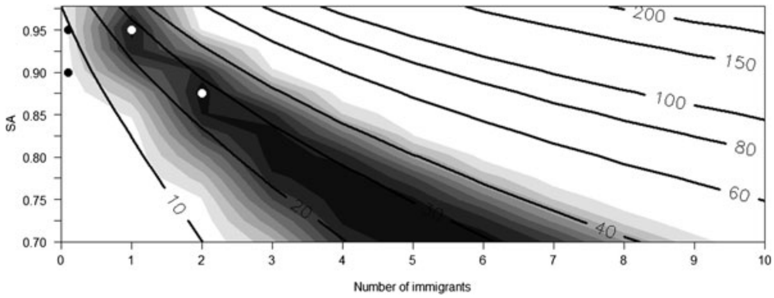

Figure 3. Likelihood estimate assuming different values of both adult survival (SA), from 0.7 to 0.975, and number of immigrants, from 0 to 10. Likelihood is represented by grey tones, from white (0) to dark grey (1). Black lines indicate the mean number of breeding pairs predicted under each scenario in 2012. White points indicate two most likely scenarios selected to perform the PVA (scenario 1 and 2). Black points indicate two closed population scenarios (3 and 4) selected to perform PVA.

As a result, the values of the metrics were much lower. However, the effect of immigration on the population growth rate was highly important, particularly between the scenarios of no immigration and of one immigrant (Figure 6).

Discussion

The Egyptian Vulture is an endangered species that has dramatically declined throughout its range (BirdLife International 2012), which includes the Iberian Peninsula, home to the bulk of the European population of this species. Despite this adverse scenario, a few local populations occupying small areas have recently grown (García-Ripollès and López-López 2006, Mateo-Tomás *et al.* 2010). We focused on one of these increasing populations in eastern Catalonia to investigate whether the observed trend could be explained simply by the establishment of individuals born in the study population or whether the arrival of immigrants was a necessary factor. To do so, we applied likelihood-based methods using a comprehensive demographic analysis. While population viability analysis is frequently applied to declining populations (Carrete *et al.* 2009), it is used much more rarely to study endangered species that are increasing in number (Ortega *et al.* 2009). We argue that identifying the demographic determinants of population increases may provide relevant information for guiding conservation managers and practitioners whose task it is to preserve populations of target species.

The results of our models strongly suggest that the increase observed in recent years in the Egyptian Vulture population in Catalonia was probably caused by a combination of higher adult survival than in other Iberian populations and the entry into the population of adults from outside the study area. Both adult survival and immigration play a key role in determining the dynamics of the population, as is to be expected for long-lived species (Real and Mañosa 1997, Saether and Bakker 2000). Our analyses were based on limited field data, a common issue when studying endangered species (Doak *et al.* 2005). Even so, the framework we applied based on likelihood methods was useful to tackle this constraint (Hernández-Matías *et al.* 2013).

Instead of adult survival or immigration, other demographic parameters could potentially affect the observed population trend. In the case of productivity, it was set at the values we estimated from the population and indeed, these values were higher than in other Spanish populations (C.R.P.R 1984, Donázar and Ceballos 1988, Fernández 1994, Donázar *et al.* 2002).

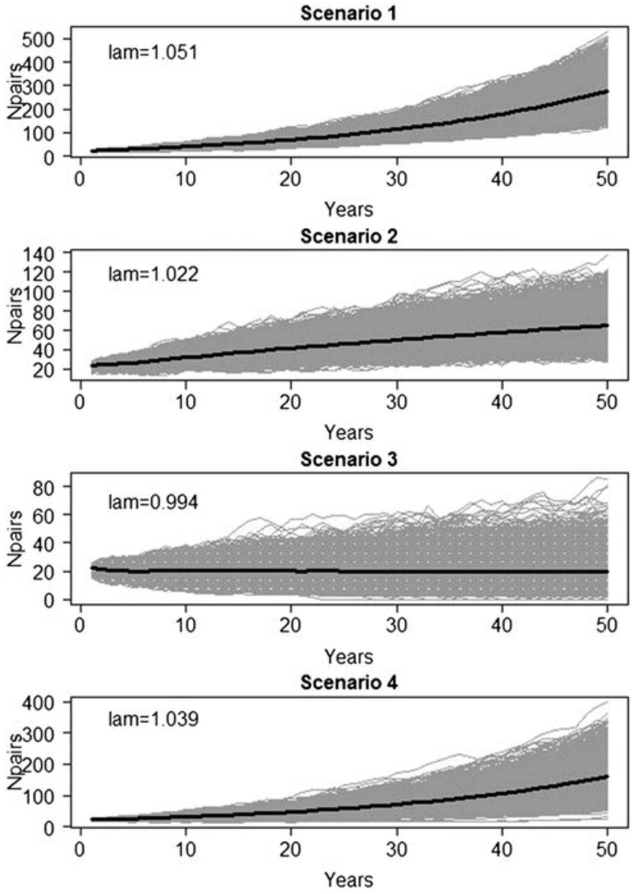

Figure 4. Projection of the population trend (in number of pairs) estimated for the next 50 years using models considered for PVA. All 5,000 replicates are represented in grey as well as the average trend represented by the black line.

Nonetheless, sensitivity and elasticity analyses indicate that, of all factors, adult survival has the strongest effect on the population growth rate and that non-adult survival and, specially, productivity are much less decisive, suggesting that the positive trend and the expansion of our population was not due simply to high levels of productivity. Regarding non-adult survival, we assumed in all models it was the same as described in the Ebro population. This assumption is reasonable if we consider that juvenile individuals from neighbouring populations share a similar life style; staying at the same wintering areas, aggregating in communal roosts and prospecting for potential breeding territories (Donázar 1993). Another assumption in our models was that all individuals recruit at five years old. Again, this seems reasonable if we bear in mind that Egyptian Vultures achieve maturity at this age (Donázar 1993) and that we studied a growing population and, therefore, no density-dependence is expected (Oro and Pradel 2000).

Unlike other areas of the Iberian Peninsula, where poisoning is the main cause of non-natural mortality in Egyptian Vultures (Hernández and Margalida 2009), the probable higher survival rates in the Catalan population could be due to the less widespread use of poison in Catalonia, especially in the study area. Support for this comes from the fact that during the period 1995–2010 only three Egyptian Vultures were found dead by poisoning in Catalonia, and all of them

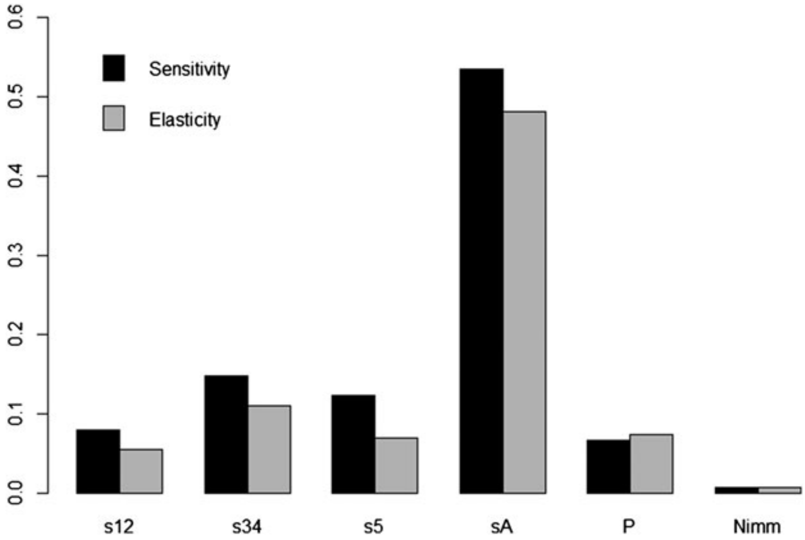

Figure 5. Sensitivity and elasticity of the population growth rate to main vital rates. S_{12}: yearly survival in the first two years of life, S_{34}: yearly survival in third and fourth years of life, S_5: survival in fifth year of life, SA: survival of territorial individuals, P: productivity (number of fledglings per territorial pair), and Nimm: number of immigrants.

outside our study area (Hernandez 2006, Programa Antídoto unpubl. data). In other neighbouring areas such as Aragon, 47 individuals were found dead during the period 1996–2006 (Hernandez 2006) which highlights the different distribution and importance of poisoning events in the Iberian Peninsula. Electrocution and collision with power lines and wind farms are also important causes of non- natural mortality of Egyptian Vulture in Spain (Donázar *et al.* 2002, Carrete *et al.* 2009), but available data coming from recovery centres report that this is not a major cause of death of the species in Catalonia since only one case of electrocution has been reported up today (Servei de Biodiversitat, Generalitat de Catalunya *in litt.* 2013). On the basis of our results, management efforts in areas where the species' populations are decreasing should be directed to improving adult survival by eliminating the human related mortality that affects the species.

Our results also suggest that our study population is regularly receiving immigrating individuals from elsewhere. The Egyptian Vulture is a philopatric species with low natal dispersal distances (median natal dispersal 19.74 km, range = 0–150 km, *n* = 26; Grande *et al.* unpubl. data). Our study area is adjacent to a nucleus in north-west Catalonia (Lleida Pyrenees), which has increased - the number of pairs rose from 35 to 55 during the period 1983–2012 (Muntaner *et al.* 1983, Servei de Biodiversitat i Protecció dels Animals, Generalitat de Catalunya 2012). The reasons for the growth of this adjacent population are not well understood although it has been argued that Egyptian Vulture benefit from some feeding points provided for vultures (Margalida *et al.* 2010) but also the lower use of poison and consequently high adult survival could be taken in account. Therefore, we hypothesise that this neighbouring population nucleus is the most likely origin of the new pairs being established in our area. Supporting this idea, the colonisation pattern inside the study area shows that new territories were always near to an existing territory (less that 50 km), although long-distant natal dispersal events are also possible in this species (0–150 km) (Grande *et al.* unpubl. data).

It is known that new sanitary policies that reduced the availability of carcass resources affected some species of vultures via reduction of their vital rates and/or dispersal of individuals

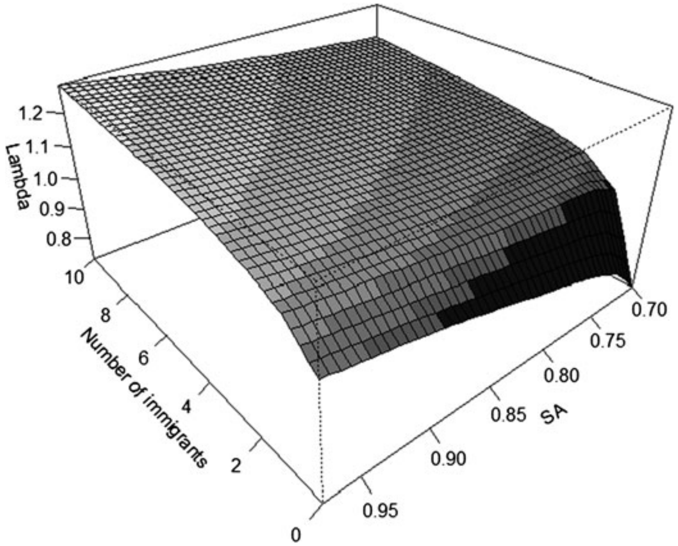

Figure 6. Contribution of immigration and adult survival to the population growth rate, assuming different values of both adult survival (SA), from 0.7 to 0.975, and number of immigrants, from 0 to 10. Values of lambda lower than 1 are represented in black, while values greater than one are represented in a grey scale, darker tones corresponding to lower values of lambda.

(Donázar *et al.* 2010, Margalida *et al.* 2010, 2014). In the case of Egyptian Vultures these policies seem not to be related to the increase in our population for two reasons; the neighbouring population of north-west Catalonia affected by the same regulations increased in the meantime and the breeding success seemed not to be affected by the possible consequent food shortages (Garcia and Margalida 2009) that is in concordance with the trends predicted by Margalida and Colomer (2012). So probably the specific lifestyle of this species (Donázar *et al.* 2010) can benefit from new environmental and human factors (landfill, extensive grazing) that occurred during the last few decades in our area favouring their colonisation and increase (Kiff 2000).

Assuming the most supported scenarios (1 and 2), both of which assume net immigration of adults, our PVA predicts continued population increase at a lower rate than that observed in the study period. However, even in the event that immigration stopped (scenario 4) the population would grow if adult survival is high. In contrast, in scenario 3 where adult survival is assumed to be equal to that estimated in the Ebro population, the population would stop growing although apparently it would remain stable. Even though the role of immigrants is commonly overlooked in population viability assessments, there is increasing evidence to suggest that immigration is a key determinant in population growth rates (Ward 2005, Schaub *et al.* 2013). It is also worth mentioning that all our predictions were made under the assumption that ecological conditions will not change. However, if declining trends in most of the species' range continue, fewer potential immigrants will be available to support the Catalan population, so it is important that future research aims to determine the ecological drivers of the vital rates of this population (e.g. Bakker *et al.* 2009). Conservation actions will thus have to take this question into account (Hernández-Matías *et al.* 2013).

Our results highlight that, despite major uncertainties, likelihood-based population analysis may provide relevant knowledge of the factors that regulate target populations. In the future, by reducing uncertainty, predictions can be refined so that advice for conservation managers can become more accurate. For example, it is essential to estimate survival, immigration and recruitment rates, to continue the long-term ringing scheme initiated in 2012 in this area and then to apply *ad hoc* statistical methods. Furthermore, the study of the ecological and/or behavioural factors driving the settlement of new territories must also be studied. It is known that conspecific attraction (Reed and Dobson 1993, Grande 2006) and environmental features, either for nesting or foraging, also play a relevant role in colonisation processes (Webb *et al.* 2011). In our study area, most breeding pairs are located near landfills and farms, or in areas where extensive livestock farming is practised, and all these features are relevant sources of food for this species (Margalida *et al.* 2007, Gangoso *et al.* 2012, unpublished data). Therefore, it is crucial for the future effective management of this species to investigate the demographic relationships of the different local populations, the environmental determinants of its demographic characteristics and whether intensive human activities may have helped drive the increase in the studied Egyptian Vulture population.

Supplementary Material

The supplementary materials for this article can be found at journals.cambridge.org/bci

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Landfills determine the distribution of an expanding breeding population of the endangered Egyptian Vulture *Neophron percnopterus*

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Human activities provide food resources for animals that are predictable in space and/or time. These resources, sometimes referred to as predictable anthropogenic food subsidies (PAFS), can be either the result of human-generated waste or provided intentionally, sometimes as a conservation measure. Some PAFS, including landfills, are used by common species. However, little information exists about the effects that these feeding points have on rarer species that feed there, some of which are of conservation concern. This study focuses on the influence of PAFS and their spatial location on the distribution of territories of the endangered Egyptian Vulture *Neophron percnopterus*. We analysed a population in the NE Iberian Peninsula that has in recent decades expanded its range. We used both null model and linear model analyses to ascertain the effect of PAFS and other covariates on the occupancy of territories by the study species. PAFS appeared to play an important role in territory selection by Egyptian Vultures, as occupied territories were nearer landfills than expected by chance. Furthermore, the distance from PAFS (landfills and vulture feeding stations, or 'restaurants') played an important role in the probability of territory occupancy by Egyptian Vultures, in addition to other environmental variables such as surface areas of rocky south-facing slopes, human settlement and the proximity of conspecifics. However, recent EU legislation aims to phase out open-air landfills to reduce the negative environmental effects of these facilities. This could have an undesired impact on the endangered species that use these feeding points. We recommend management measures that can control abundant pest species but, in the long term, other measures as supplementary feeding should be considered to counteract the probable negative effect of the disappearance of landfills on endangered species.

Keywords: null model, predictable anthropogenic food subsidies, rubbish dumps, scavengers, threatened species, vulture restaurants.

Human activities are bringing about environmental changes in natural systems that may seriously affect ecosystem components such as the dynamics of plant and animal communities over time (Burney & Flannery 2005). Thus, understanding the processes underlying the responses of species to non-natural perturbations has become a key topic in applied ecology and conservation biology (Nilsson & Grelsson 1995, Gill *et al.* 1996, Primack 2012).

One of the alterations that can seriously impact animal populations is change in the distribution and availability of food resources. This type of alteration may take the form of a drastic reduction in food supplies (Rodenhouse & Holmes 1992, Mateo-Tomás & Olea 2010) or an improvement in food availability due to the provision of food subsidies that were previously unavailable in the environment (Oro *et al.* 2013). The term 'predictable

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anthropogenic food subsidies' (PAFS) was coined to refer to resources that humans waste or intentionally offer to animals, and whose appearance in space and/or time is predictable (Oro *et al.* 2013). When referring to 'artificial' food availability, landfills are one of the most important types of PAFS and have an effect on terrestrial ecosystems, as the resources they provide in the form of waste are present in most modern human societies (Olea & Baglione 2008). In contrast, resources intentionally offered to animals (e.g. supplementary feeding stations) usually form part of specific conservation actions aimed at enhancing populations of a target (usually endangered) species (Donázar *et al.* 2009, Lieury *et al.* 2015, Cortés-Avizanda *et al.* 2016). Both landfills and resources intentionally offered to animals can have positive and negative effects on wildlife that have to be analysed if PAFS are to be managed effectively within a conservation framework (Robb *et al.* 2008, Cortés-Avizanda *et al.* 2016).

Intentionally offered resources may improve the breeding parameters of some avian species (Robb *et al.* 2008) and increase survival rates in a part of their populations, thereby aiding their recovery (Oro *et al.* 2008, Lieury *et al.* 2015). Nevertheless, a number of studies (Carrete *et al.* 2006, Robb *et al.* 2008, García-Heras *et al.* 2013) have highlighted the unintended impact of supplementary feeding on individuals, populations and communities of avian scavengers. The predictability of food availability causes an increase in the number of individuals of the target species in areas around feeding points and may cause density-dependent depression of demographic parameters (Carrete *et al.* 2006). These studies indicate that this conservation strategy must be used with caution, as it may potentially affect the viability of target species (Robb *et al.* 2008, Cortés-Avizanda *et al.* 2010). In addition, in some areas supplementary feeding in isolation has been seen to be insufficient to offset the loss of adult birds due to, for example, poisoning (Oppel *et al.* 2016). Less attention has been paid to non-target species that also use these feeding points intensively and so little information exists on how the presence of these artificial food resources affects the distribution and dynamics of the scavenger species for which these resources are not initially designed.

Landfills typically generate a series of negative environmental effects, including soil contamination, disease and/or the presence of invasive species (Ahel *et al.* 1998, Pyšek *et al.* 2003). Nevertheless, most research on the impact of landfills on animal populations has to date focused on generalist species such as gulls (Laridae), corvids (Corvidae), foxes and rats. These species benefit from predictable resources and have increased locally in areas near landfills, thereby causing problems due to their abundance and the fact that they are regarded as pests (Bosch *et al.* 1994, Boarman *et al.* 2006, Duhem *et al.* 2008, Bino *et al.* 2010). Consequently, recent modifications in legislation have aimed to close open-air landfill sites (EU Commission 2008), leading to a progressive reduction of predictable food resources that is expected to help control the populations of some abundant species (Payo-Payo *et al.* 2015). However, resources from landfills are also used by other species that are not considered pests, some of which are threatened (Viada 1994, Martina & Gallarati 1997, Newsome *et al.* 2015). The implementation of current landfill legislation may negatively affect some of these species, although there is little available information about the effects of artificial food resources on the distribution and population dynamics of the species of conservation concern that rely on landfills. Further knowledge of this topic is a prerequisite if management decisions are to be taken that will not have negative effects on the populations of these threatened species.

In this study, we address the question of whether PAFS have influenced the distribution of a territorial vulture species, the Egyptian Vulture *Neophron percnopterus*, a globally Endangered species that is able to exploit a wide range of food resources including landfills and supplementary feeding points (Donázar 2004). Vultures are useful for evaluating the effects of PAFS on spatial distribution as all vulture species are scavengers and most benefit from PAFS. Under this scenario, we focused on an Egyptian Vulture population that is currently expanding its range, an exceptional situation for this species in Europe. Unexpectedly, our study population has colonized new areas that are not thought to have been previously occupied by this species (Tauler *et al.* 2015). These newly colonized locations are found in a mountainous area with livestock where there is a network of feeding stations, termed 'vulture restaurants', that was set up primarily to help preserve the endangered Bearded Vulture *Gypaetus barbatus*, and in a more populated contiguous area that possesses a network of landfill sites.

The aim of this study was to assess whether there is an association between the spatial distribution of PAFS and the spatial distribution of Egyptian Vulture territories in central Catalonia (NE Spain), an area that has been recolonized by this species over the last two decades. To do so, we used a dual approach that combined a null model analysis (taking into account relevant environmental constraints on the locations of vulture territories) and linear models (controlling for the most relevant environmental variables known to affect habitat selection in the study species). Our main predictions were that (1) Egyptian Vulture territories would be located closer to PAFS than expected by chance, (2) breeding territories would more probably be found in remote areas with a low degree of human impact, and (3) occupied territories would be situated in areas with a high availability of livestock-derived resources.

METHODS

Study species

The Egyptian Vulture is classified as Endangered on the IUCN Red List (BirdLife International, 2015). Its Iberian population has declined by 25% over the last two decades, mainly due to poisoning and illegal persecution, but also as a result of a decline in traditional agricultural practices (Liberatori & Penteriani 2001, Donázar 2004), and in certain areas of Spain due to collision with power lines and wind farms (Donázar *et al.* 2002, Carrete *et al.* 2009). However, there are at least two populations of Egyptian Vulture in the Iberian Peninsula that are increasing and expanding their ranges (García-Ripollès & López-López 2006, Tauler *et al.* 2015).

Study area and data collection

The study area (10 500 km^2) lies in central eastern Catalonia (NE Iberian Peninsula) at altitudes of 200–1900 m asl, and encompasses an area of cliffs running from the Prelitoral Mountains in the south to the pre-Pyrenean Mountains in the north. The study population was monitored from 1988 to 2014 (Fig. 1). During this period, regular cliff-nesting raptor censuses were performed in the whole area and potential suitable habitats where newly colonizing pairs might be recorded were prospected. To determine territory occupancy, each territory was visited during the breeding season (March–August) by observers equipped with a spotting scope (20–60×). An area was considered unoccupied if no individuals were detected in the course of four visits in different days. The population grew from 1 to 25 breeding pairs between 1988 and 2014 ($\lambda = 1.13$) (Tauler *et al.* 2015). To perform the statistical analysis, we selected the period 2010–2014 because the monitoring effort during this period was more intensive, and information for the nest-sites was more accurate than for the remaining part of the period. Breeding territories included in the analyses were occupied almost all of the period (a mean of 4.2 ± 1.35 years) and if a territorial pair used more than one nest, the nest that was used for most years was selected.

Effect of PAFS on territory distribution – the null model analysis

To study the influence of PAFS on the distribution of occupied territories, we first used a null-model approach (Nitecki & Hoffman 1987, Gotelli & Graves 1996, Colwell & Lees 2000). A null model is 'a pattern-generating model that is based on randomization of ecological data or random sampling from a known or imagined distribution' (Gotelli & Graves 1996). In contrast to most other modelling approaches (Caswell 1988), the null-model strategy involves the construction of a model that deliberately excludes the mechanism being tested. The aim is to see how well the available data fit this model (Hilborn & Mangel 1997); in other words, the extent to which patterns in the data can be reproduced by a simple model that does not incorporate biologically important mechanisms, and the extent to which the data have a non-random distribution with respect to the null hypothesis. The analysis provides evidence in support of, or against, the mechanism under study (Gotelli 2001).

In our case, the mechanism being tested was whether occupied territories were located nearer PAFS than expected under the assumption that territories were not related to the presence of PAFS. We thus defined a statistic – the nearest PAFS distance (NPD) – to measure this relationship. Consequently, to describe the observed pattern, we calculated the NPD for all occupied territories in the study area using data on landfills and vulture restaurants from the Agencia de

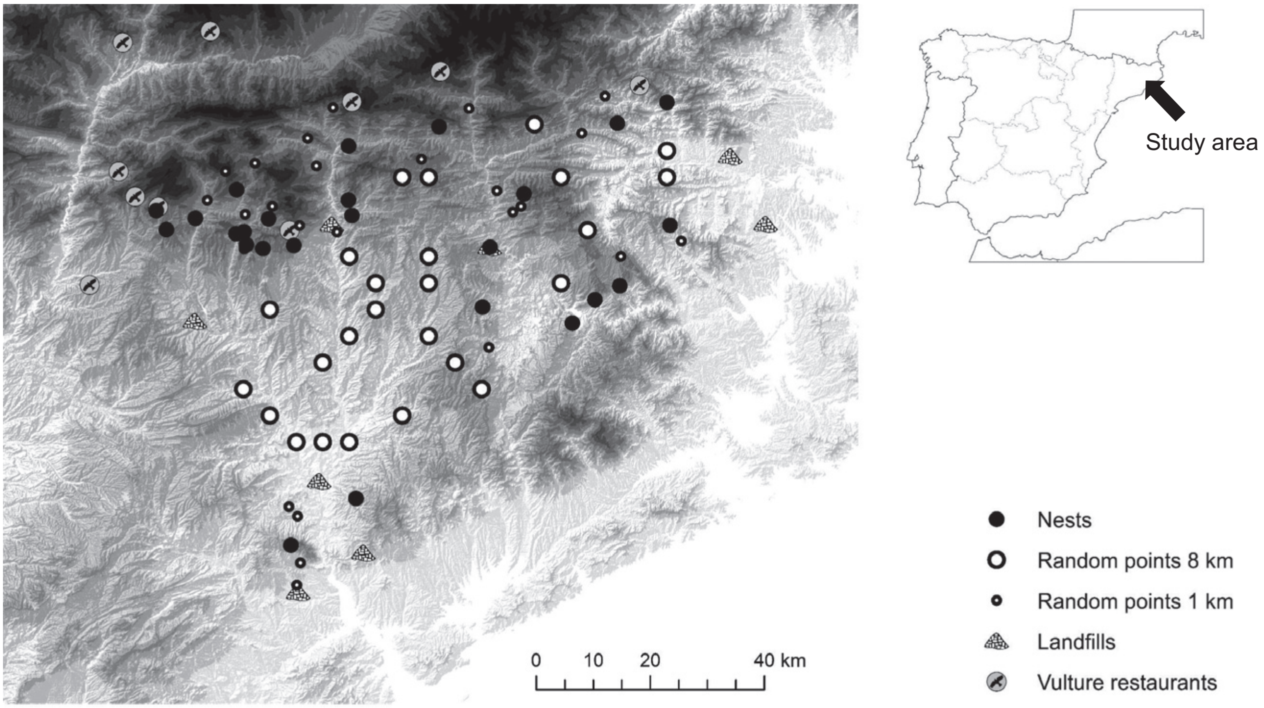

Figure 1. Distribution of occupied territories of Egyptian Vulture, landfills and vulture restaurants used in the generalized linear model (GLM) and null model analysis. Random points used in 1- and 8-km GLM analyses are also presented.

Residus de Catalunya and the Servei de Biodiversitat, Departament de Territori i Sostenibilitat, respectively (Fig. 1).

To perform the null model, the NPD of occupied nests was compared with the NPD of 1000 randomly simulated nest-sites located inside the study area on cliffs identified on a very precise land cover map (resolution of 2 m; Mapa de Cobertes del Sòl de Catalunya, Generalitat de Catalunya). By selecting only rocky areas, we imposed an ecological restriction on the null distribution that preserves some features of the real data and thus gives greater biological authenticity to the random locations of the simulated nests. The model operated in the following way: first, a sample of 25 points (i.e. the same number as the number of occupied territories) was selected randomly from the simulated set. Two calculations were then made: (1) the distance between each point and the nearest PAFS (NPD) in the study area and (2) the mean of all the obtained NPDs (MNDP). This process was repeated 999 times to generate a frequency histogram of expected MNDP values in a random distribution of nests. The position of the observed value of MNPD in occupied territories in the null distribution of the randomly generated values was then used to assign a probability value (*P*), just as in a conventional statistical analysis (Dolédec *et al.* 2000, Raes & ter Steege 2007). Finally, the 95% confidence interval (CI) of the obtained null distribution was calculated.

This model was initially performed using data from all PAFS in the study area (PAFS model), but was then also simulated separately for the distance from points to the eight landfills in the study area (MNLD; landfill model) and to the 10 vulture restaurants (MNVD; vulture restaurant model) to test for possible differential effects of these two types of predictable feeding points.

Effect of PAFS on territory selection – **generalized linear model analysis**

We performed a generalized linear model (GLM) to test the effect of PAFS on Egyptian Vulture territory selection by taking into account other environmental variables known to affect the distribution of this species (see below). Environmental variables were measured at two spatial scales to account for the fact that nest-sites may be selected by vultures on the basis of the habitat characteristics of both the immediate area around the nest and the overall home-range that vultures need to exploit to find resources. For the two spatial scales considered, we conducted a GLM using variables measured within a 1- and 8-km radius around nests, which correspond, respectively, to the core area and the home-range of breeding Egyptian Vultures according to radiotracking data from southern Spain (Carrete *et al.* 2007).

To perform GLMs, occupied territories were compared with a set of 25 randomly generated points located inside the minimum convex polygon (MCP) delimited by the 25 occupied territories. The response variable in this case was the occupation of the territories, which were either occupied or unoccupied (i.e. random points). To carry out the analysis at the breeding-area scale (1-km radius around the nest), random points were modelled that had to be inside the 8-km radius of the area of influence of occupied territories in order to highlight the characteristics of the breeding area, given that the larger area had already been selected. The points used at the home-range scale (8-km radius) were simulated randomly, but with the limitation that their areas of influence overlapped the occupied territories by a maximum of 50% to avoid replication and the overlapping of buffers of occupied and non-occupied territories.

The choice of explanatory variables to be considered in the analyses was made on the basis of previous knowledge of Egyptian Vultures (Liberatori & Penteriani 2001, Sarà & Di Vittorio 2003, Carrete *et al.* 2007, Mateo-Tomás & Olea 2010), and specific hypotheses regarding their possible effects on territorial selection were formulated (Table S1). To describe food availability, data on livestock from the national cattle census were obtained (Instituto Nacional de Estadística, 2009), and the distances between occupied territories and PAFS existing inside the study area were calculated. Variables relating to food availability were only collected for the 8-km analysis because data for livestock were not available at a 1-km scale. Topographical variables (altitude, roughness and aspect) were obtained from a digital elevation model (DEM) with a spatial resolution of 15 m. Landscape structure and land-use variables were obtained from a land-cover map (Mapa de Cobertes del Sòl de Catalunya, Generalitat de Catalunya), and the Simpson diversity index was calculated based on the information for each land-cover type. The effect of human presence and disturbance was assessed by calculating the distance to the nearest paved road, as well as by determining the total length of unpaved tracks inside the areas of influence around the nest-sites. These variables were obtained from the Topographical Map of Catalonia (1:25 000, Institut Cartogràfic de Catalunya). Finally, as the Egyptian Vulture is a territorial breeder, the effect of the presence of conspecific pairs was analysed by calculating the distance to the nearest neighbouring breeding pair. All variables were obtained using ArcGIS 10.2 (ESRI). Correlation analyses were performed to detect collinearity and non-independence between variables, and, if detected, those variables that were considered to be less biologically meaningful were not subsequently used. At the 1-km scale, we selected five variables: roughness (correlated with altitude), rocky surface, urban surface (correlated with other landscape and human presence variables), habitat diversity and aspect. At the 8-km scale, we retained seven variables: distance to PAFS, extensive and intensive livestock, urban surface (correlated with other landscape and human presence variables), roughness (correlated with aspect and rocky) and habitat diversity.

We used the logit link function to model this binomial variable (presence/pseudo-absence) and assumed a binomial error distribution. Model selection was performed by a backward–forward stepwise procedure considering the two models to be statistically different when there was a difference of two AICc units (AICc, Akaike information criterion corrected for small samples). All models that did not differ by more than two AICc points from the best model were selected if they had a lower AICc than the null model (i.e. an intercept-only model). We used full model averaging over the whole model set to calculate the parameter estimates and standard errors. Parameter estimates or predictions obtained by model averaging are robust in the sense that they reduce model selection bias and account for model selection uncertainty (Johnson & Omland 2004). For the best selected models, the relative variable importance was calculated as the sum of the Akaike weights for all models in which the variable $w + (i)$ appeared to quantify the evidence of the importance of each variable in the set (Burnham & Anderson 2002).

All variables were standardized and models were performed using the R.3.2.2 (R Development Core Team 2015) program and the package MuMIn was used to perform GLM selection and the relative variable importance.

RESULTS

Effects of PAFS on territory distribution – the null model analysis

The mean nearest PAFS distance (MNPD) observed between occupied nests and PAFS was 8.75 km. This figure is not significantly different from the expected frequency distribution under the null model (mean MNPD = 9.31 km; 95% CI 7.17–11.77) and so in this case the null hypothesis was accepted ($P = 0.330$). In the case of the landfill model, the MNLD between occupied nests and landfills (14.06 km) was significantly lower than the MNLD predicted by the null model (mean = 17.76 km, 95% CI 14.7–21.30) and so the null hypothesis was rejected, thereby supporting a non-random relationship between the locations of occupied territories and landfills ($P = 0.0013$). Finally, the MNVD between occupied territories and vulture restaurants (18.21 km) was not found to be significantly different from the expected MNVD from the null model (mean = 21.67 km, 95% CI 14.42–28.90) and so this null hypothesis was also accepted ($P = 0.178$). In summary, the landfill model was the only model that was significant when explaining the relationship between feeding points and the distribution of occupied nests (Fig. 2).

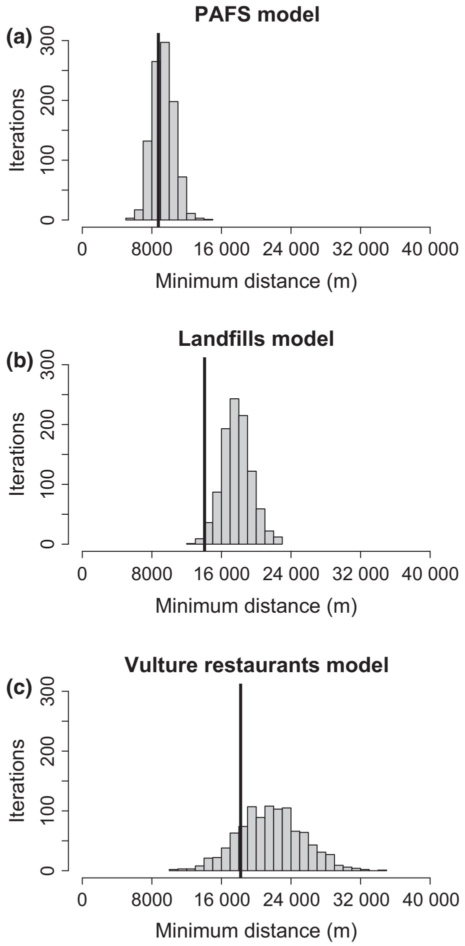

Figure 2. Frequencies of distances from simulated nests to (a) PAFS (landfills and vulture restaurants), (b) landfills and (c) vulture restaurants obtained in each null model. *N* iterations = 999. The black line indicates the distance from real nests to PAFS, landfills and vulture restaurants, respectively.

Effects of PAFS on territory selection – GLM analysis

At the breeding-area scale (1-km radius), two models were selected that did not differ by more than two AICc units from the model with the lowest AICc. All selected models at this scale included the variables 'rocky', 'aspect', 'urban' and 'roughness'. The first three variables had the same relative importance ($w + (i) = 1$), but the relative importance of roughness was 0.83 (Table 1). Occupied territories tended to have a greater proportion of rocky surface areas, were generally south-facing and had a lower proportion of built-up areas (Fig. 3). At the home-range scale (8-km radius), five models were selected. All selected models included the variable PAFS distance with a relative importance of 0.93, but the presence of conspecifics also appeared in four models with a high relative importance (0.81, Table 1). Occupied territories were situated nearer PAFS and conspecifics than were random points (Fig. 3). Roughness and intensive livestock had a lower variable importance (0.61), and were present only in three of the best models. All selected models in the two sets of analyses, at both breeding-area and home-range scales, improved the AICc relative to the null model (AICc null model = 71.39 in the two analyses).

DISCUSSION

Our study focused on an expanding population of Egyptian Vultures that has recently occupied areas situated outside its historical range. We thus assumed that territorial locations reflect preferred environmental characteristics in the study species and that other factors, including historical ones,

Table 1. Variables included in the best models (1 if included, 0 otherwise) selected at the breeding-area scale (1-km radius around the nest) and the home-range scale (8-km radius around the nest). For each variable, relative importance weights $w+(i)$ and parameter estimates and standard errors obtained from full model averaging are shown. For the best models (within 2 AICc units of the best model), the number of parameters (k), AICc, ΔAICc, AICc weight and R^2 are also presented. More information on the other models is given in Tables S3 and S4.

	$w+(i)$	1	2	3	4	5	Estimate	se
AICc top models (1-km)								
Rocky	1	1	1				1.98	0.74
Aspect	1	1	1				1.43	0.55
Urban	1	1	1				−1.77	0.75
Roughness	0.83	1	1				1.03	0.71
Habitat diversity	0.68	1	0				−1.14	1.13
k		6	5					
AICc		46.79	48.14					
ΔAICc		0	1.35					
AICc weight		0.55	0.28					
R^2		0.53	0.47					
AICc top models (8-km)								
PAFS	0.93	1	1	1	1	1	−1.11	0.57
Conspecifics	0.81	1	1	1	1	0	−0.96	0.71
Roughness	0.61	1	0	1	0	1	0.48	0.52
Intensive livestock	0.61	1	1	0	1	0	−0.57	0.62
Urban	0.43	0	0	1	0	1	−0.34	0.63
Extensive livestock	0.27	0	0	0	1	0	−0.09	0.29
Habitat diversity	0.23	0	0	0	0	0	0.04	0.25
k		5	4	5	5	4		
AICc		48.67	48.69	48.95	50.35	50.37		
ΔAICc		0	0.02	0.28	1.68	1.7		
AICc weight		0.1	0.1	0.08	0.04	0.04		
R^2		0.46	0.42	0.45	0.44	0.40		

did not confound our main findings. Our overall results indicated that PAFS played an important role in determining the current distribution of this species in the study area, which has been shaped by the presence of these artificial food resources (at least in the case of landfills). However, unlike in the linear model analysis, in the null model analysis only landfills were selected to explain territorial distribution, a finding that, as we discuss below, has serious implications for management and conservation.

The linear models allowed us to control for the effects of other environmental variables that, if not taken into account, may affect territorial choice and potentially confound the results. Model uncertainty led to large errors for some variables. Nevertheless, models including these variables ranked highly according to AICc and they explained a high proportion of variance of the response variable. Additionally, they showed relatively high Akaike weights, suggesting that they also played a relevant role in nest-site selection. PAFS had a high Akaike weight and was present in all five best models at the 8-km scale, and thus all territories were located close to a PAFS, be it a landfill or a vulture restaurant (Table 1). However, landfills and vulture restaurants did not appear as very important variables when considered separately in the linear models, as individual territories may also be situated far from either a landfill or a vulture restaurant (Table S2).

Home-range-scale analysis (8-km radius) also showed that the presence of conspecifics is important when selecting a new territory, as occupied territories tend to be close to other breeding pairs. Distance to PAFS and conspecific presence were the variables that had the lowest standard errors, hence further supporting their important role (Table 1). Additionally, the location of nests was linked to very rough terrain and the presence of intensive livestock, matching results from other studies that indicate that cattle are one of the main sources of food for Egyptian Vultures (our pers. obs., Donázar 1993, Mateo-Tomás & Olea 2009). At the breeding-area scale (i.e. 1-km radius), nests were located preferentially on south-facing cliffs

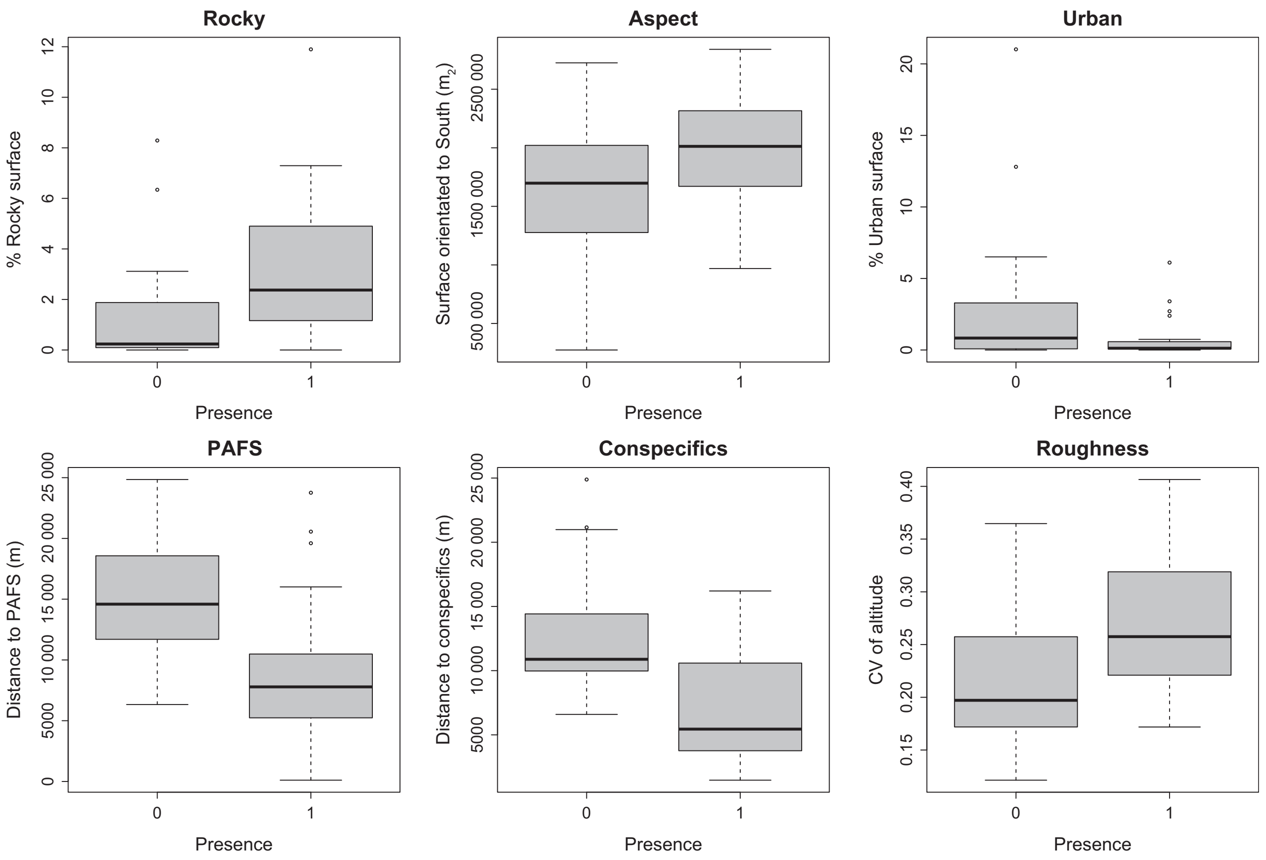

Figure 3. Boxplots of variables with the highest relative importance included in all best sets of models obtained with GLM analysis at breeding-area (1-km radius, top) and home-range (8-km radius, bottom) scales. The original data distribution of random points (occupied = 0) and occupied territories (occupied = 1) is also shown.

far from built-up areas (Fig. 3), a result that also agrees with previous studies (Liberatori & Penteriani 2001, Sarà & Di Vittorio 2003, Carrete *et al.* 2007, Mateo-Tomás & Olea 2009).

Under the null model analysis, there was an assumption that randomized territories could only exist in rocky areas (Estrada *et al.* 2005, this study), which reduced Type-I errors in all tests, as well as Type-II errors in the test for landfills, as these infrastructures are usually placed in areas of high human population density far from rocky outcrops. Nevertheless, vulture restaurants are usually placed in rocky areas corresponding to the distribution of the Bearded Vulture (Fig. 1) and so the possibility of Type-II errors increases in the vulture restaurants model. We did not detect any effect of vulture restaurants on the observed distribution of Egyptian Vultures (Fig. 2), although it is known that these sites have acted as attractants for high densities of pairs of Egyptian Vultures and other scavenger species (Carrete *et al.* 2006, Lieury *et al.* 2015). Thus, although our approach probably does not completely disentangle the effect of vulture restaurants, it does strongly support the suggestion that landfills have played an important role in the observed distribution of Egyptian Vulture territories in the study area. In this regard, our results are relevant, as landfills are important sources of food for Egyptian Vultures, as has been described in other areas (Liberatori & Penteriani 2001, Gangoso *et al.* 2013, our pers. obs.), but the implications of the presence of landfills for the territorial distribution of Egyptian Vultures have never been demonstrated before.

Implications for conservation

Feeding in landfills may have certain implications at individual and population levels that may potentially affect the conservation status of scavenging species. At an individual level, abundant and predictable food should improve body condition and breeding performance of the individuals that feed in these areas (Oro *et al.* 2013 and references therein). On the other hand, landfills can also act as a threat, as they represent a source of low-quality food with high levels of different pollutants such as heavy metals (de la Casa-Resino *et al.* 2014, Carneiro *et al.* 2015), rodenticides (Quarles 2011) and organochlorines (Martínez-López *et al.* 2015). The presence of these pollutants can have negative effects on body condition (Ortiz-Santaliestra *et al.* 2015) or even cause mortality (Sánchez-Barbudo *et al.* 2012). Moreover, the presence of the species in highly humanized areas where landfills are found could also increase the risk of fatal casualties such as collisions with power lines and other infrastructures (Martínez-Abraín *et al.* 2012, Sanz-Aguilar *et al.* 2015).

PAFS could also have positive effects at a population level by promoting the immigration of individuals attracted by the availability of food, increasing the number of Egyptian Vulture breeding pairs in Catalonia (Tauler *et al.* 2015). In this sense, the presence of breeding pairs near PAFS could also have acted as an additional attracting factor for non-breeding individuals (Carrete *et al.* 2007). Immature individuals favoured by the presence of predictable food and conspecifics could have formed new pairs that could have colonized other unoccupied territories even further from PAFS, thus continuing the population expansion. On the other hand, PAFS can act as a driver of population change, improving survival rates of both adult and immature individuals, and hence recruitment (Oro & Pradel 2000, Oro *et al.* 2008), but also could improve productivity of pairs that feed on PAFS. In this sense, the high predictability and availability of food associated with landfills have also led to steep population increases in gull, Raven *Corvus corax* and rat populations. Several studies have focused on the ecological and social consequences of this overabundance (Hario 1994, Vidal *et al.* 1998, Bosch *et al.* 2000). For example, the growth of gull populations has caused social disturbance and public health issues (Hatch 1996), thereby triggering population control measures worldwide (Bosch *et al.* 2000, Brooks & Lebreton 2001).

As a response to the negative impact of open-air landfills and to the above-mentioned concern regarding abundant problematic species, the European Commission approved a Landfill Waste Council Directive (EU Commission 2008) that aims to halt the building of open-air landfill sites. This reduction in the predictable resources available to scavenger species could have a strong negative impact on populations of vultures if compensatory measures are not implemented. Our study shows that the presence of landfills may have promoted the colonization of new areas by the Egyptian Vulture and, furthermore, that landfills provide an important proportion of the diet of some pairs in the study area (our pers. obs.). Thus, if the food resources provided by landfills disappear, some breeding failures and territory abandonment of certain breeding pairs may occur in our study area (Liberatori & Penteriani 2001). Current European legislation thus needs to promote research addressed at evaluating the potential effects on wildlife of the closure of these infrastructures. Such research can assist in the design of suitable conservation measures by considering how the predictability of food sources affects the species that feed on them and by assessing their suitability – in other words, solutions that take into account both pest and non-pest species must be found. To mitigate the potential negative effects for endangered populations of species due to the closure of landfills, other conservation measures such as supplementary feeding should be considered. However, it is worth mentioning that supplementary feeding has also had certain negative impacts on the population dynamics of some endangered species in the form of a decrease in productivity due to a rise in the number of pairs (Carrete *et al.* 2006, Lieury *et al.* 2015) and an increase in intraspecific competition (Oro *et al.* 2008). This conservation tool must thus be used with caution and its effects should be constantly assessed. In our study area, the above-mentioned EU legislation has recently been put into practice in at least one landfill and will also be implemented in other landfills in the area in the future. Thus, well-designed monitoring and conservation actions are urgently required.

Overall, this study provides highly relevant information for conservation practice given that it shows that human-induced alterations in natural food availability can have meaningful consequences that are not immediately apparent or are difficult to calculate. Such modifications may affect pest species – typically generalists in the wild – whose abundances can cause problems. However, they may also have an impact on certain threatened non-pest species and even determine their distributions and influence other possible factors that have not yet been assessed.

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SUPPORTING INFORMATION

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

Table S1. Explanatory variables used in the environmental analysis. Also given are the name of the variable, its description, the radius at which the analysis was considered (1- or 8-km radius around nests) and the source of data (INE: Instituto Nacional de Estadística, ARC: Agència de Residus de Catalunya, SB: Servei de Biodiversitat, Departament de Territori i Sostenibilitat, Generalitat de Catalunya, ICGC: Institut Cartografic i Geologic de Catalunya, MCSC: Mapa de Cobertes del Sòl de Catalunya, DEM: Digital Elevation Model). Finally, a hypothesis for the contribution of each variable to the selection of breeding areas (according to Mateo-Tomás & Olea, 2009) is given.

Table S2. Results of an additional GLM analysis performed to test the influence of PAFS, landfills and vulture restaurants separately on territory selection by Egyptian vultures. Model S1 includes PAFS, landfills and vulture restaurants along with all other variables included in the 8-km analysis; Models S2 and S3 include landfills and vulture restaurants, respectively, as well as environmental variables. In each case, variables included in the best five models (1 if included, 0 otherwise), variable relative importance weights $w+(i)$ and parameter estimate and standard error obtained from full model averaging are given. For the best five models, the number of parameters (k), AICc, ΔAICc and AICc weight are also shown.

Table S3. Additional results obtained with GLM 1-km analyses. The variables included in each model (1: Roughness, 2: Aspect, 3: Rock, 4: Habitat, 5: Urban), the number of parameters (k), AICc, ΔAICc and AICc weight obtained with the confidence model set (cumulative weights above 95%) are indicated.

Table S4. Additional results obtained with GLM 8-km analyses. The variables included in each model (1: Roughness, 2: PAFS, 3: Conspecifics, 4: Extensive, 5: Intensive, 6: Habitat, 7: Urban), and the number of parameters (k), AICc, ΔAICc and AICc weight obtained with the confidence model set (cumulative weights above 95%) are shown.