# Estudio de la ecología trófica del águila perdicera *Aquila fasciata*: efectos de la dieta sobre la condición corporal, las tasas vitales y la demografía

Jaime Resano Mayor

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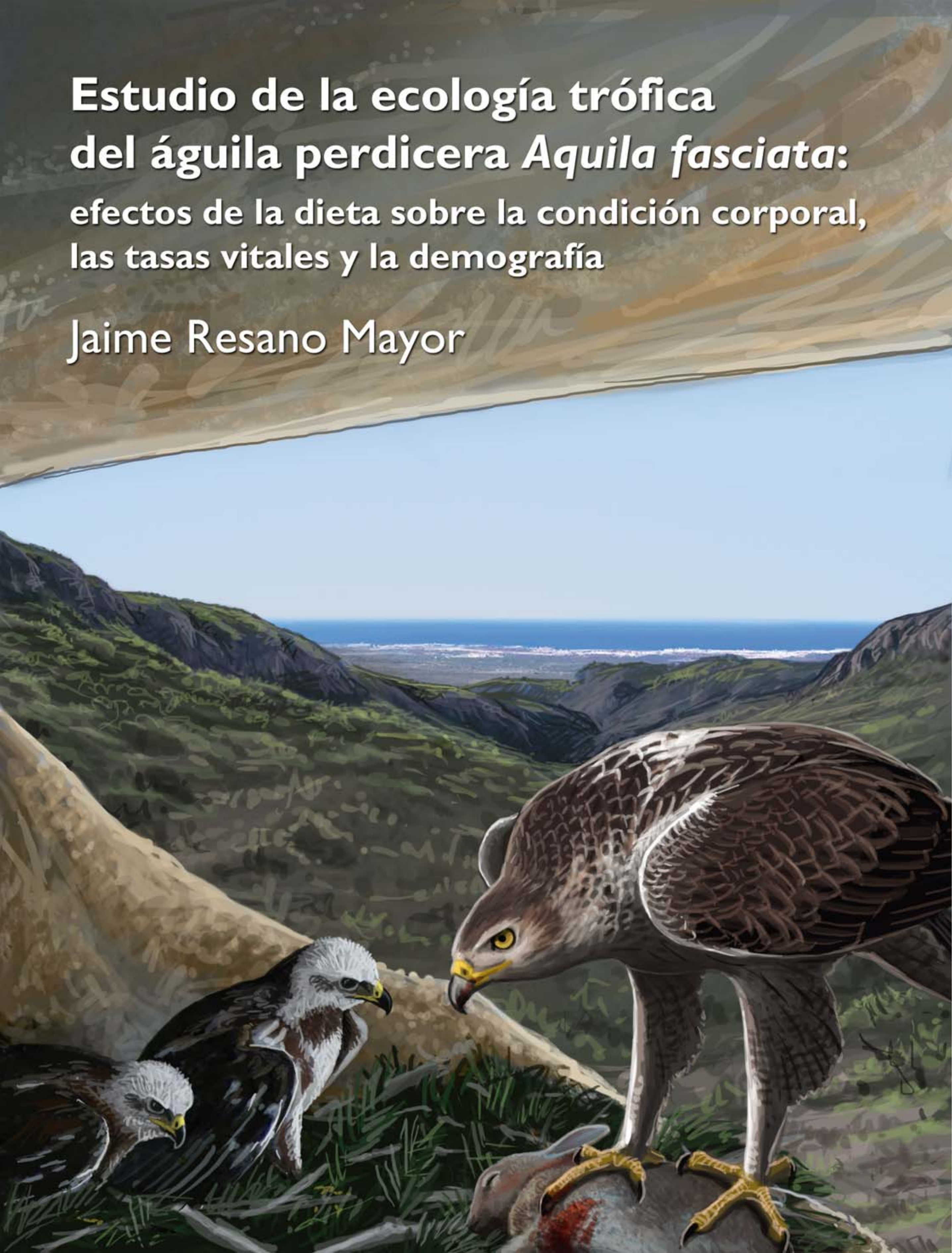

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# Study of the feeding ecology of Bonelli's Eagle *Aquila fasciata*: effects of diet on body condition, vital rates and demography

**Jaime Resano Mayor**

Barcelona, Mayo 2014

**Diseño y maquetación:** Jaime Resano Mayor

**Portada y contraportada:** Martí Franch Rodríguez

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Programa de doctorat en Biodiversitat

Facultat de Biologia - Departament de Biologia animal

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# Study of the feeding ecology of Bonelli's Eagle *Aquila fasciata*: effects of diet on body condition, vital rates and demography

Memoria presentada por
**Jaime Resano Mayor**
para optar al grado de Doctor
por la Universitat de Barcelona

Barcelona, Mayo 2014

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A mis padres, mi hermana y Álex,

por ser el sol que ilumina mis días

Para el desarrollo de esta tesis, el doctorando Jaime Resano Mayor recibió una beca predoctoral concedida por el Departamento de Educación del Gobierno de Navarra, Plan de Formación y de I+D 2008-2009

Gobierno
de Navarra

“A quien AMAS, dale ALAS para VOLAR, RAICES para VOLVER...y MOTIVOS para QUEDARSE!”

(Dalai Lama)

## El mundo

Un hombre del pueblo de Neguá, en la costa de Colombia, pudo subir al alto cielo.

A la vuelta contó. Dijo que había contemplado desde arriba, la vida humana.

Y dijo que somos un mar de fueguitos.

“El mundo es eso”- reveló- “un montón de gente, un mar de fueguitos. Cada persona brilla con luz propia entre todas las demás. No hay dos fuegos iguales. Hay fuegos grandes y fuegos chicos y fuegos de todos los colores. Hay gente de fuego sereno, que ni se entera del viento, y gente de fuego loco que llena el aire de chispas. Algunos fuegos, fuegos bobos, no alumbran ni queman; pero otros arden la vida con tanta pasión que no se puede mirarlos sin parpadear, y quien se acerca se enciende.”

(Eduardo Galeano)
El libro de los abrazos


GIRARD.06

# Contenidos

◆

Capítulos - Chapters

# Agradecimientos

# AGRADECIMIENTOS

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En primer lugar, quiero agradecer a mis padres. Papá, mamá, sólo un hijo sabe el cariño y la dedicación que unos padres pueden dedicarle. Esta tesis va dedicada a vosotros, porque sé que os hace tanta ilusión como a mí. Mil gracias por haberme apoyado en todo desde el primer día, por saberme guiar y transmitir unos valores basados en el esfuerzo y la ilusión, en el luchar por aquello que uno quiere, al tiempo que disfrutar de lo que uno tiene. Desde luego, si de algo me puedo sentir orgulloso, es de teneros a vosotros. Esta tesis también va dedicada a mi hermana y a Álex. Sin vuestro cariño, sonrisas, besos y abrazos estoy seguro de que me hubiera faltado el aire. Qué decir del resto de la familia, abuelita, abuelito, ti@s y prim@s, quienes siempre habéis estado ahí, apoyando en todo, mandándome ánimos allí donde estuviera, preguntándome por mi trabajo sin importar lo "raro" que pueda ser. Desde luego que volver a casa y veros a tod@s me causa siempre una enorme alegría.

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En una tesis como esta, en la que el trabajo de campo ha sido considerable, la ayuda de todo un equipo de personal súper cualificado ha sido importantísima a lo largo de toda un área geográfica que va desde el sur de Francia, pasando por toda Catalunya, Arribes del Duero y Portugal, hasta llegar a Andalucía. Muchas han sido las personas que han ayudado en el campo, y aunque no mencione los nombres de todas, que no os quepa duda de que os estoy enormemente agradecido. En primer lugar, comenzando por Catalunya, quiero agradecer a todos los componentes del Grup de Suport de Muntanya (GSM) del Cos d'Agents Rurals. Habéis hecho un trabajo enorme, ofreciendo apoyo incondicional e imprescindible en la recogida de muestras. Gracias por lo profesionales y buenas personas que me habéis demostrado ser. Asimismo, agradezco la ayuda a todos los miembros del Cos d'Agents Rurals que han acompañado y ayudado en las jornadas de campo. También quiero agradecer a Pere Llopart por su disponibilidad y gran ayuda durante la recogida de muestras de especies cinegéticas, así como a todos los cazadores que me proporcionaron muestras con una enorme ilusión. También a vosotros, Ignasi Toranzo, compañero de PARUS, Joan Feixas, de colombacontrol, y Gemma, de Gallecs, por vuestro tiempo y gran ayuda en la obtención de muestras de palomas en el Vallés. La recogida de presas se pudo complementar gracias al aporte de muestras de los centros de recuperación de fauna de Torreferrussa y Vallcalent, así como a Pep Nogués, a quienes agradezco mucho su ayuda. Con Joan Rodríguez y Eduard Piera compartí jornadas de campo en Catalunya de las que guardo un muy buen recuerdo, gracias cracks. Ya en Francia, llega el momento de agradecer, y mucho, a Alain Ravayrol y Nicolas Vincent-Martin. Gracias por abrirme las puertas de vuestras casas, por permitirme ir a los marcajes y a la recogida de muestras con vosotros, y por el envío de material en años sucesivos. Agradecer también al extenso grupo de naturalistas cuyo trabajo es imprescindible para el éxito del programa de seguimiento de la especie en Francia. Mi más sincero agradecimiento a Patrick Bayle, por su ayuda en el análisis de los restos de dieta recogidos en Francia y el apoyo mostrado en todo momento. Seguimos de viaje, y llega el momento de regresar a la ciudad donde pasé mis años de carrera: Salamanca. Recuerdo que sólo la idea de volver y poder realizar un muestreo para la tesis me llenaba de satisfacción. Una vez allí, el recibimiento y la coordinación de todo el equipo fue algo que no olvidaré. Comenzando por Javier, quien ayudó en la coordinación y desarrollo de todas las jornadas, y desde luego gracias a Víctor García, José Jambas, el resto del equipo de escalada, Olga Alarcia, Fran Bolaños, Ana María Martínez, Vicente López y unas cuantas personas más a quienes también os tengo en mente. Gracias por un año de muestreo inolvidable en Arribes, y queda pendiente el partido de frontenis que no pudo ser por falta de tiempo. En Salamanca ciudad, agradezco muchísimo las facilidades de los profesores Fernando Leal y Manuel Manso, de la Facultad de Biología de la USAL, quienes amablemente me cedieron un espacio en los laboratorios para poder realizar el procesado de muestras. Y ya que me encontraba en el centro de la península, lo mismo me daba subir al norte, que bajar al sur, por lo que acabé en otra de las ciudades universitarias por excelencia: Graná. Allí, el equipo de Andalucía hizo posible un muestreo muy importante para el desarrollo de esta tesis. En primer lugar quiero agradecer a Marcos, por su constante ayuda y apoyo tanto en los trabajos de campo, como en los análisis de dieta, de datos y en la redacción de artículos. Muchas gracias Marcos por hacerme sentir como en casa durante mi estancia, y por todas esas alhambras y tapas que cayeron entre pecho y espalda después de duras jornadas de trabajo. También quiero agradecer al Ninja, por su enorme ayuda en el trabajo de campo y su buen rollo. Asimismo, agradezco a los agentes de medio ambiente que nos acompañaron y ayudaron en las distintas jornadas, a los escaladores que tan buen trabajo realizaron, y a los compañeros que procesaron las muestras en el laboratorio. Finalmente, también me gustaría agradecer a todo el personal de GREFA por haber proporcionado muestras recogidas en sus jornadas de campo.

Dispuestos a viajar, llegó el momento de la estancia de la tesis. Tres meses en el sur de Inglaterra, en Penryn y Falmouth, trabajando junto a un estupendo grupo de investigadores, sirvieron de gran impulso a la tesis. En primer lugar, estoy muy agradecido a Stuart Bearhop, tanto por ofrecerme la oportunidad de trabajar en su grupo, como por todo su tiempo y dedicación en el desarrollo de los trabajos con isótopos. Para mí fue una experiencia inolvidable, comenzando por las capturas de barnaclas con redes de cañón en Strangford Lough, hasta las cenas bañadas de pintas de todos los colores y sabores. Guardo un recuerdo muy especial de todo el grupo: Rich, Tom, Matt, Bea, Mitch, Nadja, Katy y Julian, con quienes forjé una gran amistad y a los que agradezco su ayuda, así como del resto de compañeros en la oficina, quienes aportaron muy buen ambiente, risas, cenas y viajes. También quiero agradecer a Nick, por ofrecerme su casa durante mi estancia, por la buena convivencia y todos los lugares a los que me llevó de visita para que pudiera conocer la belleza de ese rincón de Inglaterra; Thank you so much! Y de vuelta a Barcelona, llegó el tiempo de ponerse a trabajar en nuevos artículos. En esta última y decisiva etapa, el apoyo del resto de coautores ha sido imprescindible para sacar adelante los diferentes trabajos. Muchísimas gracias a Rafa Mateo, Manu Ortiz, Toni Sánchez y Luís Palma por vuestra inestimable ayuda. También me gustaría agradecer a Ginés Viscor por su ayuda en la interpretación de las estimas de condición corporal de los pollos en relación a la dieta. Finalmente, quiero agradecer a Carlos Martirena, del Departamento de Educación del Gobierno de Navarra, y a Eduard Enériz, del Servei de Beques de Tercer Cicle, por su constante ayuda con los trámites administrativos de la beca. Asimismo, agradezco enormemente al Departamento de Educación del Gobierno de Navarra, por haberme concedido una ayuda predoctoral para el desarrollo de esta tesis.

Llega el momento de agradecer a todo un grupo heterogéneo de personas cuyo apoyo ha sido crucial para que este trabajo de tesis haya salido adelante. Comienzo por el tesoro de la carrera, pues si algo hace que Salamanca mereciera la pena es el grupo de amig@s que conservo. Desde primero de carrera fuisteis un ejemplo a seguir, y me satisface ver que seguimos estudiando y formándonos en aquello que nos gusta. Rebe, Laura, Ana, Mónica, Loren y Néstor, creo que de no ser por vosotros, difícilmente estaría escribiendo estas líneas. Espero que sigamos juntándonos allí donde estemos, contándonos con emoción e ilusión el paso del tiempo, a la vez que reviviendo aquellos años inolvidables. También quiero agradecer a Helena, por su amistad desde la carrera y los años que hemos compartido en Barcelona, por sus abrazos, cuentos, ánimos, y por acompañarme piscina tras piscina nadando como una flecha. Claro que todos estos años en Barcelona también dan para conocer a mucha gente, local y foránea. Me gustaría comenzar agradeciendo a los mejores compañeros de piso que he tenido: Cleo, Hedia y Cris. Qué tiempo tan feliz que compartimos, y la de risas que nos echamos con algunas historias paranormales; estoy seguro que de todo ello saldrían unas cuantas tesis. Bromas aparte, muchísimas gracias por formar parte de mi familia en Barcelona, y por todos los ánimos transmitidos que sin duda han servido para hacer realidad esta tesis. Mi otra casa, la Facultad de Biología de la UB. Muchas habéis sido las personas con las que he compartido estos últimos años, comenzando por el máster, hasta llegar a día de hoy. Nombraros a tod@s sería imposible, y correría el riesgo de dejarme a mucha gente, así que quiero que sepáis que han sido unos años increíbles, y que nunca olvidaré este tiempo compartido, toda la ayuda y los ánimos que me habéis dado, así como momentos para el recuerdo que incluyen más de una calçotada en Altafulla (gracias Alba por tu generosidad) y escapadas a mar y montaña. Desde los inicios de la tesis, la ayuda recibida dentro del departamento ha sido constante. En primer lugar, quiero agradecer a Fran y Rocío por el tiempo que dedicaron para enseñarme las técnicas de análisis de isótopos estables y la interpretación de los resultados. Desde el comienzo, vuestro trabajo fue siempre un ejemplo a seguir. También quiero agradecer la enorme ayuda ofrecida por Carola, Javi, Manolo y Raül, cediéndome material y protocolos para los análisis de isótopos, así como a Lluís por su ayuda con la estadística. Hablar de isótopos es agradecer a Pilar Teixidor, Pilar Rubio, Rosa María Marimón, Eva Aracil, Jaume y Joaquim; muchísimas gracias por hacer realidad los análisis, así como por un constante apoyo en los laboratorios. Siguiendo en el laboratorio, pero en este caso para realizar el sexado de los pollos, quiero agradecer la enorme ayuda recibida de Ester Esteban, Pedro Moral, Miquel Angel Arnedo, Enric, Gemma, Vera, Eli, Lucía y Álex. Por otro lado, de un modo, u otro, sois muchos los compañeros del departamento que me habéis ayudado y dado ánimos, y con quienes he compartido todo este tiempo: Oriol, Albert, Alberto, Mario, Owen, Fabi, Mònica, Carme, Te, Vero, Jose, Lauras, Luigi, Cesc, Edu, Irene, Chelina, Mar, Carles, Marcel, Zuzana, Eloy, Débora, Olatz, Marc, Urtxi, Eudald, Aida, Diego, Ainhoa, Jèssica, Mari Carmen, Blanca, Juan, Sergi, Sandra (te incluyo en el departamento)…y mucha más gente que quizá ahora no recuerdo, pero a la que no olvido. También quiero agradecer al estupendo personal de secretaría y administración: María José, Judit, Victòria, Isabel, Raquel y Joan, por toda la ayuda durante estos años. Y llegó el turno de la maquetación del libro, momento de agradecer una y mil veces a Isa. Muchísimas gracias por dedicar tu tiempo a enseñarme las nociones básicas de un programa del que no conocía ni el nombre, y el cual estoy seguro me ha ahorrado más de un suplicio con Word. He descubierto una nueva afición en mí, el diseño, pues he disfrutado dándole forma a las ideas de tesis que me venían a la mente. La culminación del libro ha sido posible gracias a Martí Franch, quien aceptó de muy buena gana realizar la portada y contraportada de la tesis: muchas gracias artista! Ya en el interior del documento, la creatividad de Cyril Girard se hace patente en cada una de las ilustraciones que muy amablemente me cedió para su reproducción. Anna Varea y Kiku Parés contribuyeron con magníficas fotos; muchas gracias a los dos. Concluyendo con el apartado de edición, quiero agradecer a Ana Cárcar Irujo por toda su ayuda en la revisión de textos, mostrándose en todo momento dispuesta a echar un cable.

¿Y qué sería de estos años si no hubiera compartido mi tiempo con personas tan extraordinarias como las que vienen a continuación? Comenzando por el grup PARUS. La de viajes y madrugones que nos hemos pegado en incontables jornadas de anillamiento, capturando muchas especies que las veía por primera vez, enseñándome un montón de rincones de este país, desde las cumbres del pirineo, hasta las orillas del Ebro junto a Els Ports. Si algo caracteriza al grupo pienso que es su calidad humana, y al recordar esta tesis sin duda os recordaré a tod@s vosotr@s. Gracias de corazón por todo el conocimiento y apoyo que me habéis dado, así como por las múltiples experiencias vividas (y las que nos quedan por vivir…). Saltando a los terrenos de juego, llega el momento de agradecer a mi familia futbolera, al equipo con el que he luchado y sudado la camiseta durante los últimos años de la tesis: els ÍCARS. Quiero agradecer a todo el equipo, comenzando por Oriol Canals, quien me presentó, y continuando por cada uno de los que formáis parte de esta gran familia. Ha sido un auténtico placer compartir estos años con vosotros, tanto dentro como fuera del campo. Estoy seguro de que el espíritu dels Ícars está en esta tesis: lucha, esfuerzo e ilusión. También quiero agradecer al grupo de amig@s con los que he compartido aventuras durante todos estos años, en el campo, la montaña, la playa y la ciudad, y cuyo apoyo ha sido vital para seguir trabajando día a día con las mismas ganas del primero. Sois muy grandes Iñaki, Cris, Diego, Emilio, Sergio, Iker, Edgar, Noe, Cesc y Toni. Agradecer también al equipo cigüeñas, Juan Mari y Dani, por hacer realidad un nuevo proyecto en Navarra que gracias a vuestro esfuerzo e ilusión le vamos dando continuidad y va cogiendo forma. Y como se suele decir, los últimos serán los primeros, por lo que llega el momento de agradecer a mi kuadrilla, la Kueva, mis amigos del pueblo, los de siempre, por su constante apoyo y aliento que han resultado imprescindibles durante mis largos periodos de ausencia. Con vosotros comencé todo, y con vosotros finalizo estas líneas.

# Introducción general

# INTRODUCCIÓN GENERAL

La ecología trófica es una subdisciplina de la ecología que estudia los flujos de materia y energía que se producen a través de los organismos que componen un ecosistema (Krebs 2009). Haciendo uso de energía solar, los organismos autótrofos transforman la materia inorgánica en orgánica. Por su parte, los heterótrofos consumen materia orgánica sintetizada por otros organismos, tanto autótrofos como heterótrofos, para obtener su propia energía (Krebs 2009). Todos los animales son organismos heterótrofos, y a través de la dieta obtienen la materia orgánica y energía indispensables para mantener sus procesos vitales. Debido a que los recursos tróficos son un factor limitante en la mayoría de las poblaciones de animales salvajes (White 2008), cada especie animal a lo largo de la evolución ha desarrollado estrategias de forrajeo y alimentación que le permitan maximizar la obtención de energía neta (i.e. teoría del forrajeo óptimo (TFO); Emlen 1966, Futuyma & Moreno 1988, Stephens et al. 2007). La TFO predice que un animal consumirá los recursos tróficos en función de su disponibilidad en el medio y del aporte energético neto obtenido de su búsqueda, manipulación y consumo. Los recursos de más calidad son los que proporcionan mayor energía neta al consumidor (i.e. menor relación coste/beneficio), por lo que son seleccionados preferentemente, mientras que aquellos recursos de menor calidad son incorporados conforme la abundancia de recursos de alta calidad disminuye (Emlen 1966, Pyke et al. 1977, Stephens & Krebs 1986, Futuyma & Moreno 1988). Por lo tanto, la composición de la dieta y la calidad de los recursos tróficos determinan el aporte energético obtenido por el consumidor, y en última instancia pueden afectar a parámetros vitales como el estado de condición corporal, la supervivencia o la reproducción (Stephens et al. 2007, Krebs 2009).

Tradicionalmente, en ecología se asumía que los individuos de una misma población aprovechan los recursos tróficos de modo similar (Pyke et al. 1977, Stephens & Krebs 1986). No obstante, el mayor interés en estudios de dieta a nivel individual durante los últimos años, ha evidenciado que habitualmente existe heterogeneidad entre individuos dentro de una población en cuanto al consumo de recursos (Bolnick et al. 2003, Masello et al. 2013, Catry et al. 2014). Por un lado, dichas diferencias pueden estar determinadas por factores individuales (Bolnick et al. 2003, 2011, Stephens et al. 2007). Por ejemplo, se han observado efectos de la edad, el sexo, las características fisiológicas, o la experiencia y habilidades del consumidor en la búsqueda y obtención de alimento (Jiguet 2002, Bolnick et al. 2003, 2011, Blanco-Fontao et al. 2013). A su vez, la variación de los factores ambientales en el espacio y en el tiempo puede tener un importante efecto sobre el modo en el que los individuos de una población utilizan los recursos tróficos (Bolnick et al. 2003). Por ejemplo, debido a variaciones locales en la abundancia de alimento o a consecuencia de la competencia intraespecífica e interespecífica por su consumo (Bolnick et al. 2003, Araújo et al. 2011, Laskowski & Bell 2013, L'Hérault et al. 2013). Sin embargo, todavía es incipiente el conocimiento acerca de cómo la variación individual en la dieta a nivel poblacional condiciona los parámetros vitales de los individuos y, en última instancia, la dinámica de sus poblaciones.

## Efectos de la dieta sobre los parámetros vitales a nivel individual y poblacional

Un tema de especial interés en estudios de ecología trófica ha sido si los individuos especialistas (i.e. baja diversidad de recursos tróficos en la dieta) difieren en sus parámetros vitales en comparación con los generalistas (i.e. alta diversidad de recursos tróficos) (ver Lefcheck et al. 2013). Por ejemplo, en algunas especies de aves depredadoras, una menor diversidad trófica como consecuencia de un abundante consumo de presas preferidas estuvo correlacionada con una mayor supervivencia y un mayor éxito reproductor (Korpimäki & Norrdahl 1991, Golet et al. 2000, Arroyo & García 2006). Sin embargo, estudios en otros depredadores no mostraron relación entre la diversidad trófica y los parámetros vitales de los individuos (e.g. Woo et al. 2008, Whitfield et al. 2009). Por lo tanto, las evidencias indican que diferentes estrategias de forrajeo y consumo de recursos tróficos pueden resultar más o menos ventajosas según las especies y/o los escenarios ecológicos en los que cada individuo desarrolla sus actividades vitales (MacArthur & Pianka 1966, Whitfield et al. 2009, L'Hérault et al. 2013).

La dieta también es un factor determinante del estado de condición corporal y de salud de los individuos (Brown 1996, Stevenson & Woods 2006, Labocha & Hayes 2012). Muchos han sido los métodos propuestos para estimar la condición corporal animal, entre los que destacan medidas basadas en parámetros biométricos, bioquímicos y fisiológicos (Brown 1996, Stevenson & Woods 2006, Monaghan et al. 2009, Labocha & Hayes 2012). Durante los últimos años, el análisis del metabolismo antioxidante ha recibido especial atención en estudios ecofisiológicos y, a día de hoy, el uso de biomarcadores con capacidad antioxidante, junto con medidas de daño o estrés oxidativo, ofrecen un gran potencial para evaluar la condición corporal de los individuos (Monaghan et al. 2009, Costantini et al. 2010) (ver Cuadro 1). Sin embargo, la relación entre la composición de la dieta y el estado de condición corporal de los animales en estado salvaje habitualmente resulta difícil de valorar, debido a las limitaciones a la hora de estimar la dieta a nivel individual, así como por la multitud de factores adicionales que pueden afectar a la condición corporal (Hochleithner 1994). Hasta la fecha, la mayoría de estudios que han analizado la relación entre dieta y condición se basan en pocos estimadores de condición, algo que posiblemente ha limitado la detección de los efectos de la dieta sobre la condición. Por consiguiente, para avanzar en el conocimiento sobre la relación entre la composición de la dieta y el estado de condición corporal de los individuos resulta primordial i) desarrollar nuevas metodologías que faciliten las estimas de dieta a nivel individual, y ii) integrar múltiples estimadores de condición, considerando a su vez el efecto de factores distintos a la dieta, tales como la edad, el sexo o los ritmos circadianos.

A nivel poblacional, se ha observado en diversos depredadores que las poblaciones con una menor diversidad trófica tienen mayor éxito reproductor asociado a un incremento en el consumo global de presas preferidas (Elmhagen et al. 2000, Arroyo & Garcia 2006, Watson 2010). Sin embargo, en poblaciones animales expuestas a paisajes heterogéneos o condiciones ambientales cambiantes (e.g. disponibilidad de presas), una mayor plasticidad trófica individual puede ser una estrategia ecológica más ventajosa, por ejemplo mediante la diversificación en el uso que los individuos hacen de los recursos tróficos disponibles y limitantes a nivel de la población (Bolnick et al. 2003). En este sentido, recientes investigaciones ponen de manifiesto la importancia de considerar las variaciones en la dieta entre los individuos dentro de una población, dado que pueden afectar a los parámetros vitales de los individuos y, en última instancia, modular la dinámica poblacional y contribuir en procesos evolutivos (ver Araújo et al. 2011, Bolnick et al. 2011, Schreiber et al. 2011, Tinker et al. 2012). Sin embargo, a pesar de su importancia a nivel teórico y aplicado, la correspondencia entre el uso individual de los recursos tróficos y la dinámica de las poblaciones animales sigue siendo desconocida en la mayoría de los sistemas naturales, por lo que se requieren estudios que la investiguen, considerando diversas escalas espacio-temporales.

## Estudios de ecología trófica en biología de la conservación y sus aplicaciones en bioindicación

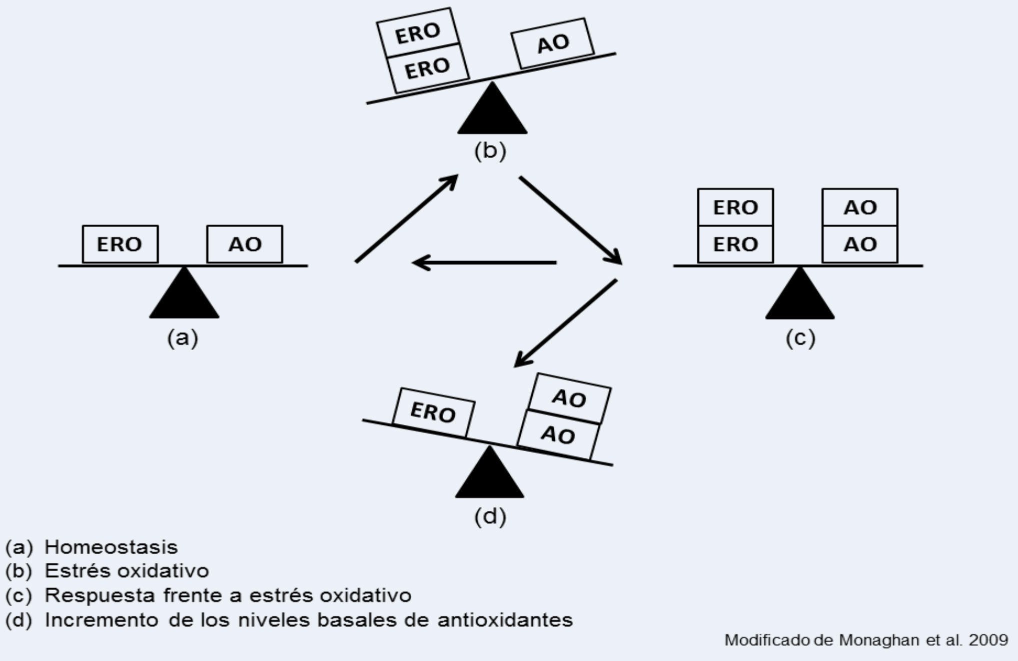
El estudio de la ecología trófica de las especies aporta información de gran relevancia en la investigación y la puesta en práctica de acciones dentro de la disciplina de la biología de la conservación. En este sentido, la dieta puede informar sobre la presencia de amenazas para las especies y sus poblaciones, por ejemplo, indicando escasez de alimento, un aporte de contaminantes y parásitos, o estando relacionada indirectamente con las tasas de mortalidad debido a los procesos de búsqueda y consumo de alimento (Daszak et al. 2000, Real et al. 2000, Becker 2003, Jørgensen & Holt 2013). Por ello, conocer los efectos de la dieta sobre los parámetros vitales en especies amenazadas resulta fundamental a la hora de planificar y ejecutar medidas de conservación, como por ejemplo acciones orientadas a la mejora de los recursos tróficos que pueden incrementar las tasas vitales. Por otro lado, el monitoreo de la composición de la dieta no solamente puede informar de los cambios en la abundancia y disponibilidad de los recursos tróficos que explotan, sino que a una escala más amplia pueden informar sobre cambios en los hábitats y ecosistemas (Becker 2003). Un claro ejemplo lo constituyen numerosas especies de depredadores, que al situarse en los niveles superiores de la red trófica, su dieta es indicativa de la estructura, composición y alteraciones en la comunidad (Furness & Camphuysen 1997, Sergio et al. 2008). Por ejemplo, el estudio de la dieta de aves marinas ha evidenciado un declive de las poblaciones de sus principales especies presa, en parte como consecuencia de la contaminación de los ecosistemas marinos y una sobreexplotación pesquera (Furness & Camphuysen 1997, Lotze & Milewski 2004, Moreno et al. 2013). Asimismo, la monitorización de la dieta de depredadores marinos ha sido útil para hacer un seguimiento, a largo plazo, de los impactos provocados por vertidos de petróleo sobre la estructura de redes tróficas marinas, y su posterior recuperación (Moreno et al. 2013). En el caso de los ecosistemas terrestres, la abundancia y predictibilidad de recursos tróficos derivados de las actividades humanas (e.g. vertederos, facilitación de carroña) ha condicionado la dieta de depredadores oportunistas y/o carroñeros facultativos, provocando cambios en sus hábitos de alimentación y favoreciendo, en muchos casos, el incremento de sus poblaciones (ver Oro et al. 2013, Moleón et al. 2014). A su vez, la dieta de depredadores terrestres puede ser indicativa de cambios en las estrategias de gestión de los recursos tróficos en un área determinada, siendo útil a la hora de valorar la efectividad de acciones de gestión y conservación (ver Hopkins et al. 2014). Por consiguiente, el estudio y la monitorización de la dieta de los depredadores ofrecen un gran potencial en bioindicación, si bien la falta de información referente a la ecología trófica de las especies y su relación con los parámetros vitales de los individuos puede limitar su utilidad y dificultar su interpretación.

## Aplicaciones del análisis de isótopos estables en estudios de ecología trófica

Durante las últimas dos décadas, el desarrollo

**Cuadro 1. Antioxidantes y estrés oxidativo**

Los organismos aeróbicos generan especies químicas reactivas de oxígeno (ERO) como subproducto de sus procesos metabólicos (Balaban et al. 2005). Debido a la elevada reactividad de las ERO, éstas pueden dañar biomoléculas clave como lípidos, proteínas o ADN, pudiendo comprometer la viabilidad celular y, por consiguiente, el correcto mantenimiento de las funciones biológicas (Halliwell & Gutteridge 2007). El estrés oxidativo ocurre cuando la producción de ERO supera a la capacidad del organismo para mitigar sus efectos nocivos a través de las defensas antioxidantes (AO) (Finkel & Holbrook 2000, Monaghan et al. 2009). Las defensas AO incluyen sustancias ingeridas en la dieta (e.g. vitaminas, carotenos), así como moléculas de producción endógena (e.g. glutatión, ácido úrico, enzimas como la glutatión peroxidasa o la superóxido dismutasa). Para una evaluación más precisa del metabolismo antioxidante es necesario integrar la medición de las defensas AO junto con medidas de daño oxidativo, ya que los niveles de antioxidantes pueden regularse en función de la exposición a ERO (ver Fig. 1).



Modificado de Monaghan et al. 2009

**Figura 1.** Ilustración simplificada y esquemática de las relaciones entre especies químicas reactivas de oxígeno (ERO), sistemas de defensa antioxidante (AO), y estrés oxidativo. (a) En estado de homeostasis, tanto los niveles de ERO como de AO son bajos y se hallan en equilibrio sin que se produzca estrés oxidativo. (b) Un aumento en la producción de ERO puede superar la capacidad de AO, lo que lleva a un periodo de estrés oxidativo. (c) Si el aumento de ERO es comedido, se producirá un despliegue de AO para impedir que el estrés oxidativo continúe, volviendo así a una situación de equilibrio. Si la producción de ERO continúa, (d) puede inducir al organismo a aumentar permanentemente sus niveles de AO (e.g. movilización desde tejidos, aumento de la expresión génica) (texto y figura modificados de Monaghan et al. 2009).

tecnológico y novedosos avances en investigación han convertido el análisis de isótopos estables (AIE) en una herramienta muy útil, y cada vez más utilizada en estudios de ecología trófica animal (Inger & Bearhop 2008, Hobson 2011, Ramos & González-Solís 2012). Su aplicabilidad se debe a que los isótopos estables de elementos comunes en la biosfera, como el carbono (C), el nitrógeno (N) o el azufre (S), actúan en los tejidos del consumidor como marcadores biogeoquímicos intrínsecos útiles a la hora de inferir sus hábitos tróficos (ver Cuadro 2). De este modo, el AIE permite mediante un muestreo puntual obtener información de la dieta asimilada durante un periodo de tiempo concreto, que viene determinado por el metabolismo del tejido muestreado a tal efecto (Hobson & Clark 1992, Inger & Bearhop 2008). Asimismo, y a diferencia de la mayoría de técnicas convencionales para el estudio de la dieta animal, el AIE no requiere un gran esfuerzo de análisis y evita sesgos relacionados con el grado de digestibilidad de cada recurso trófico (ver Real 1996, Votier et al. 2003, Marti et al. 2007, Inger & Bearhop 2008).

Una de las principales aplicaciones del AIE en estudios de ecología trófica ha sido la reconstrucción de la dieta de multitud de especies, tanto a nivel individual como poblacional (Hobson & Clark 1992, Inger & Bearhop 2008, Hobson 2011). En este sentido, el desarrollo reciente de modelos bayesianos mixtos (*Bayesian mixing models*) ha significado un gran avance, dado que permiten estimar el consumo de múltiples fuentes de alimento, a la vez que incorporan la variación e incertidumbre asociadas a los parámetros del modelo (Inger & Bearhop 2008, Moore & Semmens 2008, Parnell et al. 2010). Por lo tanto, su uso requiere obtener estimas razonables de la composición isotópica y variabilidad de las principales fuentes de alimento (e.g. presas), así como del cambio esperado en la proporción isotópica entre la dieta y los tejidos del consumidor (i.e. factor de enriquecimiento trófico). Otra de las aplicaciones del AIE en estudios de ecología trófica se debe a su utilidad como aproximación al nicho trófico poblacional (Bearhop et al. 2004, Matthews & Mazumder 2004, Newsome et al. 2007). Esto consiste en mostrar en un espacio bidimensional los valores isotópicos (habitualmente C *vs*. N) de los individuos de una población, así como los de sus principales recursos tróficos, obteniendo el denominado "nicho isotópico" (Newsome et al. 2007). De este modo, el nicho isotópico puede informarnos de la amplitud del nicho trófico en una población, del grado de solapamiento entre individuos y poblaciones, así como de posibles cambios espacio-temporales en el nicho trófico de las poblaciones objeto de estudio (Bearhop et al. 2004, Jackson et al. 2011, Jackson et al. 2012, Hopkins et al. 2014). De modo complementario, y haciendo uso de los mencionados modelos bayesianos mixtos, es posible estimar las proporciones de consumo de los principales recursos tróficos a nivel individual con el objetivo de calcular, por ejemplo, la diversidad trófica (e.g. índice de Shannon-Weaver; Shannon & Weaver 1997) de cada individuo, o su grado de especialización trófica en el contexto de la población (ver Bolnick et al. 2002, Newsome et al. 2007).

A pesar del gran potencial del AIE, su aplicación en estudios tróficos de depredadores terrestres ha sido mucho menor en comparación a depredadores marinos (ver Bearhop et al. 2001, Newsome et al. 2009, Ramos & González-Solís 2012). Por ejemplo, pocos han sido los trabajos que abordan la dieta de aves rapaces terrestres mediante AIE (ver Roemer et al. 2002, Caut et al. 2006, Eulaers et al. 2014). Sin embargo, si consideramos el esfuerzo y los posibles sesgos asociados a las técnicas de análisis convencional de dieta en rapaces (Real 1996, Marti et al. 2007), el desarrollo de nuevas aproximaciones como el AIE para estudiar su ecología trófica supone un

**Cuadro 2. Análisis de isótopos estables en estudios de ecología trófica**

Los isótopos son átomos de un mismo elemento que mientras tienen igual número de protones en su núcleo (nº atómico), difieren en cuanto al número de neutrones, considerándose estables si no se desintegran con el paso del tiempo (a diferencia de los radioactivos). Debido a que presentan la misma carga, los distintos isótopos de un mismo elemento intervienen de modo muy similar en las reacciones fisicoquímicas. Sin embargo, las diferencias entre isótopos en cuanto al número másico (suma del nº de protones y neutrones) hacen que haya isótopos "pesados" (mayor nº neutrones) y "ligeros" (menor nº neutrones), lo cual condiciona sus propiedades termodinámicas, creando pequeñas diferencias en la cinética de las reacciones bioquímicas en las que intervienen. Como consecuencia, la proporción isotópica en los tejidos del consumidor varía respecto a la de su dieta, y lo hace de un modo predecible, ocasionando lo que se denomina "factor de enriquecimiento trófico". Por lo tanto, el hecho de que la composición isotópica de la dieta quede reflejada en los tejidos del consumidor de un modo predecible es uno de los fundamentos de la aplicación del análisis de isótopos estables en estudios de ecología trófica (ver Fry 2006).

El carbono (C), el nitrógeno (N) y el azufre (S) son los elementos químicos de mayor utilidad en estudios de ecología trófica (Peterson & Fry 1987). Los dos isótopos del carbono, $^{12}C$ (98.90 %) y $^{13}C$ (1.10 %), están presentes en proteínas, grasas e hidratos de carbono, por lo que su proporción ($^{13}C/^{12}C$) en un consumidor puede informarnos de sus diferentes fuentes de alimento (Kelly 2000). El nitrógeno también posee dos isótopos, $^{14}N$ (99.63 %) y $^{15}N$ (0.37 %), presentes básicamente en las proteínas e informándonos del metabolismo proteico de la dieta. La proporción isotópica del nitrógeno ($^{15}N/^{14}N$) aumenta a lo largo de la cadena trófica como consecuencia de una menor intervención del $^{15}N$ en las reacciones bioquímicas, por lo que su análisis permite determinar el nivel trófico del consumidor (Kelly 2000). Los isótopos del azufre son cuatro: $^{32}S$ (95.02 %), $^{33}S$ (0.75 %), $^{34}S$ (4.21 %) y $^{36}S$ (0.02 %); estando presentes en algunos aminoácidos e informándonos del metabolismo proteico. Las marcadas diferencias en la proporción de ($^{34}S/^{32}S$) entre el medio marino y terrestre hacen que el análisis de azufre sea especialmente útil para discriminar entre fuentes de alimentación que puedan proceder del medio marino o terrestre (Peterson et al. 1985).

La proporción isotópica de una muestra se expresa en comparación a la de un estándar según la notación δ de la siguiente fórmula:

$$\delta X = [(R_{muestra} / R_{estándar}) - 1] * 1000\ (‰)$$

donde X es el isótopo pesado del elemento analizado (e.g. $^{13}C$, $^{15}N$, $^{34}S$), y R es la abundancia relativa del isótopo pesado respecto al ligero ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$), tanto en la muestra ($R_{muestra}$) como en el estándar ($R_{estándar}$). Los estándares establecidos a nivel internacional son PDB "Pee Dee Belemnite" para $\delta^{13}C$, nitrógeno atmosférico para $\delta^{15}N$ y CDT "Canyon Diablo Troilite" para $\delta^{34}S$. El resultado se ofrece en tanto por mil (‰) para facilitar la interpretación de las pequeñas diferencias que habitualmente existen entre la muestra y el estándar, y el signo positivo o negativo indicará, respectivamente, que la muestra posee mayor o menor proporción del isótopo pesado en relación al estándar.

avance analítico y metodológico. Por ejemplo, a diferencia de las técnicas convencionales, que informan sobre las presas consumidas, el AIE permite estimar la dieta asimilada. Otra de las ventajas que ofrece el AIE es la posibilidad de determinar la dieta a nivel individual, algo que en especies de interés para la conservación, como es el caso de numerosas aves rapaces, tiene una especial importancia. Asimismo, teniendo en cuenta que las aves rapaces se sitúan en los eslabones más altos de las redes tróficas en la mayoría de ecosistemas terrestres del planeta, el uso de biomarcadores, como los isótopos estables, ofrece una herramienta útil y práctica en bioindicación para monitorizar cambios ambientales en dichos ecosistemas, a través de estudios de dieta en depredadores.

## El águila perdicera como depredador en ecosistemas mediterráneos: demografía y ecología trófica en Europa Occidental

El águila perdicera (*Aquila fasciata*) es un ave rapaz diurna que se distribuye irregularmente por la región circummediterránea (sur de Europa, norte de África y Oriente Próximo) en su extremo más occidental, extendiéndose hacia el este por Oriente Medio, hasta alcanzar el subcontinente indio y el sureste asiático en su extremo más oriental (Cramp & Simmons 1980). Se reconocen dos subespecies, la nominal *A.f. fasciata*, cuya distribución ocupa desde Europa y norte de África hasta el sur de China e Indochina (Del Hoyo et al. 1994), y *A.f. renschi*, la cual ocupa las islas Menores de la Sonda (Trainor et al. 2013). Las poblaciones mediterráneas de águila perdicera tienen sus mayores efectivos en Europa Occidental (Península Ibérica y sureste de Francia) y los países del Magreb (Del Hoyo et al. 1994). En el último censo realizado en 2005, el número de parejas reproductoras en España se estimó en 733-768 (Del Moral 2006), constituyendo aproximadamente el 80% de la población reproductora en Europa. Debido a un importante declive poblacional en los años 80s y 90s, especialmente en la Península Ibérica (Tucker & Heath 1994, Real & Mañosa 1997, Real 2004, Del Moral 2006), la población de águila perdicera está catalogada como "En Peligro" tanto en España (Real 2004), como en Europa (BirdLife International 2004). A nivel legislativo, figura como "Vulnerable" en el "Catálogo Español de Especies Amenazadas" (Ley 42/2007 de 13 de Diciembre, Real Decreto 139/2011), y tanto a nivel estatal, como europeo (Directiva Europea 79/409/EEC), se considera especie prioritaria que deberá estar sujeta a medidas especiales para su conservación que aseguren su supervivencia y reproducción.

La regresión del águila perdicera en Europa se atribuye a tres tipos de factores no excluyentes: i) demográficos, ii) alteración del hábitat y iii) competencia con otras especies. Diversas poblaciones de águila perdicera han mostrado un marcado desequilibrio demográfico debido, sobre todo, a un aumento de la mortalidad, especialmente en ejemplares territoriales y por causas de origen antrópico (e.g. electrocución y colisión con tendidos eléctricos, persecución a tiros) (Real et al. 2001, Real 2004, Rollan et al. 2010, Hernández-Matías et al. 2013). A su vez, en algunas poblaciones se ha observado una disminución de sus tasas reproductoras (Fernández et al. 1998, Hernández-Matías et al. 2013), posiblemente a raíz de molestias o perturbaciones en las áreas de nidificación, o debido a una menor abundancia de presas. Por ejemplo, el incremento de las actividades de ocio al aire libre (e.g. escalada, senderismo, motorismo) conlleva una intensa presión antrópica en las zonas de montaña donde crían las águilas, pudiendo provocar un fracaso reproductor de forma continuada o, incluso, el abandono de las áreas de cría. A ello se suma una gestión cinegética inadecuada en muchas áreas, que convierten la caza en una actividad poco sostenible con las poblaciones silvestres de presas, reduciendo la abundancia y disponibilidad de éstas para los depredadores. En cuanto a la alteración del hábitat, por un lado, se han producido cambios irreversibles en sus áreas de nidificación y campeo (e.g. urbanizaciones, infraestructuras, canteras), provocando en algunos casos el abandono de territorios. Por otro, el abandono rural de las áreas de montaña mediterránea y sus actividades (e.g. agricultura de secano, pastoreo, explotación forestal) ha supuesto la pérdida y transformación de espacios abiertos que favorecían la presencia de presas clave para las águilas, como el conejo de monte o la perdiz roja (*Alectoris rufa*), por otros más forestales y desfavorables para sus presas. Al mismo tiempo, el desarrollo de la agricultura intensiva en las áreas de llanura (e.g. concentraciones parcelarias, uso de pesticidas, cultivos intensivos) también ha ocasionado la disminución de sus principales presas y, a su vez, la instalación de infraestructuras peligrosas para las águilas (e.g. tendidos eléctricos, balsas). Finalmente, la interacción con especies competidoras como el buitre leonado (*Gyps fulvus*), el águila real (*Aquila chrysaetos*) o el halcón peregrino (*Falco peregrinus*) ha aumentado marcadamente en algunas áreas (Gil-Sánchez et al. 2004, Real 2004, Martínez et al. 2008), lo que posiblemente ha conllevando un mayor grado de competencia interespecífica con el águila perdicera, tanto por las áreas de nidificación, como por los recursos tróficos. Todos estos factores son un buen reflejo de los importantes cambios ambientales ocurridos en los ecosistemas mediterráneos durante las últimas décadas, y han sido argüidos como negativos para la viabilidad de las poblaciones de águila perdicera en Europa Occidental (Ontiveros et al. 2004, Hernández-Matías et al. 2011, 2013).

El águila perdicera es un predador que captura presas de mediano tamaño, especialmente mamíferos y aves, y más escasamente reptiles. En Europa Occidental, sus principales presas son el conejo de monte, la perdiz roja y las palomas (*Columba* spp.). Otras presas frecuentes en su dieta son la ardilla roja (*Sciurus vulgaris*), diversos Passeriformes (especialmente Córvidos), la gaviota patiamarilla (*Larus michahellis*), Ardéidas, Anátidas y el lagarto ocelado (*Timon lepidus*). Durante las últimas tres décadas, la dieta del águila perdicera en el periodo reproductor ha sido ampliamente estudiada en sus poblaciones de Europa Occidental mediante técnicas convencionales como el análisis de egagrópilas (Real 1987, 1991, 1996, Martínez et al. 1994, Gil-Sánchez et al. 2000, 2004, Palma et al. 2006, Moleón et al. 2009a, 2012a, b). La información disponible muestra que tanto el conejo, como la perdiz, son presas preferidas por la especie, puesto que un mayor consumo se ha relacionado con una baja diversidad trófica (Real 1991, Moleón et al. 2009a, 2012a). Además, algunos estudios sugieren que el mayor consumo de estas presas aumenta las tasas reproductoras de las águilas (ver Real 1987, 1991). En las últimas décadas, se ha observado una disminución del consumo de conejo y perdiz en las poblaciones de águila perdicera, especialmente en las regiones septentrionales de su distribución (Real 1991, Moleón et al. 2009a). La causa atribuida ha sido una reducción de las poblaciones de estas presas debido a los mencionados cambios en el hábitat, la aparición de enfermedades infecciosas emergentes, como la neumonía hemorrágica vírica del conejo, y una inadecuada gestión cinegética (Real 1991, Moleón et al. 2008, 2009a, 2012b). Como consecuencia, en la actualidad existe una gran variación en las abundancias de conejo en los distintos territorios de águila perdiera, incluso dentro de una misma población local, un hecho que condiciona su consumo por parte de las águilas (Moleón et al. 2009a, 2012b). Por otro lado, en los últimos años, y a raíz de los cambios ambientales asociados a la acción del hombre, especies antiguamente ausentes o muy escasas (e.g. estorninos (*Sturnus* spp.), gaviota patiamarilla) han colonizado nuevas áreas, aumentado en gran medida sus poblaciones en algunos territorios de águila perdicera. En conjunto, esta nueva situación ha provocado cambios en la dieta de las águilas, cuyas consecuencias a nivel de conservación de la especie no han sido estudiadas. En este sentido, el estudio de la ecología trófica del águila perdicera y de la relación entre la composición de la dieta y sus principales parámetros vitales, no solo ayudaría a una mejor comprensión de las consecuencias que el cambio global en los ambientes mediterráneos ocasiona en la dieta de la especie, sino también a poder predecir los efectos de dichos cambios sobre las tasas vitales de los individuos y la dinámica de sus poblaciones. A su vez, la información obtenida sería muy útil de cara a emprender medidas de conservación dirigidas a mejorar la disponibilidad de alimento, y en particular de sus principales especies presas, siendo éste uno de los problemas argüidos en la conservación de sus poblaciones.

En esta tesis, se analiza en profundidad la ecología trófica del águila perdicera durante el periodo de cría, poniendo énfasis en el estudio de la dieta mediante nuevas metodologías, y en determinar los efectos de la composición de la dieta sobre: i) la condición corporal de los pollos, ii) las tasas vitales de los individuos territoriales y iii) la dinámica de las poblaciones. En primer lugar, para el estudio de la dieta se desarrollan técnicas novedosas basadas en el uso de biomarcadores como el AIE, pudiendo así estimar la dieta a nivel individual, territorial y poblacional. A tal efecto, se realiza una comparación entre las estimas de dieta obtenidas mediante métodos convencionales como el análisis de egagrópilas y las proporcionadas por el AIE. Una vez determinada la dieta, se estudian los efectos de su composición sobre la condición corporal de los pollos, para lo cual se hace uso de novedosos biomarcadores indicativos del metabolismo antioxidante, además de estimas convencionales basadas en índices biométricos y análisis de bioquímica plasmática. Asimismo, se analiza la relación entre la composición de la dieta y los valores de productividad, supervivencia y tasa de crecimiento, tanto a nivel territorial como poblacional. En última instancia, la información obtenida se utiliza para aportar directrices orientadas al monitoreo y a la conservación del águila perdicera en el área de estudio.

# Águila azor perdicera

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## *Clasificación*

**Reino:** *Animalia*

**Filo:** *Chordata*

**Clase:** *Aves*

**Orden:** *Accipitriformes*

**Familia:** *Accipitridae*

**Género:** *Aquila*

**Especie:** *A. fasciata* (Vieillot, 1822)

**Subespecies:** *A.f.fasciata* / *A.f.renschi*

## *Características morfológicas*

**Longitud:** 65-72 cm

**Envergadura alar:** 150-180 cm

**Peso:** 1600-2500 g

**Dimorfismo sexual:** hembra aprox. 10 % mayor que el macho

**Plumaje:** 4 plumajes diferenciados; primer año de edad (juvenil), segundo año (inmaduro), tercer año (subadulto), y cuarto año o mayor (adulto)

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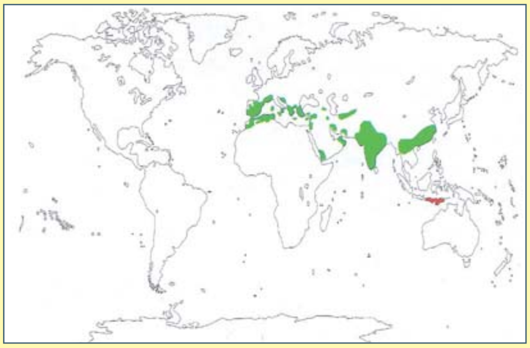
Pollo de águila perdicera en su fase de desarrollo en el nido

© Anna Varea

Juvenil de águila perdicera

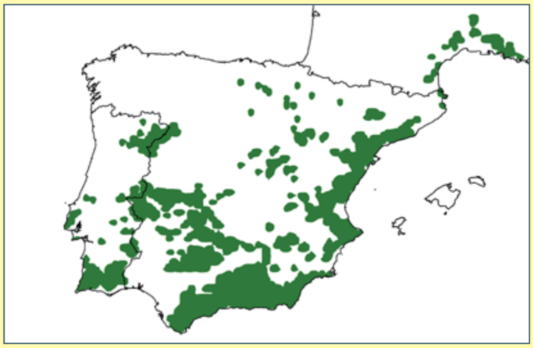
## *Distribución*

**Mundial**



Mapa de la distribución mundial del águila perdicera. En verde se muestra la distribución de la subespecie nominal *A.f.fasciata*, y en rojo el área de distribución de *A.f.renschi*

**Europa Occidental**



Mapa de la distribución de la población reproductora de águila perdicera en Europa Occidental. La distribución se extiende desde el sureste de Francia, hasta la mitad sur de la Península Ibérica, donde se encuentra el mayor núcleo poblacional.

## *Hábitat, reproducción y ciclo vital*

En Europa Occidental, los ejemplares territoriales viven en sierras mediterráneas que van desde el nivel del mar, hasta unos 1500 m.s.n.m. (Gil-Sánchez et al. 1996). Los territorios ocupan amplias áreas (mediana del kernel 95%: 36km$^2$; ver Bosch et al. 2010), especialmente cubiertas de matorrales y bosques, alternando cultivos de secano tradicionales (Real 2004). Las áreas de cría se sitúan habitualmente en cortados rocosos, aunque algunas parejas crían en árboles como alcornoques (*Quercus suber*), pinos (*Pinus* sp.) y eucaliptos (*Eucalyptus* sp.); especialmente en el sur y oeste peninsular, y muy ocasionalmente en torretas de tendidos eléctricos de transporte (Real 2004).

El águila perdicera es una especie que puede llegar a vivir hasta 32 años en libertad (Larrey et al. 2007). Su reclutamiento como territorial sucede mayoritariamente entre los 2 y los 4 años de edad (Hernández-Matías et al. 2010). Durante finales de otoño y principios de invierno, la pareja de águilas territoriales comenzará a prepararse para la cría mediante el aporte de material a los nidos, disponiendo habitualmente de varios cuyo uso para la cría puede alternar según años. En este periodo, la pareja realiza vuelos nupciales y se producen las cópulas. La puesta se da entre finales de enero y marzo, y se compone habitualmente de 1-2 huevos, raramente 3 (Real 2004). La incubación es llevada a cabo sobre todo por la hembra, mientras que el macho se encarga fundamentalmente del aporte de alimento y la vigilancia del área de cría, incubando cortos periodos de tiempo. Tras un periodo de incubación de unos 40 días, nacen los pollos, permaneciendo en el nido un promedio de 63 días (Real et al. 1998). Una vez salen del nido, estarán varias semanas por el territorio de cría aprendiendo a volar junto a sus progenitores, quienes continúan aportándoles presas durante un tiempo. Entre el segundo y tercer mes después de salir del nido, los jóvenes se independizarán emprendiendo la etapa de dispersión juvenil (Real et al. 1998, Real & Mañosa 2001, Cadahía 2007). Durante el periodo de dispersión pueden realizar movimientos de larga distancia (hasta cientos de kilómetros), asentándose temporalmente en las denominadas áreas de dispersión, que se caracterizan por poseer una elevada abundancia de presas y por la ausencia de ejemplares territoriales (Real et al. 1998, Real & Mañosa 2001, Moleón et al. 2009b). Finalmente, tras el periodo de dispersión juvenil, que puede durar varios años, los ejemplares acabarán reclutándose en un territorio para emparejarse, y posteriormente reproducirse, formando fuertes vínculos con la pareja y mostrando gran tenacidad en la defensa del territorio (ver Hernández-Matías et al. 2010, 2013).

### *Dinámica poblacional en Europa Occidental*

Las poblaciones de águila perdicera en Europa Occidental muestran considerables diferencias en cuanto a los principales parámetros vitales como son la productividad anual (i.e. varía entre 0.60 pollos/pareja en la población de Arribes y 1.42 en la población de Granada), o la supervivencia (i.e. supervivencia adulta que varía entre 0.87 en la población de Castellón y 0.94 en la de Cádiz; ver Hernández-Matías et al. 2013). En este sentido, existe un marcado gradiente norte-sur, siendo las poblaciones del norte de la Península Ibérica y sur de Francia las que muestran los peores valores de productividad y supervivencia en comparación con las poblaciones del sur peninsular (Real & Mañosa 1997, Fernández et al. 1998, Gil-Sánchez et al. 2004, Hernández-Matías et al. 2013). De hecho, en base a la información demográfica que se posee actualmente, la mayoría de las poblaciones norteñas no son autosostenibles, por lo que probablemente se mantienen gracias a la inmigración de ejemplares de poblaciones con mejor estado demográfico. Por consiguiente, se puede afirmar que las poblaciones locales en Europa Occidental pertenecen a una sola población espacialmente estructurada que actualmente funciona como un sistema de fuente-sumidero, donde las poblaciones en el sur de la Península Ibérica actúan como fuentes y, gracias a la dispersión, mantienen otras poblaciones locales, especialmente a las del norte peninsular (Hernández-Matías et al. 2013).

## Referencias

**Araújo, M.S., Bolnick, D.I. & Layman, C.A.** (2011) The ecological causes of individual specialisation. Ecology Letters, 14, 948-958.

**Arroyo, B.E. & Garcia, J.T.** (2006) Diet composition influences annual breeding success of Montagu's Harriers *Circus pygargus* feeding on diverse prey. Bird Study, 53, 73-78.

**Balaban, R.S., Nemoto, S. & Finkel, T.** (2005) Mitochondria, oxidants, and aging. Cell, 120, 483-495.

**Bearhop, S., Thompson, D.R., Phillips, R.A., Waldron, S., Hamer, K.C., Gray, C.M., Votier, S.C., Ross, B.P. & Furness, R.W.** (2001) Annual variation in great skua diets: the importance of commercial fisheries and predation on seabirds revealed by combining dietary analyses. Condor, 103, 802-809.

**Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H.** (2004) Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology, 73, 1007-1012.

**Becker, P.H.** (2003) Biomonitoring with birds. – In: Markert, B.A., Breure, A.M. & Zechmeister, H.G. (eds.), Bioindicators and biomonitors. Elsevier, pp. 677-736.

**BirdLife International** (2004) Birds in Europe: population estimates, trends and conservation status. BirdLife Conservation Series No. 12. BirdLife International, Cambridge.

**Blanco-Fontao, B., Sandercock, B.K., Obeso, J.R., McNew, L.B. & Quevedo, M.** (2013) Effects of sexual dimorphism and landscape composition on the trophic behavior of greater prairie-chicken. PLoS ONE, 8, e79986.

**Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. & Svanbäck, R.** (2002) Measuring individual-level resource specialization. Ecology, 83, 2936-2941.

**Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L.** (2003) The ecology of individuals: incidence and implications of individual specialization. American Naturalist, 161, 1-28.

**Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A.** (2011) Why intraspecific trait variation matters in community ecology. Trends in ecology & evolution, 26, 183-192.

**Bosch, R., Real, J., Tinto, A., Zozaya, E.L. & Castell, C.** (2010) Home-ranges and patterns of spatial use in territorial Bonelli's Eagles *Aquila fasciata*. Ibis, 152, 105-117.

**Brown, M.E.** (1996) Assessing body condition in birds. In: Ketterson, E.D., Val Nolan, J. (eds.) Current ornithology. Plenum Press, New York, pp 67–135.

**Cadahía, L.** (2007) Dispersión natal y estructura de la población de águila-azor perdicera *Hieraaetus fasciatus* en la Península Ibérica. Tesis doctoral. Universidad de Alicante – Estación Biológica de Doñana.

**Catry, T., Alves, J.A., Gill, J.A., Gunnarsson, T.G. & Granadeiro, J.P.** (2014) Individual specialization in a shorebird population with narrow foraging niche. Acta Oecologica, 56, 56-65.

**Caut, S., Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecological Applications, 16, 1893-1900.

**Costantini, D., Rowe, M., Butler, M.W. & McGraw, K.J.** (2010) From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. Functional Ecology, 24, 950-959.

**Cramp, S. & Simmons, K.E.L.** (1980) The Birds of the Western Palearctic. Vol. II. Oxford University Press, Oxford.

**Daszak, P., Cunningham, A.A. & Hyatt, A.D.** (2000) Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science, 287, 443-449.

**Del Hoyo, J., Elliot, A. & Sargatal, J. (eds.)** (1994) Handbook of the Birds of the World. Vol. II. New World Vultures to Guineafowl. Lynx Edicions, Barcelona.

**Del Moral, J.C. (ed.)** (2006) El águila perdicera en España. Población en 2005 y método de censo. SEO/BirdLife, Madrid.

**Elmhagen, B., Tannerfeldt, M., Verucci, P. & Angerbjörn, A.** (2000) The arctic fox (*Alopex lagopus*): an opportunistic specialist. Journal of Zoology, 251, 139-149.

**Emlen, J.M.** (1966) The role of time and energy in food preference. American Naturalist, 100, 611-617.

**Eulaers, I., Jaspers, V.L.B., Halley, D.J., Lepoint, G., Nygård, T., Pinxten, R., Covaci, A. & Eens, M.** (2014) Brominated and phosphorus flame retardants in White-tailed Eagle *Haliaeetus albicilla* nestlings: Bioaccumulation and associations with dietary proxies ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$). Science of the Total Environment, 478, 48-57.

**Fernández, A., Román, J., de la Torre, J.A., Ansola, L.M., Santamaría, J., Ventosa, R., Román, F. & Palma, C.** (1998) Demografía y conservación de una población de águila perdicera (*Hieraaetus fasciatus*) en declive. Pages 305–321 in B.U. Meyburg, R.D. Chancellor, and J.J. Ferrero, editors. Holartic birds of prey. Actas del Congreso Internacional sobre Rapaces del Holártico, Adenex-WWGBP, Mérida, Spain.

**Finkel, T. & Holbrook, N.J.** (2000) Oxidants, oxidative stress and the biology of ageing. Nature, 408, 239-247.

**Fry, B.** (2006) Stable Isotope Ecology. Springer, New York, USA.

**Furness, R.W. & Camphuysen, K.C.** (1997) Seabirds as monitors of the marine environment. ICES Journal of Marine Science, 54, 726-737.

**Futuyma, D.J. & Moreno, G.** (1988) The evolution of ecological specialization. Annual Review of Ecology and Systematics, 19, 207-233.

**Gil-Sánchez, J.M., Molino Garrido, F. & Valenzuela Serrano, G.** (1996) Selección de hábitat de nidificación por el Aguila Perdicera (*Hieraaetus fasciatus*) en Granada (SE de España). Ardeola, 43, 189-197.

**Gil-Sánchez, J.M., Molino, F., Valenzuela, G. & Moleón, M.** (2000) Demografía y alimentación del Águila-azor Perdicera (*Hieraaetus fasciatus*) en la provincia de Granada. Ardeola, 47, 69-75.

**Gil-Sánchez, J.M., Moleón, M., Otero, M., & Bautista, J.** (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biological Conservation, 118, 685-694.

**Golet, G.H., Kuletz, K.J., Roby, D.D. & Irons, D.B.** (2000) Adult prey choice affects chick growth and reproductive success in pigeon guillemots. The Auk, 117, 82-91.

**Halliwell, B. & Gutteridge, J.M.C.** (2007) Free Radicals in Biology and Medicine. Oxford University Press.

**Hernández-Matías, A., Real, J., Pradel, R., Ravayrol, A., Vincent-Martin, N., Bosca, F. & Cheylan, G.** (2010) Determinants of territorial recruitment in Bonelli's eagle (Aquila fasciata) populations. The Auk, 127, 173-184.

**Hernández-Matías, A., Real, J. & Pradel, R.** (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. Journal of Wildlife Management, 75, 856-866.

**Hernández-Matías, A., Real, J., Moleón, M., Palma, L., Sánchez-Zapata, J.A., Pradel, R., Carrete, M., Gil-Sánchez, J.M., Beja, P., Balbontín, J., Vincent-Martin, N., Ravayrol, A., Benítez, J.R., Arroyo, B., Fernández, C., Ferreiro, E. & García, J.** (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecological Monographs, 83, 239-261.

**Hobson, K.A.** (2011) Isotopic ornithology: a perspective. Journal of Ornithology, 152, 49-66.

**Hobson, K.A. & Clark, R.G.** (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor, 94, 189–197.

**Hochleithner, M.** (1994) Biochemistries. Avian medicine: principles and application (eds B.W. Ritchie, G.L. Harrison & L.R. Harrison), pp. 223-245. Wingers, Lake Worth.

**Hopkins, J.B., Koch, P.L., Ferguson, J.M. & Kalinowski, S.T.** (2014) The changing anthropogenic diets of American black bears over the past century in Yosemite National Park. Frontiers in Ecology and the Environment, 12, 107-114.

**Inger, R. & Bearhop, S.** (2008) Applications of stable isotope analyses to avian ecology. Ibis, 150, 447-461.

**Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S.** (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology, 80, 595-602.

**Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J.** (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLoS ONE, 7, e31757.

**Jiguet, F.** (2002) Arthropods in diet of Little Bustards *Tetrax tetrax* during the breeding season in western France. Bird Study, 49, 105-109.

**Jørgensen, C. & Holt, R.E.** (2013) Natural mortality: Its ecology, how it shapes fish life histories, and why it may be increased by fishing. Journal of Sea Research, 75, 8-18.

**Kelly, J.F.** (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78, 1-27.

**Korpimäki, E. & Norrdahl, K.** (1991) Numerical and functional responses of kestrels, short-eared owls, and long-eared owls to vole densities. Ecology, 72, 814-826.

**Krebs, C.J.** (2009) Ecology: the experimental analysis of distribution and abundance (6th ed.). Pearson International Edition, San Francisco, USA.

**Labocha, M.K. & Hayes, J.P.** (2012) Morphometric indices of body condition in birds: a review. Journal of Ornithology, 153, 1-22.

**Larrey, F., Roger, T., Morvan, R. & Girard, C.** (2007) Aigle de Bonelli, méditerranéen méconnu. Regard du vivant, Castelnau le Lez.

**Laskowski, K.L. & Bell, A.M.** (2013) Competition avoidance drives individual differences in response to a changing food resource in sticklebacks. Ecology Letters, 16, 746-753.

**Lefcheck, J.S., Whalen, M.A., Davenport, T.M., Stone, J.P. & Duffy, J.E.** (2013) Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology, 94, 565-572.

**L'Hérault, V., Franke, A., Lecomte, N., Alogut, A. & Bêty, J.** (2013) Landscape heterogeneity drives intra-population niche variation and reproduction in an arctic top predator. Ecology and Evolution, 3, 2867-2879.

**Lotze, H.K. & Milewski, I.** (2004) Two centuries of multiple human impacts and successive changes in a North Atlantic food web. Ecological Applications, 14, 1428-1447.

**MacArthur, R.H. & Pianka, E.R.** (1966) On optimal use of a patchy environment. American Naturalist, 100, 603-609.

**Marti, C.D., Bechard, M. & Jaksic, F.M.** (2007) Food habits. In Bird, D.M. & Bildstein, K.L. (eds.) Raptor research and management techniques: 129-151. Surrey: Hancock House Publishers LTD.

**Martínez, J.E., Sánchez, M.A., Carmona, D. & Sánchez, J.A.** (1994) Régime alimentaire de l´aigle de Bonelli *Hieraaetus fasciatus* durant la période de l´élevage des jeunes (Murcia, Espagne). Alauda, 62, 53-58.

**Martínez, J.E., Martínez, J.A., Zuberogoitia, I., Zabala, J., Redpath, S.M. & Calvo, J.F.** (2008) The effect of intra-and interspecific interactions on the large-scale distribution of cliff-nesting raptors. Ornis Fennica, 85, 13-21.

**Masello, J.F., Wikelski, M., Voigt, C.C. & Quillfeldt, P.** (2013) Distribution patterns predict individual specialization in the diet of Dolphin Gulls. PLoS ONE, 8, e67714.

**Matthews, B. & Mazumder, A.** (2004) A critical evaluation of intrapopulation variation of $\delta^{13}C$ and isotopic evidence of individual specialization. Oecologia, 140, 361-371.

**Moleón, M., Almaraz, P. & Sánchez-Zapata, J.A.** (2008) An emerging infectious disease triggering large-scale hyperpredation. PLoS ONE, 3, e2307.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Chartón, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** (2009a) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. Journal of Biogeography, 36, 1502-1515.

**Moleón, M., Bautista, J., Sánchez-Zapata, J.A. & Gil-Sánchez, J.M.** (2009b) Diet of non-breeding Bonelli's Eagles *Hieraaetus fasciatus* at settlement areas of southern Spain. Bird Study, 56, 142-146.

**Moleón, M., Sánchez-Zapata, J.A., Gil-Sánchez, J.M., Ballesteros-Duperón, E., Barea-Azcón, J.M. & Virgós, E.** (2012a) Predator–prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia, 168, 679-689.

**Moleón, M., Sebastián-González, E., Sánchez-Zapata, J.A., Real, J., Pires, M.M., Gil-Sánchez, J.M., Bautista, J., Palma, L., Bayle, P., Guimarães Jr, P.R. & Beja, P.** (2012b) Changes in intrapopulation resource use patterns of an endangered raptor in response to a disease-mediated crash in prey abundance. Journal of Animal Ecology, 81, 1154-1160.

**Moleón, M., Sánchez-Zapata, J.A., Selva, N., Donázar, J.A. & Owen-Smith, N.** (2014) Inter-specific interactions linking predation and scavenging in terrestrial vertebrate assemblages. Biological Reviews, doi: 10.1111/brv.12097.

**Monaghan, P., Metcalfe, N.B. & Torres, R.** (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology letters, 12, 75-92.

**Moore, J.W. & Semmens, B.X.** (2008) Incorporating uncertainity and prior information into stable isotope mixing models. Ecology Letters, 11, 470-480.

**Moreno, R., Jover, L., Diez, C., Sardá, F. & Sanpera, C.** (2013) Ten years after the Prestige oil spill: seabird trophic ecology as indicator of long-term effects on the coastal marine ecosystem. PLoS ONE, 8, e77360.

**Newsome, S.D., Martinez del Rio, C., Bearhop, S. & Phillips, D.L.** (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment, 5, 429-436.

**Newsome, S.D., Tinker, M.T., Monson, D.H., Oftedal, O.T., Ralls, K., Staedler, M.M., Fogel, M.L. & Estes, J.A.** (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology, 90, 961-974.

**Ontiveros, D., Real, J., Balbontín, J., Carrete, M., Ferreiro, E., Ferrer, M., Mañosa, S., Pleguezuelos, J.M. & Sánchez-Zapata, J.A.** (2004) Biología de la conservación del águila perdicera *Hieraaetus fasciatus* en España: investigación científica y gestión. Ardeola, 51, 461-470.

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

**Palma, L., Beja, P., Pais, M. & Cancela da Fonseca, L.** (2006) Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons. Journal of Applied Ecology, 43, 1075-1086.

**Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L.** (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE, 5, e9672.

**Peterson, B.J. & Fry, B.** (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18, 293-320.

**Peterson, B.J., Howarth, R.W. & Garritt, R.H.** (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science, 227, 1361-1363.

**Pyke, G.H., Pulliam, H.R. & Charnov, E.** (1977) Optimal foraging: a selective review of theory and tests. Quarterly Review of Biology, 52, 137-154.

**Ramos, R. & González-Solís, J.** (2012) Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. Frontiers in Ecology and the Environment, 10, 258-266.

**Real, J.** (1987) Evolución cronológica del regimen alimenticio de una población de *Hieraaetus fasciatus* en Catalunya: factores causantes, adaptación y efectos. Rapaci Mediterranei III, Vol. XII, 185-205.

**Real, J.** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología trófica, biología reproductora i demografía. Tesis doctoral. Universitat de Barcelona.

**Real, J.** (1996) Biases in diet study methods in the Bonelli's eagle. The Journal of Wildlife Management, 60, 632-638.

**Real, J.** (2004) Águila perdicera, *Hieraaetus fasciatus*. En, Madroño, A., González, C. & Atienza, J.C. (eds.). Libro Rojo de las aves de España. Dirección General para la Biodiversidad-SEO/Birdlife, Madrid.

**Real, J. & Mañosa, S.** (1997) Demography and conservation of western European Bonelli´s eagle *Hieraaetus fasciatus* populations. Biological Conservation, 79, 59-66.

**Real, J. & Mañosa, S.** (2001) Dispersal of juvenile and immature Bonelli's Eagles in northeastern Spain. Journal of Raptor Research, 35, 9-14.

**Real, J., Mañosa, S. & Codina, J.** (1998) Post-nestling dependence period in the Bonelli's Eagle *Hieraaetus fasciatus*. Ornis Fennica, 75, 129-137.

**Real, J., Mañosa, S., & Muñoz, E.** (2000) Trichomoniasis in a Bonelli's eagle population in Spain. Journal of Wildlife Diseases, 36, 64-70.

**Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2002) Golden eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. Proceedings of the National Academy of Sciences, 99, 791-796.

**Rollan, A., Real, J., Bosch, R., Tintó, A. & Hernández-Matías, A.** (2010) Modelling the risk of collision with power lines in Bonelli's Eagle *Hieraaetus fasciatus* and its conservation implications. Bird Conservation International, 20, 279-294.

**Schreiber, S.J., Bürger, R. & Bolnick, D.I.** (2011) The community effects of phenotypic and genetic variation within a predator population. Ecology, 92, 1582-1593.

**Sergio, F., Caro, T., Brown, D., Clucas, B., Hunter, J., Ketchum, J., McHugh, K. & Hiraldo, F.** (2008). Top predators as conservation tools: ecological rationale, assumptions, and efficacy. Annual Review of Ecology, Evolution, and Systematics, 39, 1-19.

**Shannon, C.E. & Weaver, W.** (1997) The mathematical theory of communication. University of Illinois Press, Urbana, IL, USA.

**Stephens, D.W. & Krebs, J.R.** (1986) Foraging theory. Princeton University Press.

**Stephens, D.W., Brown, J.S. & Ydenberg, R.C. (eds.)** (2007) Foraging Behavior and Ecology. Chicago: The University of Chicago Press.

**Stevenson, R.D. & Woods, W.A.** (2006) Condition indices for conservation: new uses for evolving tools. Integrative and comparative biology, 46, 1169-1190.

**Tinker, M.T., Guimarães, P.R.Jr., Novak, M., Marquitti, F.M.D., Bodkin, J.L., Staedler, M., Bentall, G. & Estes, J.A.** (2012) Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecology Letters, 15, 475-483.

**Trainor, C.R., Debus, S.J.S., Olsen, J., Norman,**

**J.A. & Christidis, L.** (2013) Bonelli's Eagle *Aquila fasciata renschi* in the Lesser Sundas, Wallacea: distribution, taxonomic status, likely origins and conservation status. Forktail, 29, 100-106.

**Tucker, G.M. & Heath, M.F.** (1994) Birds in Europe: their conservation status. Birdlife International. Birdlife Conservation Series no. 3. Cambridge.

**Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. & Furness, R.W.** (2003) Assessing the diet of Great Skuas, *Catharacta skua*, using five different techniques. Polar Biology, 26, 20-26.

**Watson, J.** (2010) The Golden Eagle. London: T & A.D Poyser, 2nd ed. 464 p.

**White, T.C.R.** (2008) The role of food, weather and climate in limiting the abundance of animals. Biological Reviews, 83, 227-248.

**Whitfield, D.P., Reid, R., Haworth, P.F., Madders, M., Marquiss, M., Tingay, R. & Fielding, A.H.** (2009) Diet specificity is not associated with increased reproductive performance of Golden Eagles Aquila chrysaetos in Western Scotland. Ibis, 151, 255-264.

**Woo, K.J., Elliott, K.H., Davidson, M., Gaston, A.J. & Davoren, G.K.** (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. Journal of Animal Ecology 77, 1082-1091.

# Objetivos

# OBJETIVOS

El **objetivo general** de esta tesis es estudiar la ecología trófica del águila perdicera mediante técnicas de análisis de dieta convencional e isótopos estables, y determinar el efecto de la dieta sobre el estado de condición corporal de los pollos, las tasas vitales de los individuos territoriales y la dinámica de las poblaciones en Europa Occidental.

Para conseguir el objetivo general, se plantean los siguientes **objetivos específicos**:

**Capítulo 1.** Determinar la aplicabilidad del análisis de isótopos estables en estudios de dieta del águila perdicera durante el periodo reproductor, haciendo una comparativa entre las estimas de consumo de presas calculadas mediante análisis de egagrópilas y las obtenidas del análisis de isótopos estables.

**Capítulo 2.** Estudiar la condición corporal de los pollos de águila perdicera, mediante el uso de múltiples indicadores de condición, y su relación con la dieta. En particular, analizar si el consumo de presas preferidas y la diversidad trófica afectan al estado de condición de los pollos.

**Capítulo 3.** Describir la amplitud y estructura del nicho trófico en varias poblaciones de águila perdicera mediante análisis de isótopos estables, y determinar su efecto sobre la productividad a nivel poblacional. Asimismo, estimar la composición de la dieta a nivel territorial, y testar si el grado de especialización en la dieta afecta a los valores de productividad de las parejas territoriales.

**Capítulo 4.** Determinar el efecto de la dieta sobre parámetros demográficos clave del águila perdicera en diversas poblaciones de Europa Occidental, siendo dichos parámetros la productividad, la supervivencia y la tasa de crecimiento, considerados tanto a nivel territorial como poblacional.

# Informe del director

# INFORME DEL DIRECTOR

Los directores de tesis, el Dr. Joan Real Ortí y el Dr. Antonio Hernández Matías, acreditamos que el doctorando Jaime Resano Mayor ha realizado las investigaciones contenidas en la presente memoria de tesis doctoral titulada "Estudio de la ecología trófica del águila perdicera *Aquila fasciata*: efectos de la dieta sobre la condición corporal, las tasas vitales y la demografía". La tesis consta de cinco trabajos de investigación en formato de artículo científico, los cuales están publicados en revistas científicas internacionales reconocidas en el *Science Citation Index* (SCI), en revisión, o pendientes de envío. A continuación se detalla la referencia de todos los artículos, indicando el factor de impacto de las revista según el *Thomson Institute for Scientific Information*, así como la contribución del doctorando en la elaboración de cada artículo:

## Capítulo 1

**Resano, J.**, Hernández-Matías, A., Real, J. & Parés, F. (2011). Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. *Journal of Raptor Research*, 45, 342-352.
Factor de impacto en los últimos 5 años (2012): 0.712

El doctorando ha participado en el diseño del muestreo, la recogida y el análisis de las muestras, el análisis de los datos y en la redacción científica.

**Resano-Mayor, J.**, Hernández-Matías, A., Real, J., Parés, F., Inger, R. & Bearhop, S. (2014). Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. *Ibis*, 156, 176-188.
Factor de impacto en los últimos 5 años (2012): 2.488

El doctorando ha participado en el diseño del muestreo, la recogida y el análisis de las muestras, el análisis de los datos y en la redacción científica.

## Capítulo 2

**Resano-Mayor, J.**, Hernández-Matías, A., Real, J., Parés, F., Moleón, M., Mateo, R. & Ortiz-Santaliestra, M.E. The influence of diet on Bonelli's Eagle *Aquila fasciata* nestling body condition: a multi-biomarker approach. En revisión en *Functional Ecology*.
Factor de impacto en los últimos 5 años (2012): 5.393

El doctorando ha participado en el diseño del muestreo, la recogida y el análisis de las muestras, el análisis de los datos y en la redacción científica.

## Capítulo 3

**Resano-Mayor, J.**, Hernández-Matías, A., Real, J., Moleón, M., Parés, F., Inger, R. & Bearhop, S. (2014). Multi-scale effects of nestling diet on breeding performance in a terrestrial top predator inferred from stable isotope analysis. *PLoS ONE*, 9, e95320. Factor de impacto en los últimos 5 años (2012): 4.244

El doctorando ha participado en el diseño del muestreo, la recogida y el análisis de las muestras, el análisis de los datos y en la redacción científica.

## Capítulo 4

**Resano-Mayor, J.**, Real, J., Moleón, M., Sánchez-Zapata, J.A., Palma, L. & Hernández-Matías, A. Large-scale spatial patterns in demographic rates in relation to diet in Bonelli's Eagle *Aquila fasciata*, a long-lived territorial predator. Pendiente de envío.

El doctorando ha participado en el análisis de los datos y en la redacción científica.

Asimismo, se hace constar que los coautores participantes en los artículos que componen esta tesis no han utilizado ni implícita ni explícitamente ninguno de estos trabajos para la elaboración de otras tesis doctorales.

Barcelona, 15 de mayo de 2014

Dr. Joan Real Ortí Dr. Antonio Hernández Matías

# Capítulos - Chapters

# Chapter 1

## Bonelli’s Eagle nestling diet inferred from stable isotope analyses

* Using stable isotopes to determine dietary patterns in Bonelli’s Eagle *Aquila fasciata* nestlings

* Comparing pellet and stable isotope analyses of nestling Bonelli’s Eagle *Aquila fasciata* diet

# Uso del análisis de isótopos estables para determinar patrones tróficos en pollos de águila perdicera *Aquila fasciata*

## Resumen

El águila perdicera (*Aquila fasciata*) es una de las rapaces más amenazadas de Europa debido a la elevada tasa de mortalidad adulta y preadulta, la degradación y pérdida del hábitat, así como una disminución de sus principales presas como el conejo de monte (*Oryctolagus cuniculus*) o la perdiz roja (*Alectoris rufa*). Durante la temporada de cría de 2008 se estudió la dieta de 15 parejas reproductoras de águila perdicera en Cataluña, noreste de la Península Ibérica, a través del análisis convencional de egagrópilas y el análisis de isótopos estables (AIE) en las plumas de los pollos. Nuestros objetivos fueron investigar la dieta de los pollos, así como determinar si el AIE permite representar con exactitud sus patrones tróficos. El análisis de egagrópilas reveló una dieta variada que incluyó palomas (*Columba* spp.; 31.1 %), conejo de monte (27.9 %), "otras aves" (16.2 %), perdiz roja (13.1 %), ardilla roja Europea (*Sciurus vulgaris*; 5.2 %), lagarto ocelado (*Timon lepidus*; 2.6 %), gaviota patiamarilla (*Larus michahellis*; 2.2 %) y "otros mamíferos" (1.7 %) como principales categorías de presas. Sin embargo, la composición de la dieta fue heterogénea y se hallaron diversos patrones tróficos entre pollos pertenecientes a diferentes territorios de cría. Asimismo, se halló una correlación positiva entre $\delta^{13}C$ y la frecuencia de ardilla en la dieta, y una correlación negativa entre $\delta^{13}C$ y la frecuencia de perdiz, especies presentes en hábitats boscosos y abiertos, respectivamente. No hubo correlación entre $\delta^{15}N$ y el consumo de presas. Sin embargo, su amplio rango de valores sugirió una dieta global con diversidad de presas pertenecientes, al menos, a dos niveles tróficos diferentes. Finalmente, $\delta^{34}S$ fue mayor en aquellos pollos que consumieron gaviotas. Este estudio aborda por vez primera la ecología trófica en pollos de águila perdicera a partir del AIE, concluyendo que $\delta^{13}C$, $\delta^{15}N$ y $\delta^{34}S$ son útiles para la evaluación de sus patrones tróficos en términos de consumo de las principales presas y el nivel trófico de las mismas.

## Referencia bibliográfica

Resano, J., Hernández-Matías, A., Real, J. & Parés, F. (2011) Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. *Journal of Raptor Research*, 45, 342-352.

# Using stable isotopes to determine dietary patterns in Bonelli's Eagle *Aquila fasciata* nestlings

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**ABSTRACT**—Bonelli's Eagle (*Aquila fasciata*) is one of the most endangered raptor species in Europe due to high adult and subadult mortality rates, habitat loss, and a decrease in populations of its most important prey, European rabbits (*Oryctolagus cuniculus*) and Red-legged Partridges (*Alectoris rufa*). During the breeding season of 2008, we studied the diet of Bonelli's Eagles at 15 breeding territories in Catalonia, northeastern Iberian Peninsula, through a conventional pellet analysis and stable isotope analyses (SIA) of nestlings' feathers. Our objectives were to investigate the diet of Bonelli's Eagle nestlings and to determine whether SIA allowed accurate representation of their dietary patterns. The pellet analysis revealed a broad diet including pigeons (*Columba* spp.; 31.1 %), European rabbits (27.9 %), ''other birds'' (16.2 %), Red-legged Partridges (13.1 %), Eurasian red squirrels (*Sciurus vulgaris*; 5.2 %), ocellated lizards (*Timon lepidus*; 2.6 %), Yellow-legged Gulls (*Larus michahellis*; 2.2 %) and ''other mammals'' (1.7 %). Diet composition was heterogeneous and varied markedly among nestlings from different breeding territories. We found a significant positive correlation between $\delta^{13}C$ and the frequency of Eurasian red squirrels in the diet, and a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges, which are species that occur in forested and open habitats, respectively. The values of $\delta^{15}N$ were not correlated with the consumption of any prey category. However, its wide range of values suggested a global diet with a broad diversity of prey species from at least two different trophic levels. Finally, $\delta^{34}S$ were higher for those nestlings that fed on Yellow-legged Gulls. Our study provided the first isotopic approach to the trophic ecology of Bonelli's Eagle nestlings, and we concluded that $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ may be useful for assessing nestlings' dietary patterns in terms of main prey consumption and prey trophic level.

# Chapter 1

## INTRODUCTION

The study of raptors' feeding habits provides meaningful information that can aid the understanding of species' dietary ecology and their trophic relationships at the community level (Jaksic & Delibes 1987, Newton 1998, Marti et al. 2007). The assessment of raptors' dietary patterns may also provide information about distribution, abundance, behavior and the vulnerability of prey species (del Hoyo et al. 1994, Johnsgard 2002). Traditionally, the diets of raptors are described using conventional methodologies that include the analysis of regurgitated pellets, food remains from nests, and stomach contents, as well as the direct observation of prey delivered to nestlings at the nests (Korpimäki & Norrdahl 1991, Salamolard et al. 2000, Katzner et al. 2006, Marti et al. 2007). Of these methods, pellet analysis is the most common approach in the study of raptors' dietary habits, both quantitatively and qualitatively, and has been shown to be an efficient and suitable method for monitoring the diet of several raptor species (Real 1996, Marti et al. 2007). The main advantage of conventional methods is that they frequently enable prey to be identified at the species or taxonomic group level. However, differences in prey sizes, digestion and consumption patterns may lead to biases such as the over- or underestimation of the proportions of prey items in a predator's diet (Real 1996, Votier et al. 2003, Marti et al. 2007, Sánchez et al. 2008). Moreover, due to the logistical difficulty in sampling regularly over an extended period of time, conventional methods may in fact reflect only short-term dietary habits (Inger & Bearhop 2008).

Over the last two decades, stable isotope analysis (SIA) has become increasingly common in avian trophic ecology as a means of studying foraging strategies and dietary specialization at both individual and population levels (Kelly 2000, Bolnick et al. 2002, Rubenstein & Hobson 2004, Araújo et al. 2009). The use of SIA in dietary studies relies on the fact that different dietary items often have different isotopic values, which are reflected in the tissue of the consumers (Pearson et al. 2003, Becker et al. 2007, Inger & Bearhop 2008). For example, metabolically inert tissues such as feathers preserve the isotopic composition of resources incorporated while growing (Hobson 1999, Bearhop et al. 2002), and the use of SIA in avian trophic ecology has been shown as a powerful means of integrating temporal dietary information, particularly when combined with conventional methods (Inger & Bearhop 2008).

Stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotopes are the most frequently used isotopes in the study of trophic relationships and food-web structures at community level (Kelly 2000). The carbon-isotope composition of a consumer enables the carbon sources of the primary production within a food web to be determined (Krouse & Herbert 1988, Crawford et al. 2008). Nitrogen isotopes are useful for diagnosing the species' trophic level position since consumers are typically enriched in $^{15}N$ by 3-5‰ in proportion to the food they consume (Post 2002, Vanderklift & Ponsard 2003). This finding has been used to provide insights into community-level phenomena such as trophic cascades, the length of food chains, and resource partitioning (Post 2002, Roemer et al. 2002). In addition, the analysis of stable sulphur isotopes ($^{34}S/^{32}S$, $\delta^{34}S$) has been recommended in dietary studies as a means of discriminating between prey from marine and terrestrial ecosystems (Peterson et al. 1985, Peterson & Fry 1987). However, despite the wide applicability of SIA in avian foraging ecology, few isotopic studies have focused on terrestrial top predators such as raptor species (but see Roemer et al. 2002, Dominguez et al. 2003, Caut et al. 2006).

Bonelli's Eagle (*Aquila fasciata)* is a medium-sized raptor distributed from Southeast Asia and the Middle East to the western Mediterranean (del Hoyo et al. 1994). Its European population has declined markedly from the 1970s to the early 1990s (Rocamora 1994, Real 2004) and this raptor is now listed as an endangered species (BirdLife International 2004). In Europe, Bonelli's Eagle occupies Mediterranean mountain ranges and lowlands, and forages mainly in scrublands and dry fields where it predates on a wide variety of species ranging from medium-sized to small mammals (Lagomorpha and Rodentia), birds (Galliformes, Columbiformes, Charadriiformes, Passeriformes and others) and occasionally reptiles (mainly lizard; Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Moleón et al. 2009a, 2009b). Furthermore, marked dietary differences may exist among territories due to heterogeneity in ecological features such as habitat coverage, prey abundance and distribution, and human pressure (Real et al. 2004). Therefore, Bonelli's Eagle is a suitable model for assessing whether territorial dietary patterns inferred by conventional techniques can also be described using isotopic data. Moreover, the ecological features of some territories have undergone great changes in recent decades (i.e., the expansion of forests as a consequence of land abandonment, an increase in human pressure that results from sprawl and greater demands for leisure activities), and the number and availability of prey species has been greatly modified. Thus, the ongoing monitoring of diet of Bonelli's Eagle may constitute a good tool to assess the prey on which this species depends during the nestling period, and also help illuminate how habitat changes may affect eagles' foraging habits.

The focus of our study was an analysis of Bonelli's Eagle diet during the breeding season via conventional pellet analysis and an evaluation of the usefulness of SIA for assessing nestlings' dietary patterns. The specific aims of this study were: (1) to assess the diet of Bonelli's Eagle nestlings from different breeding territories using conventional pellet analysis; (2) to describe stable isotope values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in nestlings' feathers; (3) to assess isotopic data in siblings as indicators of diet similarity; and (4) to test whether isotopic data from nestlings were related to their prey consumption as described by the pellet analysis.

## METHODS

### Study area

During 2008, we studied 15 territorial breeding pairs of Bonelli's Eagle in Catalonia (northeastern Spain; 01º 32' E, 41º 20' N). Sampled territories were a subset of known territories for the species in Catalonia. All sampled nests were located in cliffs, and environmental features in breeding territories varied but were representative of Mediterranean habitats, and included scrublands (*Quercus coccifera*, *Thymus vulgaris*, *Pistacia lentiscus* and *Rosmarinus officinalis*), woodland patches (mainly *Quercus ilex* and *Pinus* spp), non-irrigated cropland and built-up areas (Bosch et al. 2010). The mean altitude of nesting areas ranged from 176 to 753 m asl, with mean annual rainfall ranging from 450 to 800 mm.

### Data collection

Each breeding territory was monitored between January and July. We checked each territory using a spotting scope (20–60 X) between January and early March to assess territorial occupancy and breeding activity (i.e. displays, nest material transfer, copulation, and incubation behavior). In late March and April, we checked nests again, using a spotting scope, to detect the presence, number and approximate age of nestlings. The age of nestlings was estimated by the development of feathers and by calculating from the laying date (Real 1991, Gil-Sánchez 2000). After nestlings were approximately 37 d old, climbers accessed nests to collect 3-4 feathers from the back of each nestling for the SIA, assuming that isotopic data from nestlings' feathers were representative of the whole nestling period. At the same time, pellets were collected from the nest for the conventional diet analysis. Finally, approximately 2 wk after the nestlings had fledged, nests were visited again for a second retrieval of pellets. Therefore, we assumed that our conventional diet study based on pellet analysis was representative of nestlings' diet during their entire nestling period.

### Conventional diet study and statistical procedures

The conventional diet study was based on pellet analysis. Pellets were individually analyzed and each prey species identified in a pellet was counted as one individual (Real 1996, Gil-Sánchez et al. 2004). Pellets were visually examined and their contents (i.e., feathers, bones, hair, nails and scales) were compared with prey items from our own reference collection. For some remains such as feathers we also used a 4x magnifying glass and consulted specialized guides for the identification of macro and microscopic remains (Brom 1986). Prey were identified to species level whenever possible.

Prey items were grouped into eight different taxonomic categories: European rabbits (*Oryctolagus cuniculus*), Eurasian red squirrels (*Sciurus vulgaris*), "other mammals", pigeons (*Columba* spp.), Red-legged Partridges (*Alectoris rufa*), Yellow-legged Gulls (*Larus michahellis*), "other birds" (mainly Corvidae and Turdidae) and ocellated lizards (*Timon lepidus*). Diet data were analyzed at the territory level by comparing the frequency (%) of items in each taxonomic group relative to the total number of prey items (Palma et al. 2006, Moleón et al. 2009b). To assess the dietary patterns of nestlings at the territory level, we performed a principal component analysis (PCA) of prey frequency consumption using the varimax rotation, which keeps the rotated components orthogonal to or uncorrelated with each other after rotation (Quinn & Keough 2002). Additionally, we performed Spearman rank correlation tests ($r_s$) for all taxonomic prey consumption at the territory level.

### Stable isotope analysis and statistical procedures

Nestling feathers were frozen until they were cleaned in a solution of NaOH (0.25 M; Bearhop et al. 2002, Ramos et al. 2009) and oven-dried at 40ºC for 24 hr. Lipids were not washed off the feathers as they were shown to have negligible effects on the isotope ratios (Mizutani et al. 1992). To homogenize samples, feathers were ground into an extremely fine powder using an impactor mill (6750 Freezer/Mill, Spex Certiprep, Metuchen, NJ, USA) operating at the temperature of liquid nitrogen. Sub-samples of 0.35 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 3.7 mg (for $\delta^{34}S$) were loaded in tin recipients and crimped for combustion. Isotopic analysis were conducted using elemental analysis-isotope ratio mass spectrometry (EA-IRMS) using a Flash 1112 (for C and N)/1108 (for S) elemental analyzer coupled to a Delta C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Fisher Scientific, Bremen, Germany). Analyses were performed at the Scientific Technical Services of the University of Barcelona.

Stable isotope ratios are expressed conventionally as parts per thousand (‰), according to the following equation: $\delta X = [(Rsample / Rstandard) - 1] \times 1000$, where X is $^{13}C$, $^{15}N$ or $^{34}S$, and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. Samples were referenced against international standards: Pee Dee Belemnite (VPDB) for $^{13}$C, atmospheric nitrogen (AIR) for $^{15}$N and Canyon Diablo Troilite (CDT) for $^{34}$S. The measurement precisions for $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S were ≤ 0.15‰, ≤ 0.25‰ and ≤ 0.4‰ respectively.

Arithmetic mean values (± SD) for $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S were calculated for all nestlings. Because we expected Bonelli's Eagle nestlings raised in the same nest to have similar prey intake, we tested whether the isotopic values from siblings hatched in the same nest were more similar to each other than to isotopic values from a random sample of nestlings from the studied population. First, we applied a Spearman rank correlation test that only considered those territories where two nestlings were born ($n = 9$), and we then performed a randomization test to assess whether isotopic similarities between siblings differed from the expected random distribution. To do so, we obtained two samples of nine individuals extracted at random from the pool of the studied population ($n = 24$ nestlings) and compared their isotopic values with a Spearman rank correlation. This step was repeated 10000 times and the resulting correlation coefficients were recorded. Next, we calculated the proportion of randomized coefficients that were recorded as equal to or larger than the observed correlation coefficient in siblings. This proportion, our estimated P-value ($P$), was then used to accept or reject the assertion that isotopic values were more similar between siblings than the expected random distribution.

Finally, we analyzed whether isotopic data from nestlings was related to their diet as estimated by the pellet analysis. To do so, we performed a Spearman rank correlation test between $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S from nestlings from each breeding pair and nestlings' prey consumption as described by the pellet analysis. Nestlings from the same nest/territory were considered a single statistical observation and the isotopic values ($\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S) for each breeding territory were estimated using the means of the two siblings.

Statistical analyses were conducted using R software (R Development Core Team 2007) and SPSS 15.0 (SPSS, Chicago, Illinois).

## RESULTS

### Conventional diet

We identified 542 prey items in the 241 pellets analyzed (Table 1). In all, 62.6% of prey items were birds, 34.8% were mammals, and 2.6% were reptiles. The main prey items consumed were pigeons (31.1%), a category that included Rock Pigeon (*Columba livia*), Common Wood-pigeon (*Columba palumbus*) and Stock Dove (*Columba oenas*), followed by European rabbits (27.9%), "other birds" (16.2%), Red-legged Partridges (13.1%), Eurasian red squirrels (5.2%), ocellated lizards (2.6%), Yellow-legged Gulls (2.2%), and "other mammals" (1.7%; Fig. 1).

The PCA revealed marked dietary patterns between nestlings from different territories (Table 2 and Fig. 2). The first two components accounted for 64.6% of total diet variance. The first component, which accounted for 40.3% of diet variance, discriminated between nestlings with a high consumption of pigeons as opposed to others whose diet included more Red-legged Partridges, ocellated lizards, Yellow-legged Gulls and "other mammals". The second component explained an additional 24.3% of diet variance and discriminated between greater amounts of European rabbits as opposed to "other birds". Indeed, Spearman rank correlations between taxonomic prey consumption of nestlings at the territory level showed that intake of pigeons was negatively correlated with that of Red-legged Partridges ($r_s = -0.547$, $P < 0.05$), ocellated lizards ($r_s = -0.685$, $P < 0.005$), and Yellow-legged Gulls ($r_s = -0.465$, $P < 0.1$). Accordingly, there was also a significant negative correlation between consumption of European rabbits and "other birds" ($r_s = -0.526$, $P < 0.05$).

### Stable isotopes

The arithmetic mean isotopic values (± SD) for the 24 nestlings were -22.10‰ (± 1.03) for $\delta^{13}C$, 6.44‰ (± 1.27) for $\delta^{15}N$ and 4.30‰ (± 1.43) for $\delta^{34}S$. Isotopic values of individuals from all the different territories showed broad ranges for the three elements (Fig. 3). However, we found that those nestlings hatched and reared in the same nest/territory had significant positive correlations for $\delta^{13}C$ ($r_s = 0.93$, $P < 0.001$), $\delta^{15}N$ ($r_s = 0.98$, $P < 0.001$) and $\delta^{34}S$ ($r_s = 0.95$, $P < 0.001$), and that these correlation values were in all cases significantly higher than expected by a random distribution ($P < 0.001$).

### Conventional diet *vs.* stable isotopes

We found a significant positive correlation between $\delta^{13}C$ in nestlings and the frequency of

**Table 1.** Diet of Bonelli's Eagle nestlings during the breeding season, shown as the number of prey items and their frequencies (%), based on pellet analyses.

| PREY SPECIES | NUMBER OF ITEMS | FREQUENCY (%) |
|---|---|---|
| **Mammals** | | |
| European rabbit (*Oryctolagus cuniculus*) | 151 | 27.9 |
| Eurasian red squirrel (*Sciurus vulgaris*) | 28 | 5.2 |
| Undetermined mammal | 9 | 1.7 |
| **Total mammals** | **188** | **34.8** |
| **Birds** | | |
| Northern Goshawk (*Accipiter gentilis*) | 4 | 0.7 |
| European Honey-buzzard (*Pernis apivorus*) | 1 | 0.2 |
| Red-legged Partridge (*Alectoris rufa*) | 71 | 13.1 |
| Common Pheasant (*Phasianus colchicus*) | 1 | 0.2 |
| Galliforms (*Galliform* spp.) | 6 | 1.1 |
| Rock Pigeon (*Columba livia*) | 12 | 2.2 |
| Common Wood-pigeon (*Columba palumbus*) | 62 | 11.3 |
| Stock Dove (*Columba oenas*) | 3 | 0.6 |
| Pigeons (*Columba* spp.) | 92 | 17.0 |
| Eurasian Jay (*Garrulus glandarius*) | 12 | 2.2 |
| Black-billed Magpie (*Pica pica*) | 3 | 0.6 |
| Eurasian Blackbird (*Turdus merula*) | 7 | 1.3 |
| *Turdus* sp. | 1 | 0.2 |
| Yellow-legged Gull (*Larus michahellis*) | 12 | 2.2 |
| Common Cuckoo (*Cuculus canorus*) | 2 | 0.4 |
| Eurasian Green Woodpecker (*Picus viridis*) | 1 | 0.2 |
| *Amazona* sp. | 4 | 0.7 |
| *Anas* sp. | 1 | 0.2 |
| Undetermined bird | 45 | 8.2 |
| **Total birds** | **340** | **62.6** |
| **Reptiles** | | |
| Ocellated lizard (*Timon lepidus*) | 14 | 2.6 |

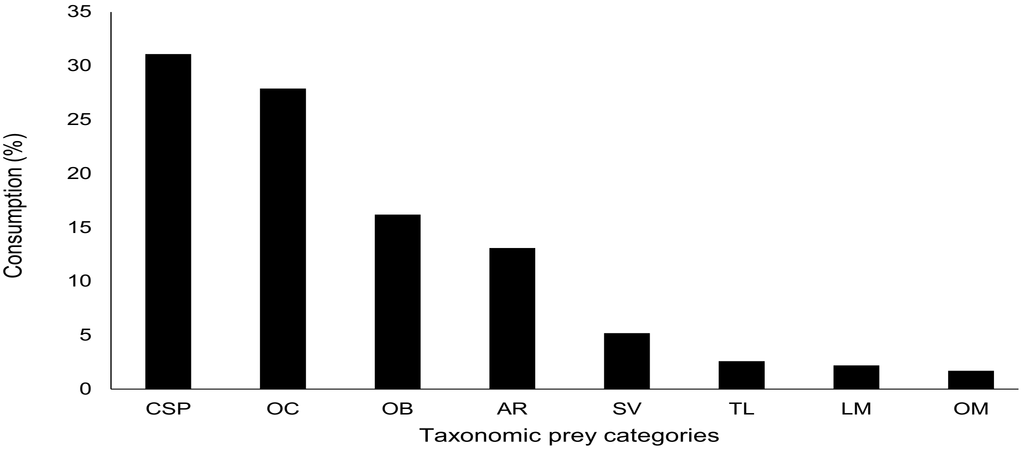

**Figure 1.** Prey consumption (%) by Bonelli's Eagle nestlings, Catalonia, Spain, as determined by pellet analysis. Taxonomic categories are ordered from greatest to lowest importance in diet: CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB ("other birds"), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM ("other mammals").

Eurasian red squirrels in their diet ($r_s = 0.565$, $P < 0.05$), as well as a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges ($r_s = -0.688$, $P \leq 0.005$) (Table 3). Despite not correlating with any particular prey item, high levels of $\delta^{34}S$ were found in the nestlings hatched in the two territories where Yellow-legged Gulls was consumed.

**Table 2.** Prey category consumption (%) of nestlings at the territory level, based on pellet analyses. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB ("other birds"), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM ("other mammals").

| TERR | CSP | OC | OB | AR | SV | TL | LM | OM |
|---|---|---|---|---|---|---|---|---|
| 1 | 33.3 | 33.3 | 13.3 | 0 | 20.1 | 0 | 0 | 0 |
| 2 | 25.6 | 33.3 | 20.5 | 7.7 | 7.7 | 2.6 | 0 | 2.6 |
| 3 | 47.4 | 10.5 | 23.7 | 15.8 | 0 | 0 | 0 | 2.6 |
| 4 | 3.8 | 34.2 | 6.3 | 26.6 | 0 | 10.1 | 12.7 | 6.3 |
| 5 | 45.7 | 8.6 | 31.3 | 2.9 | 8.6 | 2.9 | 0 | 0 |
| 6 | 66.7 | 33.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 29 | 38.7 | 6.5 | 25.8 | 0 | 0 | 0 | 0 |
| 8 | 54.9 | 13.7 | 9.8 | 7.8 | 11.8 | 0 | 0 | 2 |
| 9 | 29 | 38.8 | 16.1 | 16.1 | 0 | 0 | 0 | 0 |
| 10 | 35.7 | 35.7 | 14.4 | 7.1 | 7.1 | 0 | 0 | 0 |
| 11 | 13.6 | 18.2 | 31.8 | 22.7 | 4.6 | 9.1 | 0 | 0 |
| 12 | 23.6 | 20.6 | 35.3 | 5.9 | 8.8 | 2.9 | 0 | 2.9 |
| 13 | 37.1 | 44.4 | 14.8 | 3.7 | 0 | 0 | 0 | 0 |
| 14 | 26.5 | 20.6 | 23.5 | 11.8 | 8.8 | 2.9 | 5.9 | 0 |
| 15 | 31.4 | 37.2 | 5.7 | 20 | 5.7 | 0 | 0 | 0 |

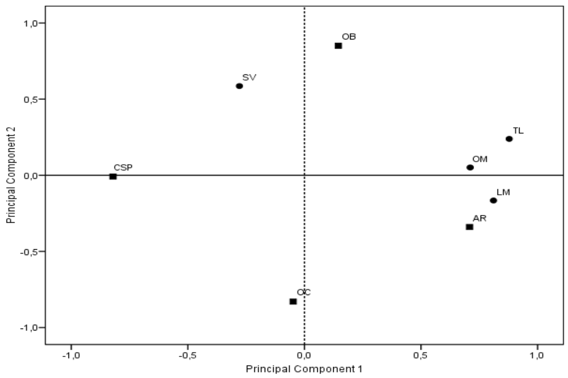

**Figure 2.** Principal component analysis of taxonomic prey category consumption at territory level. Component 1 and 2 (X-axis and Y-axis, respectively) provide information regarding the rotated and dimensionally reduced diet data. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB ("other birds"), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM ("other mammals"). Solid black squares represent frequently consumed prey and solid black circles represent less frequently consumed prey.

## DISCUSSION

The diet of Bonelli's Eagle in Catalonia during the nestling period primarily included medium-sized birds such as pigeons and Red-legged Partridges, mammals including European rabbits and Eurasian red squirrels, as well as a variety of less frequently consumed birds (Yellow-legged Gulls, Corvidae and Turdidae) and a single reptile (ocellated lizard). This diet composition agreed with the general patterns found in other western European populations, where, overall, rabbits, pigeons, partridges, and corvids were the most frequently eaten prey (Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Moleón et al. 2009b), and was particularly similar to diets described for the Mediterranean coastal strip of Spain and France, where rabbits are more scarce and the consumption of pigeons and "other birds" is greater (Moleón et al. 2009b).

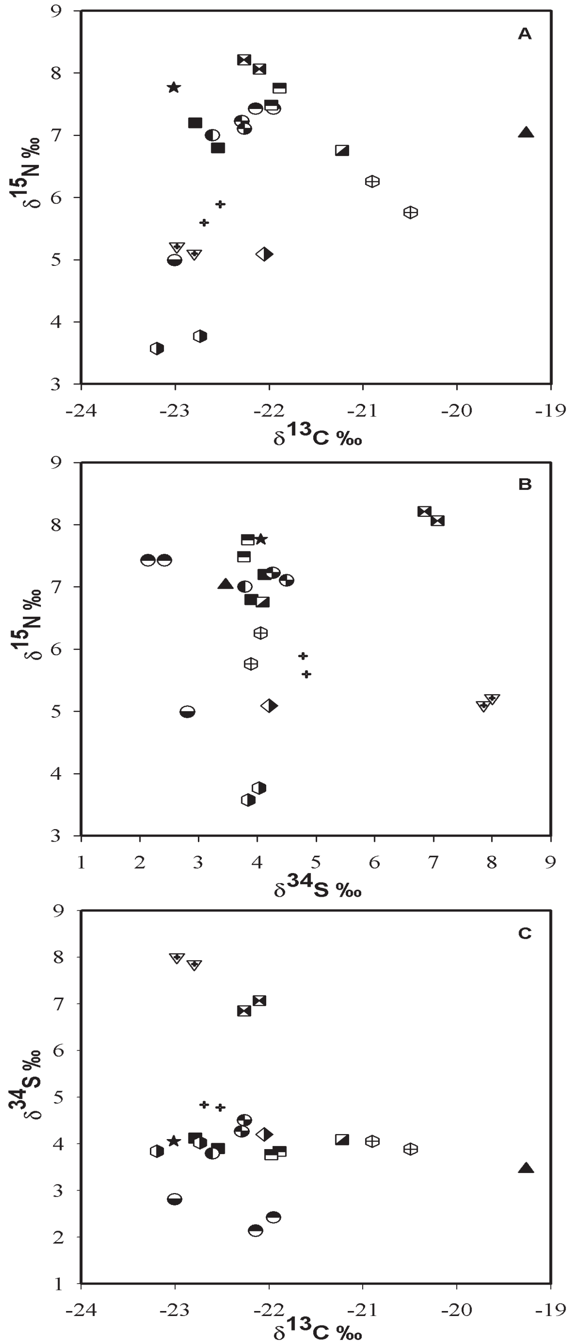

**Figure 3.** Isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of Bonelli's Eagle nestlings. Different symbols are associated with different territories ($n$ = 15); nine territories had two nestlings. (a) $\delta^{15}N$ vs. $\delta^{13}C$, (b) $\delta^{15}N$ vs. $\delta^{34}S$ and (c) $\delta^{34}S$ vs. $\delta^{13}C$.

**Table 3.** Spearman correlation values ($r_s$) for correlations between diet of nestlings as determined by pellet analysis at the territory level and nestlings' isotopic values. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB ("other birds"), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM ("other mammals"). Significant correlations ($P < 0.05$) are shown in bold type.

| | CSP | OC | OB | AR | SV | TL | LM | OM |
|---|---|---|---|---|---|---|---|---|
| $\delta^{13}C$ | 0.411 | -0.197 | 0.057 | **-0.688** | **0.565** | -0.258 | -0.199 | -0.183 |
| *P*-value | 0.128 | 0.480 | 0.840 | **0.005** | **0.028** | 0.353 | 0.477 | 0.514 |
| $\delta^{15}N$ | 0.025 | -0.228 | 0.350 | 0.091 | 0.347 | -0.036 | -0.042 | -0.441 |
| *P*-value | 0.930 | 0.414 | 0.201 | 0.747 | 0.205 | 0.898 | 0.881 | 0.100 |
| $\delta^{34}S$ | 0.021 | 0.196 | 0.057 | 0.039 | -0.338 | 0.060 | 0.157 | -0.460 |
| *P*- value | 0.940 | 0.485 | 0.840 | 0.889 | 0.218 | 0.831 | 0.576 | 0.085 |

In our study, the PCA suggested that the consumption of the two dominant prey types (pigeons and rabbits) determined the intake of other prey species. For example, those territories with low consumption of pigeons had greater intake of alternative prey species such as partridges or, less frequently, Yellow-legged Gulls, ocellated lizard, and small mammals. Similarly, in those territories where rabbits were not frequently consumed, other medium-sized bird species were more important. Variations in diet of Bonelli's Eagle in western Europe seem to be a function of spatio-temporal variation in the abundance of rabbits and the presence of alternative prey species, in conjunction with territorial environmental features (Moleón et al. 2009b). Consequently, the different dietary patterns found in our study at the territory level were likely influenced by the high heterogeneity in ecological features within territories, including habitat, and prey density and distribution.

Stable isotope signatures from nestlings exhibited broad ranges for the three elements we measured ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$), a finding that agreed with the high diversity of taxonomic prey items revealed by the conventional pellet analysis. Consumers incorporate carbon into their tissues with an increase of around 1‰ in $^{13}C$ relative to their food (Kelly 2000) and so the wide range of $\delta^{13}C$ observed in our study (3.76‰) is probably due to a heterogeneous intake of prey species with different carbon isotopic signatures (Gu et al. 1997). Additionally, we found a significant positive correlation between $\delta^{13}C$ and the frequency of Eurasian red squirrels in nestlings' diet, as well as a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges. Interestingly, abundances of these two prey species at territory level are dependent on habitat types, with squirrels more common in forested territories and partridges more abundant in open habitats in our study area (Real et al. 1995, Mañosa 2004); these associations suggest that the analysis of $\delta^{13}C$ may be a good indicator of prey consumption and habitat features at the territory level. In the case of nitrogen, consumers are typically enriched in $^{15}N$ by 3-5‰ relative to their prey (Post 2002, Vanderklift & Ponsard 2003), a fact that allows the trophic level position of the prey species to be assessed (Kelly 2000). In our study, $\delta^{15}N$ ranged from 3.57 to 8.21‰, what suggested that the total diet within our study sample included prey species from at least two different trophic levels. This was supported by the wide range of prey species detected by the conventional pellet analysis, including herbivores (rabbits), granivores (pigeons), secondary consumers (thrushes and Corvidae), and even potential scavengers (Yellow-legged Gulls). Finally, the use of $\delta^{34}S$ in dietary studies has been recommended as a means of distinguishing between terrestrial and marine prey species (Peterson et al. 1985, Moreno et al. 2009). In our study, higher signatures of $\delta^{34}S$ were found at two territories where Yellow-legged Gulls were consumed, and that species was the only marine prey species identified in the pellet analysis. Accordingly, $\delta^{34}S$ signatures of this gull species from the same study area (Ramos et al. 2009) showed similar signatures to those found in Bonelli's Eagle nestlings that consumed it. The lack of significant correlation between $\delta^{34}S$ and the consumption of Yellow-legged Gulls probably resulted from the fact that it was consumed at only 2 of 15 territories.

Our interpretation of the SIA based on the diet composition of Bonelli's Eagle nestlings may be potentially constrained by a number of biases. A basic assumption when using SIA in the assessment of animal diets is that the main prey species have different isotopic composition (Bearhop et al. 2004, Matthews & Mazumder 2004). However, we did not analyze isotopic composition of prey species and instead used indirect evidence to evaluate the suitability of SIA as a means of inferring diet. First, the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of nestlings hatched and raised in the same nest were more similar than would be randomly expected. Given that Bonelli's Eagle nestlings share prey items (Real 1996), our results indicated that the isotopic signatures of nestlings were related to the prey consumed (see also Angerbjörn et al. 1994, Gu et al. 1997, Araújo et al. 2009). Second, we tested whether $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were correlated with prey consumption. In fact, we found significant correlations between $\delta^{13}C$ and two prey species, as well as other dietary patterns for $\delta^{15}N$ and $\delta^{34}S$ (above).

In recent decades, the use of stable isotopes in avian foraging studies has been increasingly used as a robust tool for providing long-term information on birds' foraging habits and degree of dietary specialization at both the individual and population level (Kelly 2000, Bolnick et al. 2002, Rubenstein & Hobson 2004, Inger & Bearhop 2008, Araújo et al. 2009). However, few isotopic studies have focused on raptors' dietary habits (but see Roemer et al. 2002, Dominguez et al. 2003 or Caut et al. 2006), so the advantages of SIA in studies of raptors' trophic ecology are sometimes underestimated. Our study provided the first reference values for isotopic signatures in Bonelli's Eagle nestlings. One advantage of isotopic analyses is that they may overcome some of the biases traditionally associated with conventional procedures. For example, isotopic data from nestlings' feathers are representative of the nestlings' diet over the entire period of tissue development (Inger & Bearhop 2008), whereas pellets may be representative of a shorter period if they are not collected regularly. Moreover, isotopic data inform about prey digested and absorbed, and may overcome the over or under-representation of certain prey items associated with conventional diet analyses (Inger & Bearhop 2008). In terms of effort, the pellet analysis is more time-consuming than isotopic analysis. SIA may also allow assessment of individual's diets, as, for example, when comparing the diet between siblings or between parents and nestlings. Finally, temporal changes or spatial heterogeneity in diet composition can be addressed with SIA (Bearhop et al. 2001, Rubenstein & Hobson 2004, Chiaradia et al. 2010); by analyzing the isotopic composition of nestlings' feathers, we may be able to monitor temporal variations in prey abundance at the territory level. The major disadvantage of SIA in dietary studies where we do not know the isotopic prey signatures is that we cannot distinguish individual prey species in the predators' diet.

Mediterranean landscapes have undergone important changes in terms of human activity and the extent of different types of land use (Meeus 1993, Butet et al. 2010), and such changes have influenced the distribution and abundance of Bonelli's Eagle prey and hence the conservation of this raptor species (Ontiveros et al. 2005, Moleón et al. 2009b). In our study, SIA proved useful for monitoring nestling Bonelli's Eagles' diets, which may reflect the abundance and distribution of prey at the territory level. Thus, the implementation of SIA on a regular basis at the territory level may be a valuable tool for monitoring not only the biological relationship between Bonelli's Eagle and its prey, but also temporal changes in Mediterranean habitats and ecosystems. Future isotopic analyses will provide further insights and a deeper understanding of the trophic ecology of Bonelli's Eagles.

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## REFERENCES

**Angerbjörn, A., Hersteinsson, P., Lidén, K. & Nelson, E.** (1994) Dietary variation in arctic foxes (*Alopex lagopus*) - an analysis of stable carbon isotopes. Oecologia, 99, 226-232.

**Araújo, M.S., Bolnick, D.I., Martinelli, L.A., Giaretta, A.A. & Dos Reis, S.F.** (2009) Individual-level diet variation in four species of Brazilian frogs. Journal of Animal Ecology, 78, 848-856.

**Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H.** (2004) Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology, 73, 1007-1012.

**Bearhop, S., Thompson, D.R., Phillips, R.A., Waldron, S., Hamer, K.C., Gray, C.M., Votier, S.C., Ross, B.P. & Furness, R.W.** (2001) Annual variation in Great Skua diets: the importance of commercial fisheries and predation on seabirds revealed by combining dietary analyses. Condor, 103, 802-809.

**Bearhop, S., Waldron, S., Votier, S.C. & Furness, R.W.** (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiological and Biochemical Zoology, 75, 451-458.

**Becker, B.H., Newman, S.H., Inglis, S. & Beissinger, S.R.** (2007) Diet-feather stable isotope ($\delta^{15}N$ and $\delta^{13}C$) fractionation in Common Murres and other seabirds. Condor, 109, 451-456.

**BirdLife International** (2004) Birds in Europe: population estimates, trends and conservation status. BirdLife Conservation Series No. 12, Cambridge, U.K.

**Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. & Svanbäck, R.** (2002) Measuring individual-level resource specialization. Ecology, 83, 2936-2941.

**Bosch, R., Real, J., Tinto, A., Zozaya, E.L. & Castell, C.** (2010) Home-ranges and patterns of spatial use in territorial Bonelli's Eagles *Aquila fasciata*. Ibis, 152, 105-117.

**Brom, T.G.** (1986) Microscopic identification of feathers and feather fragments of palearctic birds. Bijdragen tot de Dierkunde, 56, 181-204.

**Butet, A., Michel, N., Rantier, Y., Comor, V., Hubert-Moy, L., Nabucet, J. & Delettre, Y.** (2010) Responses of Common Buzzard (*Buteo buteo*) and Eurasian Kestrel (*Falco tinnunculus*) to land use changes in agricultural landscapes of western France. Agriculture, Ecosystems and Environment, 138, 152-159.

**Caut, S., Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecological Applications, 16, 1893-1900.

**Chiaradia, A., Forero, M.G., Hobson, K.A. & Cullen, J.M.** (2010) Changes in diet and trophic position of a top predator 10 years after a mass mortality of a key prey. ICES Journal of Marine Science, 67, 1710-1720.

**Crawford, K., Mcdonald, R.A. & Bearhop, S.** (2008) Applications of stable isotope techniques to the ecology of mammals. Mammal Review, 38, 87-107.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds) (**1994) Handbook of the birds of the world, Vol.2: New World Vultures to Guineafowl. Lynx Editions, Barcelona, Spain.

**Dominguez, L., Montevecchi, W.A., Burgess, N.M., Brazil, J. & Hobson, K.A.** (2003) Reproductive success, environmental contaminants, and trophic status of nesting Bald Eagles in eastern Newfoundland, Canada. Journal of Raptor Research, 37, 209-218.

**Gil-Sánchez, J.M.** (2000) Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). Ardeola, 47, 1-8.

**Gil-Sánchez, J.M., Moleón, M., Otero, M. & Bautista, J.** (2004) A nine-year study of successful breeding in a Bonelli's Eagle population in southeast Spain: a basis for conservation. Biological Conservation, 118, 685-694.

**Gu, B., Schelske, C.L. & Hoyer, M.V.** (1997) Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. Ecology, 78, 2263-2266.

**Hobson, K.A.** (1999) Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: implications for evaluating trophic relationships and breeding origins. Condor, 101, 799-805.

**Iezekiel, S., Bakaloudis, D.E. & Vlachos, C.G.** (2004) The diet of the Bonelli's Eagle *Hieraaetus fascitus* in Cyprus. Pages 581-587 *in* R.D. Chancellor and B.U. Meyburg (eds), Raptors worldwide: proceedings of the World Working Group on Birds of Prey / MME, Berlin, Germany.

**Inger, R. & Bearhop, S.** (2008) Applications of stable isotope analyses to avian ecology. Ibis, 150, 447-461.

**Jaksic, F.M. & Delibes, M.** (1987) A comparative analysis of food-niche relationships and trophic guild structure in two assemblages of vertebrate predators differing in species richness: causes, correlations, and consequences. Oecologia, 71, 461-472.

**Johnsgard, P.A.** (2002) North American owls. Smithsonian Institution Press, Washington, DC, U.S.A.

**Katzner, T.E., Bragin, E.A., Knick, S.T. & Smith, A.T.** (2006) Spatial structure in the diet of Imperial Eagles *Aquila heliaca* in Kazakhstan. Journal of Avian Biology, 37, 594-600.

**Kelly, J.F.** (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78, 1-27.

**Korpimäki, E. & Norrdahl, K.** (1991) Numerical and functional responses of kestrels, Short eared Owls, and Long-eared Owls to vole densities. Ecology, 72, 814-826.

**Krouse, H.R. & Herbert, H.K.** (1988) Sulphur and carbon isotope studies of food webs. Pages 315-322 *in* B.V. Kennedy and G.M. LeMoine (eds), Diet and subsistence: current archaeological perspectives. University of Calgary Archaeology Association, Calgary, AB, Canada.

**Mañosa, S.** (2004) Perdiu roja *Alectoris rufa.* Pages 108-109 *in* J. Estrada, V. Pedrocchi, L. Brotons, and S. Herrando (eds), Atles dels ocells nidificants de Catalunya (1999-2002). Institut Català d'Ornitologia (ICO) and Lynx Editions, Barcelona, Spain.

**Marti, C.D., Bechard, M. & Jaksic, F.M.** (2007) Food habits. Pages 129-151 *in* D.M. Bird and K.L. Bildstein (eds), Raptor research and management techniques. Hancock House Publishers LTD, Surrey, BC, Canada.

**Martínez, J.E., Sánchez, M.A., Carmona, D. & Sánchez, J.A.** (1994) Régime alimentaire de l'aigle de Bonelli *Hieraaetus fasciatus* durant la période de l'élevage des jeunes (Murcia, Espagne). Alauda, 62, 53-58.

**Matthews, B. & Mazumder, A.** (2004) A critical evaluation of intrapopulation variation of $\delta^{13}C$ and isotopic evidence of individual specialization. Oecologia, 140, 361-371.

**Meeus, J.H.A.** (1993) The transformation of agricultural landscapes in western Europe. The Science of Total Environment, 129, 171-190.

**Mizutani, H., Fukuda, M. & Kabaya, Y.** (1992) $^{13}C$ and $^{15}N$ enrichment factors of feathers of 11 species of adult birds. Ecology, 73, 1391-1395.

**Moleón, M., Bautista, J., Sánchez-Zapata, J.A. & Gil-Sánchez, J.M.** (2009a) Diet of non breeding Bonelli's Eagles *Hieraaetus fasciatus* at settlement areas of southern Spain. Bird Study, 56, 142-146.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Charton, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** (2009b) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. Journal of Biogeography, 36, 1502-1515.

**Moreno, R., Jover, L., Munilla, I., Velando, A. & Sanpera, C.** (2009) A three-isotope approach to disentangling the diet of a generalist consumer: the Yellow-legged Gull in northwest Spain. Marine Biology, 157, 545-553.

**Newton, I.** (1998) Population limitation in birds. Academic Press, San Diego, CA, U.S.A.

**Ontiveros, D., Pleguezuelos, J.M. & Caro, J.** (2005) Prey density, prey detectability and food habits: the case of Bonelli's Eagle and the conservation measures. Biological Conservation, 123, 19-25.

**Palma, L., Beja, P., Pais, M. & Cancela da Fonseca, L.** (2006) Why do raptors take domestic prey? The case of Bonelli's Eagle and pigeons. Journal of Applied Ecology, 43, 1075-1086.

**Pearson, S.F., Levey, D.J., Greenberg, C.H. & Martínez del Rio, C.** (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon and isotopic signatures in an omnivorous songbird. Oecologia, 135, 516-523.

**Peterson, B.J. & Fry, B.** (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18, 293-320.

**Peterson, B.J., Howarth, R.W. & Garritt, R.H.** (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science, 227, 1361-1363.

**Post, D.M.** (2002) Using stable isotopes to estimate trophic position: models, methods, and assumption. Ecology, 83, 703-718.

**Quinn, G.P. & Keough, M.J. (eds)** (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, U.K.

**Ramos, R., Ramírez, F., Sanpera, C., Jover, L.**

**& Ruiz, X.** (2009) Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a compartative assessment using conventional and isotopic methods. Marine Ecology Progress Series, 377, 289-297.

**Real, J.** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía. Ph.D. thesis, University of Barcelona, Barcelona, Spain.

**Real, J.** (1996) Biases in diet study methods in the Bonelli's Eagle. Journal of Wildlife Management, 60, 632-638.

**Real, J.** (2004) Águila azor-perdicera, *Hieraaetus fasciatus.* Pages 154-157 *in* A. Madroño, C. González, and J.C. Atienza (eds), Libro rojo de las aves de España. Dirección General para la Biodiversidad-SEO/Birdlife, Madrid.

**Real, J., Piqué, J. & Rodríguez-Teijeiro. J.D.** (1995) Esquirol *Sciurus vulgaris.* Pages 45-50 *in* J. Ruiz-Olmo and A. Aguilar (eds), Els grans mamífers de Catalunya i Andorra. Lynx Editions, Barcelona, Spain.

**Real, J., Tintó, A., Borau, A., Beneyto, A. & Parellada, X.** (2004) Àliga perdiguera *Hieraaetus fasciatus.* Pages 182-183 *in* J. Estrada, V. Pedrocchi, L. Brotons, and S. Herrando (eds), Atles dels ocells nidificants de Catalunya (1999-2002). Institut Català d'Ornitologia (ICO) and Lynx Editions, Barcelona, Spain.

**Rocamora, G.** (1994) Bonelli's Eagle *Hieraaetus fasciatus.* Pages 184-185 *in* G.M. Tucker and M.F. Heath (eds), Birds in Europe: their conservation status. BirdLife International, Cambridge, U.K.

**Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2002) Golden Eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. Proceedings of the National Academy of Science of the United States of America, 99, 791-796.

**Rubenstein, D.R. & Hobson, K.A.** (2004) From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology and Evolution, 19, 256-263.

**Salamolard, M., Butet, A., Leroux, A. & Bretagnolle, V.** (2000) Responses of an avian predator to variations in prey density at a temperate latitude. Ecology, 81, 2428-2441.

**Sánchez, R., Margalida, A., González, L.M. & Oria, J.** (2008) Biases in diet sampling methods in the Spanish Imperial Eagle *Aquila adalberti*. Ornis Fennica, 85, 82-89.

**Vanderklift, M.A. & Ponsard, S.** (2003) Sources of variation in consumer-diet $\delta^{15}$N enrichment: a meta-analysis. Oecologia, 136, 169-182.

**Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. & Furness, R.W.** (2003) Assessing the diet of Great Skuas, *Catharacta skua*, using five different techniques. Polar Biology, 26, 20-26.

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# Stable isotopes in Bonelli's Eagles

# Comparativa de las estimas de dieta según análisis de egagrópilas e isótopos estables en pollos de águila perdicera *Aquila fasciata*

## Resumen

Los análisis de dieta son fundamentales para el estudio de la ecología trófica de las aves, y la contribución a tal efecto de los análisis de isótopos estables ha sido creciente en las últimas dos décadas. Pocos estudios isotópicos han evaluado la dieta de aves rapaces, que habitualmente se ha analizado mediante métodos convencionales como el análisis de egagrópilas. En este estudio, se comparan las estimas de consumo de presas de pollos de águila perdicera *Aquila fasciata* calculadas mediante análisis de egagrópilas (en términos de ítems y biomasa) y modelos isotópicos mixtos (SIAR) utilizando $\delta^{13}C$, $\delta^{15}N$ y $\delta^{34}S$ medidos en las plumas. El análisis de egagrópilas mostró que el conejo europeo *Oryctolagus cuniculus*, las palomas (principalmente paloma torcaz *Columba palumbus* y paloma bravía *Columba livia* dom.), la perdiz roja *Alectoris rufa*, varias especies de paseriformes, la gaviota patiamarilla *Larus michahellis* y la ardilla roja europea *Sciurus vulgaris* fueron las principales categorías de presas, por lo que fueron seleccionadas para la reconstrucción de la dieta en SIAR. A nivel de población, las estimas promedio de consumo de presas fueron similares según el análisis de egagrópilas (tanto ítems como biomasa) y SIAR. A nivel de territorio, el coeficiente kappa ponderado mostró buena concordancia en la escala ordinal del consumo de presas entre ítems o biomasa y SIAR. Aunque el coeficiente de correlación intraclase mostró poca concordancia entre métodos al considerar todas las presas en el mismo análisis, los coeficientes de correlación intraclase para cada categoría de presa por separado mostraron una concordancia significativa en el consumo de conejos, palomas y gaviotas, con una menor concordancia para las estimas de paseriformes y ardillas. Finalmente, para las estimas de consumo de perdiz no hubo concordancia entre métodos. Nuestros resultados sugieren una buena concordancia global en las estimas de la dieta de los pollos de águila perdicera según análisis de egagrópilas y SIAR a nivel poblacional y, en menor medida, a nivel territorial, apoyando la utilidad de los modelos isotópicos mixtos para identificar las presas terrestres y marinas en la dieta de aves rapaces.

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# Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet

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**ABSTRACT—** Diet analyses are central to the study of avian trophic ecology, and stable isotope analyses have made an increasing contribution in the last two decades. Few isotopic studies have assessed the diet of raptor species, which are more frequently analysed by conventional diet methods such as pellet analysis. In this study, we compare prey consumption estimates of nestling Bonelli's Eagles *Aquila fasciata* from conventional pellet analysis (in terms of items and biomass) and stable isotopic mixing models (SIAR) using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of feathers. The pellet analysis showed that European Rabbits *Oryctolagus cuniculus*, pigeons (mainly Common Wood Pigeons *Columba palumbus* and Domestic Pigeons *Columba livia* dom.), Red-legged Partridges *Alectoris rufa*, passerines, Yellow-legged Gulls *Larus michahellis* and Eurasian Red Squirrels *Sciurus vulgaris* were the main prey, so they were selected for diet reconstructions in SIAR. At the population level, mean prey consumption estimates were similar for pellets (both items and biomass) and SIAR. At the territory level, the weighted kappa statistic showed good ordinal scale agreement in main prey consumption between items or biomass and SIAR. Although the intraclass correlation coefficient showed poor method agreement when considering all prey in the same analysis, the intraclass correlation coefficients for each prey category showed significant agreement between pellets and SIAR when estimating the consumption of Rabbits, pigeons and Gulls, with lower agreement for passerines and Squirrels. Lastly, there was poor method agreement for estimates of Partridges. Our results suggest an overall agreement between the pellet analysis and SIAR when estimating nestling Bonelli's Eagle diet at both the population and, to a lesser extent, the territory level, supporting the usefulness of isotopic mixing models when identifying the terrestrial and marine components of raptor diets.

# Chapter 1

## INTRODUCTION

Animal foraging ecology explains much of the observed variation among individual fitness correlates such as body condition, survival and breeding success (Schoener 1971, Pyke 1984, Inger et al. 2008, Terraube et al. 2012). As such, it can also explain population dynamics, prey-predator relationships and species distributions (Newton 1998, Moleón et al. 2009, Cortés-Avizanda et al. 2011). Nevertheless, measuring diet is often challenging due to the difficulty of making direct observations of feeding events over long periods, with consequent reliance on indirect methods and their potential biases (see Real 1996, Votier et al. 2003, Huang et al. 2006).

The most common methods of diet assessment in birds are direct observations of feeding habits, and analyses of nest food remains, individual stomach contents, faecal droppings and regurgitated pellets (Marti et al. 2007, Maziarz & Wesolowski 2010, Michalski et al. 2011, Bourass et al. 2012), although these methods have a number of limitations (Real 1996, Votier et al. 2003). For instance, they usually involve a great effort in terms of data collection and analysis. Moreover, they often reflect only a snapshot of a consumer's diet (Inger & Bearhop 2008) and present potential biases linked to prey sizes or digestibility (Brown & Ewins 1996, Real 1996, Votier et al. 2003, Marti et al. 2007).

Over the last two decades, the use of stable isotope analysis (SIA) to study avian trophic ecology has increased considerably (Kelly 2000, Inger & Bearhop 2008, Hobson 2011). The isotopic ratios in bird tissue reflect its diet at the time of tissue synthesis in a predictable manner. The shift in isotope ratio between diet and consumer tissue is known as the trophic enrichment factor (TEF) and can be used in isotopic mixing models to quantify the relative contributions of isotopically distinct sources to the diet of individuals or populations (Inger et al. 2006, Moreno et al. 2010). More recently, Bayesian isotopic mixing models have been developed to account for uncertainty and variation in model estimates, allow for multiple dietary sources, and generate potential dietary solutions as true probability distributions (Moore & Semmens 2008, Jackson et al. 2009, Parnell et al. 2010). Nevertheless, the use of isotopic mixing models requires accurate prior information regarding the trophic ecology of the studied species, and dietary estimates from mixing models would be only as good as the assumptions and parameters on which they depend (Bond & Diamond 2011, Hobson 2011).

Despite the applicability of both conventional diet analyses and isotopic mixing models to determine avian diets, and the caveats and potential biases associated with each, few studies have compared these methods (but see Doucette et al. 2011, Steenweg et al. 2011, Weiser & Powell 2011). Moreover, although conventional methods have been used traditionally to assess raptor food habits (Real 1996, Marti et al. 2007, Sánchez et al. 2008, Bakaloudis et al. 2012), to date few isotopic studies have focused on assessing the diets of avian terrestrial predators, including most raptor species (but see Roemer et al. 2002, Caut et al. 2006). Consequently, the potential applicability of isotopic mixing models to assess raptor foraging ecology is still poorly understood. The fact that isotopic data inform about assimilated rather than just ingested prey is a major advantage of using isotopic analysis to study raptor diet. Moreover, isotopic mixing models provide a powerful tool to estimate the foraging ecology of individuals to test the incidence and implications of individual resource use (Bolnick et al. 2003). Finally, isotopic analysis may constitute a homogeneous sampling procedure to monitor temporal or spatial variation in raptor diets.

Bonelli's Eagle *Aquila fasciata* is distributed from the western Mediterranean to southeast

Asia (del Hoyo et al. 1994). The European population is now classified as endangered after a marked decline in number and range in recent decades (BirdLife International 2004), related to unnaturally high mortality rates, habitat degradation, and decline of their main prey species (Real 2004, Hernández-Matías et al. 2011). The diet of Bonelli's Eagle in the Mediterranean has been widely studied by conventional methods, showing that the species mainly predates European Rabbits *Oryctolagus cuniculus*, partridges *Alectoris spp.*, pigeons *Columba spp.*, passerines (mainly corvids and thrushes), and lizards (Real 1991, Moleón et al. 2009). More recently, an isotopic approach showed that $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S are useful to assess both terrestrial and marine prey consumption of Bonelli's Eagle nestlings (Resano et al. 2011). Consequently, this species is a suitable model to test whether conventional diet analysis and isotopic mixing models provide similar information when assessing the diet of avian predators.

The aim of this study was to compare prey consumption estimates of nestling Bonelli's Eagles using conventional pellet analysis and Bayesian isotopic mixing models by (1) performing a comprehensive pellet analysis in terms of prey item consumption and prey assimilated biomass, (2) characterizing the isotopic composition of main prey types and (3) comparing main prey consumption estimates obtained from the pellet analysis and the isotopic mixing models.

## METHODS

### Study area and data collection

From 2008 to 2010 we monitored 43 successful breeding attempts of 28 territorial pairs of Bonelli's Eagle in Catalonia (41º20'N 01º32'E). Habitat characteristics differed between territories but all showed Mediterranean landscape features (Carrascal & Seoane 2009, Bosch et al. 2010), with an average annual rainfall ranging from 425 to 664 mm. All sampled nests were located on cliffs, and the altitude of nesting areas ranged from 30 to 776 m asl.

From January to early March, we monitored breeding territories to assess occupancy and breeding activity. In late March and April, occupied nests were checked to detect the number of nestlings and their age, which was estimated by feather development and laying date (Real 1991, Gil-Sánchez 2000). To minimise the risk of disturbance, observations were always carried out from long distances using 10x binoculars and 20-60x spotting scopes. Once nestlings were approximately 37 days old, we caught them with the assistance of experienced climbers and sampled 3–4 mantle feathers from each individual for SIA. Pellets were collected from the nests after the breeding season and analysed to determine nestling diet by conventional methods (Real 1996).

To characterise isotopically the main prey of Bonelli's Eagle, we collected muscle samples from 215 individuals of the following species or species groups during 2008-2011: European Rabbits ($n$ = 42), Red-legged Partridges *Alectoris rufa* ($n$ = 38), Common Wood Pigeons *Columba palumbus* ($n$ = 39), Domestic Pigeons *Columba livia dom.* ($n$ = 45), passerines (Corvidae, Sturnidae and Turdidae) ($n$ = 40), Yellow-legged Gulls *Larus michahellis* ($n$ = 4) and Eurasian Red Squirrels *Sciurus vulgaris* ($n$ = 7). All individuals were obtained from the studied Eagles' breeding territories, either in the nests or their surroundings, except most passerines and some Squirrels, which came from rehabilitation centres located in the study area.

### Pellet analysis

Each prey item identified in each pellet was counted as one item (Real 1996, Gil-Sánchez et al. 2004). Pellet contents (i.e. feathers, bones, hair, nails and scales) were identified with the help of a reference collection, a 4x magnifying glass, and consulting specialised guides (Brom 1986, Brown et al. 2003). Prey items were identified to species level whenever possible.

Prey consumption was estimated for any given territory and year (hereafter referred to as territory level; *n* = 43) as percentages of total items and total biomass, as is common in raptor diet studies (Real 1996, Sánchez-Zapata & Calvo 1998). To calculate the biomass of each prey type we used the weights of each prey species, corrected for the degree of consumption by adults at the nest before delivering prey to the chicks. Mean weights of prey species were obtained from the literature (Brom 1986, Real 1991, del Hoyo et al. 1997), most estimates being from measurements of individuals from the study area (see Supporting Information Table S1). Consumption of each prey type was estimated on the basis of field observations of feeding events from a hide (*n* = 182 prey items; J. Real unpubl. data). Therefore our final prey net biomass estimates (Table S1) were representative of nestlings' ingested biomass rather than total prey biomass. More than 20 prey items in each territory and year were included to ensure reliability in the pellet analysis (Ontiveros et al. 2005).

Based on the results of pellet analysis, the most-consumed prey categories in terms of items or biomass (hereafter main prey categories) were selected for comparison using the two diet assessment methods: European Rabbits, pigeons (mostly Common Wood Pigeons and Domestic Pigeons), Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels. Main prey consumption values from either the items or the biomass approaches were re-scaled relative to their global percentage in each territory to ensure that main prey categories accounted for the 100% of the diet in each territory. These re-scaled values were used for comparison with estimates obtained from the isotopic mixing models.

### Prey type isotopic characterisation

Isotopic signatures of species may be influenced by their local environment (Connolly et al. 2004, Choi et al. 2007), and hence isotopic values of main prey categories may differ between Bonelli's Eagle territories. To test this, prey samples of the most widely consumed prey (rabbits, pigeons and partridges) were characterised as a function of proximity to the sea and to habitat in the eagle territory from which they came. Territories located on coastal cliffs or in coastal mountain ranges were classified as marine, and those farther inland as terrestrial. Territory habitat was measured in a 3.3 km radius around the nest to represent the home range used by Bonelli's Eagles (Bosch et al. 2010), and each territory was classified according to its predominant habitat as either forest, scrubland or agricultural. Habitat predominance was estimated using MiraMon v6.4 software (Pons 2002) from land cover data available at a scale of 1 : 3000 and updated in December 2009.

### Stable isotope analysis

A 3-cm$^3$ piece of muscle from the chest or leg of each sampled prey animal was lyophilized for 48 hours. Samples were lipid extracted using several chloroform-methanol (2:1) rinses following Folch et al. (1957). Muscle was ground into fine powder using an impactor mill (Freezer/mill 6750 Spex Certiprep), and subsamples of approximately 0.32 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 5.6 mg (for $\delta^{34}S$) were loaded in tin receptacles and crimped for combustion. Nestling feathers were first cleaned in a solution of NaOH (0.25M) and oven-dried at 40ºC for 24hr. Feathers were ground into fine powder and subsamples of 0.35

mg (for $\delta^{13}C$ and $\delta^{15}N$) and 3.7 mg (for $\delta^{34}S$) were loaded in tin receptacles before combustion. Isotopic measurements of both prey and nestlings were performed at the Scientific and Technological Centers of the University of Barcelona using the methods of Resano et al. (2011).

Stable isotope ratios are reported as δ values and expressed in ‰, according to the following equation: $\delta X = [(Rsample / Rstandard) - 1] \times 1000$, where X is $^{13}C$, $^{15}N$ or $^{34}S$ and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. Rstandard is the ratio of the international standards: Pee Dee Belemnite (PDB) for $^{13}C$, atmospheric nitrogen (AIR) for $^{15}N$ and Canyon Diablo Troilite (CDT) for $^{34}S$. Measurement precisions for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were ≤ 0.15‰, ≤ 0.25‰ and ≤ 0.40‰, respectively.

### Bayesian isotopic mixing models

We used the SIAR package for R (Parnell et al. 2010) to estimate the relative contribution of main prey categories to the diet of Bonelli's Eagle nestlings at the territory level. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from nestlings and prey were included in the models. Each nest and year was considered a single statistical observation by estimating the mean isotopic values of sampled siblings. Prey isotopic values were selected for each territory: either the overall mean prey values when no effect of environmental features on prey signature was detected, or different values for a single prey when their isotopic values were affected by environmental features (see below). The TEFs for $\delta^{13}C$ (2.1‰ ± 0.08 sd) and $\delta^{15}N$ (2.7‰ ± 0.5 sd) were those obtained for feathers of Peregrine Falcons *Falco peregrinus* fed on muscle of Japanese Quail *Coturnix japonica* (Hobson & Clark 1992). We selected those values because the consumer in that experiment was taxonomically related to our consumer species, and the tissues analysed from both consumers and prey also matched those we studied. The TEF for $\delta^{34}S$ (0‰ ± 0.5 sd) was also obtained from the literature (Michener & Lajtha 2007), where it is commonly assumed that there is no enrichment in $^{34}S$ in animal diets. Common Wood Pigeon and Domestic Pigeon were included as separate sources within the models, and their consumption estimates from SIAR were summed *a posteriori* to allow for direct comparison with the pellet data.

### Statistical analyses

Prey isotopic data were checked for departures from normality using the Kolmogorov-Smirnov test and Q-Q plots. We performed a multivariate analysis of variance (MANOVA) to assess whether prey isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ as the dependent variables) differed by species (fitted as a single factor with Rabbits, Common Wood Pigeons, Domestic Pigeons, Partridges, passerines, Gulls and Squirrels as the group categories; $n = 215$). Additionally, we performed a second MANOVA to assess the effect of species, sea proximity and habitat (fitted as fixed effects) on prey isotopic values, but only including the most widely consumed prey (Rabbits, Common Wood Pigeons, Domestic Pigeons and Partridges; $n = 164$). Passerines, Gulls and Squirrels were excluded from this analysis because they were rare in some territory categories. Those factors with a significant effect on the MANOVAs were subjected to one-way analysis of variance (ANOVA) to test the factor effect on each dependent variable ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) separately (Quinn & Keough 2002). Levene's test was used to detect heteroscedasticity and Welch's correction was applied accordingly. Post-hoc pairwise analysis included Tukey's procedure or the Tamhane test when variances were heterogeneous. Prey isotopic values are reported as means ± sd.

To estimate prey consumption at the

population level by the pellet analysis, we first calculated mean diet for each territory (*n* = 28), and then a mean was calculated across all territories. Re-scaled values of the main prey categories (for both items and biomass) and SIAR estimates were used for method's comparisons (*n* = 43 territory-years) both at the population and the territory levels. These prey consumption percentages were arcsine transformed and checked for normality using the Kolmogorov-Smirnov test and Q-Q plots. At the population level, a two-way ANOVA was used to assess whether prey consumption estimates differed by prey category and dietary method, with prey consumption estimates from all territories as the dependent variable, and both prey category and dietary method as fixed factors. Additionally, separate one-way ANOVAs were used to test the method effect on each prey category. At the territory level, the weighted kappa statistic (Kw) was used to assess agreement between methods on an ordinal scale by ordering main prey categories from higher to lower rates of consumption, and the intraclass correlation coefficient (ICC) was used to test for the agreement of two methods in their quantitative prey consumption estimates. In this regard, we first calculated the ICCs using a three-way mixed effects model (Zhou et al. 2011) with prey consumption values as the dependent variable, territory and method as random effects, and prey category as a fixed factor. Bland-Altman plots (Bland & Altman 1986) were created to represent the method's repeatability in prey estimates. Secondly, ICCs were calculated by a two-way mixed effects model (McGraw & Wong 1996) for each prey category.

Statistical analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL, USA) and MedCalc 12.3.0 (MedCalc Software, Mariakerke, Belgium). SIAR was run using R software (R Development Core Team 2007).

## RESULTS

### Pellet analysis

We identified 2254 prey items in the 979 pellets analysed, corresponding to at least 31 prey species (Table S1). Birds accounted for 59.3%, mammals for 33.6% and reptiles for 7.1% of prey items, and 55.2%, 40.8% and 4% of biomass, respectively. At the population level, the most frequently consumed prey were pigeons (26.3%), Rabbits (21.1%), passerines (10.7%) and Red-legged Partridges (10.6%), which together accounted for 68.7% of dietary items. In terms of biomass, Rabbits were the main prey item (30.9%), followed by pigeons (26.9%), Yellow-legged Gulls (8.7%), Partridges (8.1%) and Squirrels (4.9%), together accounting for 79.5% of total biomass ingested.

### Prey isotopic characterization

There was a significant difference between main prey items in isotopic values (MANOVA: Wilk's lambda, $F_{18,583} = 17.56$, $P < 0.001$). There were overall differences between prey categories in $\delta^{13}C$ (one-way ANOVA: $F_{\text{Welch } 6,30} = 48.63$, $P < 0.001$), but Red-legged Partridges, Common Wood Pigeons and passerines, and Domestic Pigeons, Eurasian Red Squirrels and Yellow-legged Gulls formed two sub-groups of prey within which pairwise differences were not significant. Overall significant differences in $\delta^{15}N$ (one-way ANOVA: $F_{8,206} = 22.20$, $P < 0.001$) were related to prey trophic level. For instance, $\delta^{15}N$ in Rabbits was significantly lower than in other prey except Squirrels, and Yellow-legged Gulls had significantly higher $\delta^{15}N$ than most prey. Fewer pairwise differences in $\delta^{15}N$ were found between Squirrels, Partridges, pigeons or passerines. Yellow-legged Gulls showed the highest $\delta^{34}S$ values, and most of the significant differences in $\delta^{34}S$ (one-way ANOVA: $F_{8,36}$ =

**Table 1.** $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S (mean ± sd; ‰) in main prey types of Bonelli's Eagle in Catalonia: European Rabbits (OC), Common Wood Pigeons (CP), Red-legged Partridges (AR), Domestic Pigeons (CL), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Within prey types, significant values differing either by sea proximity, habitat or both, are shown in bold type. Samples from marine or terrestrial territories are shown as (m) or (t), respectively. Samples from different habitat types are shown as forest (1), scrubland (2) or agricultural (3). Mean prey values were considered when there was no significant influence of environmental features. Prey $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S values listed in this table were those included into the SIAR, accordingly selected for each territory depending on their sea proximity or habitat type.

| Prey | $\delta^{13}$C | $\delta^{15}$N | $\delta^{34}$S |
|---|---|---|---|
| OCm | -25.33 ± 1.02 | 3.17 ± 2.50 | **8.81 ± 2.62** |
| Oct | -25.33 ± 1.02 | 3.17 ± 2.50 | **5.22 ± 2.07** |
| CP | -23.98 ± 0.88 | 5.54 ± 1.75 | 5.33 ± 1.68 |
| AR | -23.32 ± 1.30 | 5.81 ± 1.77 | 6.10 ± 2.59 |
| CLm1 | -21.28 ± 3.88 | 7.15 ± 2.03 | **6.38 ± 0.73** |
| CLt1 | -21.28 ± 3.88 | 7.15 ± 2.03 | **4.74 ± 1.34** |
| CLm2 | -21.28 ± 3.88 | **6.85 ± 0.77** | **6.38 ± 0.73** |
| CLt2 | -21.28 ± 3.88 | **6.85 ± 0.77** | **4.74 ± 1.34** |
| CLm3 | -21.28 ± 3.88 | **8.06 ± 1.37** | **6.38 ± 0.73** |
| CLt3 | -21.28 ± 3.88 | **8.06 ± 1.37** | **4.74 ± 1.34** |
| PAS | -23.36 ± 0.72 | 7.25 ± 1.24 | 6.51 ± 0.90 |
| LM | -20.50 ± 0.55 | 9.35 ± 0.55 | 11.97 ± 3.88 |
| SV | -19.36 ± 1.60 | 3.23 ± 1.99 | 5.47 ± 2.08 |

7.45, P < 0.001) seemed to be related to a marine influence (see below). Mean prey isotopic values included into the isotopic mixing models are summarised in Table 1. Isotope biplots ($\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S) showed that Bonelli's Eagle nestlings lay within the space delineated by main prey categories previously corrected by TEFs (Fig. 1).

In the second MANOVA we found a significant overall effect of species (MANOVA: Wilk's lambda, $F_{9,355}$ = 16.11, *P* < 0.001), sea proximity (MANOVA: Wilk's lambda, $F_{3,146}$ = 9.92, *P* < 0.001), and habitat (MANOVA: Wilk's lambda, $F_{6,292}$ = 7.90, *P* < 0.001) on isotopic prey values, with a

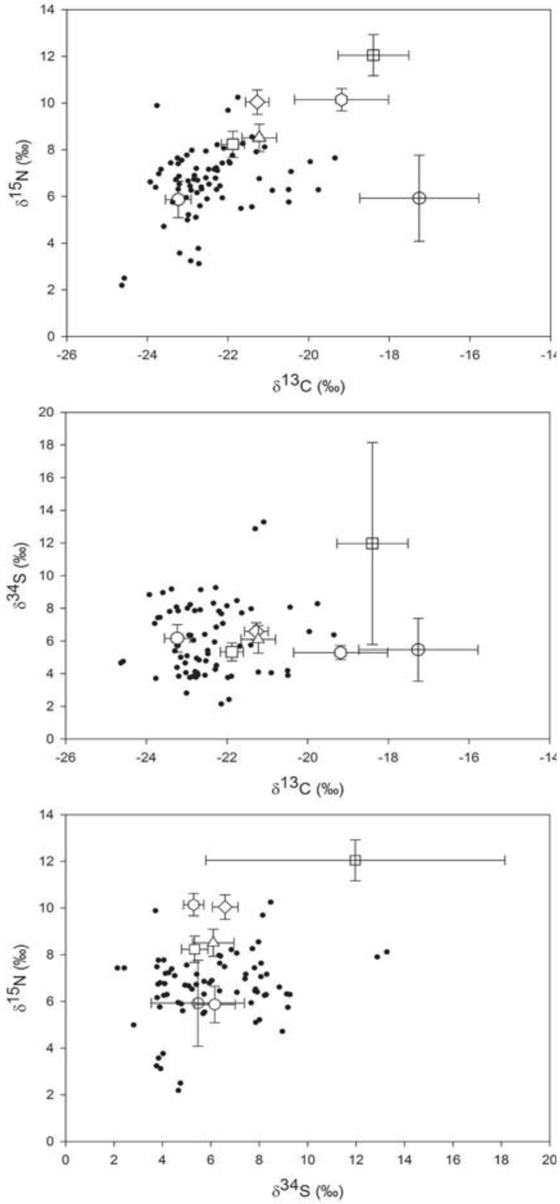

**Figure 1.** Isotopic values ($\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S) of Bonelli's Eagle nestlings and main prey types (mean ± 95% CI) in Catalonia. Open symbols represent main prey types: European Rabbits (open circle), Common Wood Pigeons (open square), Red-legged Partridges (open triangle), Domestic Pigeons (open hexagon), passerines (open rhombus), Yellow-legged Gulls (crossed square) and Eurasian Red Squirrels (crossed circle). Closed dots represent Bonelli's Eagle nestlings. Prey isotopic values are corrected by TEFs (2.1‰ for $\delta^{13}$C, 2.7‰ for $\delta^{15}$N and 0‰ for $\delta^{34}$S).

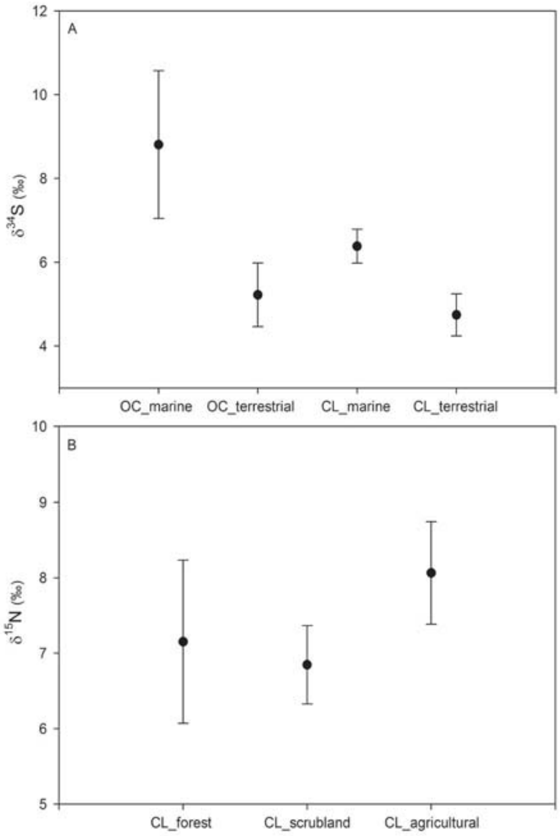

**Figure 2.** Influence of territorial environmental features on prey isotopic values. (A) Significant differences for $\delta^{34}$S in European Rabbits (OC) and Domestic Pigeons (CL) from marine and terrestrial territories. (B) Significant differences for $\delta^{15}$N in Domestic Pigeons (CL) from territories with scrubland and agricultural predominance. Domestic Pigeons from territories with forest predominance are shown, although they were not significantly different from any other habitat category. Prey isotopic values are represented as mean ± 95% CI.

significant interaction between species and both sea proximity (MANOVA: Wilk's lambda, $F_{9,355} = 3.97$, $P < 0.001$) and habitat (MANOVA: Wilk's lambda, $F_{18,413} = 1.78$, $P < 0.05$). European Rabbits and Domestic Pigeons from marine territories had higher $\delta^{34}$S than those from terrestrial territories (one-way ANOVA: $F_{1,40} = 21.12$, $P < 0.001$ and $F_{1,43} = 19.28$, $P < 0.001$, respectively) (Fig. 2). Moreover, Domestic Pigeons from agricultural territories had higher $\delta^{15}$N than those from scrubland territories (one-way ANOVA: $F_{\text{Welch } 2,27} = 4.60$, $P < 0.05$) (Fig. 2). On the other hand, isotopic values of Rabbits were not influenced by habitat, and neither sea proximity nor habitat features influenced isotopic values of Common Wood Pigeons or Red-legged Partridges.

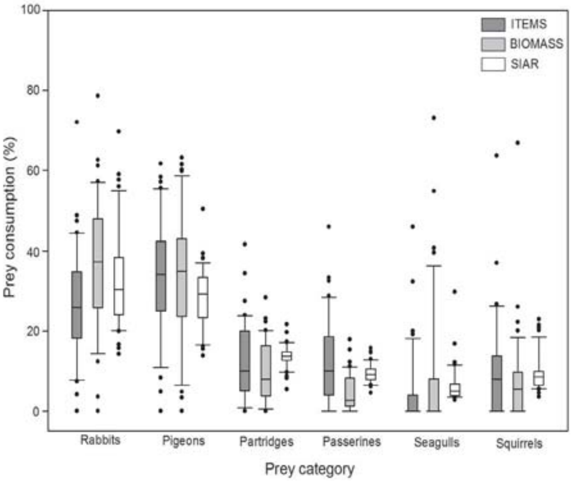

**Figure 3.** Boxplot with the consumption of main prey categories estimated by ITEMS (dark grey), BIOMASS (light grey) and SIAR (white) at the population level.

### Comparison of pellet analysis and SIAR

At the population level, mean consumption estimates differed by prey category (two-way ANOVA: $F_{5,\ 756} = 145.76$, $P < 0.001$), and dietary method (two-way ANOVA: $F_{2,756} = 8.01$, $P < 0.001$). Although there was a significant interaction between prey category and the dietary method effects (two-way ANOVA: $F_{10,\ 756} = 4.38$, $P < 0.001$), all methods estimated a similar dietary pattern of higher consumption of Rabbits and pigeons, and lower consumption of Partridges, passerines, Gulls and Squirrels (Fig. 3, Supporting Information Table S2). When comparing methods for each prey

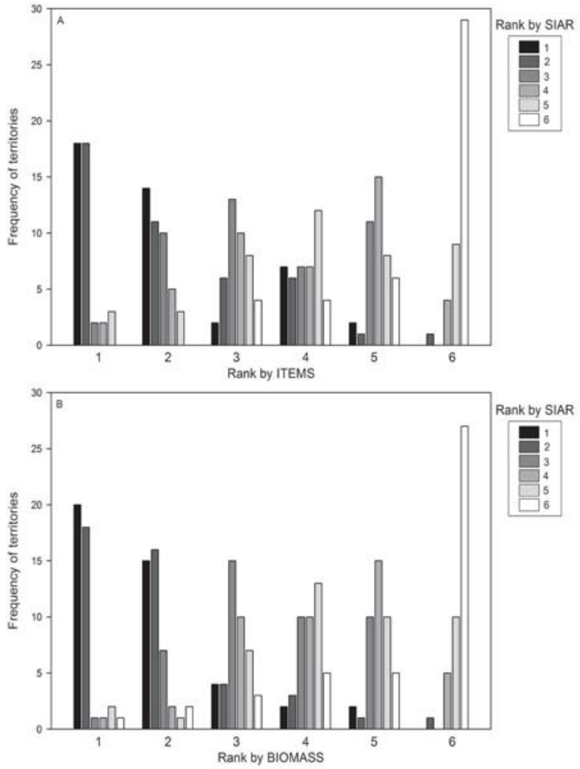

**Figure 4.** Agreement between ITEMS and SIAR (A) or BIOMASS and SIAR (B) in main prey consumption estimates when these are ranked in an ordinal scale (from higher to lower consumption) at the territory-year level. For the ITEMS and BIOMASS approaches, main prey categories are scaled from higher (1) to lower (6) consumption. According to SIAR, prey categories are scaled from higher (black) to lower (white) consumption. The x-axis shows all the rank combinations between methods (i.e. colours and numbers). The y-axis shows the number of territories (frequency) in which any of the colour-number combinations occurred.

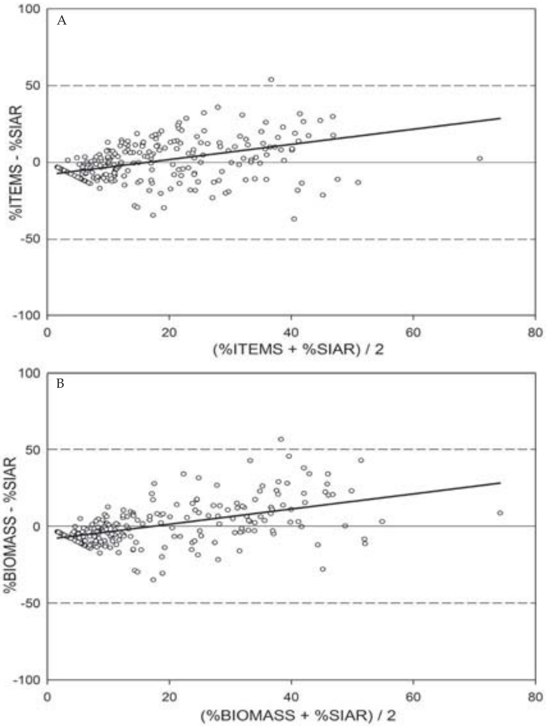

**Figure 5.** Bland-Altman plots showing the agreement between ITEMS and SIAR (A) or BIOMASS and SIAR (B) in main prey consumption estimates (%) at the territory-year level. The y-axis shows the difference in prey consumption estimates between ITEMS and SIAR (A) or BIOMASS and SIAR (B). The x-axis shows mean prey consumption estimates from both methods: (ITEMS+SIAR)/2 (A) or (BIOMASS+SIAR)/2 (B). Solid black lines at 0 indicate total method's agreement (i.e. both methods estimated the same prey consumption percentage), whereas dashed lines at ±50 indicate a disagreement in the method's prey estimates higher or lower than 50%. The linear trend between the variables plotted is shown.

category (i.e. prey item counts from pellets vs. biomass estimation from pellets vs. SIAR), there were significant differences between prey item counts and biomass estimation in Rabbits (one-way ANOVA: $F_{2,\ 126} = 4.47$, $P < 0.05$), between biomass estimation and SIAR in Partridges (one-way ANOVA: $F_{\text{Welch}\ 2,\ 64} = 9.09$, $P < 0.001$), between biomass estimation and both prey item counts and SIAR in passerines (one-way ANOVA: $F_{\text{Welch}\ 2,\ 64} = 22.06$, $P < 0.001$), between prey item counts and SIAR in Gulls (one-way ANOVA: $F_{\text{Welch}\ 2,\ 67} = 12.80$, $P < 0.001$), and between biomass estimation and SIAR in Squirrels (one-way ANOVA: $F_{\text{Welch}\ 2,\ 68} = 3.87$, $P < 0.05$).

At the territory level, we found good agreement when ordering main prey categories from higher to lower levels of consumption between both prey item counts and SIAR (Kw = 0.47, 95% CI = 0.40-0.54) and between biomass estimation and SIAR (Kw = 0.53, 95% CI = 0.46-0.59). In both comparisons, the highest agreement was found when estimating the most- or least-consumed prey categories (1 or 6), with lower agreement for the other prey categories (Fig. 4).

The overall ICC showed low agreement among prey estimates when comparing prey item counts and SIAR (ICC = 0.30, $P = 0.13$) or biomass estimation and SIAR (ICC = 0.29, $P = 0.14$; Table 2). Bland-Altman plots illustrated a significant positive correlation between the difference in prey estimates between methods (items – SIAR or biomass – SIAR) and the mean prey consumption values obtained from both methods ($r_s = 0.35$, $P < 0.001$ for prey item counts vs. SIAR and $r_s = 0.47$, $P < 0.001$ for biomass estimation vs. SIAR; Fig. 5). In other words, the pellet analysis (in terms of both items and biomass) estimated lower consumption rates than SIAR for less-consumed prey, whereas the opposite was true for more-consumed prey.

When assessing agreement between methods for each prey category, we found agreement between prey item counts and SIAR estimates for Rabbits (ICC = 0.42, $P < 0.05$), pigeons (ICC = 0.44, $P < 0.05$) and Gulls (ICC = 0.55, $P < 0.01$), but no significant agreement between methods for passerines (ICC = 0.21, $P = 0.22$) or Squirrels (ICC = 0.27, $P = 0.16$). Similarly, the biomass estimation and SIAR approaches showed a significant agreement for pigeons (ICC = 0.43, $P < 0.05$) and Gulls (ICC = 0.43, $P < 0.05$), but not for Rabbits (ICC = 0.31, $P = 0.11$), passerines (ICC = 0.29, $P = 0.14$) or Squirrels (ICC = 0.29, $P = 0.14$). Lastly, there was poor agreement for estimates of Partridges, both between prey item counts and SIAR (ICC = -0.32, $P = 0.81$) and between biomass estimation and SIAR (ICC = -0.18, $P = 0.71$; Table 2).

**Table 2.** Intraclass correlation coefficients (ICCs) and P-values (P) when comparing prey consumption estimates at the territory level between ITEMS and SIAR, or between BIOMASS and SIAR. Results include both the overall intraclass correlation (n = 516), and the intraclass correlation done by prey (n = 43). Prey categories are European Rabbits (OC), pigeons (CSP), Red-legged Partridges (AR), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Significant P-values are shown in bold type.

| | ICCs | *P* |
|---|---|---|
| Overall intraclass correlation | | |
| ITEMS vs. SIAR | 0.301 | 0.125 |
| BIOMASS vs. SIAR | 0.286 | 0.139 |

| | ICCs | *P* |
|---|---|---|
| Intraclass correlation by prey | | |
| OC – ITEMS vs. SIAR | 0.418 | **0.042** |
| CSP – ITEMS vs. SIAR | 0.437 | **0.033** |
| AR – ITEMS vs. SIAR | -0.316 | 0.812 |
| PAS – ITEMS vs. SIAR | 0.210 | 0.224 |
| LM – ITEMS vs. SIAR | 0.549 | **0.006** |
| SV – ITEMS vs. SIAR | 0.267 | 0.159 |
| OC – BIOMASS vs. SIAR | 0.314 | 0.113 |
| CSP – BIOMASS vs. SIAR | 0.430 | **0.036** |
| AR – BIOMASS vs. SIAR | -0.183 | 0.706 |
| PAS – BIOMASS vs. SIAR | 0.286 | 0.140 |
| LM – BIOMASS vs. SIAR | 0.432 | **0.035** |
| SV – BIOMASS vs. SIAR | 0.289 | 0.137 |

## DISCUSSION

We assessed the diet of Bonelli's Eagle nestlings in Catalonia, which included both marine and terrestrial prey, using conventional pellet analysis and Bayesian isotopic mixing models based on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. The pellet analysis revealed that European Rabbits, pigeons, Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels were the main prey items, and these were sampled for SIA. Our prey isotopic characterization accounted for the effect of environmental features (Table 1), and allowed reliable use of SIAR to estimate prey consumption at the territory level.

Our results show an overall agreement in main prey consumption estimates between the pellet analysis and SIAR both at the population level and, to a lesser extent, at the territory level, where prey consumption was nonetheless similarly ranked by both methods (pellets vs. SIAR), especially in terms of the most- and least-consumed prey. Method comparisons through intraclass correlation for each prey category showed reasonable similarities, except in the case of Partridges. Overall, our results suggest that a combination of pellet analysis and SIA can be a useful way to assess the diet of predator species, and can add important insights with the application of isotopic analysis to study raptor food habits.

Conventional and isotopic methods each have advantages and disadvantages. Pellet analysis is non-invasive and allows detailed prey identification. However, it has potential biases related to prey size or digestibility (Votier et al. 2003, Marti et al. 2007), and may involve great effort in terms of both data collection and analysis of pellet contents. In contrast, SIA generates data about assimilated rather than ingested prey. Furthermore, isotopic analysis has the advantage that it provides diet estimates from the sampled individuals, which is frequently unachievable through conventional dietary methods, for example, when several chicks are raised in the same nest. However, the use of isotopic mixing models to estimate prey consumption requires accurate prior information of the species' feeding ecology to select the right prey for tissue analysis, as well as suitable TEFs estimates of each element. Moreover, tissue collection for isotopic analysis requires handling of both consumer and prey, and laboratory analyses are more expensive than for conventional diet analyses. Therefore, conventional pellet and isotopic analyses can be considered complementary methods to monitor dietary patterns in territorial birds.

Our dietary results accord with other studies of Bonelli's Eagle in northeast Iberia, where the species takes more pigeons and fewer Rabbits and Partridges than populations in the southern Iberian Peninsula (Moleón et al. 2009). Moreover, in our study area, near the northern limit of the species' distribution in western Europe, local environmental conditions are more heterogeneous among territories than in southern populations, and this probably translates into greater dietary differences between territories, with some territorial pairs preying disproportionally on prey species that may be considered secondary or suboptimal elsewhere. For example, we show that Yellow-legged Gulls may constitute an important prey for some territorial pairs located in coastal areas, probably due the high abundance of Gulls in those territories (see also Resano et al. 2012).

Despite the importance of a comprehensive isotopic characterisation of prey as the basis for SIA, logistical difficulties generally constrain prey sample collection. Therefore, published studies often present low prey sample sizes and rarely consider spatial heterogeneity in prey isotopic values (but see Hebert et al. 1999, Ramos et al. 2009, Moreno et al. 2010). In our study, we obtained large samples of individuals in most prey categories and achieved this across the whole study area, to assess whether individuals differed in their isotopic values due to environmental variation caused by proximity to the sea or habitat variation. For Rabbits and Domestic Pigeons we found that individuals collected in Eagle territories close to the sea showed higher $\delta^{34}S$ than individuals from inland territories (Fig. 2), in accordance with the general trend of higher $\delta^{34}S$ in species inhabiting marine ecosystems (Thode 1991, Deegan & Garritt 1997, Connolly et al. 2004). Moreover, Yellow-legged Gulls, the only marine prey species detected in the diet of Bonelli's Eagle in our study, showed the highest $\delta^{34}S$ values of all analysed prey. This supports the use of $\delta^{34}S$ in distinguishing among the terrestrial and marine components in the diet of predator species foraging in both marine and terrestrial ecosystems (Moreno et al. 2010, Ramos et al. 2013). Conversely, prey isotopic values did not vary across habitat types, except in the case of Domestic Pigeons from agricultural habitats, which showed significantly higher $\delta^{15}N$ than those from scrubland habitats (Fig. 2). This may be related to the use of nitrate-based fertilisers in agricultural areas (Choi et al. 2007) and the tendency of Domestic Pigeons to forage on agricultural crops in the study area (Authors pers. obs.). Based on these results, prey isotopic values included in SIAR for European Rabbits and Domestic Pigeons were selected according to environmental features (i.e. proximity to the sea and habitat type) of Eagle territories (Table 1), thus allowing consideration of spatial heterogeneity in prey isotopic values and increasing model accuracy when estimating Bonelli's Eagle nestling diet. The fact that the isotopic values of nestlings generally lie within the δ-space delineated by main prey categories previously corrected by TEF's (Fig. 1) suggested both that main prey categories were representative of nestling diet, and that prey isotopic values and TEFs were reasonable. Overall, our isotopic characterisation of prey highlights the importance of an extensive prey sampling strategy to avoid equivocal interpretations from isotopic prey base values and to resolve mixing models with higher reliability.

There was an overall agreement between pellet analysis (in terms of both items and biomass) and SIAR when estimating prey consumption by Bonelli's Eagles at the population level. Both methods estimated similar means and ranges for prey consumption, and showed that Rabbits and pigeons were consumed more than Partridges, passerines, Gulls and Squirrels (Fig. 3). Our results therefore suggest that both pellet analysis and SIAR are suitable methods to assess the diet of avian predators at the population level (Real 1996, Resano et al. 2011). At the territory level, we also found broad agreement in the relative rankings of prey consumption rates; that is, the most-consumed prey as assessed by pellet analysis was the same prey category identified as most-consumed by SIAR, and similarly for the least-consumed prey category (Fig. 4). This result supports the applicability of isotopic mixing models to infer main prey consumption patterns in territorial raptor species. In contrast, the intraclass correlation coefficient showed poor agreement when comparing consumption estimates of all prey categories in the same analysis, probably due to differences in agreement between methods for individual prey categories. For instance, the prey item counts and SIAR showed agreement in their consumption estimates of Rabbits, pigeons and Gulls, and biomass estimation and SIAR did the same for pigeons and Gulls (Table 2). We did not find significant agreement between methods for passerines and Squirrels; however, the ICCs showed certain similarities between methods for those prey, especially between the biomass estimation and SIAR. The fact that passerines and Squirrels were the main prey categories with the lowest biomass could make them susceptible to be underestimated by the pellet analysis. Finally, we found poor agreement between methods in consumption estimates for Partridges, although we could not identify any evidence or possible causes to explain this.

Despite an overall agreement between the pellet analysis and SIAR in terms of main prey estimates, noticeable method discrepancies were found in the estimated percentages of some prey, especially at the territory level. This was not related to the origin of pellets (i.e. adults vs. nestlings) because adults rarely leave pellets in the nest (J. Real pers. obs.), but could be related to the fact that pellets and nestling feathers were temporally mismatched. Pellets represented nestling diet during the whole rearing period, whereas the isotopic composition of nestling feathers represented diet during feather growth (i.e. approximately half of the whole rearing period). Nevertheless, this would only affect our results in those cases where nestling diet changed during the second half of the rearing period.

In conclusion, our results support the potential of intrinsic biogeochemical markers (i.e. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) to infer the main prey consumption of raptor nestlings by analysing the isotopic composition of their feathers. Moreover, and in accordance with other isotopic studies of predator species, the use of $\delta^{34}S$ could serve to assess the marine prey components in those raptor species foraging on both terrestrial and marine ecosystems (see Chamberlain et al. 2005). The use of isotopic mixing models to assess nestling diet would also allow individual diet estimates, thus offering a valuable approach to investigate the foraging ecology of individuals within a population, its ecological causes and fitness or evolutionary consequences. Nevertheless, the use of isotopic mixing models requires previous information of the species' feeding ecology, usually assessed by conventional diet analysis, a comprehensive prey isotopic characterisation, and reliable TEF's estimates, which are usually available for some model species (e.g. Peregrine Falcon) but may be difficult to obtain for a particular species of interest. Future empirical research will contribute to a deeper understating of the applicability and potential biases associated with isotopic analyses in avian predators, and we particularly encourage research to evaluate the usefulness of isotopic approaches to study the foraging ecology and its ecological implications in raptor species worldwide.

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## REFERENCES

**Bakaloudis, D.E., Iezekiel, S., Vlachos, C.G., Bontzorlos, V.A., Papakosta, M. & Birrer, S.** (2012) Assessing bias in diet methods for the long-legged Buzzard *Buteo rufinus*. J. Arid Environ., 77, 59-65.

**BirdLife International** (2004) Birds in Europe: population estimates, trends and conservation status. BirdLife Conservation Series No. 12. Cambridge: BirdLife International.

**Bland, J.M. & Altman, D.G.** (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 1, 307-310.

**Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L.** (2003) The ecology of individuals: incidence and implications of individual specialization. Am. Nat., 161, 1-28.

**Bond, A.L. & Diamond, A.W.** (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol. Appl., 21, 1017-1023.

**Bosch, R., Real, J., Tintó, A., Zozaya, E.L. & Castell, C.** (2010) Home-ranges and patterns of spatial use in territorial Bonelli's Eagles *Aquila fasciata*. Ibis, 152, 105-117.

**Bourass, K., Leger, J.F., Zaime, A., Qninba, A., Rguibi, H., El Agbani, M.A., Benhoussa, A. & Hingrat, Y.** (2012) Observations on the diet of the North African houbara bustard during the non-breeding season. J. Arid Environ., 82, 53-59.

**Brom, T.G.** (1986) Microscopic identification of feathers and feather fragments of palearctic birds. Bijdr. Dierkd., 56, 181-204.

**Brown, K.M. & Ewins, P.J.** (1996) Technique-dependent biases in determination of diet composition: An example with Ring-Billed Gulls. Condor, 98, 34-41.

**Brown, R., Ferguson, J., Lawrence, M. & Lees, D.** (2003) Huellas y señales de las aves de España y Europa. Barcelona: Ediciones Omega.

**Carrascal, L.M. & Seoane, J.** (2009) Factors affecting large-scale distribution of the Bonelli's eagle *Aquila fasciata* in Spain. Ecol. Res., 24, 565-573.

**Caut, S., Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecol. Appl., 16, 1893-1900.

**Chamberlain, C.P., Waldbauer, J.R., Fox-Dobbs, K., Newsome, S.D., Koch, P.L., Smith, D.R., Church, M.E., Chamberlain, S.D., Sorenson, K.J. & Risebrough, R.** (2005) Pleistocene to recent dietary shifts in California condors. PNAS, 102, 16707-16711.

**Choi, W.J., Han, G.H., Lee, S.M., Lee, G.T., Yoon, K.S., Choi, S.M. & Ro, H.M.** (2007) Impact of land-use types on nitrate concentration and $\delta^{15}$N in unconfined groundwater in rural areas of Korea. Agr. Ecosyst. Environ., 120, 259-268.

**Connolly, R.M., Guest, M.A., Melville, A.J. & Oakes, J.M.** (2004) Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia, 138, 161-167.

**Cortés-Avizanda, A., Almaraz, P., Carrete, M., Sánchez-Zapata, J.A., Delgado, A., Hiraldo, F. & Donázar, J.A.** (2011) Spatial heterogeneity in resource distribution promotes facultative sociality in two trans-Saharan migratory birds. PLoS ONE, 6, e21016.

**Deegan, L.A. & Garritt, R.H.** (1997) Evidence for spatial variability in estuarine food webs. Mar. Ecol. Prog. Ser., 147, 31-47.

**Doucette, J.L., Wissel, B. & Somers, C.M**. (2011) Cormorant-fisheries conflicts: Stable isotopes reveal a consistent niche for avian piscivores in diverse food webs. Ecol. Appl., 21, 2987-3001.

**Folch, J., Lees, M. & Sloane-Stanley, G.H.** (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497–509.

**Gil-Sánchez, J.M.** (2000) Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). Ardeola, 47, 1-8.

**Gil-Sánchez, J.M., Moleón, M., Otero, M. & Bautista, J.** (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biol. Conserv., 118, 685-694.

**Hebert, C.E., Shutt, J.L., Hobson, K.A. & Weseloh, D.C.** (1999) Spatial and temporal differences in the diet of Great Lakes herring gulls (*Larus argentatus*): evidence from stable isotope analysis. Can. J. Fish. Aquat. Sci., 56, 323-338.

**Hernández-Matías, A., Real, J. & Pradel, R.** (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. J. Wildlife Manage., 75, 856-866.

**Hobson, K.A.** (2011) Isotopic ornithology: a perspective. J. Ornithol., 152, S49-S66.

**Hobson, K.A. & Clark, R.G**. (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor, 94, 189–197.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds)** (1994) Handbook of the Birds of the World, Vol. 2. Barcelona: Lynx Edicions.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds)** (1997) Handbook of the Birds of the World, Vol. 4. Barcelona: Lynx Edicions.

**Huang, K.Y., Lin, Y.S. & Severinghaus, L.L.** (2006) Comparison of three common methods for studying the diet of nestlings in two Accipiter species. Zool. Stud., 45, 234-243.

**Inger, R., Ruxton, G.D., Newton, J., Colhoun, K., Robinson, J.A., Jackson, A.L. & Bearhop, S.** (2006) Temporal and intrapopulation variation in prey choice of wintering geese determined by stable isotope analysis. J. Anim. Ecol., 75, 1190-1200.

**Inger, R. & Bearhop, S.** (2008) Applications of stable isotope analyses to avian ecology. Ibis, 150, 447-461.

**Inger, R., Gudmundsson, G.A., Ruxton, G.D., Newton, J., Colhoun, K., Auhage, S. & Bearhop, S.** (2008) Habitat utilisation during staging affects body condition in a long distance migrant, *Branta bernicla hrota*: potential impacts on fitness? J. Avian Biol., 39, 704-708.

**Jackson, A.L., Inger, R., Bearhop, S. & Parnell, A.** (2009) Erroneous behavior of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). Ecol. Lett., 12, E1-E5.

**Kelly, J.F.** (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool., 78, 1-27.

**Marti, C.D., Bechard, M. & Jaksic, F.M.** (2007) Food habits. In Bird, D.M. & Bildstein, K.L. (eds) Raptor research and management techniques: 129-151. Surrey: Hancock House Publishers LTD.

**Maziarz, M. & Wesolowski, T.** (2010) Timing of breeding and nestling diet of Wood Warbler *Phylloscopus sibilatrix* in relation to changing food supply. Bird Study, 57, 540-552.

**McGraw, K.O. & Wong, S.P.** (1996) Forming inferences about some intraclass correlation coefficients. Psychol. Methods., 1, 30-46.

**Michalski, M., Nadolski, J., Marciniak, B., Loga, B. & Banbura, J.** (2011) Faecal analysis as a method of nestling diet determination in insectivorous birds: a case study in Blue Tits *Cyanistes caeruleus* and Great Tits *Parus major*. Acta Ornithol., 46, 164-172.

**Michener, R. & Lajtha, K.** (2007) Stable isotopes in ecology and environmental science. 2nd ed. Boston: Blackwell Publishers.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Charton, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. J. Biogeogr., 36, 1502-1515.

**Moore, J.W. & Semmens, B.X.** (2008) Incorporating uncertainity and prior information into stable isotope mixing models. Ecol. Lett., 11, 470-480.

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

**Newton, I.** (1998) Population limitation in birds. San Diego: Academic Press.

**Ontiveros, D., Pleguezuelos, J.M. & Caro, J.** (2005) Prey density, prey detectability and food habits: the case of Bonelli's eagle and the conservation measures. Biol. Conserv., 123, 19-25.

**Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L.** (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE, 5, e9672.

**Pons, X.** (2002) MiraMon. Sistema d'Informació Geogràfica i software de Teledetecció. Bellaterra: Centre de Recerca Ecològica i Aplicacions Forestals, CREAF.

**Pyke, G. H.** (1984) Optimal foraging theory: a critical review. Ann. Rev. Ecol. Syst., 15, 523-575.

**Quinn, G.P. & Keough, M.J.** (2002) Experimental Design and Data Analysis for Biologists. Cambridge: Cambridge University Press.

**Ramos, R., Ramírez, F., Sanpera, C., Jover, L. & Ruiz, X.** (2009) Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods. Mar. Ecol. Prog. Ser., 377, 289-297.

**Ramos, R., Ramírez, F. & Jover, L.** (2013) Trophodynamics of inorganic pollutants in a wide-range feeder: The relevance of dietary inputs and biomagnification in the Yellow-legged gull (*Larus michahellis*). Environ. Pollut., 172, 235-242.

**Real, J.** (1991) L´àliga perdiguera Hieraaetus fasciatus a Catalunya: status, ecología tròfica, biología reproductora i demografía. PhD Thesis, University of Barcelona.

**Real, J.** (1996) Biases in diet study methods in the Bonelli's Eagle. J. Wildlife Manage., 60, 632-638.

**Real, J.** (2004) Águila azor-perdicera, Hieraaetus fasciatus. In Madroño, A., González, C. & Atienza, J.C. (eds) Libro rojo de las aves de España: 154-157. Madrid: Dirección General para la Biodiversidad-SEO/Birdlife.

**Resano, J., Hernández-Matías, A., Real, J. & Parés, F.** (2011) Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. J. Raptor. Res., 45, 342-352.

**Resano, J., Bayle, P., Real, J., Hernández, A., Vincent-Martin, N. & Ravayrol, A.** (2012) Analyse du régime alimentaire de l'Aigle de Bonelli *Hieraaetus fasciatus* (Vieillot, 1822) pendant la saison de reproduction 2010 en France. Nature de Provence, 1, 95-101.

**Roemer, G.W., Donlan, C.J. & Courchamp, F.** (2002) Golden eagles, feral pigs, and insular carnivores: How exotic species turn native predators into prey. Proc. Natl. Acad. Sci. USA, 99, 791-796.

**Sánchez, R., Margalida, A., González, L.M. & Oria, J.** (2008) Biases in diet sampling methods in the Spanish Imperial Eagle *Aquila adalberti*. Ornis Fennica, 85, 82-89.

**Sánchez-Zapata, J.A. & Calvo, J.F.** (1998) Importance of birds and potential bias in food habit studies of Montagu's Harriers (*Circus pygargus*) in southeastern Spain. J. Raptor. Res., 32, 254-256.

**Schoener, T.W.** (1971) Theory of feeding strategies. Ann. Rev. Ecol. Syst., 11, 369-404.

**Steenweg, R.J., Ronconi, R.A. & Leonard, M.L.** (2011) Seasonal and age-dependent dietary partitioning between the Great Black-Backed and Herring Gulls. Condor, 113, 795-805.

**Terraube, J., Arroyo, B.E., Bragin, A., Bragin, E. & Mougeot, F.** (2012) Ecological factors influencing the breeding distribution and success of a nomadic, specialist predator. Biodivers. Conserv., 21, 1835-1852.

**Thode, H.G.** (1991) Sulfur isotopes in nature and the environment: an overview. In Krouse, H.R. & Grinenko, V.A. (eds) Stable isotopes: natural and anthropogenic sulphur in the environment: 1-26. Chichester: Wiley.

**Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. & Furness, R.W.** (2003) Assessing the diet of Great Skuas, *Catharacta skua*, using five different techniques. Polar Biol., 26, 20-26.

**Weiser, E.L. & Powell, A.N.** (2011) Evaluating gull diets: a comparison of conventional methods and stable isotope analysis. J. Field Ornithol., 82, 297-310.

**Zhou, H., Muellerleile, P., Ingram, D. & Wong, S.P.** (2011) Confidence intervals and F test for intraclass correlation coefficients based on three-way mixed effects models. J. Educ. Behav. Stat., 36, 638-671.

## SUPPORTING INFORMATION

Additional supporting information is shown in the following pages: Tables S1 and S2.

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# Chapter 1

## SUPPORTING INFORMATION

**Table S1.** Diet of Bonelli's Eagle nestlings in Catalonia based on the pellet analysis (n = 979 pellets analysed). Those items unidentified at the species level are included in different undetermined prey categories. The table gives the number of items (nº), the gross biomass per individual prey (g), the net biomass per individual prey after partial consumption by adults before delivering prey to the chicks (g), the total net consumed biomass (g), and the consumption percentages (%) based on both items and biomass. Gross and net biomasses were considered the same for prey species with a weight under 101 g (i.e. no adult prey consumption).

| Prey species | Items (nº) | Gross biomass (g) | Net biomass (g) | Consumed biomass (g) | Consumption of items (%) | Consumption of biomass (%) |
|---|---|---|---|---|---|---|
| **Mammals** | | | | | | |
| *Oryctolagus cuniculus* | 511 | 857 | 494 | 252434 | 22,67 | 33,34 |
| *Sciurus vulgaris* | 173 | 241 | 208 | 35984 | 7,68 | 4,75 |
| *Mustela nivalis* | 6 | 100 | 100 | 600 | 0,27 | 0,08 |
| *Apodemus sylvaticus* | 1 | 25 | 25 | 25 | 0,04 | < 0,01 |
| Undetermined mammal | 67 | 500 | 295 | 19765 | 2,97 | 2,61 |
| **Total Mammals** | **758** | | | **308808** | **33,63** | **40,79** |
| **Birds** | | | | | | |
| *Ardea cinerea* | 3 | 1555 | 1258 | 3774 | 0,13 | 0,50 |
| *Anas platyrhynchos* | 16 | 1126 | 911 | 14576 | 0,71 | 1,93 |
| *Pernis apivorus* | 6 | 705 | 571 | 3426 | 0,27 | 0,45 |
| *Accipiter gentilis* | 7 | 680 | 550 | 3850 | 0,31 | 0,51 |
| *Falco tinnunculus* | 7 | 170 | 148 | 1036 | 0,31 | 0,14 |
| *Alectoris rufa* | 218 | 425 | 252 | 54936 | 9,67 | 7,26 |
| *Phasianus colchicus* | 2 | 970 | 575 | 1150 | 0,09 | 0,15 |
| *Gallus gallus* | 1 | 2000 | 1186 | 1186 | 0,04 | 0,16 |
| Undetermined galliform | 4 | 420 | 249 | 996 | 0,18 | 0,13 |
| *Rallus aquaticus* | 1 | 136 | 118 | 118 | 0,04 | 0,02 |
| *Larus michahellis* | 75 | 1119 | 906 | 67950 | 3,33 | 8,97 |
| *Columba livia* dom. | 88 | 385 | 314 | 27632 | 3,90 | 3,65 |
| *Columba oenas* | 4 | 300 | 243 | 972 | 0,18 | 0,13 |
| *Columba palumbus* | 144 | 425 | 344 | 49536 | 6,39 | 6,54 |
| *Columba* spp. | 401 | 405 | 329 | 131929 | 17,79 | 17,42 |
| *Streptopelia* sp. | 5 | 135 | 117 | 585 | 0,22 | 0,08 |
| *Cuculus canorus* | 3 | 114 | 99 | 297 | 0,13 | 0,04 |
| *Strix aluco* | 2 | 430 | 348 | 696 | 0,09 | 0,09 |
| *Asio otus* | 1 | 251 | 203 | 203 | 0,04 | 0,03 |
| *Caprimulgus europaeus* | 1 | 85 | 85 | 85 | 0,04 | 0,01 |
| *Picus viridis* | 18 | 175 | 152 | 2736 | 0,80 | 0,36 |
| *Turdus merula* | 39 | 90 | 90 | 3510 | 1,73 | 0,46 |
| *Turdus philomelos* | 4 | 70 | 70 | 280 | 0,18 | 0,04 |
| *Turdus* sp. | 8 | 90 | 90 | 720 | 0,35 | 0,10 |
| *Garrulus glandarius* | 51 | 170 | 148 | 7548 | 2,26 | 1,00 |
| *Pica pica* | 24 | 170 | 148 | 3552 | 1,06 | 0,47 |
| *Sturnus unicolor* | 13 | 80 | 80 | 1040 | 0,58 | 0,14 |
| *Sturnus* sp. | 30 | 80 | 80 | 2400 | 1,33 | 0,32 |
| Undetermined passerine | 57 | 127 | 110 | 6270 | 2,53 | 0,83 |
| *Melopsittacus undulatus* | 2 | 27 | 27 | 54 | 0,09 | 0,01 |
| *Psittacine* sp. | 4 | 438 | 354 | 1416 | 0,18 | 0,19 |
| Undetermined bird | 98 | 300 | 243 | 23814 | 4,35 | 3,15 |
| **Total Birds** | **1337** | | | **418273** | **59,32** | **55,24** |
| **Reptiles** | | | | | | |
| *Timon lepidus* | 159 | 228 | 189 | 30051 | 7,05 | 3,97 |
| **TOTAL** | **2254** | | | **757132** | **100** | **100** |

## SUPPORTING INFORMATION

**Table S2.** Prey consumption (mean ± sd; %) of main prey categories estimated by each dietary approach: ITEMS, BIOMASS and SIAR. Prey categories were European Rabbits (OC), pigeons (CSP), Red-legged Partridges (AR), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Note that prey consumption values from either the items or biomass approaches were re-scaled to main prey categories.

| Prey | ITEMS (%) | BIOMASS (%) | SIAR (%) |
|---|---|---|---|
| **OC** | 26.37 ± 14.48 | 36.63 ± 16.88 | 32.62 ± 12.27 |
| **CSP** | 33.78 ± 15.37 | 33.68 ± 16.29 | 28.32 ± 7.59 |
| **AR** | 12.74 ± 9.69 | 9.58 ± 7.28 | 13.67 ± 2.84 |
| **PAS** | 12.45 ± 10.95 | 4.65 ± 4.56 | 9.45 ± 2.36 |
| **LM** | 4.11 ± 9.55 | 7.86 ± 16.73 | 6.46 ± 4.70 |
| **SV** | 10.56 ± 12.14 | 7.61 ± 11.15 | 9.49 ± 4.63 |

# Chapter 2

## The influence of diet on Bonelli's Eagle nestling body condition

* The influence of diet on Bonelli's Eagle *Aquila fasciata* nestling body condition: a multi-biomarker approach

# Efectos de la dieta sobre la condición corporal de los pollos de águila perdicera *Aquila fasciata*: aproximación mediante múltiples biomarcadores

## Resumen

El concepto de condición corporal animal hace referencia al estado de salud y fisiológico de los individuos, habiéndose propuesto múltiples parámetros para su estimación. La dieta es uno de los principales determinantes de la condición corporal individual, y el modo en el que la dieta influye sobre la condición corporal ha sido objeto de un intenso debate. La mayoría de estudios, sin embargo, se centran en individuos en cautividad, debido a la dificultad para evaluar la composición de la dieta en animales de poblaciones salvajes. Afortunadamente, recientes avances metodológicos basados en el análisis de isótopos estables permiten reconstruir la dieta a nivel individual. En este estudio, investigamos la relación entre la dieta y la condición corporal de pollos de águila perdicera *Aquila fasciata* muestreados en libertad en dos poblaciones geográficamente distantes situadas en España. El consumo de las principales presas se estimó a nivel individual mediante análisis de isótopos estables. El uso de múltiples biomarcadores, que incluyeron parámetros morfométricos y análisis de bioquímica sanguínea (i.e. hematocrito, bioquímica plasmática y biomarcadores de estrés oxidativo), permitió obtener una estima integrada de condición corporal. Los resultados mostraron que el mayor consumo de una presa preferida (i.e. conejo europeo *Oryctolagus cuniculus*) mejoró el estado de condición corporal de los pollos, como indican los niveles reducidos de colesterol en plasma, una mayor actividad de las enzimas que intervienen en el catabolismo proteico, niveles más altos de tocoferol y glutatión, así como la reducida actividad de la glutatión peroxidasa, lo que a su vez sugiere una menor exposición a estrés oxidativo. Por otra parte, una mayor diversidad de la dieta correlacionó positivamente con los niveles de glutatión oxidado, lo que sugiere peor condición corporal de estos pollos. Además de la dieta, otros factores afectaron a las estimas de condición corporal, como indica la relación positiva entre el hematocrito y la altitud de las áreas de nidificación, diferencias en los niveles de glucosa entre sexos (mayores en machos), aumento de la actividad de las enzimas plasmáticas fosfatasa alcalina y creatina quinasa con la edad, los ritmos circadianos de la creatinina, o el efecto de la ingesta reciente de alimento en los niveles de triglicéridos, ácido úrico o palmitato de retinol. En resumen, este estudio pone de manifiesto la utilidad de emplear múltiples biomarcadores para evaluar la condición corporal animal, y revela un efecto de la dieta sobre la condición corporal de los pollos de águila perdicera. Además, nuestros resultados concluyen la importancia de considerar la posible influencia de otros factores intrínsecos y extrínsecos al evaluar la condición corporal animal. A pesar de que un gran número de estimas de condición corporal puede dificultar en parte una interpretación directa, recomendamos el uso de múltiples biomarcadores para una mejor comprensión de las relaciones entre dieta y condición corporal. Por último, nuestro estudio puede servir para orientar futuros programas de monitoreo cuyo objetivo sea evaluar el estado de salud tanto de los pollos de águila perdicera, como de otras rapaces amenazadas.

# The influence of diet on Bonelli’s Eagle *Aquila fasciata* nestling body condition: a multi-biomarker approach

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**ABSTRACT—** Animal body condition refers to the health and physiologic state of individuals, and multiple parameters have been proposed to quantify this key concept. Food intake is one of the main determinants of individual body condition and much debate has been generated in the scientific community. Most studies, however, have been based on captive animals due to the difficulty to assess individual food composition in wild populations. Fortunately, recent methodologies based on isotopic analyses allow for individual diet reconstructions of wild animals. In this study, we investigate the relationships between diet and body condition in free-living Bonelli’s eagle *Aquila fasciata* nestlings sampled at two geographically distant populations in Spain. Nestlings’ main prey consumption was estimated using isotopic analyses. A multi-biomarker approach, including morphometric and blood biochemical measures (i.e. haematocrit, plasma biochemistry and oxidative stress biomarkers), enabled us to integrate all the body-condition measures taken. Our results showed that a greater consumption of a preferred prey item (i.e. the European rabbit *Oryctolagus cuniculus*) improved nestling body condition, as indicated by lower levels of cholesterol in plasma, greater activity of enzymes mediating in protein catabolism, higher levels of tocopherol and glutathione, and less glutathione peroxidase activity, which also suggest less exposure to oxidative stress. On the other hand, greater diet diversity was positively correlated with levels of oxidized glutathione, which suggests that these nestlings had poorer body condition. Several factors other than diet also had an effect on certain body condition measures, as indicated by the positive relationship between nestlings’ haematocrit and the altitude of nesting areas, sex differences in glucose levels (higher in males), increases in the activity of the plasmatic enzymes alkaline phosphatase and creatine kinase with age, circadian rhythms in creatinine, and the effects of recent food ingestion on levels of triglycerides, uric acid and retinyl palmitate. Overall, this study highlights the usefulness of a multi-biomarker approach when assessing animal body condition, and reveals a measurable effect of diet on Bonelli’s eagle nestling body condition. Moreover, our results demonstrate the importance of considering the potential influence of multiple intrinsic and extrinsic factors when assessing animal body condition. Even though an over-large number of body condition estimates may somewhat hamper a straightforward interpretation, we still recommend the use of a multi-biomarker approach for a more realistic understanding of the relationships between diet and body condition. Finally, our study may serve to guide future monitoring programs aiming to evaluate nestlings’ health in the Bonelli’s eagle and other long-lived, endangered birds of prey.

## Chapter 2

### INTRODUCTION

Body condition is a key concept used in animal biology to quantify the health and physiologic state of individuals (Brown 1996, Stevenson & Woods 2006, Labocha & Hayes 2012). Internal and external factors influence animal body condition (e.g. Zera & Harshman 2001, Clinchy et al. 2004, Bonal & Aparicio 2008) and the potential influence of both should be considered when attempting to disentangle the complexity underlying this concept.

Diet is one of the most important determinants of body condition and may affect individual growth rate and survival and, ultimately, individual fitness (Pothoven et al. 2001, Sorensen et al. 2009, Harrison et al. 2011). The diet-body condition association has been studied in several species differing in their feeding strategies and under a variety of ecological scenarios (Stevenson & Woods 2006, Lefcheck et al. 2013). For instance, the relationship between individual body condition and generalist versus specialist diets (i.e. diets composed of many versus few food types – e.g. species –, respectively) has been much debated in recent decades (see Bowen et al. 1995, Lefcheck et al. 2013). Most of the current scientific knowledge on this topic is, however, based on microcosm studies performed in laboratories under controlled conditions. Thus, our knowledge of how trophic composition and diversity relates to the body condition of wild animals is still at a preliminary stage. In this regard, the difficulty in assessing the food intake of free-living organisms, especially at individual level, may have hampered research on this topic. Fortunately, recently developed techniques based on stable isotope analyses (SIA) allow for individual diet reconstruction by converting isotopic composition (most frequently the carbon and nitrogen in consumer tissues and main food resources) into dietary proportions (Parnell et al. 2010). Therefore, SIA offer wildlife ecologists a useful tool for performing applied studies of animal nutritional and functional ecology. This is particularly interesting in the case of endangered species, in which the study of individual body condition in natural populations can help to detect potential threats to population viability and, at the same time, provide a guide for management actions (see Ferrer & Dobado-Berrios 1998, Balbontín & Ferrer 2002, Hernández & Margalida 2010).

Despite the importance of the individual body condition concept in ecological and evolutionary studies, its assessment is not straightforward. A range of indices – from morphological to biochemical and physiological – that measures different levels of biological organization can be used to estimate animal body condition (Brown 1996, Stevenson & Woods 2006, Labocha & Hayes 2012). Nevertheless, the large number of factors that influence individual body condition makes the interpretation of results difficult, particularly when only one or a few body condition parameters are analyzed. In this regard, assessments of body condition based on integrated analyses of multiple body condition parameters are recommendable, although this type of analysis is still uncommon.

Traditionally, morphological indices have been used to assess animal body condition (Brown 1996, Stevenson & Woods 2006). These indices are based on the premise that once corrected for the structural size of an individual, its 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). Thus, to establish a meaningful comparison between individuals that differ in size, morphological indices must account for the effects of growth, development and sex differences on body mass-morphometric relationships (see Peig & Green 2009, 2010). This critical point has generated discussion since the violation of the basic assumptions on which indices are based (e.g. that mass increases linearly with size) will lead to inaccurate estimates of body condition (Green 2001, Peig & Green 2009, Labocha & Hayes 2012). Despite controversy, numerous studies have associated morphological indices with fitness parameters such as survival rates or reproductive success, thereby offering ecologists a practical methodology for addressing animal fitness-related hypotheses (Brown 1996, Schulte-Hostedde et al. 2005).

Measuring blood parameters is another common procedure for estimating animal body condition (Milner et al. 2003, Owen 2011). Most studies, however, use captive animals in rehabilitation or research centers and reference values for free-living organisms are much scarce. Plasma constituents are among the most common blood parameters analyzed. For instance, some plasma components are indicative of the activity in or damage to specific tissues (Harrison & Harrison 1986), while other plasma metabolites such as ion, carbohydrate, fat, total amount of protein and nitrogen waste concentrations may be more directly related with diet. These metabolites can be synthesized and obtained from ingested food, or mobilized from endogenous body reserves in tissues (Hochleithner 1994, Alonso-Alvarez 2005, Stevenson & Woods 2006). Apart from diet, factors such as age, sex, circadian rhythm and recent food ingestion may also influence plasma biochemistry (see Hochleithner 1994, Dobado-Berrios et al. 1998, Alonso-Alvarez 2005) and thus hinder parameter interpretation. For instance, uric acid increases after food intake, especially in species with high protein diets (Hochleithner 1994). Yet, uric acid is also a by-product of protein catabolism, which can increase with food scarcity once lipid reserves have been consumed and so higher uric acid levels may also be indicative of trophic stress and muscle damage (Hochleithner 1994). Therefore, the proper interpretation of plasmatic parameters for each individual requires an integrated assessment of multiple body condition parameters.

In recent years, analyses of antioxidant (AO) defenses and oxidative stress (OS) have received considerable attention in studies of animal ecology as proxies of individual fitness (Monaghan et al. 2009, Costantini et al. 2010). Aerobic organisms generate reactive oxygen species (ROS) as a by-product of normal metabolic processes (Balaban et al. 2005), which can damage key biomolecules such as lipids, proteins and DNA due to their high reactivity. OS occurs as a result of an imbalance between the production of ROS and the body's ability to mitigate its harmful effects through AO defenses (Finkel & Holbrook 2000, Monaghan et al. 2009). Antioxidants include diet-derived (e.g. vitamins and carotenoids) and endogenous molecules that prevent or minimize oxidative damage through interrelated mechanisms. Because animals obtain some of their AO defenses from their diet, which also determines energy and nutrient income, an individual's nutritional state can potentially affect the delicate balance that generally exists between ROS production and AO defenses (see Catoni et al. 2008, Monaghan et al. 2009). It has been observed, however, that growing individuals facing temporary food restrictions, including AO defenses or their precursors, are capable of adjusting their metabolism to a lower level of development that is conductive to lower values of OS (Alonso-Alvarez et al. 2006, Noguera et al. 2011). Therefore, measures of AO defenses alone are not enough to make inferences regarding OS, as these substances can be regulated by an animal's exposure to ROS (Costantini & Verhulst 2009, Monaghan et al. 2009). Overall, although AO and OS biomarkers offer a powerful tool for assessing individual body condition and for detecting developmental problems or stress in growing individuals, their interpretation requires the integration of the presence of AO defenses with measures of oxidative damage.

Bonelli's eagle *Aquila fasciata* is a long-lived raptor distributed from south-east Asia and the Middle East to the western Mediterranean (del Hoyo et al. 1994). The European population is listed as endangered (BirdLife International 2004) in light of a marked decline in number and range in recent decades, related to unnaturally high mortality rates, habitat degradation and the decline of its main prey species (Real 2004, Hernández-Matías et al. 2011, Hernández-Matías et al. 2013). In Western Europe, Bonelli's eagle mainly predates on European rabbits *Oryctolagus cuniculus*, red-legged partridges *Alectoris rufa*, pigeons *Columba* spp., other birds and lizards (Real 1991, Moleón et al. 2009). In this geographical area, rabbits and, to a lesser extent, partridges are considered to be the preferred prey item for this raptor and are preferentially consumed wherever they are abundant, thereby reducing this eagle's diet diversity (Real 1991, Moleón et al. 2009, 2012). Greater consumption of preferred prey is assumed to provide more efficiency in terms of energy intake in this species and so it is expected to benefit eagles' body condition. For instance, a recent study has shown that Bonelli's eagle productivity at both territorial and local population levels is positively and negatively influenced, respectively, by greater preferred prey consumption and greater diet diversity (see Resano-Mayor et al. 2014a). Nevertheless, how diet affects nestling body condition is almost unknown in this and most other long-lived birds.

By using Bonelli's eagle as a study model, here we explore the relationships between nestling diet and body condition, estimated, respectively, by isotopic analyses and a multi-biomarker approach including morphometric and blood biochemical measures. The specific objectives were: 1) to provide reference values for body condition measures (morphometric index, plasmatic biochemistry and antioxidant metabolism) in free-living Bonelli's eagle nestlings, 2) to test whether or not the consumption of preferred prey and diet diversity correlates with nestling body condition, and 3) to assess the potential influence of intrinsic and extrinsic factors other than prey consumption on body condition estimates. Our main prediction is that greater consumption of preferred prey will positively correlate with nestling body condition, while greater diet diversity (to be expected when the preferred prey is not abundantly consumed) will have the opposite effect.

## METHODS

### Study area

During 2010-2011 we monitored 43 Bonelli's eagle breeding territories located in two local populations in Spain: 33 in Catalonia (41º20'N, 01º32'E; northeast Spain; $n = 23$ and 24 in 2010 and 2011, respectively) and 10 in Andalusia in 2011 (37º76'N, 03º85'W; southern Spain). Breeding nests were located on cliffs; the altitude of nesting areas ranged from 30 to 790 m a.s.l in Catalonia and from 500 to 1500 m a.s.l in Andalusia. All territories are typical Mediterranean landscapes (see Gil-Sánchez et al. 2004, Carrascal & Seoane 2009, Bosch et al. 2010) characterized by marked variation in habitat coverage, habitat structure and prey abundances, especially in Catalonia.

### Sample collection

Breeding territories were monitored between January and March to check for breeding activity (i.e. incubation behaviour). In late March and April, occupied nests were observed to detect the presence, number and age of nestlings, which were estimated by feather development and backdating from laying dates (Real 1991, Gil-Sánchez 2000). To avoid disturbance, observations were always carried out at long distance from the nests using 10× binoculars and 20-60× spotting scopes. Once nestlings were around 35-45 days old, experienced climbers accessed the nests to capture the chicks, which were then carefully transported to the top of the cliff for morphometric measurements and tissue 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 (see Mañosa et al. 1995). Four mantle feathers were then sampled from each chick for individual diet estimates via isotopic analyses (see below) as feather isotopic composition indicates nestling diet at the time of tissue development (i.e. the previous few weeks). Finally, before 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 heparinised tubes of 1 ml, from which two haematocrit capillaries were filled. The remaining blood was collected in an Eppendorf tube 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 ºC in a portable fridge until processed in the laboratory within 12 h. Once in the laboratory, haematocrit capillaries were centrifuged at 9000 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) using the same electronic calliper. Heparinised tubes were centrifuged at 10 000 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 10 000 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 ºC until analysis. The blood samples in ethanol were directly frozen at -20 ºC until sex determination.

### Individual diet estimates

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 following the procedure described by Resano-Mayor et al. (2014b). Isotopic measurements were conducted at the *Centres Científics i Tecnològics* in the University of Barcelona using the methods in Resano et al. (2011).

The Bayesian mixing model SIAR (Parnell et al. 2010) was used to estimate the relative contribution of the main prey categories in the diet of each chick. The main prey categories included in the SIAR were European rabbits, red-legged partridges, wood pigeons *Columba palumbus*, domestic pigeons *C. livia* dom. (distinguishing between pigeons that forage on crops in the wild and those that are associated with dovecotes and are fed with corn *Zea mays*; see Resano-Mayor et al. 2014a), passerines (Corvidae, Sturnidae and Turdidae), Eurasian red squirrels *Sciurus vulgaris*, ocellated lizards *Timon lepidus* and yellow-legged gulls *Larus michahellis* (only in Catalonian given that this prey was not consumed in Andalusian). Prey isotopic values were obtained from the most recently published studies (see Resano-Mayor et al. 2014a). The trophic discrimination factors (TDFs) included in the SIAR were 2.1‰ ± 0.08 for $\delta^{13}C$ and 2.7‰ ± 0.5 for $\delta^{15}N$, described for feathers from peregrine falcons *Falco peregrinus* fed on the flesh of Japanese quails *Coturnix japonica* (Hobson & Clark 1992). Mean prey consumption estimates from SIAR were selected for subsequent analyses. These prey consumption percentages were also used to estimate the diet diversity by means of the Shannon-Weaver index (H') (ln; Shannon & Weaver 1949), including all pigeon groups as a single prey category.

### Morphometric body condition index

To obtain an estimate of body condition based on nestling morphometry we calculated the scaled mass index (SMI), which scales each individual's body mass to the value expected if all nestlings had the same body size, by using the inherent power relationship between mass and size modelled from the data (Peig & Green 2009). The SMI was estimated separately for males and females given morphometric sexual dimorphism. We scaled the body masses of each chick to the mean tarsus length (110.60 and 113.10 mm for males and females, respectively) using a secondary major axis slope of 2.78 for males and 2.50 for females (see Peig & Green 2009).

### Plasma biochemistry

The plasma fraction stored at -80 ºC was used to measure calcium, magnesium, phosphorus, glucose, cholesterol, triglycerides, creatinine, urea, uric acid, total proteins and the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK), 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).

Levels of vitamin A (free retinol in alcoholic form) and their esterified forms with fatty acids (retinyl palmitate), vitamin E ($\alpha$-tocopherol) and carotenes (lutein and zeaxanthin) were determined in plasma by high-pressure liquid chromatography (HPLC, Agilent Technologies1100Series), as described in Rodríguez-Estival et al. (2011). All these plasmatic components are important antioxidant defenses obtained from diet and their levels indicate individual capacity in the event of oxidative stress.

### Red blood cell analysis

Several 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 Mateo et al. (2003) and Reglero et al. (2009). Firstly, the activities of two antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD), were determined spectrophotometrically with an A25 autoanalyzer and 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 relative to milligrams of protein in the homogenates calculated spectrophotometrically. Membrane lipid peroxidation in erythrocytes was estimated as thiobarbituric acid reactive substances (TBARS). Determination of TBARS was performed colorimetrically with a spectrophotometer (Ultrospec2100pro, UV/vis, Amersham Biosciences). Levels of total glutathione (tGSH) and GSH in oxidized form (oxGSH; 2 oxGSH = 1 GSSG) were obtained by a reaction coupled to GSH reductase as described by Reglero et al. (2009) with an A25 autoanalyzer. The oxGSH was expressed as a molar concentration and as a percentage of the tGSH (%oxGSH).

### Statistical analysis

Descriptive parameters of all body condition measures (i.e. scaled mass index, haematocrit, plasma biochemistry, antioxidants and oxidative stress biomarkers) were calculated as the mean, standard deviation (±SD) and minimum and maximum values. Descriptive parameters are shown for nestlings for each local population-year (Catalonia 2010: n = 33 nestlings; 2011: n = 41; and Andalusia 2011: n = 18), as well as for all the monitored nestlings (n = 92).

To test the effect of diet on nestling body condition we applied Generalized Linear Mixed Models (GLMMs), which allowed us to account for the potential non-independence of clustered observations from the same territory, year and population. Each body condition measure was modelled as the response variable using the identity link function and errors were assumed to be normally distributed. Rabbit consumption (OC), diet diversity (H') and the sum OC + H' were selected as explanatory variables in all the models in order to test our main prediction that greater consumption of preferred prey (i.e. OC) and an increase in diet diversity correlate positively and negatively, respectively, with nestling body condition. Given that other variables (diet-related or otherwise) may have a relevant effect on our body condition measures, previous GLMMs were used to select the most feasible variables for each body condition measure, that is, those which reduced the null model $AIC_c$ value by 2 or more points. Potential explanatory variables included in the previous GLMMs were as follows: the consumption of partridges (AR), wood pigeons (CP), domestic pigeons (CL) and passerines (PAS), brood size (categorical variable: 1 *vs.* 2), nestling age, sex, the additive effects of age + sex, altitude of nesting areas, nestling circadian rhythm (categorical variable: whether nestlings were sampled before or after midday), and nestling food ingestion (categorical variable: whether nestlings had been recently fed – i.e. had a full crop or there was fresh prey in the nest – or not). When assessing the scaled mass index as a response variable, all models included sex as a fixed factor to control for sexual size dimorphism. GLMMs were fitted using the *lmer* function from the *lme4* package of R (Bates et al. 2012). Model selection was based on Akaike's Information Criterion adjusted for sample size ($AIC_c$), the Akaike weights ($AIC_{cw}$) being computed to assess the probability that each candidate model was the best of the proposed set (Burnham & Anderson 2002). The goodness-of-fit of each model was estimated from marginal ($R^2_m$) and conditional ($R^2_c$) coefficients of determination, following (Nakagawa & Schielzeth 2013). The $R^2_m$ value shows the proportion of the variance in the raw data explained by the fixed effects only, while the $R^2_c$ value shows the proportion of the variance explained by the full model, including both fixed and random effects.

## RESULTS

### Reference values

Descriptive values of body condition measures (i.e. scaled mass index, haematocrit, plasma biochemistry, antioxidants and oxidative stress biomarkers) for nestlings from each local population-year, and for the overall 92 monitored nestlings (40 males and 52 females), are given in Table 1.

### Effects of diet and other parameters on body condition

A summary of the main effects of diet composition and diversity, brood size, nestling age, nestling sex, altitude of nesting areas, nestling circadian rhythm and nestling recent food ingestion on body condition measures is given in Table 2. All these interactions are described below (see Tables S1-4 for details of the models used for each body condition measure).

**Table 1.** Descriptive values of body condition measures for nestlings in each local population and for each year: Catalonia in 2010 (n = 33 nestlings) and 2011 (n = 41) and Andalusia in 2011 (n = 18). Values for the overall monitored nestlings are also provided (n = 92). The parameters shown are the scaled mass index, haematocrit, plasma metabolites (calcium, magnesium, phosphorus, glucose, cholesterol, triglycerides, creatinine, urea, uric acid and total proteins), plasma enzyme activities (alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase and creatine kinase), plasma antioxidants (retinol, retinyl palmitate, tocopherol, lutein and zeaxanthin), and oxidative stress biomarkers analyzed in red blood cells (antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD), thiobarbituric acid-reactive substances (TBARS), levels of total glutathione (tGSH) and GSH in oxidized form (oxGSH and %oxGSH)). The mean, standard deviation (±SD), minimum and maximum (min/max) values are shown for each measure; units of measurement are specified.

| Body condition measures | Catalonia 2010 | Catalonia 2011 | Andalusia 2011 | Overall |
|---|---|---|---|---|
| **Scaled mass index** | 1666.81 ± 212.60 | 1690.75 ± 186.42 | 1611.29 ± 224.25 | 1666.62 ± 203.55 |
| **(g)** | 1287.56 / 2021.90 | 1382.70 / 1956.46 | 1355.12 / 2085.33 | 1287.56 / 2085.33 |
| **Haematocrit** | 29.66 ± 2.30 | 29.45 ± 2.18 | 32.34 ± 2.37 | 30.04 ± 2.48 |
| **(%)** | 23.16 / 35.27 | 25.10 / 33.53 | 28.71 / 37.26 | 23.16 / 37.26 |
| **Plasma biochemistry** | | | | |
| **Calcium** | 10.61 ± 1.26 | 10.11 ± 1.00 | 10.96 ± 1.29 | 10.45 ± 1.19 |
| **(mg/dl)** | 6.78 / 13.20 | 5.68 / 12.15 | 7.68 / 12.51 | 5.68 / 13.20 |
| **Magnesium** | 1.37 ± 0.24 | 1.37 ± 0.39 | 1.49 ± 0.44 | 1.39 ± 0.35 |
| **(mg/dl)** | 1.01 / 1.88 | 0.83 / 2.77 | 0.95 / 2.92 | 0.83 / 2.92 |
| **Phosphorus** | 6.52 ± 0.88 | 6.61 ± 1.24 | 6.76 ± 0.90 | 6.61 ± 1.05 |
| **(mg/dl)** | 5.34 / 9.69 | 5.14 / 12.59 | 5.20 / 8.36 | 5.14 / 12.59 |
| **Glucose** | 317.30 ± 29.95 | 319.88 ± 19.69 | 319.28 ± 22.60 | 318.84 ± 24.14 |
| **(mg/dl)** | 266 / 421 | 278 / 371 | 277 / 347 | 266 / 421 |
| **Cholesterol** | 183.82 ± 27.81 | 178.76 ± 19.60 | 184.72 ± 32.97 | 181.74 ± 25.52 |
| **(mg/dl)** | 110 / 236 | 132 / 238 | 141 / 241 | 110 / 241 |
| **Triglycerides** | 90.88 ± 70.44 | 104.76 ± 106.29 | 122.94 ± 85.29 | 103.34 ± 90.57 |
| **(mg/dl)** | 6 / 324 | 14 / 595 | 5 / 272 | 5 / 595 |
| **Creatinine** | 0.33 ± 0.10 | 0.35 ± 0.10 | 0.34 ± 0.06 | 0.34 ± 0.09 |
| **(mg/dl)** | 0.06 / 0.50 | 0.13 / 0.80 | 0.25 / 0.44 | 0.06 / 0.80 |
| **Urea** | 18.35 ± 7.46 | 12.31 ± 4.83 | 20.91 ± 9.36 | 16.16 ± 7.69 |
| **(mg/dl)** | 5.50 / 34.80 | 5.80 / 25.80 | 12.20 / 53.90 | 5.50 / 53.90 |
| **Uric acid** | 13.13 ± 5.41 | 11.85 ± 4.34 | 13.61 ± 4.99 | 12.65 ± 4.88 |
| **(mg/dl)** | 6.84 / 28.73 | 6.01 / 26.87 | 6.71 / 23.00 | 6.01 / 28.73 |
| **Total proteins** | 32.30 ± 3.08 | 31.82 ± 3.03 | 34.49 ± 2.50 | 32.52 ± 3.09 |
| **(g/l)** | 23.20 / 38.30 | 26.40 / 42.70 | 28.80 / 39.60 | 23.20 / 42.70 |
| **Alkaline phosphatase** | 785.88 ± 139.19 | 897.39 ± 162.08 | 1024.06 ± 270.56 | 882.17 ± 198.71 |
| **(U/l)** | 400 / 1001 | 629 / 1359 | 610 / 1602 | 400 / 1602 |
| **Aspartate aminotransferase** | 222.03 ± 26.69 | 214.24 ± 25.59 | 209.61 ± 28.06 | 216.13 ± 26.61 |
| **(U/l)** | 184 / 318 | 173 / 280 | 178 / 291 | 173 / 318 |
| **Lactate dehydrogenase** | 2207.88 ± 882.50 | 2352.51 ± 455.56 | 2382.56 ± 409.70 | 2306.51 ± 634.09 |
| **(U/l)** | 597 / 4780 | 1797 / 3786 | 1935 / 3657 | 597 / 4780 |
| **Creatine kinase** | 4233.45 ± 781.71 | 4274.83 ± 698.68 | 4352.11 ± 1844.40 | 4275.11 ± 1032.84 |
| **(U/l)** | 3068 / 5784 | 2916 / 5988 | 2172 / 7868 | 2172 / 7868 |

**Table 1.** Continued from page before.

| Body condition measures | Catalonia 2010 | Catalonia 2011 | Andalusia 2011 | Overall |
|---|---|---|---|---|
| **Antioxidants and oxidative stress biomarkers** | | | | |
| **Retinol** | 4.72 ± 0.96 | 5.30 ± 0.58 | 7.56 ± 1.43 | 5.53 ± 1.39 |
| **(uM)** | 2.88 / 6.53 | 3.82 / 6.29 | 4.61 / 10.18 | 2.88 / 10.18 |
| **Retinyl palmitate** | 0.17 ± 0.19 | 0.15 ± 0.19 | 0.33 ± 0.50 | 0.19 ± 0.28 |
| **(uM)** | 0.00 / 0.89 | 0.00 / 1.06 | 0.00 / 2.17 | 0.00 / 2.17 |
| **Tocopherol** | 17.39 ± 3.86 | 21.51 ± 4.61 | 22.27 ± 4.66 | 20.18 ± 4.81 |
| **(uM)** | 7.78 / 27.53 | 13.79 / 35.77 | 13.81 / 28.80 | 7.78 / 35.77 |
| **Lutein** | 2.72 ± 0.78 | 3.03 ± 0.85 | 3.72 ± 1.25 | 3.05 ± 0.97 |
| **(uM)** | 1.27 / 4.12 | 1.02 / 4.47 | 0.77 / 5.91 | 0.77 / 5.91 |
| **Zeaxanthin** | 1.23 ± 0.42 | 1.33 ± 0.42 | 1.47 ± 0.52 | 1.32 ± 0.45 |
| **(uM)** | 0.48 / 2.22 | 0.43 / 2.30 | 0.18 / 2.05 | 0.18 / 2.30 |
| **GPx** | 0.28 ± 0.09 | 0.28 ± 0.09 | 0.32 ± 0.10 | 0.29 ± 0.09 |
| **(U/mg protein)** | 0.12 / 0.48 | 0.08 / 0.51 | 0.15 / 0.54 | 0.08 / 0.54 |
| **SOD** | 1.35 ± 0.21 | 1.07 ± 0.30 | 1.55 ± 0.25 | 1.26 ± 0.32 |
| **(U/mg protein)** | 1.01 / 1.98 | 0.62 / 2.02 | 1.18 / 2.10 | 0.62 / 2.10 |
| **TBARS** | 0.06 ± 0.02 | 0.06 ± 0.02 | 0.06 ± 0.01 | 0.06 ± 0.02 |
| **(umol/g pellet)** | 0.04 / 0.09 | 0.02 / 0.09 | 0.04 / 0.09 | 0.02 / 0.09 |
| **tGSH** | 4.79 ± 0.90 | 5.03 ± 0.83 | 4.61 ± 0.60 | 4.87 ± 0.83 |
| **(mmol/g pellet)** | 3.18 / 6.54 | 3.35 / 7.71 | 3.45 / 5.59 | 3.18 / 7.71 |
| **oxGSH** | 838.86 ± 504.68 | 938.52 ± 597.56 | 598.53 ± 512.63 | 836.25 ± 558.13 |
| **(umol/g pellet)** | 98.58 / 1914.65 | 148.16 / 1979.97 | 28.89 / 1611.68 | 28.89 / 1979.97 |
| **%oxGSH** | 8.47 ± 4.61 | 9.47 ± 5.83 | 6.40 ± 5.33 | 8.51 ± 5.39 |
| | 1.17 / 19.24 | 0.96 / 19.56 | 0.27 / 17.36 | 0.27 / 19.56 |

### Scaled mass index

The best-fitted model for the SMI included the consumption of rabbits, H′ and nestling sex (Table S1), although the relationship between nestling diet and this index was weak and showed no clear pattern.

### Haematocrit

We did not find any effect of diet on nestlings' haematocrit, which was, however, highly and positively correlated with the altitude of nesting areas (Fig. 1). The $R^2_m$ of the best-fitted model indicated that the 41.4% of the variance in haematocrit was explained by altitude, while random effects (i.e. territory, year and population) slightly improved the model explanatory power (see Table S2).

### Plasma biochemistry

We found no clear effect of diet on either calcium or phosphorus levels. Despite the fact that the best-fitted models for these parameters included the consumption of passerines and pigeons, these prey categories had little explanatory power, i.e. very low $R^2_m$. No diet effect was detected for magnesium levels since the null model was the best fit (see Table S3).

Although glucose levels tended to increase with age, the main factor explaining plasmatic glucose was nestling sex, with males having higher levels than females (Fig. 2a). Despite being included in the best-fitted model, neither rabbit consumption nor H′ had any strong effect on glucose levels, as suggested by the low $R^2_m$ of this model (see Table S3).

Cholesterol and triglyceride values increased as rabbit consumption fell and H′ rose (Fig. 3a,b). In the case of triglycerides, however, nestlings that had recently ingested food had considerably higher levels than nestlings that had not recently fed (Fig. 2b). In fact, this factor explained triglyceride levels better than any other dietary parameter as shown by the higher $R^2_m$ of the model (see Table S3).

Creatinine was not affected by diet. However, it was the only parameter that showed a strong variation in relation to nestling circadian rhythm, as nestlings sampled before midday had lower creatinine levels than those sampled after midday (Fig. 2c) (see Table S3).

Urea levels slightly increased with lower H′ (Fig. 3c), although the $R^2_m$ of the best-fitted model that included the consumption of rabbits and H′ reveals the weak explanatory power of these dietary parameters (see Table S3). Nestling food ingestion had the same effect on uric acid as described above for triglycerides, with higher uric acid levels in nestlings that had recently ingested food (Fig. 2d). Despite the fact that the best-fitted models for total proteins included the consumption of partridges and domestic pigeons, we detected no clear relationship between these dietary parameters and nestlings' total protein levels (see Table S3).

The plasmatic activities of ALP and CK tended to be higher in males than in females (Fig. 2e,f), although nestling age was the main determinant as their levels increased with age (Fig. 3d,g). Rabbit consumption and H′ were the main dietary parameters affecting the activity of AST and increased with greater consumption of rabbits and lower H′. The same pattern was found for LDH activity, although in a weaker relationship (Fig. 3e,f) (see Table S3).

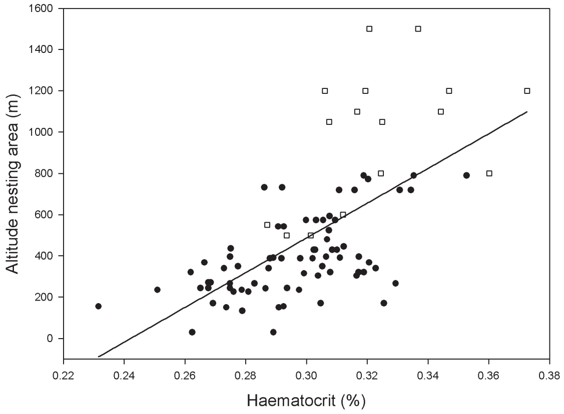

**Figure 1.** Relationship between haematocrit values (%) in Bonelli's eagle nestlings and the altitude of nesting areas (m a.s.l.). The trend line of the relationship between the variables is shown. Black dots refer to nestlings from Catalonia and white squares to nestlings from Andalusia.

Antioxidants and oxidative stress

When detected, the effects of diet on plasmatic antioxidant defenses varied according to the parameter. We detected no clear effect of diet on either retinol or lutein. Retinyl palmitate, however, increased in nestlings that had recently ingested food (Fig. 4). Tocopherol levels were slightly higher in nestlings with more rabbit consumption and lower diet diversity (Fig. 5a); the greater consumption of wood pigeons tended to increase the levels of zeaxanthin (Fig. 5d), although the $R^2_m$ values in these cases were low (see Table S4).

The activity of the enzyme GPx increased with greater consumption of passerines and decreased with greater rabbit consumption (Fig. 5b). In the case of the SOD, greater consumption of wood pigeons reduced this enzyme's activity, which increased with lower diet diversity (Fig.

**Table 2.** Summary of the main effects of diet composition, brood size, nestling age, nestling sex, altitude of nesting area, nestling circadian rhythm and nestling recent food ingestion (in the first column) on body condition measures (in the first line). Diet parameters analyzed were the consumption of rabbits, partridges, wood pigeons, domestic pigeons, passerines and diet diversity. Body condition measures are the same as described in Table 1: the scaled mass index, haematocrit, plasma metabolites (calcium, magnesium, phosphorus, glucose, cholesterol, triglycerides, creatinine, urea, uric acid and total proteins), plasma enzyme activities (alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK)), plasma antioxidants (retinol, retinyl palmitate, tocopherol, lutein and zeaxanthin), and oxidative stress biomarkers analyzed in red blood cells (antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD), thiobarbituric acid-reactive substances (TBARS), levels of total glutathione (tGSH) and GSH in oxidized form (oxGSH and %oxGSH)). Symbols denote whether the parameter had an effect on body condition estimates; symbols indicate a strong "*" or a weak "=" effect.

| | Scaled mass index | Haematocrit | Calcium | Magnesium | Phosphorus | Glucose | Cholesterol | Triglycerides | Creatinine | Urea | Uric acid | Total proteins | ALP | ASP | LDH | CK | Retinol | Retinyl palmitate | Tocopherol | Lutein | Zeaxanthin | GPx | SOD | TBARS | tGSH | oxGSH | %oxGSH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbits | = | | | | | = | * | = | | = | | | | * | = | | | | * | | | * | = | | * | = | = |
| Partridges | | | | | | | | | | | | = | | | | | = | | = | = | | | | | | | |
| Wood pigeons | | | = | | = | | | | | | | | | | | | = | | = | = | * | | * | | | | |
| Dom. pigeons | | | | | = | | | | | | | = | | | | | | | = | | | | | | | | |
| Passerines | | | = | | = | | | | | | | | | | | | = | | = | = | | * | | | | | |
| Diet diversity | = | | | | | = | * | = | | = | | | | * | = | | | | * | | | | * | | = | * | * |
| Brood size | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nestling age | | | | | | = | | | | | | | * | | | * | | | | | | | | | | | |
| Nestling sex | = | | | | | * | | | | | | | = | | | = | | | | | | | | | | | |
| Altitude nesting area | | * | | | | | | | | | | | | | | | | | | | | | | | | | |
| Circadian rhythm | | | | | | | | | * | | | | | | | | | | | | | | | | | | |
| Recent food ingestion | | | | | | | | * | | | * | | | | | | | * | | | | | | | | | |

5c); rabbit consumption showed no clear effect on the activity of this enzyme. We detected no effect of diet on TBARS. By contrast, tGSH increased with greater consumption of rabbits (Fig. 5e), but diet diversity had no effect. Both oxGSH and %oxGSH increased with greater H' (Fig. 5f); rabbit consumption showed no clear effect (see Table S4).

## DISCUSSION

The assessment of individual body condition is crucial in studies of animal ecology and conservation biology because animal health may determine fitness parameters (Brown 1996, Stevenson & Woods 2006). In this study, we describe the effects of diet on the body condition of free-living Bonelli's eagle nestlings as estimated by morphometric and blood biochemical indicators. We also analyzed whether or not nestling body condition was influenced by other intrinsic or extrinsic factors such as sex, age, time of day at sampling, or recent food intake. Additionally, our study provides reference values for multiple body condition measures in nestlings from two geographically distant populations (Catalonia and Andalusia; see also Balbontín & Ferrer 2002), which, in the case of

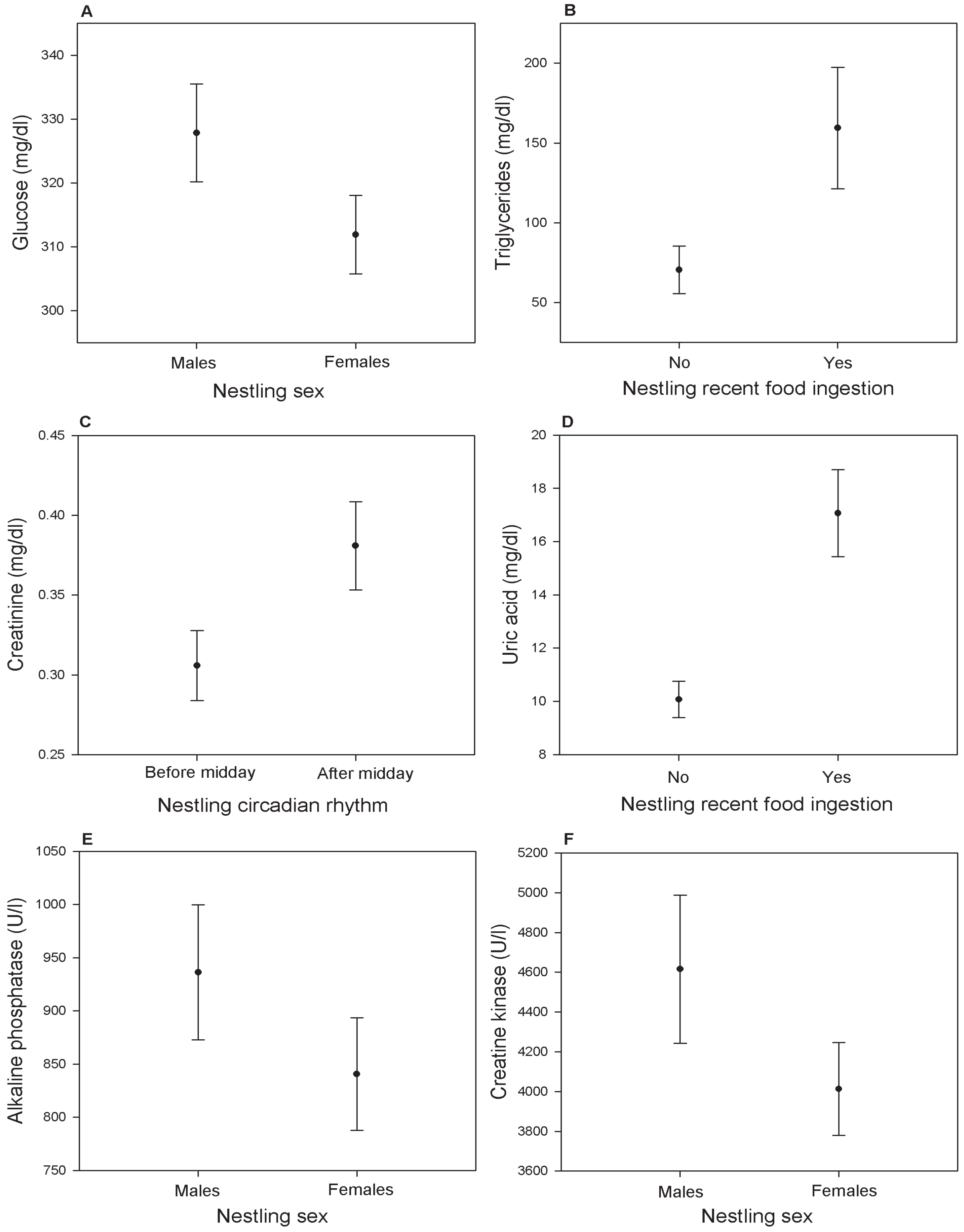

**Figure 2.** Effects of Bonelli's eagle nestling sex, recent food ingestion and circadian rhythm on plasma biochemical parameters: glucose (A), triglycerides (B), creatinine (C), uric acid (D), alkaline phosphatase (E) and creatine kinase (F).

the antioxidant metabolism, constitutes the first such data for this species and contributes to the hitherto scarce literature on the subject in other raptors (see e.g. Galvan et al. 2010, Sternalski et al. 2010, Casagrande et al. 2011).

Morphological indices are among the most commonly used methods to assess body condition in birds since they offer an easy and straightforward way of estimating an animals' nutritional status (Brown 1996, Stevenson & Woods 2006). Their usefulness, however, depends on how accurately they can compensate for variation in mass due to body size; if this is not accomplished, results may be misleading (Brown 1996, Green 2001, Peig & Green 2009). In this study, we scaled nestling body mass to the mean tarsus length of all individuals (accounting for sexual dimorphism) as if they were of identical skeletal size. This allowed us to obtain body condition estimates that were uncorrelated with nestling body size (i.e. tarsus length) and so we are confident that the scaled mass index did not reflect differences in nestling body size. Even so, we found no relationship between the scaled mass index and dietary parameters. This result suggests that body condition parameters based on nestling biometry are not sensitive enough to detect diet effects during the rearing period in Bonelli's eagle, especially in comparison with results from the blood biochemical level (see below).

The traditional use of haematocrit values as an indicator of body condition in wild birds has recently been questioned due to the fact that multiple factors such as age, sex, geographical elevation, energy expenditure, parasitism, dehydration, nutrition and genetics may influence these values (see Fair et al. 2007). In our study, nestling haematocrit was mainly explained by the altitude of nesting areas; on the other hand, neither age nor dietary parameters showed any clear effect on this parameter. Several studies have shown that haematocrit is either independent of or increases with elevation (Carpenter 1975, Borras et al. 2010). Accordingly, we found that Bonelli's eagle nestlings raised in more elevated nests had higher haematocrit values, which will enable them to ensure adequate oxygen delivery to tissues at lower partial oxygen pressures. The wide altitudinal range of nestling sites in our study area, which ranges from coastal cliffs to mountain ranges up to 1500 m a.s.l., possibly facilitated the detection of this pattern. In general, breeding territories in Andalusia were located at higher altitude than in Catalonia and so most nestlings in the former region had higher haematocrit values. We thus recommend that the effect of altitude be accounted for in studies using haematocrit as an indicator of nestling body condition.

Descriptive reference values of plasma metabolites are available for numerous raptor species, including Bonelli's eagle, and provide insights into the health of both captive and free-living individuals (Polo et al. 1992, Ferrer & Dobado-Berrios 1998, Balbontín & Ferrer 2002, Hernández & Margalida 2010). Overall, the Bonelli's eagle nestlings in our study showed similar plasma parameter values to free-living nestlings analyzed in western Andalusia by Balbontín & Ferrer (2002), albeit with a higher range for most parameters. This was probably because we analyzed a considerably larger number of nestlings (n = 92 *vs.* 28) from two geographically distant populations (Catalonia and eastern Andalusia), which, in the case of nestlings from Catalonia, had important dietary differences between territories (see Resano-Mayor et al. 2014a). We also detected slightly higher values in phosphorus, glucose and triglycerides in nestlings in our study; nevertheless, the mean and range of values for alkaline phosphatase reported by Balbontín & Ferrer (2002) were much higher. When comparing our results with published results for free-living nestlings of other raptor species

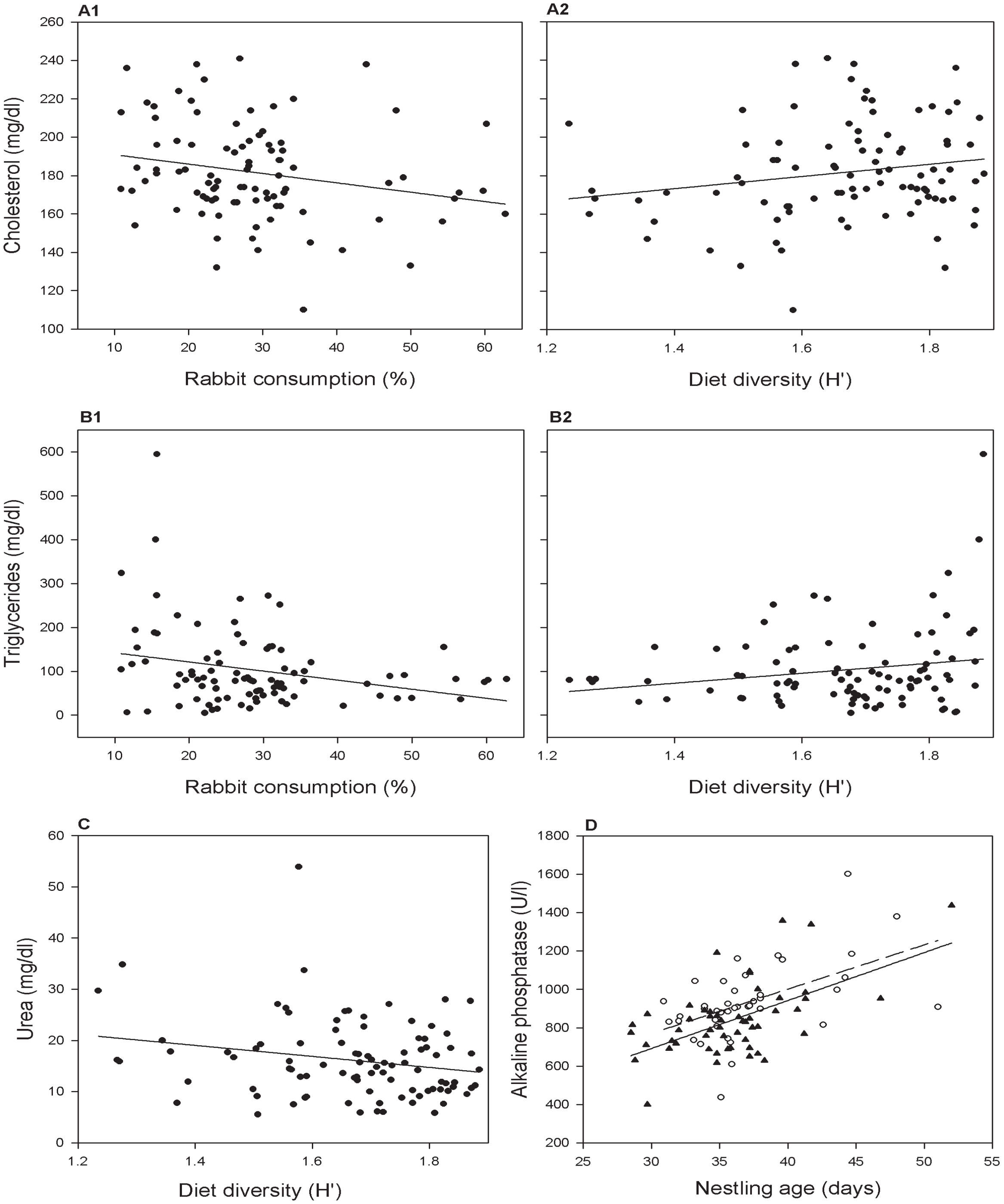

**Figure 3.** Effects of rabbit consumption (%), diet diversity (H’) and Bonelli’s eagle nestling age (days) on nestling plasma biochemical parameters: cholesterol (A1,2), triglycerides (B1,2), urea (C), alkaline phosphatase (D), aspartate aminotransferase (E1,2), lactate dehydrogenase (F1,2) and creatine kinase (G). In the case of the relationships shown in D and G, open circles denote males and filled triangles females. Trend lines are shown.

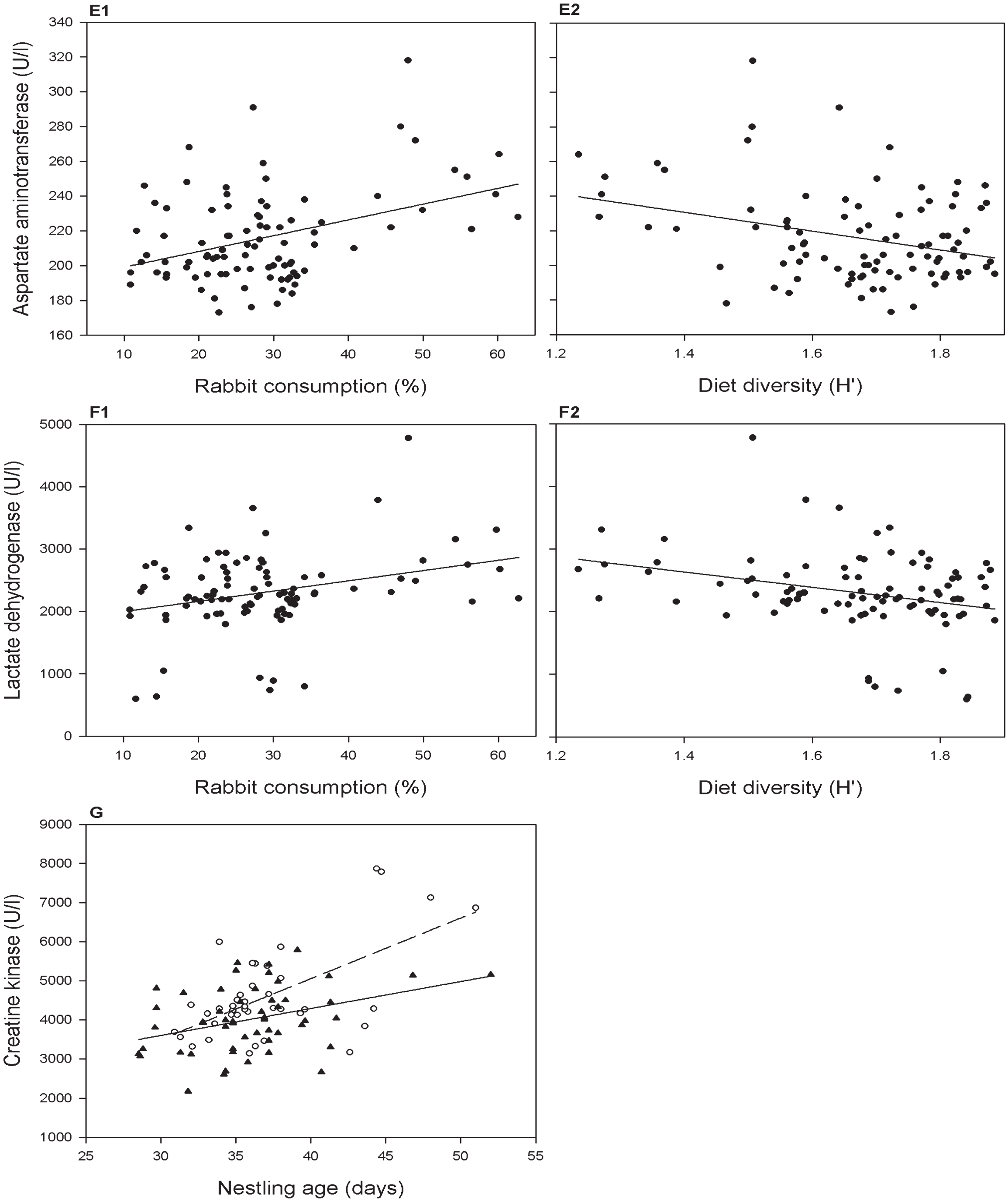

**Figure 3.** Continued from page before.

in Spain, Bonelli's eagle nestlings had (a) higher mean glucose concentration than nestlings of either Spanish Imperial eagle *Aquila adalberti*, Booted eagle *Aquila pennata*, Egyptian vulture *Neophron percnopterus* or Bearded vultures *Gypaetus barbatus*; (b) similar cholesterol and triglycerides to the eagle species but slightly lower values than the vultures; (c) similar total protein concentration to Imperial eagles and the two vulture species (nestlings of Booted eagle had very low plasma protein values); and (d) similar urea and uric acid to eagles, higher values for these two metabolites than Egyptian vultures, and similar urea levels but much higher uric acid than Bearded vultures (Dobado-Berrios et al. 1998, Ferrer & Dobado-Berrios 1998, Casado et al. 2002, Hernández & Margalida 2010). All these differences in blood parameters may be explained by, among other factors, specific characters such as growth patterns, metabolism and diet, as well as variations in sampling procedures.

Although avian blood parameters (including plasma biochemistry and antioxidant metabolism) provide information about individual nutritional status, few studies have integrated a multiple blood parameter approach with individual diet estimates into an interpretation of bird body condition. In this study, we hypothesized that greater consumption of rabbits (i.e. preferred prey) and greater diet diversity (i.e. a proxy of low availability of preferred prey) would positively and negatively affect, respectively, Bonelli's eagle nestling body condition. Both greater rabbit consumption and lower diet diversity reduced cholesterol and triglycerides in plasma. In birds, an increase in these lipid reserves has been related to starvation due to the mobilization of endogenous fats during fasting (García-Rodríguez et al. 1987a, Hochleithner 1994, Rubio et al. 2014). Thus, high rabbit consumption possibly indicates that nestlings' ever-increasing energetic demands are fulfilled, while an increase in diet diversity could lead to an energy shortage that would trigger the use of endogenous lipid reserves. Greater diet diversity was also related with lower urea levels. Suitable ingestion rates of protein-rich diets can increase the urea levels in birds (Koutsos et al. 2001, Salahuddin et al. 2012) as protein breakdown into amino acids releases urea (Hochleithner 1994). Thus, nestlings with a more diverse diet could have an overall lower protein intake leading to a subsequent reduction in urea levels. This idea is supported by lower AST activity (an enzyme involved in amino acid catabolism) in nestlings with greater diet diversity and lower rabbit consumption (see Das & Waterlow 1974). Therefore, based on conventional plasma parameters, nestlings with greater diet diversity seem to have poorer nutritional status as they have to mobilize endogenous fat reserves and also receive a lower intake of protein-rich food (see Parker et al. 2005). On the other hand, nestlings with greater rabbit consumption have overall better body condition, thereby supporting our assumption that this is an optimal prey item for the species.

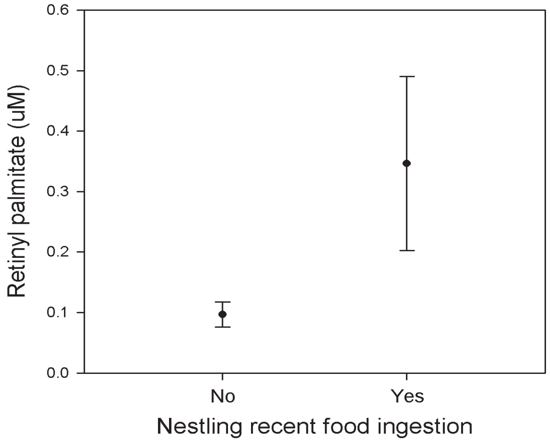

**Figure 4.** Effects of recent food ingestion by Bonelli's eagle nestlings on levels of plasma retinyl palmitate.

The analyses of AO defenses and OS biomarkers in an organism can be informative of the physiological functioning of the body and individual redox status. Vitamins and carotenoids are important plasmatic AO obtained from diet (Meydani et al. 1995, Møller et al. 2000). In our study, the Bonelli's eagle nestlings that consumed more rabbits had higher tocopherol levels, which fell as diet diversity increased. Tocopherol co-occurs with chlorophyll in chloroplasts and is abundant in leafy wild vegetables in our study area (Morales et al. 2011); thus, the greater consumption of a herbivorous prey (rabbits) will lead to higher tocopherol levels. Moreover, greater wood pigeon consumption increased nestlings' zeaxanthin levels, which is to be expected in the case of a granivorous prey item given the high content of this carotenoid in grain (Liu 2007). Given that both these AO substances prevent cell membranes from lipid peroxidation (Burton & Ingold 1981, Sujak et al. 1999), the greater consumption of these prey items could enhance nestling AO capacity. On the other hand, GSH is a key intracellular AO peptide that has vital functions in animals (Wu et al. 2004). Protein malnutrition and OS can markedly reduce GSH concentrations (Lu 2009). In our study, nestling tGSH increased with greater rabbit consumption, suggesting that this prey item enhanced nestlings' ability to scavenge ROS since it supplies higher protein levels (and GSH precursors) and also other AO such as tocopherol. This result supports our previous interpretation that an optimal diet enhances nutritional body condition, thereby suggesting that nutritional and antioxidant states are closely related. Nevertheless, an integrative approach for assessing animal OS should also measure the activity of AO enzymes such as GPx and SOD, which interact with the AO substances and regulate the overall ROS levels to maintain physiological homeostasis (Finkel & Holbrook 2000). Nestling GPx activity increased with greater passerine consumption and decreased with greater rabbit consumption. Given that GPx catalyzes the glutathione-dependent reduction of hydrogen peroxide ($H_2O_2$) and other peroxides (Wu et al. 2004), increased enzyme activity could be related with greater abundance of ROS. Thus, greater passerine consumption may imply nestling food shortage and increasing ROS levels, and may ultimately activate GPx to avoid OS. On the other hand, greater rabbit consumption not only increased nestling tGSH (i.e. AO capacity to directly scavenge ROS) but also reduced the activity of GPx, suggesting an overall better redox balance (i.e. less ROS to be scavenged) associated with greater consumption of this preferred prey. Moreover, in this enzyme reaction GSH becomes oxidized (oxGSH), which, if measured relative to the tGSH (%oxGSH), provides an estimate of oxidative damage. Both the oxGSH and the %oxGSH increased with greater diet diversity, suggesting greater OS in nestlings with more diverse diets. In the case of the SOD, greater consumption of wood pigeons and greater diet diversity reduce this enzyme's activity. SOD speeds the conversion of superoxide anion ($O_2^-\cdot$) to $H_2O_2$, which subsequently serves as a substrate of GPx and catalase. Thus, contrary to expectations, we did not detect any increase in this enzyme's activity with lower rabbit consumption or greater diet diversity, probably because other routes aside from the activation of SOD synthesis were used to cope with the increase in ROS (e.g. AO defenses could play an important role in scavenging ROS before activating SOD synthesis). Finally, TBARS did not correlate with any dietary parameter, suggesting that nestling diet had no effect on the oxidative damage of the erythrocyte membrane, probably due to the existence of a compensatory mechanism aimed at avoiding lipid peroxidation and hence OS damage (see Dimova et al. 2008).

Hematological values, however, are influenced by many factors other than individual nutritional condition. For instance, age-related differences have been described for several blood parameters (Dobado-Berrios et al. 1998, Alonso-Alvarez 2005). In our study, the levels of plasmatic enzymes ALP and CK increased with nestling age. An increase in ALP activity with age has been reported in the nestlings of other

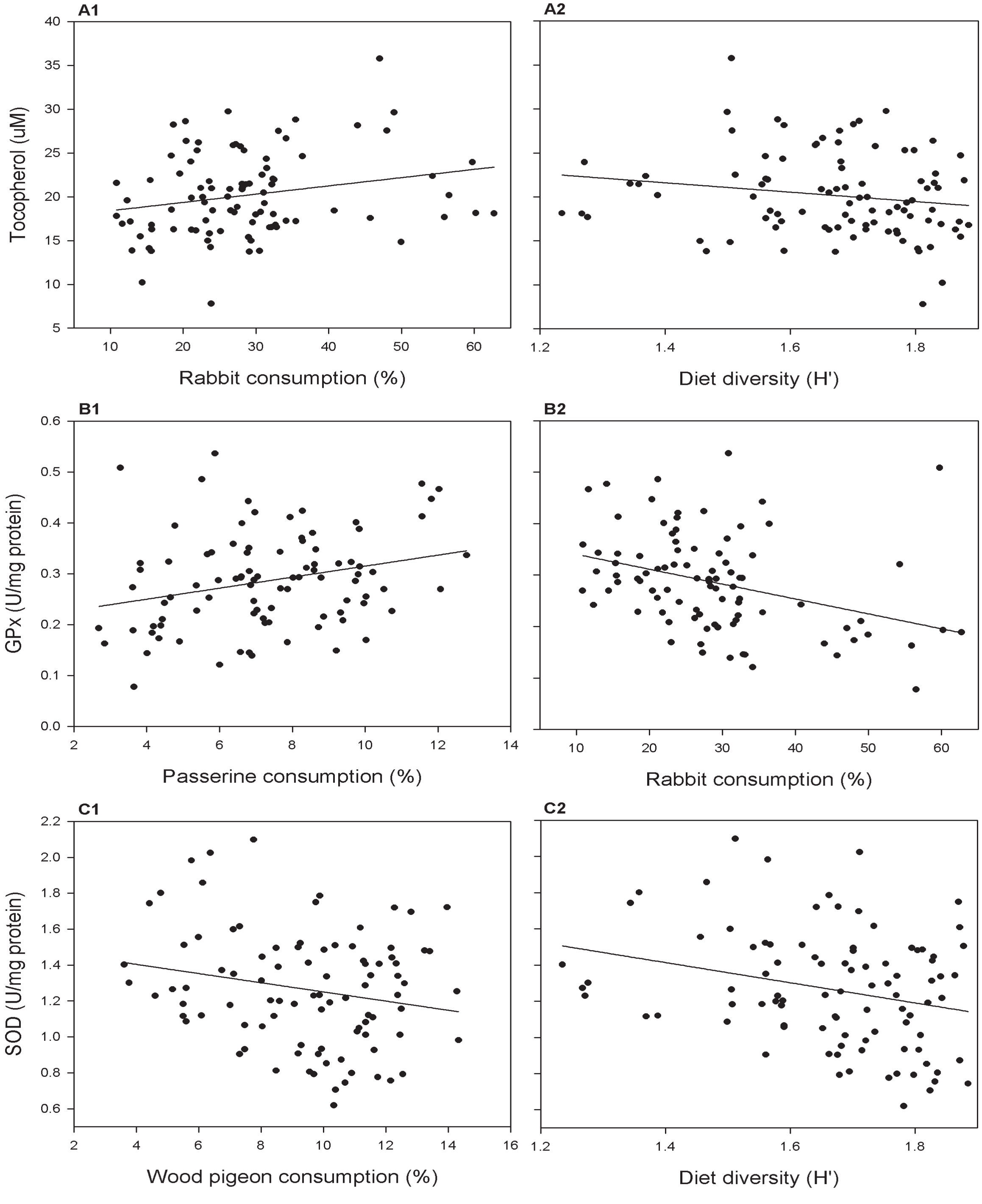

**Figure 5.** Effects of rabbit consumption (%), passerines consumption, wood pigeons consumption and diet diversity (H') on plasma antioxidants and oxidative stress biomarkers: tocopherol (A1,2), glutathione peroxidase (GPx) (B1,2), superoxide dismutase (SOD) (C1,2), zeaxanthin (D), total glutathione (tGSH) (E), oxidized glutathione (oxGSH) (F1) and the percentage of oxGSH (%oxGSH) (F2). Trend lines are shown.

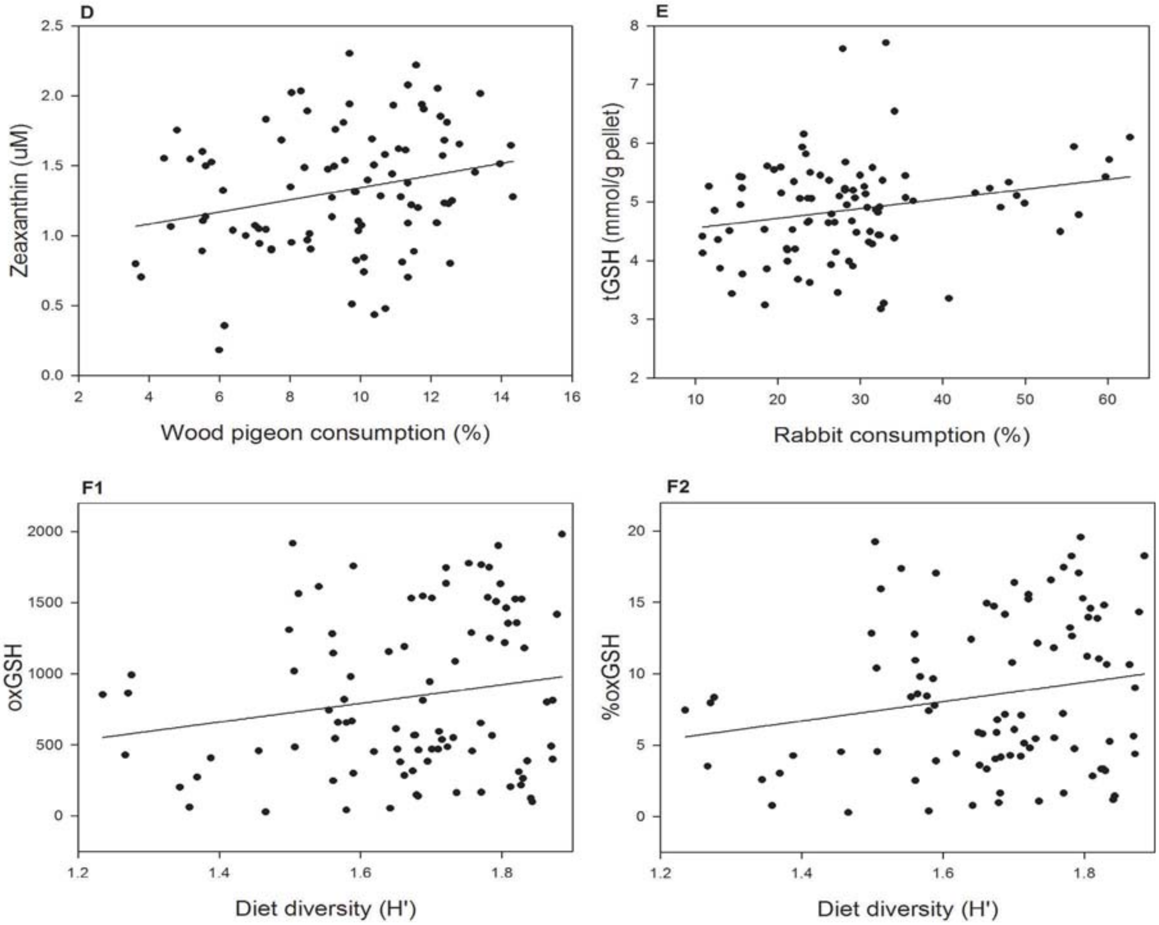

**Figure 5.** Continued from page before.

raptor species (Hoffman et al. 1985, Viñuela et al. 1991) as this enzyme is related with osteogenesis (Dobado-Berrios & Ferrer 1997). For instance, Viñuela et al. (1991) found that ALP levels in nestlings of both red and black kites (*Milvus milvus* and *Milvus migrans*, respectively) increased until an age of approximately 34 and 38 days, respectively, and then decreased. We found, however, a continuous increase in ALP with age, which may indicate that Bonelli's eagle nestlings progressively increase their ossification until they fledge. Despite being also involved in osteogenesis, in our study inorganic phosphorus was uncorrelated with nestling age and levels of ALP. The positive relationship between CK and nestling age was explained by an increase in muscle formation while growing, along with greater physical activity. Given that CK mediates in muscle contraction and increases with exercise-related muscle damage (Hochleithner 1994), we expect the oldest nestlings to have the highest enzyme levels due to increased wing-flapping activity before fledging. Sex differences in blood parameters have been also reported (Hochleithner 1994). Here, we found that male nestlings had higher plasmatic levels of glucose than females; nevertheless, despite the fact that males tended to have greater ALP and CK activity than females, sex differences were not as evident as in the case of glucose. Glucose differences could be explained by different metabolic rates between sexes due to sexual size dimorphism. Females are larger and may allocate more glucose for tissue formation than males. This idea has been previously suggested by Balbontín & Ferrer (2002) and Casado et al. (2002), who, respectively, reported higher glucose levels in both Bonelli's and Booted eagle male nestlings and suggested that this was because males have lower energy demands for growth than females. Blood parameter concentrations can also change after food ingestion (Hochleithner 1994). In this regard, Bonelli's eagle nestlings had a postprandial increase in plasma triglycerides, uric acid and retinyl palmitate. Given that triglyceride and uric acid levels may increase either shortly after food consumption (Lumeij & Remple 1991, Hochleithner 1994) or due to severe tissue damage or starvation (García-Rodríguez et al. 1987a, Hochleithner 1994), taking into account the recent food intake helped us appreciate that the higher levels of these parameters were indicative of recent food intake rather than the consequence of a pathology or disease. Creatinine was the only blood parameter that underwent a circadian rhythm in the sampled nestlings, being higher in nestlings sampled after than before midday. This result contrasts with the absence of diurnal rhythms for this plasmatic parameter in either Common buzzards *Buteo buteo* or Eagle owls *Bubo bubo* (García-Rodríguez et al. 1987b). Overall, our results highlight the importance of considering factors such as individual age, sex, time of last food intake and circadian rhythms when interpreting avian blood parameters in order to avoid reaching misleading conclusions regarding body condition.

In conclusion, by controlling for the potential influence of other intrinsic and extrinsic factors, our study reveals the usefulness of a multi-biomarker approach in assessing the body condition of Bonelli's eagle nestlings in relation to diet. Different body condition measures supported our prediction that greater consumption of a preferred prey item (rabbit) improved nestling body condition, whereas an increase in diet diversity had the opposite effect. In this regard, the most informative parameters were plasmatic levels of cholesterol, AST activity, tocopherol and zeaxanthin, as well as several oxidative stress biomarkers such as GPx, tGSH, oxGSH and %oxGSH. On the other hand, a single body condition measure such as the scaled mass index provided little information. Overall, these results highlight the importance of a multi-parameter approach when attempting to reach a deeper understanding of the relationships between diet and animal body condition, even though such an approach may preclude the possibility of a straightforward interpretation. The assessment of the relationships between nestling diet and body condition are crucial in endangered species such as the Bonelli's eagle because they can guide and focus management actions (e.g. improving local environmental characteristics as a means of enhancing the abundance of preferred prey items such as rabbits) in territories in which nestlings are in poorer health. The further application of multi-biomarker approaches to these and other populations within the distribution range of this species might help test optimal foraging theory assumptions (MacArthur & Pianka 1966, Pyke et al. 1977) and ascertain the contribution of nestlings' body condition to local (territories and populations) demographic fates. Finally, our study may serve as a practical starting point for future monitoring programs aiming to evaluate nestling health in Bonelli's eagles and other long-lived raptors, which could also be used as indicators of environmental changes (i.e. prey abundances and assemblages) in ecosystems highly influenced by human activities such as many in the Mediterranean Basin.

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## REFERENCES

**Alonso-Alvarez, C.** (2005) Age-dependent changes in plasma biochemistry of yellow-legged gulls (*Larus cachinnans*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 140, 512-518.

**Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O. & Sorci, G.** (2006) An experimental manipulation of life-history trajectories and resistance to oxidative stress. Evolution, 60, 1913-1924.

**Balaban, R.S., Nemoto, S. & Finkel, T.** (2005) Mitochondria, oxidants, and aging. Cell, 120, 483-495.

**Balbontín, J. & Ferrer, M.** (2002) Plasma chemistry reference values in free-living Bonelli’s Eagle (*Hieraaetus fasciatus*) nestlings. Journal of Raptor Research, 36, 231-235.

**Bates, D., Maechler, M. & Bolker, B.** (2012) lme4: linear mixed-effects models using S4 classes. R package version 0.999999-0. http://cran.r-project.org/web/packages/lme4/index.html.

**BirdLife International** (2004) Birds in Europe. Population estimates, trends and conservation status. In: BirdLife Conservation Series No. 12. Cambridge: BirdLife International, p. 374.

**Borras, A., Cabrera, J. & Senar, J.C.** (2010) Hematocrit variation in response to altitude changes in wild birds: a repeated-measures design. Condor, 112, 622-626.

**Bosch, R., Real, J., Tintó, A., Zozaya, E.L. & Castell, C.** (2010) Home-ranges and patterns of spatial use in territorial Bonelli’s Eagles *Aquila fasciata*. Ibis, 152, 105-117.

**Bowen, S.H., Lutz, E.V. & Ahlgren, M.O.** (1995) Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology, 76, 899-907.

**Brown, M.E.** (1996) Assessing body condition in birds. In: Ketterson, E.D., Val Nolan, J. (eds) Current ornithology. Plenum Press, New York, pp 67–135.

**Burnham, K.P. & Anderson, D.R.** (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed., Springer.

**Burton, G.W. & Ingold, K.U.** (1981) Autoxidation of biological molecules. 1. Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. Journal of the American Chemical Society, 103, 6472-6477.

**Carpenter, F.L.** (1975) Bird hematocrits: effects of high altitude and strength of flight. Comparative Biochemistry and Physiology Part A: Physiology, 50, 415-417.

**Carrascal, L.M. & Seoane, J.** (2009) Factors affecting large-scale distribution of the Bonelli's eagle Aquila fasciata in Spain. Ecological Research, 24, 565-573.

**Casado, E., Balbontin, J. & Ferrer, M.** (2002) Plasma chemistry in booted eagle (*Hieraaetus pennatus*) during breeding season. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 131, 233-241.

**Casagrande, S., Dell'Omo, G., Costantini, D., Tagliavini, J. & Groothuis, T.** (2011) Variation of a carotenoid-based trait in relation to oxidative stress and endocrine status during the breeding season in the Eurasian kestrel: a multi-factorial study. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 160, 16-26.

**Catoni, C., Peters, A. & Martin Schaefer, H.** (2008) Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. Animal Behaviour, 76, 1107-1119.

**Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J.C., & Smith, J.N.** (2004) Balancing food and predator pressure induces chronic stress in songbirds. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271, 2473-2479.

**Costantini, D. & Verhulst, S.** (2009) Does high antioxidant capacity indicate low oxidative stress? Functional Ecology, 23, 506-509.

**Costantini, D., Rowe, M., Butler, M.W. & McGraw, K.J.** (2010) From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. Functional Ecology, 24, 950-959.

**Das, T.K. & Waterlow, J.C.** (1974) The rate of adaptation of urea cycle enzymes, aminotransferases and glutamic dehydrogenase to changes in dietary protein intake. British Journal of Nutrition, 32, 353-373.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds)** (1994) Handbook of the Birds of the World, Vol. 2. Barcelona: Lynx Edicions.

**Dimova, E.G., Bryant, P.E. & Chankova, S.G.** (2008) Adaptive response: some underlying mechanisms and open questions. Genetics and Molecular Biology, 31, 396-408.

**Dobado-Berrios, P.M. & Ferrer, M.** (1997) Age-related changes of plasma alkaline phosphatase and inorganic phosphorus, and late ossification of the cranial roof in the Spanish imperial eagle (*Aquila adalberti* CL Brehm, 1861). Physiological Zoology, 70, 421-427.

**Dobado-Berrios, P.M., Tella, J.L., Ceballos, O. & Donázar, J.A.** (1998) Effects of age and captivity on plasma chemistry values of the Egyptian vulture. Condor, 100, 719-725.

**Fair, J., Whitaker, S. & Pearson, B.** (2007) Sources of variation in haematocrit in birds. Ibis, 149, 535-552.

**Ferrer, M. & Dobado-Berrios, P.** (1998) Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 120, 209-217.

**Finkel, T. & Holbrook, N.J.** (2000) Oxidants, oxidative stress and the biology of ageing. Nature, 408, 239-247.

**Fridolfsson, A.K. & Ellegren, H.** (1999) A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology, 30, 116-121.

**Galván, I., Gangoso, L., Grande, J.M., Negro, J.J., Rodríguez, A., Figuerola, J. & Alonso-Alvarez, C.** (2010) Antioxidant machinery differs between melanic and light nestlings of two polymorphic raptors. PLoS ONE, 5, e13369.

**García-Rodríguez, T., Ferrer, M., Carrillo, J.C. & Castroviejo, J.** (1987a) Metabolic responses of *Buteo buteo* to long-term fasting and refeeding. Comparative Biochemistry and Physiology Part A: Physiology, 87, 381-386.

**García-Rodríguez, T., Ferrer, M., Recio, F. & Castroviejo, J.** (1987b) Circadian rhythms of determined blood chemistry values in buzzards and eagle owls. Comparative Biochemistry and Physiology Part A: Physiology, 88, 663-669.

**Gil-Sánchez, J.M.** (2000) Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). Ardeola, 47, 1-8.

**Gil-Sánchez, J.M., Moleón, M., Otero, M. & Bautista, J.** (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biological Conservation, 118, 685-694.

**Green, A.J.** (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology, 82, 1473-1483.

**Harrison, G.L. & Harrison, L.R.** (1986) Clinical Avian Medicine and Surgery. London: W.B. Saunders.

**Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S.** (2011) Carry-over effects as drivers of fitness differences in animals. Journal of Animal Ecology, 80, 4-18.

**Hernández-Matías, A., Real, J. & Pradel, R.** (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. Journal of Wildlife Management, 75, 856-866.

**Hernández-Matías, A., Real, J., Moleón, M., Palma, L., Sánchez-Zapata, J.A., Pradel, R., Carrete, M., Gil-Sánchez, J.M., Beja, P., Balbontín, J., Vincent-Martin, N., Ravayrol, A., Benítez, J.R., Arroyo, B., Fernández, C., Ferreiro, E. & García, J.** (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecological Monographs, 83, 239-261.

**Hernández, M., & Margalida, A.** (2010) Hematology and blood chemistry reference values and age-related changes in wild bearded vultures (*Gypaetus barbatus*). Journal of Wildlife Diseases, 46, 390-400.

**Hobson, K.A. & Clark, R.G.** (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor, 94, 189-197.

**Hochleithner, M.** (1994) Biochemistries. In: Ritchie, B.W., Harrison, G.L. & Harrison, L.R. (eds) Avian medicine: principles and application. Wingers, Lake Worth, pp. 223-245.

**Hoffman, D.J., Franson, J.C., Pattee, O.H., Bunck, C.M. & Murray, H.C.** (1985) Biochemical and hematological effects of lead ingestion in nestling American kestrels (*Falco sparverius*). Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 80, 431-439.

**Koutsos, E.A., Smith, J., Woods, L.W. & Klasing, K.C.** (2001) Adult cockatiels (*Nymphicus hollandicus*) metabolically adapt to high protein diets. The Journal of Nutrition, 131, 2014-2020.

**Labocha, M.K. & Hayes, J.P.** (2012) Morphometric indices of body condition in birds: a review. Journal of Ornithology, 153, 1-22.

**Lefcheck, J.S., Whalen, M.A., Davenport, T.M., Stone, J.P. & Duffy, J.E.** (2013) Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology, 94, 565-572.

**Liu, R.H.** (2007) Whole grain phytochemicals and health. Journal of Cereal Science, 46, 207-219.

**Lu, S.C.** (2009) Regulation of glutathione synthesis. Molecular Aspects of Medicine, 30, 42-59.

**Lumeij, J.T. & Remple, J.D.** (1991) Plasma urea, creatinine and uric acid concentrations in relation to feeding in peregrine falcons (*Falco peregrinus*). Avian Pathology, 20, 79-83.

**MacArthur, R.H. & Pianka, E.R.** (1966) On optimal use of a patchy environment. The American Naturalist, 100, 603-609.

**Mañosa, S., Real, J. & Codina, J.** (1995) Age estimation and growth patterns in nestling Bonelli's Eagles. Journal of Raptor Research, 29, 273-275.

**Martinez-Haro, M., Green, A.J. & Mateo, R.** (2011) Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. Environmental Research, 111, 530-538.

**Mateo, R., Beyer, W.N., Spann, J., Hoffman, D. & Ramis, A.** (2003) Relationship between oxidative stress, pathology, and behavioral signs of lead poisoning in mallards. Journal of Toxicology and Environmental Health Part A, 66, 1371-1389.

**Meydani, S.N., Wu, D., Santos, M.S. & Hayek, M.G.** (1995) Antioxidants and immune response in aged persons: overview of present evidence. The American Journal of Clinical Nutrition, 62, 1462S-1476S.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Charton, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. Journal of Biogeography, 36, 1502-1515.

**Moleón, M., Sánchez-Zapata, J.A., Gil-Sánchez, J.M., Ballesteros-Duperón, E., Barea-Azcón, J.M. & Virgós, E.** (2012) Predator–prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia, 168, 679-689.

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

**Monaghan, P., Metcalfe, N. B., & Torres, R.** (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecology Letters, 12, 75-92.

**Morales, P., Carvalho, A.M., Sánchez-Mata, M.C., Cámara, M., Molina, M. & Ferreira, I.C.** (2012) Tocopherol composition and antioxidant activity of Spanish wild vegetables. Genetic Resources and Crop Evolution, 59, 851-863.

**Nakagawa, S. & Schielzeth, H.** (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution, 4, 133-142.

**Noguera, J.C., Lores, M., Alonso-Álvarez, C. & Velando, A.** (2011) Thrifty development: early-life diet restriction reduces oxidative damage during later growth. *Functional Ecology*, 25, 1144-1153.

**Owen, J.C.** (2011) Collecting, processing, and storing avian blood: a review. Journal of Field Ornithology, 82, 339-354.

**Parker, K.L., Barboza, P.S. & Stephenson, T.R.** (2005) Protein conservation in female caribou (*Rangifer tarandus*): effects of decreasing diet quality during winter. Journal of Mammalogy, 86, 610-622.

**Parnell, A.C, Inger, R., Bearhop, S. & Jackson, A.L.** (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE, 5, e9672.

**Peig, J. & Green, A.J.** (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos, 118, 1883-1891.

**Peig, J. & Green, A.J.** (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Functional Ecology, 24, 1323-1332.

**Polo, F.J., Celdran, J.F., Peinado, V.I., Viscor,**

**G. & Palomeque, J.** (1992) Hematological values for four species of birds of prey. Condor, 94, 1007-1013.

**Pothoven, S.A., Nalepa, T.F., Schneeberger, P.J. & Brandt, S.B.** (2001) Changes in diet and body condition of lake whitefish in southern Lake Michigan associated with changes in benthos. North American Journal of Fisheries Management, 21, 876-883.

**Pyke, G.H., Pulliam, H.R. & Charnov, E.L.** (1977) Optimal foraging: a selective review of theory and tests. Quarterly Review of Biology, 52, 137-154.

**Real, J.** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía [PhD thesis]. Barcelona: University of Barcelona. 241 p.

**Real, J.** (2004) Águila-Azor Perdicera, *Hieraaetus fasciatus*. In: Madroño A, González C, Atienza JC, editors. Libro rojo de las aves de España. Madrid: DGB-SEO/Birdlife. pp.154-157.

**Reglero, M.M., Taggart, M.A., Monsalve-González, L. & Mateo, R.** (2009) Heavy metal exposure in large game from a lead mining area: effects on oxidative stress and fatty acid composition in liver. Environmental Pollution, 157, 1388-1395.

**Resano, J., Hernández-Matías, A., Real, J. & Parés, F.** (2011) Using stable isotopes to determine dietary patterns in Bonelli's eagle (*Aquila fasciata*) nestlings. Journal of Raptor Research, 45, 342-352.

**Resano-Mayor, J., Hernández-Matías, A., Real, J., Moleón, M., Parés, F., Inger, R. & Bearhop, S.** (2014a) Multi-Scale Effects of Nestling Diet on Breeding Performance in a Terrestrial Top Predator Inferred from Stable Isotope Analysis. PLoS ONE, 9, e95320.

**Resano-Mayor, J., Hernández-Matías, A., Real, J., Parés, F., Inger, R. & Bearhop, S.** (2014b) Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. Ibis, 156, 176-188.

**Rodríguez-Estival, J., Taggart, M.A. & Mateo, R.** (2011) Alterations in vitamin A and E levels in liver and testis of wild ungulates from a lead mining area. Archives of Environmental Contamination and Toxicology, 60, 361-371.

**Rubio, M.D., Ildefonso, N., Agüera, E.I., Almaraz, P., De Miguel, R.J. & Escribano, B. M.** (2014) Plasma biochemistry and haematology of crested coots (*Fulica cristata*) and common coots (*Fulica atra*) from Spain. Comparative Clinical Pathology, 23, 385-391.

**Salahuddin, M., Miah, M.A. & Ahmad, N.** (2012) Effects of protein and vitamin ade on growth performance and haemato-biochemical profile in broiler. Bangladesh Journal of Veterinary Medicine, 10, 9-14.

**Shannon, C.E. & Weaver, W.** (1949) The mathematical theory of communication. Univ. of Illinois Press.

**Sorensen, M.C., Hipfner, J.M., Kyser, T.K. & Norris, D.R.** (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. Journal of Animal Ecology, 78, 460-467.

**Sternalski, A., Mougeot, F., Eraud, C., Gangloff, B., Villers, A. & Bretagnolle, V.** (2010) Carotenoids in nestling Montagu's harriers: variations according to age, sex, body condition and evidence for diet-related limitations. Journal of Comparative Physiology B, 180, 33-43.

**Stevenson, R.D. & Woods, W.A.** (2006) Condition indices for conservation: new uses for evolving tools. Integrative and Comparative Biology, 46, 1169-1190.

**Sujak, A., Gabrielska, J., Grudziński, W., Borc, R., Mazurek, P. & Gruszecki, W.I.** (1999) Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. Archives of Biochemistry and Biophysics, 371, 301-307.

**Viñuela J., Ferrer, M. & Recio, F.** (1991) Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. Comparative Biochemistry and Physiology Part A: Physiology, 99, 49-54.

**Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R. & Turner, N.D.** (2004) Glutathione metabolism and its implications for health. The Journal of Nutrition, 134, 489-492.

**Zera, A.J. & Harshman, L.G.** (2001) The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics, 32, 95-126.

**Zera, A.J. & Harshman, L.G.** (2001) The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics, 32, 95-126.

## SUPPORTING INFORMATION

Additional supporting information is shown in the following pages: Tables S1, S2, S3 and S4.

## SUPPORTING INFORMATION

**Table S1.** Models used for the scaled mass index as estimates of body condition. The best-fitted model is shown in bold type.

| Scaled mass index | ΔAICc | AICcw | R²m | R²c |
|---|---|---|---|---|
| **1. Sex + OC + H′** | **0.000** | **0.888** | **0.641** | **0.769** |
| 2. Sex + PAS | 6.542 | 0.034 | 0.644 | 0.766 |
| 3. Sex + CP | 6.982 | 0.027 | 0.644 | 0.764 |
| 4. Sex + CL | 7.971 | 0.016 | 0.644 | 0.764 |
| 5. Sex + AR | 9.052 | 0.010 | 0.643 | 0.765 |
| 6. Sex + Food Ingestion | 9.199 | 0.009 | 0.652 | 0.786 |
| 7. Sex + OC | 9.668 | 0.007 | 0.643 | 0.769 |
| 8. Sex + H′ | 10.479 | 0.005 | 0.643 | 0.766 |
| 9. Sex + Circadian Rhythm | 11.466 | 0.003 | 0.654 | 0.767 |
| 10. Sex + Brood Size | 12.496 | 0.002 | 0.644 | 0.766 |
| 11. Sex | 19.228 | 0.000 | 0.647 | 0.763 |

Best model AICc = 1112.249

**Table S2.** Models used for haematocrit as estimates of body condition. The best-fitted model is shown in bold type.

| Haematocrit | ΔAICc | AICcw | R²m | R²c |
|---|---|---|---|---|
| **1. Altitude** | **0.000** | **0.736** | **0.414** | **0.455** |
| 2. Null model | 2.371 | 0.225 | 0.000 | 0.602 |
| 3. OC | 6.375 | 0.030 | 0.037 | 0.604 |
| 4. H′ | 9.290 | 0.007 | 0.017 | 0.553 |
| 5. OC + H′ | 12.659 | 0.001 | 0.040 | 0.672 |

Best model AICc = -414.770

## SUPPORTING INFORMATION

**Table S3.** Models used for plasma biochemistry parameters as estimates of body condition. Best-fitted models are shown in bold type.

| Calcium | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. PAS** | **0.000** | **0.375** | **0.002** | **0.265** |
| **2. CP** | **0.320** | **0.319** | **0.001** | **0.266** |
| 3. OC + H′ | 2.614 | 0.101 | 0.001 | 0.266 |
| 4. Null model | 3.181 | 0.076 | 0.000 | 0.262 |
| 5. OC | 3.298 | 0.072 | 0.000 | 0.268 |
| 6. H′ | 3.781 | 0.057 | 0.000 | 0.263 |

Best model AICc = 298.730

| Magnesium | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. Null model** | **0.000** | **0.572** | **0.000** | **0.377** |
| 2. OC | 2.019 | 0.209 | 0.004 | 0.388 |
| 3. H′ | 2.866 | 0.137 | 0.000 | 0.388 |
| 4. OC + H′ | 3.865 | 0.083 | 0.005 | 0.393 |

Best model AICc = 75.589

| Phosphorus | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. PAS** | **0.000** | **0.408** | **0.015** | **0.179** |
| **2. CP** | **1.338** | **0.209** | **0.000** | **0.170** |
| **3. CL** | **1.905** | **0.157** | **0.005** | **0.173** |
| 4. OC + H′ | 3.286 | 0.079 | 0.011 | 0.177 |
| 5. Null model | 3.970 | 0.056 | 0.000 | 0.163 |
| 6. OC | 4.032 | 0.054 | 0.003 | 0.177 |
| 7. H′ | 4.851 | 0.036 | 0.000 | 0.171 |

Best model AICc = 276.445

| Glucose | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.367** | **0.028** | **0.446** |
| **2. Sex + Age** | **0.012** | **0.365** | **0.134** | **0.414** |
| **3. Sex** | **1.999** | **0.135** | **0.096** | **0.364** |
| 4. PAS | 4.686 | 0.035 | 0.001 | 0.344 |
| 5. CP | 4.924 | 0.031 | 0.000 | 0.346 |
| 6. CL | 5.802 | 0.020 | 0.001 | 0.346 |
| 7. AR | 6.585 | 0.014 | 0.005 | 0.343 |
| 8. Brood size | 6.600 | 0.014 | 0.046 | 0.430 |
| 9. OC | 7.794 | 0.007 | 0.000 | 0.344 |
| 10. H′ | 7.985 | 0.007 | 0.007 | 0.348 |
| 11. Circadian rhythm | 10.462 | 0.002 | 0.007 | 0.348 |
| 12. Age | 10.867 | 0.002 | 0.052 | 0.380 |
| 13. Food ingestion | 10.886 | 0.002 | 0.002 | 0.326 |
| 14. Null model | 13.987 | 0.000 | 0.000 | 0.332 |

Best model AICc = 834.136

| Cholesterol | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.501** | **0.054** | **0.412** |
| 2. AR | 2.271 | 0.161 | 0.107 | 0.502 |
| 3. CP | 2.514 | 0.143 | 0.066 | 0.372 |
| 4. PAS | 2.598 | 0.137 | 0.063 | 0.366 |
| 5. CL | 5.960 | 0.025 | 0.029 | 0.404 |
| 6. OC | 6.629 | 0.018 | 0.053 | 0.406 |
| 7. H′ | 7.999 | 0.009 | 0.039 | 0.396 |
| 8. Food ingestion | 10.601 | 0.003 | 0.029 | 0.391 |

| | | | | |
|---|---|---|---|---|
| 9. Circadian rhythm | 11.636 | 0.001 | 0.018 | 0.411 |
| 10. Brood size | 12.425 | 0.001 | 0.003 | 0.395 |
| 11. Sex | 13.179 | 0.001 | 0.001 | 0.389 |
| 12. Null model | 16.107 | 0.000 | 0.000 | 0.384 |
| Best model AICc = 838.896 | | | | |
| **Triglycerides** | **ΔAICc** | **AICcw** | **R$^2$m** | **R$^2$c** |
| **1. OC + H′** | **0.000** | **0.687** | **0.105** | **0.529** |
| **2. Food ingestion** | **1.686** | **0.296** | **0.191** | **0.490** |
| 3. CL | 9.771 | 0.005 | 0.064 | 0.520 |
| 4. OC | 10.229 | 0.004 | 0.091 | 0.505 |
| 5. AR | 11.400 | 0.002 | 0.056 | 0.556 |
| 6. PAS | 12.037 | 0.002 | 0.009 | 0.449 |
| 7. CP | 12.830 | 0.001 | 0.000 | 0.450 |
| 8. Sex + Age | 12.933 | 0.001 | 0.050 | 0.480 |
| 9. H′ | 14.319 | 0.001 | 0.037 | 0.494 |
| 10. Sex | 15.481 | 0.000 | 0.036 | 0.482 |
| 11. Brood size | 16.731 | 0.000 | 0.021 | 0.489 |
| 12. Circadian rhythm | 16.800 | 0.000 | 0.028 | 0.448 |
| 13. Age | 22.261 | 0.000 | 0.013 | 0.455 |
| 14. Null model | 24.493 | 0.000 | 0.000 | 0.446 |
| Best model AICc = 1053.415 | | | | |
| **Creatinine** | **ΔAICc** | **AICcw** | **R$^2$m** | **R$^2$c** |
| **1. Circadian rhythm** | **0.000** | **0.980** | **0.183** | **0.312** |
| 2. Null model | 8.036 | 0.018 | 0.000 | 0.163 |
| 3. OC | 13.024 | 0.001 | 0.002 | 0.175 |
| 4. H′ | 13.792 | 0.001 | 0.000 | 0.172 |
| 5. OC + H′ | 17.356 | 0.000 | 0.007 | 0.176 |
| Best model AICc = -170.813 | | | | |
| **Urea** | **ΔAICc** | **AICcw** | **R$^2$m** | **R$^2$c** |
| **1. OC + H′** | **0.000** | **0.443** | **0.013** | **0.783** |
| 2. CP | 2.588 | 0.121 | 0.003 | 0.803 |
| 3. PAS | 2.617 | 0.120 | 0.002 | 0.796 |
| 4. Circadian rhythm | 3.297 | 0.085 | 0.031 | 0.799 |
| 5. CL | 3.842 | 0.065 | 0.001 | 0.795 |
| 6. AR | 4.940 | 0.037 | 0.000 | 0.799 |
| 7. H′ | 5.060 | 0.035 | 0.012 | 0.790 |
| 8. OC | 5.242 | 0.032 | 0.005 | 0.807 |
| 9. Food ingestion | 5.292 | 0.031 | 0.026 | 0.738 |
| 10. Brood size | 5.683 | 0.026 | 0.016 | 0.778 |
| 11. Null model | 9.759 | 0.003 | 0.000 | 0.798 |
| Best model AICc = 613.658 | | | | |
| **Uric acid** | **ΔAICc** | **AICcw** | **R$^2$m** | **R$^2$c** |
| **1. Food ingestion** | **0.000** | **1.000** | **0.398** | **0.649** |
| 2. Circadian rhythm | 23.981 | 0.000 | 0.141 | 0.715 |
| 3. OC + H′ | 25.772 | 0.000 | 0.063 | 0.712 |
| 4. CP | 27.413 | 0.000 | 0.049 | 0.710 |
| 5. PAS | 29.666 | 0.000 | 0.019 | 0.684 |
| 6. CL | 32.078 | 0.000 | 0.001 | 0.690 |
| 7. AR | 33.060 | 0.000 | 0.002 | 0.687 |
| 8. H′ | 33.215 | 0.000 | 0.027 | 0.685 |
| 9. OC | 33.987 | 0.000 | 0.000 | 0.689 |
| 10. Null model | 36.997 | 0.000 | 0.000 | 0.687 |
| Best model AICc = 490.434 | | | | |

| Total proteins | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. AR** | **0.000** | **0.397** | **0.046** | **0.593** |
| **2. CL** | **1.896** | **0.154** | **0.016** | **0.456** |
| 3. PAS | 2.065 | 0.141 | 0.000 | 0.447 |
| 4. CP | 2.269 | 0.128 | 0.000 | 0.449 |
| 5. OC + H′ | 2.465 | 0.116 | 0.001 | 0.452 |
| 6. OC | 5.145 | 0.030 | 0.000 | 0.450 |
| 7. H′ | 5.665 | 0.023 | 0.000 | 0.448 |
| 8. Null model | 7.112 | 0.011 | 0.000 | 0.434 |

Best model AICc = 463.291

| Alkaline phosphatase | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. Sex + Age** | **0.000** | **0.957** | **0.241** | **0.574** |
| 2. Age | 6.649 | 0.034 | 0.237 | 0.589 |
| 3. OC + H′ | 9.500 | 0.008 | 0.010 | 0.664 |
| 4. PAS | 16.502 | 0.000 | 0.017 | 0.627 |
| 5. CL | 16.954 | 0.000 | 0.025 | 0.632 |
| 6. CP | 17.966 | 0.000 | 0.003 | 0.616 |
| 7. AR | 20.006 | 0.000 | 0.001 | 0.606 |
| 8. OC | 20.838 | 0.000 | 0.003 | 0.618 |
| 9. H′ | 21.581 | 0.000 | 0.000 | 0.616 |
| 10. Circadian rhythm | 22.860 | 0.000 | 0.014 | 0.635 |
| 11. Food ingestion | 23.393 | 0.000 | 0.008 | 0.638 |
| 12. Brood size | 23.824 | 0.000 | 0.001 | 0.618 |
| 13. Sex | 24.337 | 0.000 | 0.002 | 0.590 |
| 14. Null model | 31.330 | 0.000 | 0.000 | 0.612 |

Best model AICc = 1180.668

| Aspartate aminotransferase | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.922** | **0.123** | **0.613** |
| 2. OC | 6.662 | 0.033 | 0.124 | 0.608 |
| 3. CL | 7.220 | 0.025 | 0.074 | 0.594 |
| 4. PAS | 8.990 | 0.010 | 0.032 | 0.569 |
| 5. CP | 10.643 | 0.005 | 0.008 | 0.581 |
| 6. H′ | 11.270 | 0.003 | 0.065 | 0.602 |
| 7. AR | 12.989 | 0.001 | 0.002 | 0.600 |
| 8. Brood size | 16.269 | 0.000 | 0.007 | 0.596 |
| 9. Circadian rhythm | 17.105 | 0.000 | 0.001 | 0.597 |
| 10. Food ingestion | 17.177 | 0.000 | 0.002 | 0.601 |
| 11. Sex | 17.479 | 0.000 | 0.001 | 0.595 |
| 12. Null model | 20.309 | 0.000 | 0.000 | 0.598 |

Best model AICc = 823.677

| Lactate dehydrogenase | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.993** | **0.077** | **0.466** |
| 2. PAS | 12.918 | 0.002 | 0.022 | 0.460 |
| 3. OC | 13.218 | 0.001 | 0.074 | 0.463 |
| 4. CP | 14.216 | 0.001 | 0.004 | 0.470 |
| 5. H′ | 14.389 | 0.001 | 0.061 | 0.459 |
| 6. Sex + Age | 14.763 | 0.001 | 0.012 | 0.471 |
| 7. Circadian rhythm | 14.965 | 0.001 | 0.074 | 0.517 |
| 8. CL | 15.067 | 0.001 | 0.005 | 0.472 |
| 9. AR | 16.423 | 0.000 | 0.001 | 0.480 |
| 10. Brood size | 18.643 | 0.000 | 0.017 | 0.483 |
| 11. Food ingestion | 19.577 | 0.000 | 0.012 | 0.484 |
| 12. Sex | 20.032 | 0.000 | 0.008 | 0.484 |

| | | | | |
|---|---|---|---|---|
| 13. Age | 24.647 | 0.000 | 0.006 | 0.459 |
| 14. Null model | 30.086 | 0.000 | 0.000 | 0.475 |

Best model AICc = 1403.763

| Creatine Kinase | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. Sex + Age** | **0.000** | **0.999** | **0.276** | **0.680** |
| 2. OC + H′ | 15.075 | 0.001 | 0.029 | 0.601 |
| 3. Age | 17.683 | 0.000 | 0.219 | 0.645 |
| 4. CL | 24.681 | 0.000 | 0.065 | 0.609 |
| 5. Sex | 26.905 | 0.000 | 0.064 | 0.589 |
| 6. PAS | 27.446 | 0.000 | 0.006 | 0.558 |
| 7. CP | 27.868 | 0.000 | 0.002 | 0.564 |
| 8. AR | 28.087 | 0.000 | 0.028 | 0.577 |
| 9. OC | 29.272 | 0.000 | 0.028 | 0.579 |
| 10. H′ | 30.929 | 0.000 | 0.010 | 0.556 |
| 11. Food ingestion | 32.109 | 0.000 | 0.022 | 0.618 |
| 12. Brood size | 33.565 | 0.000 | 0.004 | 0.577 |
| 13. Circadian rhythm | 33.659 | 0.000 | 0.006 | 0.569 |
| 14. Null model | 44.622 | 0.000 | 0.000 | 0.572 |

Best model AICc = 1473.948

**Table S4.** Models used for antioxidants and oxidative stress biomarkers as estimates of body condition. Best-fitted models are shown in bold type.

| Retinol | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. PAS** | **0.000** | **0.395** | **0.005** | **0.851** |
| **2. CP** | **1.102** | **0.228** | **0.002** | **0.859** |
| **3. AR** | **1.939** | **0.150** | **0.006** | **0.875** |
| 4. OC + H′ | 2.921 | 0.092 | 0.005 | 0.873 |
| 5. Null model | 4.087 | 0.051 | 0.000 | 0.856 |
| 6. OC | 4.240 | 0.047 | 0.000 | 0.854 |
| 7. H′ | 4.755 | 0.037 | 0.000 | 0.861 |

Best model AICc = 253.888

| Retinyl palmitate | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. Food ingestion** | **0.000** | **0.910** | **0.174** | **0.316** |
| 2. AR | 5.346 | 0.063 | 0.054 | 0.474 |
| 3. Null model | 7.934 | 0.017 | 0.000 | 0.348 |
| 4. OC | 10.596 | 0.005 | 0.002 | 0.363 |
| 5. H′ | 10.928 | 0.004 | 0.005 | 0.377 |
| 6. OC + H′ | 12.463 | 0.002 | 0.008 | 0.401 |

Best model AICc = 30.732

| Tocopherol | ΔAICc | AICcw | $R^2$m | $R^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.282** | **0.013** | **0.687** |
| **2. PAS** | **0.370** | **0.234** | **0.006** | **0.709** |
| **3. CP** | **1.151** | **0.159** | **0.000** | **0.710** |
| **4. AR** | **1.680** | **0.122** | **0.016** | **0.721** |
| **5. CL** | **1.912** | **0.108** | **0.002** | **0.702** |
| 6. OC | 3.250 | 0.056 | 0.011 | 0.689 |
| 7. H′ | 4.475 | 0.030 | 0.001 | 0.705 |
| 8. Null model | 6.795 | 0.009 | 0.000 | 0.708 |

Best model AICc = 514.543

| Lutein | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. PAS** | **0.000** | **0.303** | **0.006** | **0.768** |
| **2. CP** | **0.712** | **0.212** | **0.000** | **0.766** |
| **3. AR** | **1.061** | **0.178** | **0.020** | **0.811** |
| 4. OC + H′ | 2.316 | 0.095 | 0.017 | 0.758 |
| 5. OC | 2.526 | 0.086 | 0.015 | 0.758 |
| 6. H′ | 3.055 | 0.066 | 0.017 | 0.754 |
| 7. Null model | 3.202 | 0.061 | 0.000 | 0.767 |

Best model AICc = 220.745

| Zeaxanthin | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. CP** | **0.000** | **0.727** | **0.030** | **0.784** |
| 2. Null model | 3.182 | 0.148 | 0.000 | 0.784 |
| 3. OC | 5.081 | 0.057 | 0.000 | 0.784 |
| 4. H′ | 5.729 | 0.041 | 0.000 | 0.786 |
| 5. OC + H′ | 6.631 | 0.026 | 0.003 | 0.790 |

Best model AICc = 70.112

| GPx | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. PAS** | **0.000** | **0.470** | **0.076** | **0.395** |
| **2. OC** | **0.572** | **0.353** | **0.122** | **0.383** |
| 3. Null model | 3.437 | 0.084 | 0.000 | 0.370 |
| 4. H′ | 4.344 | 0.054 | 0.077 | 0.449 |
| 5. OC + H′ | 5.014 | 0.038 | 0.126 | 0.375 |

Best model AICc = -169.460

| SOD | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. CP** | **0.000** | **0.663** | **0.021** | **0.693** |
| **2. OC + H′** | **1.780** | **0.272** | **0.077** | **0.568** |
| 3. Null model | 5.709 | 0.038 | 0.000 | 0.673 |
| 4. H′ | 7.123 | 0.019 | 0.010 | 0.652 |
| 5. OC | 9.006 | 0.007 | 0.000 | 0.671 |

Best model AICc = 31.353

| TBARS | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. Null model** | **0.000** | **0.825** | **0.000** | **0.218** |
| 2. H′ | 3.480 | 0.145 | 0.078 | 0.269 |
| 3. OC | 7.218 | 0.022 | 0.019 | 0.245 |
| 4. OC + H′ | 9.390 | 0.008 | 0.091 | 0.262 |

Best model AICc = -475.140

| tGSH | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.615** | **0.097** | **0.109** |
| 2. OC | 3.505 | 0.107 | 0.051 | 0.116 |
| 3. AR | 3.718 | 0.096 | 0.041 | 0.118 |
| 4. CP | 4.125 | 0.078 | 0.009 | 0.148 |
| 5. PAS | 4.275 | 0.072 | 0.005 | 0.129 |
| 6. Null model | 6.940 | 0.019 | 0.000 | 0.127 |
| 7. H′ | 7.673 | 0.013 | 0.010 | 0.158 |

Best model AICc = 230.734

| oxGSH | ΔAICc | AICcw | R$^2$m | R$^2$c |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.993** | **0.073** | **0.257** |
| 2. CP | 12.023 | 0.002 | 0.028 | 0.297 |
| 3. AR | 12.352 | 0.002 | 0.069 | 0.205 |
| 4. PAS | 14.027 | 0.001 | 0.002 | 0.283 |
| 5. CL | 15.237 | 0.000 | 0.001 | 0.287 |
| 6. Sex + Age | 15.289 | 0.000 | 0.004 | 0.325 |

| | | | | |
|---|---|---|---|---|
| 7. H′ | 15.794 | 0.000 | 0.033 | 0.270 |
| 8. OC | 17.087 | 0.000 | 0.003 | 0.288 |
| 9. Circadian rhythm | 19.108 | 0.000 | 0.015 | 0.273 |
| 10. Brood size | 19.204 | 0.000 | 0.009 | 0.250 |
| 11. Food ingestion | 20.247 | 0.000 | 0.001 | 0.274 |
| 12. Sex | 20.347 | 0.000 | 0.002 | 0.290 |
| 13. Age | 24.473 | 0.000 | 0.003 | 0.311 |
| 14. Null model | 29.584 | 0.000 | 0.000 | 0.273 |

Best model AICc = 1391.404

| **%oxGSH** | **ΔAICc** | **AICcw** | **$R^2$m** | **$R^2$c** |
|---|---|---|---|---|
| **1. OC + H′** | **0.000** | **0.602** | **0.063** | **0.252** |
| 2. CP | 2.976 | 0.136 | 0.021 | 0.276 |
| 3. AR | 3.728 | 0.093 | 0.050 | 0.206 |
| 4. PAS | 4.213 | 0.073 | 0.003 | 0.268 |
| 5. CL | 5.398 | 0.040 | 0.003 | 0.274 |
| 6. H′ | 5.701 | 0.035 | 0.040 | 0.254 |
| 7. OC | 7.102 | 0.017 | 0.006 | 0.273 |
| 8. Null model | 10.581 | 0.003 | 0.000 | 0.259 |

Best model AICc = 566.121

# Chapter 3

## The isotopic niche and productivity of Bonelli's Eagle populations

* Multi-scale effects of nestling diet on breeding performance in a terrestrial top predator inferred from stable isotope analysis

# Efectos multiescala de la dieta de los pollos en la reproducción de un depredador terrestre inferidos mediante análisis de isótopos estables

## Resumen

La variación en la dieta entre individuos dentro de las poblaciones probablemente tiene importantes implicaciones ecológicas y evolutivas. Sin embargo, las relaciones entre dieta y eficacia biológica a nivel individual, y los procesos poblacionales derivados de esta relación son poco conocidos para la mayoría de aves depredadoras que habitan en ecosistemas terrestres complejos. En este estudio, utilizamos el análisis de isótopos estables para evaluar la ecología trófica de los pollos de una rapaz de vida larga, el águila perdicera *Aquila fasciata*, e investigamos si la amplitud del nicho trófico y el consumo de las principales presas afectan a la reproducción de la especie en dos escalas espaciales: los territorios dentro de las poblaciones, y las poblaciones en una amplia área geográfica. A nivel territorial, las parejas reproductoras cuyos pollos tuvieron una dieta similar a la del conjunto de la población (i.e. consumo moderado de presas preferidas, y complementado por categorías de presas alternativas), o una dieta con un gran consumo de presas preferidas, mostraron mayor probabilidad de criar exitosamente dos pollos. Sin embargo, un aumento en la diversidad trófica de los pollos estuvo relacionado con una menor productividad de las parejas reproductoras. Las edades y los recambios en los miembros de la pareja reproductora también tuvieron un efecto sobre la productividad, que fue mayor en parejas adultas con pocos recambios, como es de esperar en especies de vida larga. A nivel poblacional, la productividad media fue mayor en aquellas poblaciones-año con menor amplitud de nicho trófico y una mayor similitud en la dieta entre territorios, lo cual se relacionó con un mayor consumo global de presas preferidas. Por lo tanto, nuestro estudio pone de manifiesto una correspondencia en las relaciones dieta y eficacia biológica en dos escalas espaciales: territorios y poblaciones. En base a nuestros resultados, sugerimos que los análisis de isótopos estables pueden ser una herramienta muy útil para monitorizar la dieta de aves depredadoras en ecosistemas terrestres en una amplia escala espacio-temporal, sirviendo a su vez para detectar posibles cambios en la disponibilidad de presas de las que estos depredadores dependen para criar. Por ello, animamos tanto a ecólogos como biólogos evolutivos y de conservación, que se ocupan de estudiar las consecuencias a múltiples escalas de la variación interindividual en el uso de recursos dentro de la población, a emplear enfoques metodológicos similares basados en análisis de isótopos estables. Este tipo de estudios podrá aplicarse con éxito en ecosistemas complejos, como los mediterráneos.

## Referencia bibliográfica

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# Multi-scale effects of nestling diet on breeding performance in a terrestrial top predator inferred from stable isotope analysis

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**ABSTRACT—** Inter-individual diet variation within populations is likely to have important ecological and evolutionary implications. The diet-fitness relationships at the individual level and the emerging population processes are, however, poorly understood for most avian predators inhabiting complex terrestrial ecosystems. In this study, we use an isotopic approach to assess the trophic ecology of nestlings in a long-lived raptor, the Bonelli's eagle *Aquila fasciata*, and investigate whether nestling dietary breath and main prey consumption can affect the species' reproductive performance at two spatial scales: territories within populations and populations over a large geographic area. At the territory level, those breeding pairs whose nestlings consumed similar diets to the overall population (i.e. moderate consumption of preferred prey, but complemented by alternative prey categories) or those disproportionally consuming preferred prey were more likely to fledge two chicks. An increase in the diet diversity, however, related negatively with productivity. The age and replacements of breeding pair members had also an influence on productivity, with more fledglings associated to adult pairs with few replacements, as expected in long-lived species. At the population level, mean productivity was higher in those population-years with lower dietary breadth and higher diet similarity among territories, which was related to an overall higher consumption of preferred prey. Thus, we revealed a correspondence in diet-fitness relationships at two spatial scales: territories and populations. We suggest that stable isotope analyses may be a powerful tool to monitor the diet of terrestrial avian predators on large spatio-temporal scales, which could serve to detect potential changes in the availability of those prey on which predators depend for breeding. We encourage ecologists and evolutionary and conservation biologists concerned with the multi-scale fitness consequences of inter-individual variation in resource use to employ similar stable isotope-based approaches, which can be successfully applied to complex ecosystems such as the Mediterranean.

## INTRODUCTION

The trophic niche of a species refers to the range of food sources it uses and is a key component of the n-dimensional hypervolume niche concept [1]. Classical models based on the optimal foraging theory assume that individuals within populations respond similarly to spatial and temporal heterogeneity in resource availability [2-4]. According to this theory, individual consumers are expected to take preferred resources when food availability is high, or expand their food range by adding suboptimal resources when resource availability lowers [5-7]. More recently, variations in trophic resource use among individuals within a population have been highlighted to be a widespread phenomenon in nature, what may result from a broad range of mechanisms [8]. Apart from sex, age or individual's phenotype, individual diet variation may arise from their differences in dominance, experience or foraging ability [8-12]. Consequently, both extrinsic ecological factors and intrinsic organismal traits may generate a dietary spectrum within the population.

Despite the increasing understanding of the causes of individual diet variation within animal populations, its implications in terms of individual fitness are still poorly understood. Differences in trophic resource use among individuals are likely to affect their energy income and, therefore, to have an impact on fitness [13-15]. In this regard, individual diet variation has been shown to differently influence breeding success in several avian species, an issue particularly studied in seabirds [16-19]. For instance, individuals with lower diet diversity may increase their breeding success provided that low trophic diversity is the result of specialization on preferred prey [16,20]. The superior fitness of individuals with specialized diets, however, is not a universal pattern, and numerous studies have documented the fitness benefits of a generalized diet [19,21]. Therefore, the fitness consequences of individual dietary variation are not always easy to predict as different feeding strategies may be advantageous for different species and/or ecological scenarios, or even between different individuals within a population, depending on multiple factors like the nutritional quality of food, the physiology of the consumer, or the spatial and temporal availability of prey [21-24].

Several important drivers of the structure and dynamics of populations and communities, such as intraspecific competition, predation risk or parasitism, are linked to individual's resource use. Consequently, theoretical studies have highlighted the importance of intraspecific diet variation in shaping populations and communities [25-27]. In this regard, several studies have linked lower dietary diversity with higher breeding success at the population level, what has been explained by a higher consumption of preferred prey in those populations with lower diet diversity [28-30]. Conversely, populations exposed to heterogeneous landscapes or changing environmental conditions (e.g. food availability) can perform better if individuals within the population diversify in food resource use [8]. In fact, the correspondence between both scales (i.e. intra-population resource use and population processes) remains largely unknown in most natural systems so further studies are required to investigate how the relationship between diet and fitness at the individual level translates to the population scale.

Stable isotope analyse (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has been increasingly used by animal ecologists to assess individual resource use and intra-population niche partitioning [31-33], as it provides insightful dietary information at the individual level difficult to obtain by conventional procedures. In particular, the isotopic composition in metabolically inert tissues (e.g. feathers), represents consumer's diet at the time of deposition (e.g. feather growth), so SIA are a powerful tool to assess animal (e.g. avian) temporal and spatial dietary information at both the individual and population levels [34,35]. Consumer isotopic data delineated in δ-space (e.g. $\delta^{13}C$-$\delta^{15}N$ bi-plot), has been termed the "isotopic niche" [32], and is thought to be closely aligned with the true trophic niche. Recently developed metrics based on δ-space also allow for a species' trophic structure and diet diversity to be quantified not only at the individual, but also at the population and community levels [36,37]. Complementarily, the use of isotopic mixing models allow for individual diet reconstructions by converting isotopic data of consumers and main food resources into dietary proportions (p-space) [38-40], that can be translated into niche-width metrics commonly used by ecologists [41]. Surprisingly, the use of SIA to assess the trophic ecology of terrestrial avian top predators, either at the territory or the population levels, has rarely been done (but see [24,42]).

In this study, we use an isotopic approach to assess the trophic ecology of a long-lived raptor, the Bonelli's eagle *Aquila fasciata*, to explore the relationship between nestling diet and reproductive performance at both the territorial and population scales. Bonelli's eagle is a suitable model species because it shows marked intra- and inter-population demographic variations across its western European distribution range [43]. Moreover, the marked population decline occurred in this area in recent decades has been partly related to habitat degradation and main prey scarcity [44]. Here, both European rabbits *Oryctolagus cuniculus* (mostly) and red-legged partridges *Alectoris rufa* have been suggested to be optimal prey for Bonelli's eagle, so that they are preferentially consumed wherever they are abundant, and their consumption reduces eagle's diet diversity [45-49]. The dietary variation among territories is however substantial, and is thought to be influenced by habitat heterogeneity linked to crashes in rabbit numbers around two decades ago due to outbreaks of rabbit haemorrhagic disease, which indirectly impacted on the abundance of partridges and possibly other prey [46,48,50,51]. In the post-disease period, the average consumption of rabbits and partridges has been relatively low in most populations of western Europe, particularly in those located in the north of the Iberian Peninsula and France [48], what could reveal unprecedented effects of eagles' diet on fitness components such as breeding success (see [46]).

The specific objectives of this study were to i) describe the isotopic niche width and structure (as a proxy of trophic niche) of Bonelli's eagle nestlings in three populations of western Europe, ii) estimate nestling prey consumption at the intra-population (i.e. territory) level using isotopic mixing models, and iii) test the relationship between the dietary estimates and isotopic niche metrics with breeding success at both the territory and population levels. Our main prediction is that those territories and populations where nestlings have narrower trophic niches, what is expected to occur where nestling diets largely rely on rabbits and/or partridges, will perform better in terms of breeding success.

## METHODS

### Study area

From 2008 to 2011 we monitored the main vital rates in 131 territorial Bonelli's eagle pairs located in three populations across the species' western European range, north to south, Provence and Languedoc-Roussillon (43º58'N, 03º20'E; southeast France; *n* = 30 and 31 pairs in 2010 and 2011, respectively), Catalonia (41º20'N, 01º32'E; northeast Spain; *n* = 52, 40, 44 and 45 in 2008, 2009, 2010 and 2011, respectively) and Andalusia (37º76′N, 03º85′W; southeast Spain; *n* = 45 in 2011) (Figure 1). The population in France had a breeding density of 0.29 territorial pairs/100km$^2$, it showed a mean productivity of 0.98 fledglings/pair, an adult survival of 0.880, and the mean percentage of non-adult birds in territorial pairs was 8.39. The population in Catalonia had a breeding density of 0.64, it showed slightly higher values of productivity (1.13) and adult survival (0.889), and the mean percentage of non-adult birds was 10.81. The population in Andalusia had a breeding density of 0.85, it showed the highest values of productivity (1.23) and adult survival (0.926), and the mean percentage of non-adult birds was 3.86 [43]. All breeding nests were located on cliffs, and territories varied markedly with respect to habitat features, prey abundances or human activity [44].

### Data collection

To assess the breeding success and productivity of monitored territorial pairs, known breeding areas were yearly visited a minimum of five days during the whole breeding period. Between January and March we checked the presence of territorial birds and breeding activity (i.e. incubation behaviour). In late March and April, occupied nests were checked to detect the presence, number, and age of nestlings, which was estimated by feather development and backdating from laying date [52]. Nestlings at the age of ≥ 50 days old were assumed to have fledged successfully [53].

For each territorial pair, individual turnover events or replacements (i.e. if the same individuals/pairs occupied the same territories across years) were estimated by comparing the plumage-ages of the male and the female in two consecutive years [54]. Each pair was classified as adult (both individuals with an adult plumage) or non-adult (at least one individual with a non-adult plumage) [55].

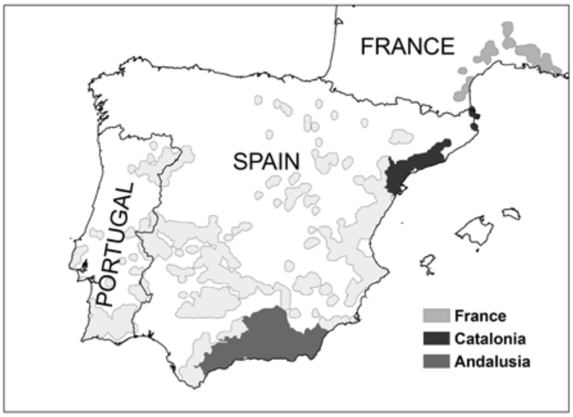

**Figure 1.** Distribution area (shaded polygons) of Bonelli's eagle in western Europe (modified from [43]). In darker grey we show the monitored populations, north to south, southern France, Catalonia and Andalusia (see legend).

Once nestlings were on average 35-40 days old, we accessed breeding nests with the assistance of experienced climbers to sample four mantle feathers from each chick for SIA purposes. Nestlings were sampled in a subset of the monitored territories, involving 21 different breeding territories in France (*n* = 20 and 12 in 2010 and 2011, respectively), 38 in Catalonia (*n* = 20, 17, 25, and 24 in 2008, 2009, 2010 and 2011, respectively) and 12 in Andalusia 2011. The isotopic composition of sampled feathers reflects nestling diet during tissue development and was used to estimate both the isotopic niche metrics at the population level and the proportional contribution of main prey categories to nestling diets at the territory-year level (see below).

To characterize main prey isotopic values we obtained samples from the three studied populations by collecting a small piece of muscle from carcasses found at the nests at the same time as chick feathers were sampled. Prey tissue collections were supplemented with dead individuals collected in the surroundings of eagles' breeding territories in Catalonia (see [42]). We did not find any evidence of prey isotopic differences among the three populations when comparing the main prey categories, i.e. rabbits, partridges and pigeons. Therefore, we combined all samples of each prey group to calculate mean ± SD prey isotopic values for the whole study area.

### Stable isotope analyses

The isotopic ratios of Carbon ($^{13}C$:$^{12}C$) and Nitrogen ($^{15}N$:$^{14}N$) were measured for both nestling feathers and prey muscle samples following the procedure described in [42]. Subsamples of approximately 0.35 mg of feathers and 0.32 mg of muscle were loaded in tin recipients and crimped for combustion. All isotopic measurements were conducted at the "Centres Científics i Tecnològics, Universitat de Barcelona".

Stable isotope values are reported following the conventional δ notation, where $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} / R_{standard}) - 1] * 1000$ (‰), and R is the corresponding ratio $^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$. International $R_{standards}$ were Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$. The measurement precisions were ≤ 0.15‰ and ≤ 0.25‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

### Population isotopic niche metrics

Our population approach was based on each population-year sampled (n=7; France 2010-2011, Catalonia 2008-2011 and Andalusia 2011). To address Bonelli's eagle nestling trophic structure at the population-year level we used the isotopic metrics originally proposed by Layman et al. [36], and recently extended by Jackson et al. [37]. Each territory-year was a single observation; in territories with two chicks, we calculated mean isotopic values of siblings because they showed similar isotopic values (see [56]). The following isotopic niche metrics were calculated based on the $\delta^{13}C$-$\delta^{15}N$ bi-plot (i.e. δ-space) as described in [37]: $\delta^{13}C$ range ($CR_b$) and $\delta^{15}N$ range ($NR_b$) to assess the total carbon and nitrogen ranges in the consumed prey; mean distance to centroid ($CD_b$) as a measure of population trophic diversity; and standard deviation of nearest neighbour distance ($SDNND_b$) to infer population trophic evenness. All these metrics were bootstrapped ('$_b$'; $n$ = 10000) based on the minimum number of territories ($n$ = 12) in the data set of population-years, which allows comparisons among population-years despite different territorial sample sizes [57]. Finally, we calculated the corrected standard ellipse area ($SEA_c$) to estimate the isotopic niche width of each population-year. The $SEA_c$ measures the core isotopic niche area (ca. 40% of the data) and corrects for bias associated with small sample sizes [37].

### Nestling prey consumption estimates

We used the Bayesian mixing model SIAR (Stable Isotope Analysis in R; [40,58]) to estimate the relative contribution of main prey categories to nestling diets at the territory-year level. Main prey categories included into SIAR were European rabbits, red-legged partridges, wood pigeons *Columba palumbus*, domestic pigeons *C. livia* dom., passerines (Corvidae, Sturnidae and Turdidae), Eurasian red squirrels *Sciurus vulgaris*, and ocellated lizards *Timon lepidus* [48]. Based on $\delta^{13}C$ values, domestic pigeons were divided in two categories to account for the marked isotopic differences observed between individuals foraging on crops (i.e. lower $\delta^{13}C$) and those associated with dovecotes and fed with corn *Zea mays* (i.e. higher $\delta^{13}C$) (see Table S1 for prey isotopic values). The $\delta^{13}C$-$\delta^{15}N$ bi-plot with nestling and main prey isotopic values corrected for trophic discrimination

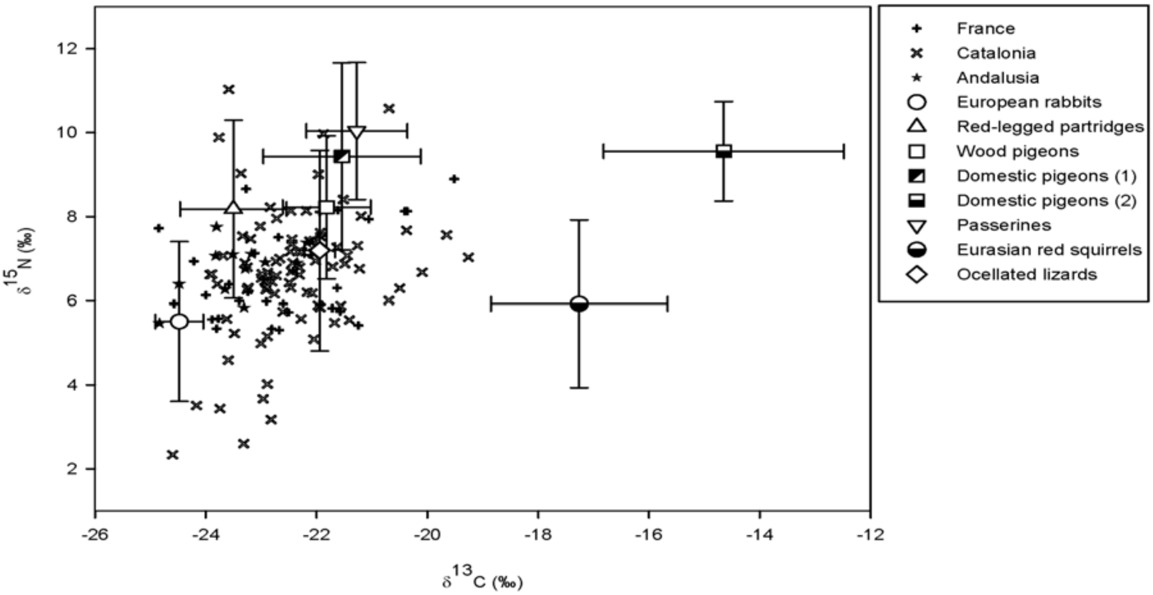

**Figure 2.** Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) in Bonelli's eagle nestlings and their main prey. Prey isotopic values were corrected for trophic discrimination factors (i.e. 2.1 and 2.7‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively; [59]). Single data points represent nestlings of each territory-year, with different symbols for France, Catalonia and Andalusia. Prey categories (mean ± 95% SD) included: European rabbits, red-legged partridges, wood pigeons, domestic pigeons wildly foraging in crops (1), domestic pigeons from dovecotes and fed with corn (2), passerines, Eurasian red squirrels, and ocellated lizards (see figure legend).

factors (TDFs) is shown in Figure 2. We used TDFs values of 2.1‰ ± 0.08 for $\delta^{13}C$ and 2.7‰ ± 0.5 for $\delta^{15}N$, calculated from feathers of peregrine falcons *Falco peregrinus* fed on muscle of Japanese quail *Coturnix japonica* [59]. Mean prey consumption estimates from SIAR were selected for subsequent analyses. To investigate the sensitivity of prey consumption estimates to inaccuracy in our TDFs, we varied 1‰ those values used for $\delta^{13}C$ (1.6-2.6‰) and those used for $\delta^{15}N$ (2.2-3.2‰) by maintaining the SD of 0.08 and 0.5 for $\delta^{13}C$ and $\delta^{15}N$, respectively [59]. On average, main prey consumption estimates varied less than 4% for the mean contributions to the diet. Therefore, we were confident on our previous results based on the TDFs obtained from the literature since even changes in 1‰ in the TDFs do not considerably affect our mean prey consumption estimates from SIAR.

### Data analysis

Main prey consumption estimates from SIAR were used to assess nestlings' diet diversity and prey consumption specificity at the territory-year level. The Shannon-Weaver index (H') was used to calculate the diet diversity [60]. To estimate nestlings' prey consumption specificity we used the proportional similarity index ($PS_i$) [41], which measures the diet overlap between an individual (i.e. nestlings in a territory-year in our approach) and its population (i.e. mean diet in the whole set of territory-years sampled in the study area). $PS_i$ tends towards 1 in those territories where nestling prey consumption is similar to the mean population diet, and is increasingly lower when prey consumption differs from the mean diet. In order to enhance the interpretation of our results, we also assessed the relationship between H' and $PS_i$. Moreover, we tested the existence of preferred prey in our study area by relating mean consumption of each prey category and H' at the territory level through Spearman rank correlation tests [49], as higher consumption of preferred prey has been suggested to be inversely related with predator's H' [6].

To test the diet-fitness relationship at the territory-year level we applied Generalized Linear Mixed Models (GLMMs), which allowed accounting for the potential non-independence of clustered observations from the same territories, years and populations. Due to limitations imposed by the isotopic analysis (i.e. nestlings can only be sampled if breeding pairs are successful in hatching and rearing chicks), our territorial diet estimates only included successful breeding pairs. In our study area, spatial autocorrelation in productivity is absent between populations [43] and, therefore, we included territory and year nested by population as random categorical factors. In this analysis, productivity of breeding successful pairs (i.e. probability of producing two chicks instead of one) was modeled as a binomial response variable using a logit link function. Error distributions were assumed to be binomially distributed. We evaluated a set of models including the following explanatory variables: age of the breeding pair and mate replacement (categorical factors); consumption of rabbits, consumption of partridges, H' and $PS_i$ (continuous variables). We also included the interaction between H' and $PS_i$, and the quadratic effect of these two variables to test whether a parabolic trend fitted the model response (i.e. higher and lower values of either H' or $PS_i$ imply an advantage/disadvantage in terms of productivity compared with intermediate values of these variables) (see Table S2 for details on model parameters and their interactions). Ages of the breeding pair and mate replacements were included in all the models due to their potential influence on reproductive parameters [61,62]. GLMMs were fitted using the lmer function from the lme4 package of R [63]. Model selection was based on Akaike's Information Criterion adjusted for sample size ($AIC_c$), computing the Akaike weights ($AIC_{cw}$) to assess the probability that each candidate model was the best of the proposed set [64]. The goodness-of-fit of each model was estimated from marginal ($R^2_{GLMM(m)}$) and conditional ($R^2_{GLMM(c)}$) coefficients of determination, following [65]. The $R^2_{GLMM(m)}$ value shows the proportion of the variance in the raw data explained by the fixed effects only, while the $R^2_{GLMM(c)}$ value shows the proportion of the variance explained by the full model, including both fixed and random effects.

Regarding the diet-fitness relationship at the population-year level, we used Spearman rank correlation tests to assess for any relationship between mean productivity and either $SEA_c$ or $SDNND_b$. Mean productivity for each population-year was calculated either as the mean number of fledglings in the successful breeding pairs or the mean productivity in all monitored territorial pairs (i.e. successful breeders or not). In this second analysis we assumed that the $SEA_c$ and the $SDNND_b$ obtained from successful breeders were representative of the trophic niche structure in the whole population-year.

Statistical analyses were conducted using the R statistics platform (CRAN 2009) and SPSS (PASW 18.0).

## RESULTS

### Population isotopic niche metrics

Overall, the isotopic values of Bonelli's eagle nestlings ranged from -24.85 to -19.26‰ for $\delta^{13}C$ (mean ± SD: -22.56 ± 1.08‰; *n* = 130 nests), and from 2.34 to 11.03‰ for $\delta^{15}N$ (6.65 ± 1.36‰). France 2010 had the largest $CR_b$, Catalonia 2009 the largest $NR_b$, while Andalusia 2011 showed the lowest values for both $CR_b$ and $NR_b$. The $CD_b$ and $SDNND_b$ in Andalusia 2011 was substantially lower than all other population-years, suggesting higher trophic diversity and lower even distribution of nestling trophic niches in France and Catalonia if compared with Andalusia. Accordingly, the $SEA_c$ in Andalusia 2011 was notably lower than any other population-year, being four times lower than Catalonia 2009, the highest $SEA_c$ obtained in our study (Table 1; Figure 3).

**Prey consumption estimates**

Rabbits and partridges were the prey categories that most varied in terms of consumption among population-years, with Andalusia 2011 showing the highest mean consumption of these two prey, and Catalonia 2008 showing the lowest values (Table 2). Considerable variation in prey consumption was found among territories within the same population in a given year both in France or Catalonia, especially in the consumption of rabbits, partridges, domestic pigeons from dovecotes and squirrels. On the other hand, territories in Andalusia showed more homogeneous diets, with higher variation in the consumption of partridges than rabbits (Table 2).

At the territory level, H' was strongly negatively correlated with the consumption of rabbits ($r_s$ = -0.92, $P < 0.001$) and partridges ($r_s$ = -0.82, $P < 0.001$; Figure S1), but positively correlated with the consumption of wood pigeons ($r_s$ = 0.90, $P < 0.001$), domestic pigeons from dovecotes ($r_s$ = 0.95, $P < 0.001$), passerines ($r_s$ = 0.89, $P < 0.001$), squirrels ($r_s$ = 0.90, $P < 0.001$), and ocellated lizards ($r_s$ = 0.81, $P < 0.001$). We did not find a significant linear correlation between H' and $PS_i$ ($r_s$ = 0.205, $P > 0.05$). Nevertheless, we found that the mean population diet (i.e. highest $PS_i$) coincided with intermediate H' values, so either higher H' (i.e. more generalized diets) or

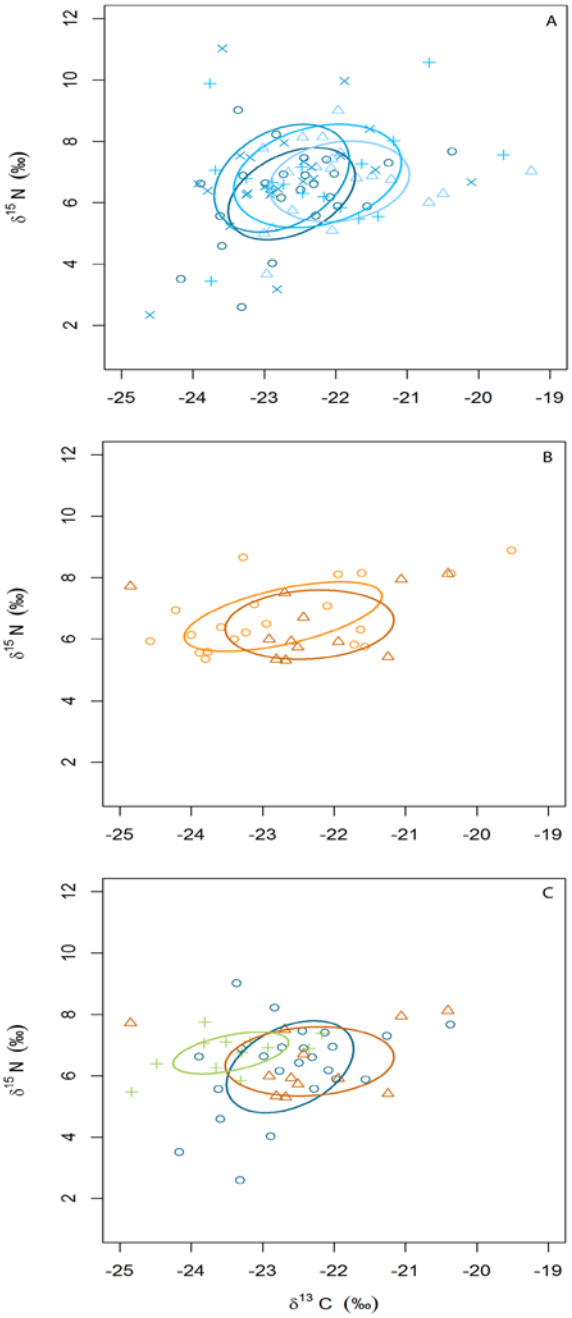

**Figure 3.** Corrected standard ellipse areas ($SEA_c$) estimated from $\delta^{13}C$ and $\delta^{15}N$ values (‰) in Bonelli's eagle nestlings. A) $SEA_c$ in Catalonia 2008-2011 (light to dark blue), B) $SEA_c$ in France 2010-2011 (light to dark orange), and C) $SEA_c$ in France, Catalonia and Andalusia 2011 (dark orange, dark blue and green, respectively). Same symbols represent nestlings from the same population-year.

**Table 1.** Isotopic niche metrics of Bonelli's eagle nestlings at the population-year level.

| Population-year | Mean $\delta^{13}C$ | Mean $\delta^{15}N$ | $CR_b$ | $NR_b$ | $CD_b$ | $SDNND_b$ | $SEA_c$ | n |
|---|---|---|---|---|---|---|---|---|
| France-2010 | -22.71 | 6.73 | 3.95 | 3.09 | 1.47 | 0.49 | 4.01 | 20 |
| France-2011 | -22.34 | 6.47 | 3.46 | 2.68 | 1.28 | 0.50 | 4.15 | 12 |
| Catalonia-2008 | -21.95 | 6.69 | 2.88 | 3.97 | 1.29 | 0.49 | 3.99 | 20 |
| Catalonia-2009 | -22.25 | 6.87 | 3.38 | 5.29 | 1.56 | 0.64 | 5.98 | 17 |
| Catalonia-2010 | -22.76 | 6.80 | 2.99 | 5.82 | 1.43 | 0.74 | 4.92 | 25 |
| Catalonia-2011 | -22.62 | 6.29 | 2.76 | 4.72 | 1.35 | 0.51 | 3.86 | 24 |
| Andalusia-2011 | -23.45 | 6.75 | 2.32 | 1.94 | 0.81 | 0.29 | 1.49 | 12 |

Note: $CR_b$ = $\delta^{13}C$ range; $NR_b$ = $\delta^{15}N$ range; $CD_b$ = mean centroid distance; $SDNND_b$ = standard deviation of nearest neighbour distance; $SEA_c$ = corrected standard ellipse area; n = number of sampled territories. All metrics except $SEA_c$ were bootstrapped ('$_b$'; n = 10000).

lower H' (i.e. more specialized diets) reduced $PS_i$ values (see Figure S2).

### Influence of nestling diet on productivity

The GLMMs showed that the productivity at the territory-year level was best explained by the age of pair and mate replacement, and by the age of pair and mate replacement together with the quadratic effect of $PS_i$ or the negative effect of H' (Table 3, Table S3). That is, either adult breeding pairs feeding their chicks with similar diets to the overall population (highest $PS_i$ values and intermediate H') or pairs disproportionally exploiting a single or few preferred prey types (lowest values of both $PS_i$ and H') were more likely to fledge two chicks than pairs with nestling showing intermediate $PS_i$ values and higher H' (see Figures 4 and S2). Nevertheless, the coefficient of determination of best fitted models indicated that the models have in general rather low explanatory power (see Table 3).

At the population-year level, mean productivity of successful pairs were not correlated neither with $SEA_c$ ($r_s$ = -0.54, $P$ = 0.215) nor with $SDNND_b$ ($r_s$ = -0.40, $P$ = 0.379). We found, however, a significant and negative correlation between mean productivity in the whole population-year and the $SEA_c$ ($r_s$ = -0.86, $P$ = 0.014), and a marginally significant negative correlation between mean productivity and the $SDNND_b$ ($r_s$ = -0.72, $P$ = 0.068), suggesting that those population-years with more heterogeneous territories in terms of diet were less productive than population-years with territories showing more homogeneous prey consumption patterns (Figure 5).

## DISCUSSION

Inter-individual diet variation is a widespread phenomenon within animal populations, but traditionally underappreciated [8]. Theory and empirical evidence of its ecological causes and eco-evolutionary consequences have been intensively addressed in recent years [8,10,19,27]. In the case of terrestrial territorial predators, spatial differences in the abundance and availability of prey have been highlighted as an important factor influencing individual (e.g. territorial) diet differences [21,24], but its fitness consequences to avian predators inhabiting terrestrial complex ecosystems like the Mediterranean region are poorly known. In this study, we used an isotopic approach to assess inter-individual (i.e. inter-territorial) diet variation within three Bonelli's eagle populations

**Table 2.** Mean and range (maximum-minimum) prey consumption (%) of Bonelli's eagle nestlings on each monitored population-year calculated from the dietary estimates at the territory-year level obtained from Bayesian isotopic mixing models (SIAR).

| **Population** | France | France | Catalonia | Catalonia | Catalonia | Catalonia | Andalusia |
|---|---|---|---|---|---|---|---|
| **Year** | 2010 | 2011 | 2008 | 2009 | 2010 | 2011 | 2011 |
| **Prey category** | Mean (range) % | | | | | | |
| OC | 26.9 (34.5) | 23.4 (22.5) | 21.9 (36.6) | 23.4 (49.1) | 27.4 (52.3) | 28.2 (45.6) | 29.6 (16.7) |
| AR | 20.4 (27.2) | 17.5 (26.0) | 15.2 (12.1) | 17.2 (21.7) | 19.0 (21.8) | 17.6 (17.3) | 24.6 (24.1) |
| CP | 10.2 (08.3) | 11.3 (07.2) | 12.0 (06.3) | 11.3 (07.2) | 11.0 (09.7) | 11.0 (08.4) | 09.6 (8.7) |
| CLw | 11.8 (06.6) | 12.0 (14.2) | 11.4 (07.9) | 12.3 (15.5) | 12.2 (21.5) | 11.1 (11.7) | 12.7 (4.7) |
| CLd | 05.1 (19.1) | 05.4 (13.2) | 06.4 (16.9) | 05.8 (16.4) | 04.2 (13.4) | 04.4 (13.0) | 02.8 (3.3) |
| PAS | 08.3 (09.4) | 08.7 (8.2) | 10.0 (09.5) | 09.2 (11.3) | 08.9 (12.8) | 08.6 (08.3) | 07.3 (8.2) |
| SV | 06.8 (11.8) | 08.4 (14.9) | 09.6 (16.0) | 08.3 (14.1) | 06.0 (16.0) | 07.0 (11.8) | 04.2 (5.4) |
| TL | 10.5 (10.5) | 13.2 (10.8) | 13.5 (07.0) | 12.5 (09.4) | 11.4 (10.4) | 12.1 (10.5) | 09.3 (9.4) |

Note: OC = European rabbits; AR = red-legged partridges; CP = wood pigeons; CLw = domestic pigeons wildly foraging in crops; CLd = domestic pigeons from dovecotes and fed with corn; PAS = passerines; SV = Eurasian red squirrels; TL = ocellated lizards.

of western Europe, and how different prey consumption patterns affected both the territory and the population breeding performance.

The use of SIA to assess the trophic niche width and individual resource partitioning within and among populations has proved a powerful tool in recent years [31,32,34,35]. The sensitivity analysis on the TDFs supported our dietary results at the territory level, which, overall, highlighted diet variation among territories in the French and Catalonian populations (the northernmost populations of Bonelli's eagle in Europe), while territories in the Andalusian population showed more homogeneous diets (Table 2). Differences in the intra-population diet variation could be explained by higher heterogeneity in prey availability among French and Catalonian territories compared with those in Andalusia, especially in terms of rabbits, partridges, domestic pigeons from dovecotes or squirrels, which were the most variable prey in the diet (Table 2). We also found a significant negative correlation between territorial consumption of rabbits or partridges and H', suggesting these prey are optimal for Bonelli's eagle in the study area [3,6] as found in previous studies that used traditional diet examination methods [46,48,49]. Thus, rabbits and partridges could be positively selected by Bonelli's eagle when available, reducing the diet diversity, while higher consumption of other prey like pigeons, other birds, squirrels or lizards could compensate a shortage of the former prey, especially in some territories in France and Catalonia (see [48,49,66]). The challenge then was to assess whether differences in prey consumption at both the territory and population levels could affect breeding performance.

The fitness consequences of individual diet variation are the result of a complex interplay between individual foraging behavior and abilities, variation in resource preferences, and heterogeneity in resource availability. Thus, different feeding strategies may be advantageous for different species and/or ecological scenarios, an issue mostly addressed in some colonial seabirds [16,17,19]. Consumers may increase their breeding success by specializing when capturing prey for their chicks, probably because they become more efficient foragers [16]. Conversely,

**Table 3.** Model selection to assess the effects of age, mate replacements and diet on the productivity of breeding successful pairs.

| **Model definition** | **$\Delta AIC_c$** | **$AIC_{cw}$** | **$R^2_{GLMM(m)}$** | **$R^2_{GLMM(c)}$** |
|---|---|---|---|---|
| **1. Age + Replacement** | **0.000*** | **0.215** | **0.036** | **0.109** |
| **2. Age + Replacement + $PS_i$ + $PS_i^2$** | **0.301** | **0.185** | **0.107** | **0.206** |
| **3 Age + Replacement + H′** | **1.774** | **0.088** | **0.042** | **0.114** |
| 4. Age + Replacement + (OC+AR) | 2.074 | 0.076 | 0.038 | 0.111 |
| 5. Age + Replacement + OC | 2.129 | 0.074 | 0.037 | 0.105 |
| 6. Age + Replacement + $PS_i$ | 2.149 | 0.073 | 0.037 | 0.108 |
| 7. Age + Replacement + AR | 2.173 | 0.072 | 0.037 | 0.119 |
| 8. Age + Replacement + H′ + $H'^2$ | 3.331 | 0.041 | 0.058 | 0.118 |
| 9. Age + Replacement + (OC + AR) + H′ | 3.693 | 0.034 | 0.048 | 0.122 |
| 10. Age + Replacement + OC + H′ | 3.852 | 0.031 | 0.045 | 0.132 |
| 11. Age + Replacement + AR + H′ | 4.078 | 0.028 | 0.042 | 0.110 |
| 12. Age + Replacement + (OC + AR) + $PS_i$ | 4.320 | 0.025 | 0.039 | 0.109 |
| 13. Age + Replacement + OC + $PS_i$ | 4.362 | 0.024 | 0.038 | 0.104 |
| 14. Age + Replacement + AR + $PS_i$ | 4.399 | 0.024 | 0.038 | 0.115 |
| 15. Age + Replacement + H′ + $PS_i$ + (H′ * $PS_i$) | 6.185 | 0.010 | 0.047 | 0.120 |

Model definition enumerates the fixed effects considered in the GLMMs. All models included territory and year nested by population as random effects.

*Best model $AIC_c$ = 174.899.

Note: OC = rabbit consumption; AR = partridge consumption; H′ = diet diversity; $PS_i$ = prey consumption specificity. Parameters' interactions are denoted by (*), while ($^2$) indicates a quadratic effect. $\Delta AIC_c$ refers to the difference in the corrected Akaike Information Criteria ($AIC_c$) between model i and the model with the lowest ($AIC_c$) (i.e. the best model). Models with $\Delta AIC_c < 2$ are shown in bold type. The Akaike weights ($AIC_{cw}$) explains the probability that a given candidate model is the best of the proposed set, so the sum of all the models is 1.0. $R^2_{GLMM(m)}$ estimates model fit using fixed effects only, while $R^2_{GLMM(c)}$ estimates model fit including both fixed and random effects.

differences in reproductive success between specialists and generalists not always emerge, presumably because both foraging strategies can be advantageous at different levels of prey abundance or predictability [19]. Nevertheless, studies on terrestrial territorial avian predators have been scarce (but see [20,21,24]), probably due to the difficulty in monitoring predator's main vital rates and their dietary patterns on large spatial scales.

At the territory level, Bonelli's eagle productivity was higher in those territories showing few individual turnover rates, as expected in long-lived species [61,62], and according with the fact that among birds, reproductive success often increases with age [67]. The lower productivity we found in non-adult breeding pairs could be related with their intrinsic lower quality (e.g. breeding inexperience, lower foraging efficiency or competitive ability) (see [61,68]). Diet had also an effect on the productivity of breeding successful pairs, and both prey consumption specificity ($PS_i$) and diet diversity (H′) allowed predicting the number of chicks fledged (Figure 4). Pairs disproportionally exploiting a single or few preferred prey types (lowest values of $PS_i$ and H′) were more likely to fledge two chicks. Nevertheless, there were few territories where nestlings disproportionally consumed rabbits and/or partridges, suggesting that the scenario of superabundance of preferred

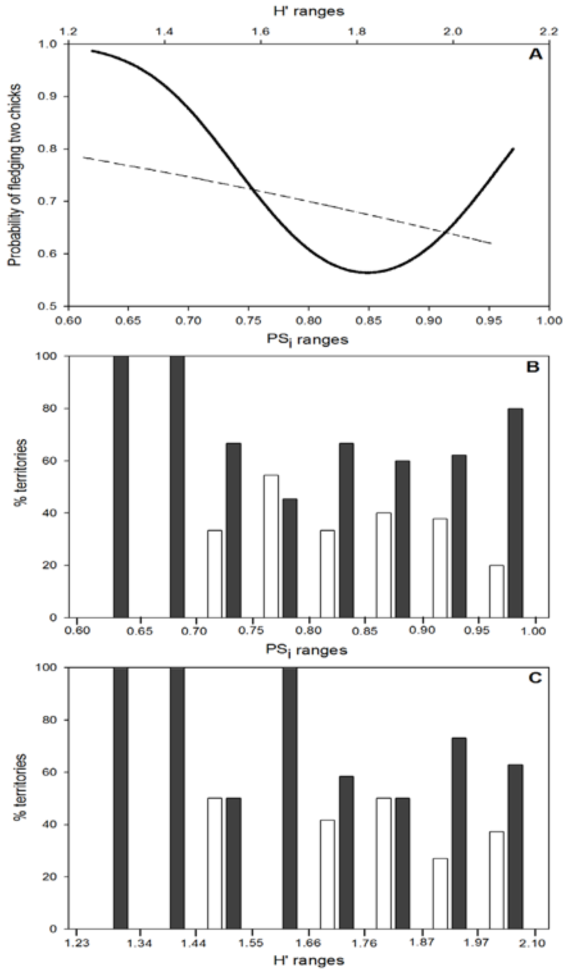

**Figure 4.** Responses of the second and third best models based on the GLMM approach. The productivity of successful breeding pairs (i.e. probability of producing two chicks instead of one) was the response variable, and age of breeding pairs, individual replacement, nestling prey consumption specificity ($PS_i$) or diet diversity (H’) are the explanatory variables. The graphic (A) represents the modeled probability of fledging two chicks (y-axis, probabilities between 0.5 and 1.0) if only considering adult pairs with no individual replacement, and using the range of observed $PS_i$ values (solid line and lower x-axis, $PS_i$ between 0.62 and 0.97) or H’ values (dashed line and upper x-axis, H’ between 1.23 and 2.08). The graphics (B and C) shows the real data used in the GLMM, with the x-axis representing $PS_i$ (B) or H’ (C) values of each territory, and the y-axis the proportion of territories fledging one (light bars) or two (dark bars) chicks.

prey is rare in the study area (see [48]). Furthermore, pairs whose nestlings consumed prey in similar proportions than the overall population (highest $PS_i$ values and intermediate H’) were also more likely to fledge two chicks. In this case, mean population diet included moderate consumption of rabbits, pigeons and partridges, which possibly corresponds to the more common scenario in suitable habitats for the Bonelli’s eagle in our study area. Our results thus suggest that Bonelli’s eagles may benefit in terms of productivity either from high consumption of preferred prey like rabbits and/or partridges, but also from moderate intake of these prey provided that they are abundantly complemented by some key alternative prey, such as pigeons. High values of diet diversity, however, had a negative effect on productivity, possibly as a consequence of higher consumption of a variety of suboptimal prey triggered by the scarcity of preferred prey (see [5]). It is worth to mention that our best fitted models had low explanatory power. This fact could be related to low variation in diet composition due to the exclusion of data from unsuccessful pairs. Nevertheless, other causes apart from diet may have great impact on productivity (e.g. human disturbances, inter-specific competition, etc.), which can difficult our ability to detect the diet effects on productivity. Additionally, we do not discard that high turnover rates may generate covariation between diet diversity and productivity [see 69].

At the population-year level, we found a negative correlation between mean productivity and the $SEA_c$ (Figure 5), suggesting a link between higher productivity and lower trophic niche width. The productivity of successful pairs, however, was not correlated with any isotopic niche metric, a finding that can be explained because temporal variance in productivity of successful pairs is very low in our study area (unpublished data). For instance, in Catalonia

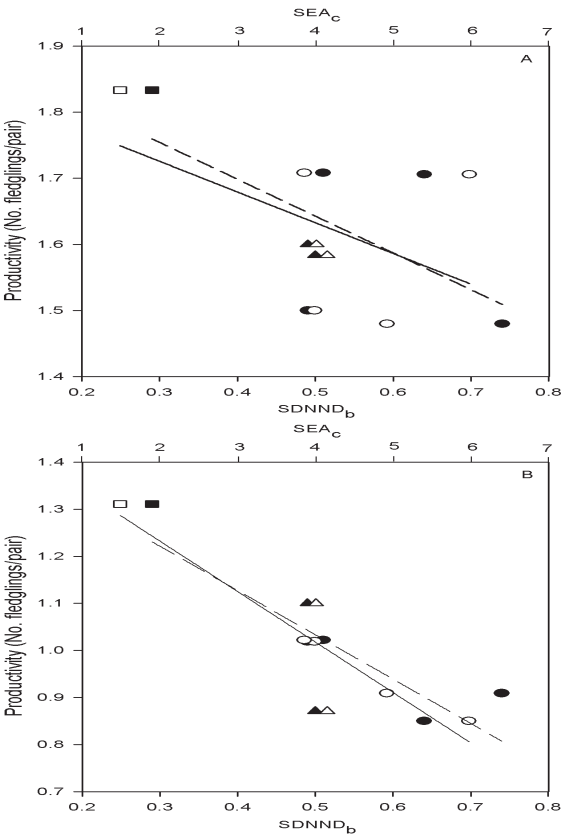

**Figure 5.** Correlations between mean productivity and both the SEA$_c$ and the SDNND$_b$ at the population-year level. Mean productivity refers to A) only successful pairs and B) the whole monitored population (either successful or not successful breeding pairs). In both cases, the upper x-axis shows the SEA$_c$ (corrected standard ellipse area; open symbols), and the lower x-axis shows the SDNND$_b$ (standard deviation of nearest neighbour distance bootstrapped; filled symbols). Trend lines of the relationship between productivity and either SEA$_c$ (solid line) or SDNND$_b$ (dashed line) are shown. Different symbol shapes represent France (triangles), Catalonia (circles) and Andalusia (squares).

there were poor environmental conditions (e.g. cold and rainy days) in spring 2009 that negatively affected the productivity of Bonelli's eagle, which was remarkably low (0.85, n = 40), while the productivity of successful pairs on that breeding season (1.71, n = 17) was similar or even higher than in other years. Apparently, pairs holding good territories are able to rear chicks even in bad years [70], but this does not necessarily implies that the environmental and food supply conditions that these pairs experience are the same over years, as suggested by our results. In particular, the population-year with the highest mean productivity (i.e. Andalusia 2011) showed the lowest SEA$_c$, while the population-year with the lowest mean productivity (i.e. Catalonia 2009) showed the highest SEA$_c$. In the case of Andalusia, lower SEA$_c$ was concordant with an overall higher consumption of preferred prey, which ultimately could have increased mean productivity [29,71]. Assuming that consumers' diet diversity usually increase as food becomes limiting [5], our results suggest higher heterogeneity in preferred prey availability among territories in France or Catalonia compared with Andalusia. In contrast, higher consumptions of preferred prey at the population level increased diet homogeneity among territories, as we found in the case of Andalusia that showed the lowest SDNND$_b$ value. Indeed, this population shows the highest values of main vital rates over the western European range of Bonelli's eagle [43]. Differences in individual's foraging abilities may also influence diet variation [8]. Nonetheless, inter-individual differences in foraging behaviour were expected to be similar in the three populations so we did not expect that this effect may explain the suggested differences in main dietary patterns of France and Catalonia compared with Andalusia, which are indeed inter-connected each other through dispersal processes [43]. Therefore, we concur with [21] that within population diet variation in our case study could primarily be a consequence of variation in prey availability among territories. However, variation in productivity could also arise from differences in the percentage of non-adult pairs among populations. In this regard, Andalusia holds the larger mean percentage of adult pairs and Catalonia the lowest (see “Study area” in “Materials and Methods”). Thus, age of breeders, turnover rates and territory quality could simultaneously affect reproductive output within a population (as suggested by our previous results at the territory level), and ultimately drive productivity at the population level (see [61, 69, 72, 73]).

Our study has relevant conservation implications because Bonelli’s eagle is a threatened raptor in Mediterranean countries, and is listed as “endangered” in Europe [74]. We suggest that SIA could be a useful tool to monitor the species’ diet on large spatio-temporal scales to detect potential changes in the main prey on which Bonelli’s eagle depends for breeding. In some Bonelli’s eagle populations, the low productivity prevents an adequate demographic balance and recruitment of birds [43]. Thus, improving the availability of optimal prey as European rabbit and red-legged partridge in certain Bonelli’s eagle territories can be an important conservation tool to enhance their viability. Increasing the populations of alternative prey such as pigeons should be also considered to compensate the potential shortage of preferred prey, especially in highly degraded environments and where rabbit haemorrhagic disease has drastically depleted rabbit abundances (see [48,66]).

The rapid development of quantitative analytical approaches for applying stable isotope data in studies of individual animal foraging ecology offers a new perspective to test hypotheses under the framework of the optimal foraging theory [2,3]. While dietary information at the individual level is difficult to obtain for large avian predator species by conventional diet analysis, isotopic derived metrics based on both δ- and p-space can be used to address diet variation at the intra-population level, as well as its eco-evolutionary consequences (see [8,25]). Nevertheless, the application of stable isotope analyses to assess nestling trophic ecology is obviously limited to successful breeding territories. A solution to explore diet-fitness relationships at the territory level could be to study the diet of parents instead of nestlings, but field sampling effort (e.g. blood samples for isotopic analysis, pellet collection, etc.) would notably increase. Another shortcoming of using stable isotope analyses was that they do not provide information on biomass consumed, which may also influence productivity. In our case, however, it has been described a direct correspondence in both the qualitative and quantitative representation of the different prey groups in the diet of the Bonelli’s eagle (at least in part of our study area, [75]), which support our general conclusions. Overall, in this study we have illustrated that, despite limitations, monitoring diet and fitness of individuals and populations distributed over large geographical ranges has indeed a great potential to further understand the fitness consequences of variation in resource use within- and between-populations. Also, we have shown the success of this approach in complex terrestrial ecosystems like the Mediterranean, where the number of potential confounding factors is greater than in other predator-prey systems [49]. Thus, we encourage the use of similar approaches to ecologists as well as evolutionary and conservation biologists concerned with the multi-scale fitness consequences (not only breeding success, but also other indicators such as body condition or survival) of inter-individual variation in resource use.

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## REFERENCES

**1. Hutchinson GE** (1957) Concluding Remarks. Cold Spring Harbor Symposia on Quant Biol, 22, 415-427.

**2. Pyke GH, Pulliam HR, Charnov EL** (1977) Optimal foraging: a selective review of theory and tests. Q. Rev. Biol., 52, 137-154

**3. Stephens DW, Krebs JR** (1986) Foraging theory. Princeton: Princeton University Press. 262 p.

**4. Stephens DW, Brown JS, Ydenberg RC, editors.** (2007) Foraging Behavior and Ecology. Chicago: The University of Chicago Press.

**5. Roughgarden J** (1972) Evolution of niche width. Am. Nat., 106, 683-718.

**6. Futuyma DJ, Moreno G** (1988) The evolution of ecological specialization. Annu. Rev. Ecol. Syst., 19, 207-233.

**7. Thompson ID, Colgan PW** (1990) Prey choice by marten during a decline in prey abundance. Oecologia, 83, 443-451.

**8. Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, et al.** (2003) The ecology of individuals: incidence and implications of individual specialization. Am. Nat., 161, 1-28.

**9. Svanbäck R, Bolnick DI** (2005) Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. Evol. Ecol. Res., 7, 993-1012.

**10. Svanbäck R, Bolnick DI** (2007) Intraspecific competition drives increased resource use diversity within a natural population. Proc. R. Soc. Lond. B Biol. Sci., 274, 839-844.

**11. Araújo MS, Bolnick DI, Martinelli LA, Giaretta AA, dos Reis SF** (2009) Individual-level diet variation in four species of Brazilian frogs. J. Anim. Ecol., 78, 848-856.

**12. Pires MM, Guimarães PRJr, Araújo MS, Giaretta AA, Costa JCL, et al.** (2011) The nested assembly of individual-resource networks. J. Anim. Ecol., 80, 896-903.

**13. Inger R, Gudmundsson GA, Ruxton GD,**

**Newton J, Colhoun K, et al.** (2008) Habitat utilisation during staging affects body condition in a long distance migrant, *Branta bernicla hrota*: potential impacts on fitness? J. Avian Biol., 39, 704-708.

**14. Inger R, Harrison XA, Ruxton GD, Newton J, Colhoun K, et al.** (2010) Carry-over effects reveal reproductive costs in a long-distance migrant. J. Anim. Ecol., 79, 974-982.

**15. Sorensen MC, Hipfner JM, Kyser TK, Norris DR** (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. J. Anim. Ecol., 78, 460-467.

**16. Golet GH, Kuletz KJ, Roby DD, Irons DB** (2000) Adult prey choice affects chick growth and reproductive success in pigeon guillemots. Auk, 117, 82-91.

**17. Suryan RM, Irons DB, Benson J** (2000) Prey switching and variable foraging strategies of black-legged kittiwakes and the effect on reproductive success. Condor, 102, 374-384.

**18. Votier SC, Bearhop S, Ratcliffe N, Furness RW** (2004) Reproductive consequences for great skuas specializing as seabird predators. Condor, 106, 275-287.

**19. Woo KJ, Elliott KH, Davidson M, Gaston AJ, Davoren GK** (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. J. Anim. Ecol., 77, 1082-1091.

**20. Katzner TE, Bragin EA, Knick ST, Smith AT** (2005) Relationship between demographics and diet specificity of Imperial Eagles *Aquila heliaca* in Kazakhstan. Ibis, 147, 576-586.

**21. Whitfield DP, Reid R, Haworth PF, Madders M, Marquiss M, et al.** (2009) Diet specificity is not associated with increased reproductive performance of Golden Eagles *Aquila chrysaetos* in Western Scotland. Ibis, 151, 255-264.

**22. MacArthur RH, Pianka ER** (1966) On optimal use of a patchy environment. Am. Nat., 100, 603–609.

**23. Lefcheck JS, Whalen MA, Davenport TM, Stone JP, Duffy JE** (2013) Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology, 94, 565-572.

**24. L'Hérault V, Franke A, Lecomte N, Alogut A, Bêty J** (2013) Landscape heterogeneity drives intra-population niche variation and reproduction in an arctic top predator. Ecol. Evol., 3, 2867-2879.

**25. Araújo MS, Bolnick DI, Layman CA** (2011) The ecological causes of individual specialisation. Ecol. Lett., 14, 948-958.

**26. Schreiber SJ, Bürger R, Bolnick DI** (2011) The community effects of phenotypic and genetic variation within a predator population. Ecology, 92, 1582-1593.

**27. Tinker MT, Guimarães PRJr, Novak M, Marquitti FMD, Bodkin JL, et al.** (2012) Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecol. Lett., 15, 475-483.

**28. Elmhagen B, Tannerfeldt M, Verucci P, Angerbjörn A** (2000) The arctic fox (*Alopex lagopus*): an opportunistic specialist. J. Zool., 251, 139-149.

**29. Arroyo BE, Garcia JT** (2006) Diet composition influences annual breeding success of Montagu's Harriers *Circus pygargus* feeding on diverse prey. Bird Study, 53, 73-78.

**30. Watson J** (2010) The Golden Eagle. London: T & A D Poyser, 2nd ed. 464 p.

**31. Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H** (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol., 73, 1007-1012.

**32. Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL** (2007) A niche for isotopic ecology. Front. Ecol. Environ., 5, 429-436.

**33. Quevedo M, Svanbäck R, Eklöv P** (2009) Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology, 90, 2263-2274.

**34. Inger R, Bearhop S** (2008) Applications of stable isotope analyses to avian ecology. Ibis, 150, 447-461.

**35. Hobson KA** (2011) Isotopic ornithology: a perspective. J. Ornithol., 152, 49-66.

**36. Layman CA, Arrington DA, Montaña CG, Post DM** (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology, 88, 42-48.

**37. Jackson AL, Inger R, Parnell AC, Bearhop S** (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol., 80, 595-602.

**38. Moore JW, Semmens BX** (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett., 11, 470-480.

**39. Jackson AL, Inger R, Bearhop S, Parnell A** (2009) Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). Ecol. Lett., 12, E1-E5.

**40. Parnell AC, Inger R, Bearhop S, Jackson AL** (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE, 5, e9672.

**41. Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R** (2002) Measuring individual-level resource specialization. Ecology, 83, 2936-2941.

**42. Resano-Mayor J, Hernández-Matías A, Real J, Parés F, Inger R, et al.** (2013) Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. Ibis, 156, 176-188.

**43. Hernández-Matías A, Real J, Moleón M, Palma L, Sánchez-Zapata JA, et al.** (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecol. Monogr., 83, 239-261.

**44. Real J** (2004) Águila-Azor Perdicera, *Hieraaetus fasciatus*. In: Madroño A, González C, Atienza JC, editors. Libro rojo de las aves de España. Madrid: DGB-SEO/Birdlife. pp.154-157.

**45. Real J** (1987) Evolución cronológica del régimen alimenticio de una población de *Hieraaetus fasciatus* en Cataluña: factores causantes, adaptaciones y efectos. Rapaci Mediterranei III. Vol XI: 185-205. Instituto Nazionale di Biologia della Selvaggina. Bologna.

**46. Real J** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía [PhD thesis]. Barcelona: University of Barcelona. 241 p.

**47. Moleón M, Gil-Sánchez JM, Real J, Sánchez-Zapata JA, Bautista J, et al.** (2007) Non-breeding feeding ecology of territorial Bonelli's eagles *Hieraaetus fasciatus* in the Iberian Peninsula. Ardeola, 54, 135-143.

**48. Moleón M, Sánchez-Zapata JA, Real J, García-Charton JA, Gil-Sánchez JM, et al.** (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. J. Biogeogr., 36, 1502–1515.

**49. Moleón M, Sánchez-Zapata JA, Gil-Sánchez JM, Ballesteros-Duperón E, Barea-Azcón JM, et al.** (2012) Predator-prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia, 168, 679–689.

**50. Moleón M, Almaraz P, Sánchez-Zapata JA** (2008) An emerging infectious disease triggering large-scale hyperpredation. PLoS ONE, 3, e2307.

**51. Moleón M, Sebastián-González E, Sánchez-Zapata JA, Real J, Pires MM, et al.** (2012) Changes in intrapopulation resource use patterns of an endangered raptor in response to a disease-mediated crash in prey abundance. J. Anim. Ecol., 81, 1154–1160.

**52. Gil-Sánchez JM** (2000) Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del Águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). Ardeola, 47, 1-8.

**53. Gil-Sánchez JM, Moleón M, Otero M, Bautista J** (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biol. Conserv., 118, 685-694.

**54. Hernández-Matías A, Real J, Pradel R** (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. J. Wildl. Manage., 75, 856-866.

**55. Forsman D** (1999) The Raptors of Europe and the Middle East. London: T & A D Poyser. 589 p.

**56. Resano J, Hernández-Matías A, Real J, Parés F** (2011) Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. J. Raptor Res., 45, 342-352.

**57. Jackson MC, Donohue I, Jackson AL, Britton JR, Harper DM, et al.** (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLoS ONE, 7, e31757.

**58. Parnell A, Inger R, Bearhop S, Jackson AL** (2008) SIAR: Stable isotope analysis in R.

**59. Hobson KA, Clark RG** (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor, 94, 189-197.

**60. Shannon CE, Weaver W** (1997) The Mathematical Theory of Communication. Urbana, IL, USA: University of Illinois Press.

**61. Carrete M, Sánchez-Zapata JA, Tella JL, Gil-Sánchez JM, Moleón M** (2006) Components of breeding performance in two competing species: habitat heterogeneity, individual quality and density-dependence. Oikos, 112, 680-690.

**62. Hernández-Matías A, Real J, Pradel R, Ravayrol A, Vincent-Martin N** (2011) Effects of age, territoriality and breeding on survival of Bonelli's Eagle *Aquila fasciata*. Ibis, 153, 846-857.

**63. Bates D, Maechler M, Bolker B** (2012) lme4: linear mixed-effects models using S4 classes. R package version 0.999999-0. *http://cran.r-project.org/web/packages/lme4/index.html.*

**64. Burnham KP, Anderson DR** (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. Springer.

**65. Nakagawa S, Schielzeth H** (2013) A general and simple method for obtaining $R^2$ from generalized linear mixed-effects models. Methods Ecol. Evol., 4, 133-142.

**66. Palma L, Beja P, Pais M, Cancela da Fonseca L** (2006) Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons. J. Appl. Ecol., 43, 1075-1086.

**67. Forslund P, Pärt T** (1995) Age and reproduction in birds—hypotheses and tests. Trends Ecol. Evol., 10, 374-378.

**68. Real J, Mañosa S, Muñoz E** (2000) Trichomoniasis in a Bonelli's eagle population in Spain. J. Wildl. Dis., 36, 64-70.

**69. Margalida A, Benítez JR, Sánchez-Zapata JA, Ávila E, Arenas R, et al.** (2012) Long-term relationship between diet breadth and breeding success in a declining population of Egyptian Vultures *Neophron percnopterus*. Ibis, 154, 184-188.

**70. Krüger O, Chakarov N, Nielsen JT, Looft V, Grünkorn T, et al.** (2012) Population regulation by habitat heterogeneity or individual adjustment? J. Anim. Ecol., 81, 330-340.

**71. Korpimäki E** (1992) Diet composition, prey choice, and breeding success of long-eared owls: effects of multiannual fluctuations in food abundance. Can. J. Zool., 70, 2373-2381.

**72. Penteriani V, Balbontin J, Ferrer M** (2003) Simultaneous effects of age and territory quality on fecundity in Bonelli's Eagle *Hieraaetus fasciatus*. Ibis, 145, E77-E82.

**73. Margalida A, González LM, Sánchez R, Oria J, Prada L, et al.** (2007) A long-term large-scale study of the breeding biology of the Spanish imperial eagle (*Aquila adalberti*). J. Ornithol., 148, 309-322.

**74. BirdLife International** (2004) Birds in Europe. Population estimates, trends and conservation status. BirdLife Conservation Series No. 12. Cambridge: BirdLife International. 374 p.

**75. Moleón M, Sánchez-Zapata JA, Gil-Sánchez JM, Barea-Azcón JM, Ballesteros-Duperón E, et**

**al.** (2011) Laying the foundations for a human-predator conflict solution: assessing the impact of Bonelli's eagle on rabbits and partridges. PLoS ONE, 6, e22851.

## SUPPORTING INFORMATION

Additional supporting information is shown in the following pages: Figures S1, S2 and Tables S1, S2 and S3.

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## SUPPORTING INFORMATION

**Figure S1.** Relationship between the diet diversity (H’) and the consumption percentage (%) of rabbits (A) and partridges (B) at the territory level (*n* = 71).

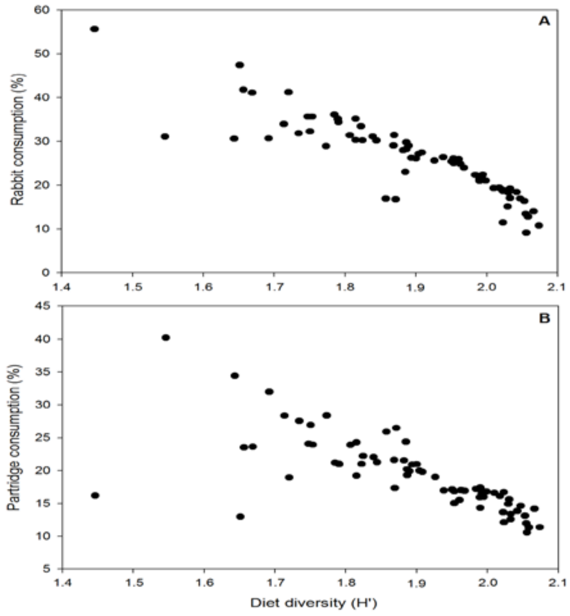
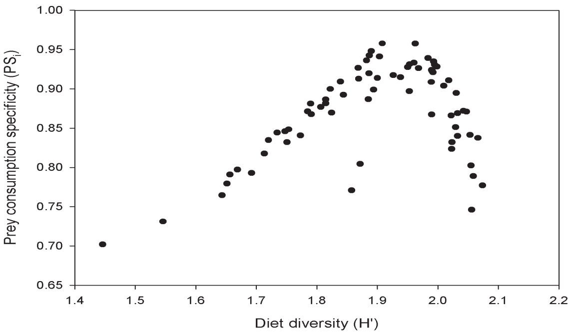


**Figure S2.** Relationship between the diet diversity (H’) and the prey consumption specificity ($PS_i$) at the territory level (n = 71).



## SUPPORTING INFORMATION

**Table S1.** Mean ± SD (‰) values of $\delta^{13}C$ and $\delta^{15}N$ in the Bonelli's eagle prey categories included in SIAR.

| Prey category | $\delta^{13}C$ Mean ± SD (‰) | $\delta^{15}N$ Mean ± SD (‰) | n |
|---|---|---|---|
| **OC** | -26.58 ± 0.43 | 2.81 ± 1.90 | 14 |
| **AR** | -25.60 ± 0.96 | 5.48 ± 2.12 | 10 |
| **CP** | -23.92 ± 0.79 | 5.52 ± 1.70 | 42 |
| **CLw** | -23.64 ± 1.42 | 6.73 ± 2.23 | 41 |
| **CLd** | -16.75 ± 2.17 | 6.85 ± 1.19 | 19 |
| **PAS** | -23.36 ± 0.72 | 7.25 ± 1.24 | 40 |
| **SV** | -19.36 ± 1.60 | 3.23 ± 1.99 | 7 |
| **TL** | -24.04 ± 0.28 | 4.50 ± 2.38 | 3 |

Note: OC = European rabbits; AR = red-legged partridges; CP = wood pigeons; CLw = domestic pigeons wildly foraging in crops; CLd = domestic pigeons from dovecotes and fed with corn; PAS = passerines; SV = Eurasian red squirrels; TL = ocellated lizards; n = number of individuals analysed for each prey category.

**Table S2.** Explanatory variables used in the GLMMs to assess their potential effect on Bonelli's eagle productivity, classified either as spatiotemporal parameters, breeding pair parameters or diet parameters.

| |
|---|
| ***Spatiotemporal parameters*** (categorical factors) |
| TERRITORY: territory code. |
| POPULATION: Catalonia, France or Andalusia. |
| YEAR: 2008-2011. |
| ***Breeding pair parameters*** (categorical factors) |
| AGE OF THE BREEDING PAIR: adult (i.e. both individuals with an adult plumage) or non-adult pair (i.e. at least one individual with a non-adult plumage). |
| MATE REPLACEMENT: presence or absence of replacement (based on individual plumage patterns) of at least one member of the breeding pair in two consecutive years. |
| ***Diet parameters*** (continuous variables) |
| RABBIT CONSUMPTION: mean percentage of European rabbit in the fledglings' diet per territory estimated by SIAR. |
| PARTRIDGE CONSUMPTION: mean percentage of red-legged partridge in the fledglings' diet per territory estimated by SIAR. |
| DIET DIVERSITY (H'): Shannon-Weaver diversity index using the mean consumption percentage of main prey categories in the fledglings' diet per territory estimated by SIAR. |
| PREY CONSUMPTION SPECIFICITY ($PS_i$): measure of diet overlap between a given territory and the population as a whole. The territorial diet represents the mean percentage of main prey categories in the fledglings' diet estimated by SIAR. |
| ***Interaction of parameters*** |
| (H' * $PS_i$): interaction between H' and $PS_i$. |
| $H' + H'^2$: quadratic effect of H'. |
| $PSi + PS_i^2$: quadratic effect of PSi. |

## SUPPORTING INFORMATION

**Table S3.** Summary of model parameter estimates and standard error of parameter estimates for each model included in the GLMMs. Models are showed following the same order as in Table 3.

**Model 1 <- Age + Replacement + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 0.663 | 0.218 |
| Age | -1.067 | 0.860 |
| Replacement | -0.142 | 0.807 |

**Model 2 <- Age + Replacement + PSi + (PSi$^2$) + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 55.660 | 29.170 |
| Age | -1.116 | 0.884 |
| Replacement | -0.008 | 0.831 |
| PSi | -130.500 | 68.970 |
| PSi$^2$ | 76.890 | 40.580 |

**Model 3 <- Age + Replacement + H' + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 2.448 | 2.486 |
| Age | -1.045 | 0.861 |
| Replacement | -0.176 | 0.808 |
| H' | -0.942 | 1.304 |

**Model 4 <- Age + Replacement + (OC+AR) + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 0.344 | 0.705 |
| Age | -1.053 | 0.860 |
| Replacement | -0.167 | 0.809 |
| OC+AR | 0.007 | 0.015 |

**Model 5 <- Age + Replacement + OC + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 0.445 | 0.562 |
| Age | -1.037 | 0.859 |
| Replacement | -0.150 | 0.806 |
| OC | 0.008 | 0.020 |

**Model 6 <- Age + Replacement + PSi + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 1.549 | 2.343 |
| Age | -1.067 | 0.860 |
| Replacement | -0.146 | 0.806 |
| PSi | -1.021 | 2.678 |

**Model 7 <- Age + Replacement + AR + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 0.446 | 0.668 |
| Age | -1.087 | 0.866 |
| Replacement | -0.174 | 0.812 |
| AR | 0.012 | 0.034 |

**Model 8 <- Age + Replacement + H′ + ($H'^2$) + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 21.221 | 23.830 |
| Age | -1.076 | 0.858 |
| Replacement | -0.132 | 0.805 |
| H′ | -21.854 | 26.141 |
| $H'^2$ | 5.769 | 7.139 |

**Model 9 <- Age + Replacement + (OC + AR) + H′ + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 8.462 | 9.790 |
| Age | -1.043 | 0.863 |
| Replacement | -0.157 | 0.810 |
| OC+AR | -0.030 | 0.047 |
| H′ | -3.399 | 4.092 |

**Model 10 <- Age + Replacement + OC + H′ + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 5.508 | 6.370 |
| Age | -1.097 | 0.874 |
| Replacement | -0.195 | 0.813 |
| OC | -0.023 | 0.043 |
| H′ | -2.237 | 2.813 |

**Model 11 <- Age + Replacement + AR + H′ + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 2.917 | 3.859 |
| Age | -1.028 | 0.863 |
| Replacement | -0.163 | 0.808 |
| AR | -0.007 | 0.045 |
| H′ | -1.120 | 1.720 |

**Model 12 <- Age + Replacement + (OC + AR) + PSi + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 1.079 | 2.605 |
| Age | -1.055 | 0.860 |
| Replacement | -0.167 | 0.808 |
| OC+AR | 0.006 | 0.015 |
| PSi | -0.802 | 2.731 |

**Model 13 <- Age + Replacement + OC + PSi + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 1.216 | 2.513 |
| Age | -1.041 | 0.860 |
| Replacement | -0.153 | 0.806 |
| OC | 0.007 | 0.020 |
| PSi | -0.857 | 2.713 |

**Model 14 <- Age + Replacement + AR + PSi + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 1.259 | 2.550 |
| Age | -1.084 | 0.865 |
| Replacement | -0.172 | 0.810 |
| AR | 0.010 | 0.035 |
| PSi | -0.897 | 2.718 |

**Model 15 <- Age + Replacement + H’ + PSi + (H’ * PSi) + (1|Terr) + (Popu|Year)**

| Parameter | Estimate | Standard Error |
|---|---|---|
| Intercept | 15.116 | 25.294 |
| Age | -1.051 | 0.862 |
| Replacement | -0.167 | 0.809 |
| H’ | -7.692 | 13.508 |
| PSi | -16.259 | 32.107 |
| H’*PSi | 8.628 | 17.01 |

Note: models are ordered from higher to lower $AIC_{cw}$ values following the classification and nomenclature shown in Table 3.

# Effects of nestling diet on breeding

# Chapter 4

## The influence of diet on demographic parameters of Bonelli's Eagle in Western Europe

* Large-scale spatial patterns in demographic rates in
relation to diet in Bonelli's Eagle *Aquila fasciata*,
a long-lived territorial predator

# Patrones espaciales a gran escala en las tasas demográficas de un depredador territorial de vida larga, el águila perdicera *Aquila fasciata*, en relación con la dieta

## Resumen

La comprensión de los mecanismos que determinan la dinámica de las poblaciones animales tiene especial interés en ecología, evolución y biología de la conservación. La disponibilidad de alimentos es un importante factor limitante en la mayoría de poblaciones animales, y puede tener consecuencias demográficas. La teoría del forrajeo óptimo predice que un depredador consumirá más presas preferidas y tendrá una dieta menos diversa cuando el alimento es abundante, lo cual podrá beneficiar en términos de reproducción o supervivencia. Sin embargo, la correspondencia entre el uso de los recursos a nivel individual, y los procesos demográficos emergentes en las poblaciones de aves rapaces que habitan amplias regiones con ecosistemas complejos como los de la cuenca mediterránea, en gran parte se desconoce. En base a un programa de monitoreo a largo plazo de la dieta y la demografía del águila perdicera *Aquila fasciata* en Europa Occidental, en este estudio testamos la hipótesis de que la dieta de un depredador tiene un efecto sobre la productividad, la supervivencia y el crecimiento a nivel territorial haciendo que, en última instancia, tenga consecuencias sobre la tasa de crecimiento de la población. A nivel territorial, la productividad aumentó con un mayor consumo de conejos *Oryctolagus cuniculus*, una de las presas preferidas del águila perdicera, y disminuyó con el aumento del consumo de presas no preferidas, como varias especies de aves de mediano a gran tamaño. A su vez, la supervivencia de las parejas territoriales fue menor al aumentar la diversidad de la dieta, que también afectó negativamente a la tasa de crecimiento territorial. A nivel poblacional, los efectos de la dieta sobre los parámetros demográficos fueron similares, aunque más notables que a nivel territorial. En este sentido, un mayor consumo de presas preferidas (sobre todo conejo, pero también perdiz roja *Alectoris rufa*) aumentó la productividad, mientras que una mayor diversidad de la dieta incrementó la mortalidad y redujo la tasa de crecimiento poblacional. En general, nuestro estudio muestra que la dieta de un depredador puede tener un efecto sobre parámetros vitales clave a nivel individual y, en última instancia, determinar la dinámica de sus poblaciones en una amplia escala espacial.

# Large-scale spatial patterns in demographic rates in relation to diet in Bonelli's Eagle *Aquila fasciata*, a long-lived territorial predator

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**ABSTRACT—** Understanding the mechanisms shaping animal population dynamics is of fundamental interest in ecology, evolutionary and conservation biology. Food supply is an important limiting factor in most animal populations and may have demographic consequences. Optimal foraging theory predicts greater consumption of preferred prey and less diet diversity when food is abundant, which may benefit key fitness parameters such as productivity and survival. Nevertheless, the correspondence between individual resource use and demographic processes in populations of avian predators inhabiting large geographic areas in complex ecosystems such as those of the Mediterranean basin remains largely unexplored. Based on a long-term monitoring program of the diet and demographics of Bonelli's eagle *Aquila fasciata* in Western Europe, here we test the hypothesis that a predator's diet is correlated to its breeding productivity, survival and growth rate at territorial level and, ultimately, to its population growth rate. At territorial level, we found that productivity increased with greater consumption of European rabbits *Oryctolagus cuniculus*, one of this species' preferred prey items, but decreased with greater consumption of non-preferred prey items such as medium-to-large avian species. The survival of territorial pairs was negatively affected by diet diversity, which also had a negative effect on the territorial growth rate. Diet effects at population level were similar but more noticeable than at territorial level, i.e., a greater consumption of preferred prey (first rabbits and second red-legged partridges *Alectoris rufa*) increased productivity, while greater diet diversity increased mortality and reduced the population growth rate. Overall, our study illustrates how the diet of a predator species can be closely related to key individual vital rates, which, in turn, leave a measurable fingerprint on population dynamics within and between populations across large spatial scales.

# Chapter 4

## INTRODUCTION

Understanding the mechanisms shaping animal population dynamics is of fundamental interest in ecology, evolutionary and conservation biology (Begon et al. 2005). On a simple level, births, movements and deaths are the main demographic parameters conditioning animal population dynamics (Krebs et al. 2001) and, in turn, several abiotic and biotic factors potentially affect these parameters (Dempster 1975). A universal property of life is that organisms need energy and resources for reproduction and survival and so food availability and consumption are expected to affect individual vital rates and hence are an important limiting factor in animal populations (Stephens and Krebs 1986, White 2008). In addition, the way resource consumption by individuals ultimately affects population demographic trends is closely related to the characteristics of species' life histories. In animals, although fecundity has an important impact on population growth rates in short-lived species, survival rates are more relevant to population dynamics in long-lived species (Sæther and Bakke 2000). Thus, the effects of diet on individual vital rates within a population, along with the species' life-history traits, will determine how individual resource consumption ultimately affects the population growth rate.

Avian predators have been well studied as a way of assessing how prey consumption influences consumers' key demographic parameters such as breeding success and survival (e.g. Korpimäki and Norrdahl 1991, Millon and Bretagnolle 2008). In the case of territorial species whose populations range over large geographic areas, habitat heterogeneity is expected to influence prey abundance and availability, which will ultimately affect individual diet variation within a population (Whitfield et al. 2009) since organisms prefer environments in which their reproductive success and survival is good (Orians and Wittenberger 1991). Foraging theory predicts that animals will feed most efficiently by consuming a narrow range of preferred prey items when they are abundant, but will expand their range of food and consume less-preferred prey items when food becomes scarce (Emlen 1966, Futuyma and Moreno 1988). In this sense, species will prefer prey that benefits their individual fitness and key vital rates (Schoener 1971, Pyke et al. 1977), a choice that will seriously shape predator population dynamics (Vucetich and Peterson 2004, Millon and Bretagnolle 2008). For instance, high prey abundance may increase preferred prey consumption and enhance predator breeding success and survival (Korpimäki and Norrdahl 1991, Millon and Bretagnolle 2008). By contrast, the scarcity of the preferred prey will force predators to expand their diet and to consume alternative prey (Schoener 1971, Pyke et al. 1977), which might constrain reproductive success and survival (Arroyo and Garcia 2006, White 2008). Nevertheless, the correspondence between individual resource use and demographic processes in populations (e.g. growth rates) remains largely unexplored in complex natural systems.

Numerous studies have assessed the relationship between temporal variations in diet and demographic parameters in avian predators, particularly in boreal and other northern ecosystems. These systems are characterized by cyclical prey regimes and both coupled and lagged variations in predator-prey population dynamics have been reported (e.g. Korpimäki and Krebs 1996, Krebs et al. 2001). By contrast, studies assessing the relationship between spatial variation in prey consumption patterns and demographic parameters of avian predators are comparatively scarce, particularly in temperate Mediterranean ecosystems that are more complex since they do not have clearly marked cyclical prey regimes (but see Salamolard et al. 2000, Fargallo et al. 2009). In such systems, research on whether patterns of prey consumption at large spatial scales are related to the demographic fate of local populations in spatially structured populations necessarily requires large investment in long-term monitoring of resource consumption and demography over large spatio-temporal scales that is unavailable for most avian predator species.

Based on a long-term monitoring program in France, Spain and Portugal of Bonelli's eagle *Aquila fasciata*, a long-lived territorial raptor, here we test the hypothesis that a predator's diet is correlated to its breeding productivity and survival at territorial level and, ultimately, to its population growth rate. In Western Europe, Bonelli's eagle predates on a wide range of prey that includes small-to-medium sized mammals (Lagomorpha and Rodentia), birds (Columbiformes, Galliformes, Passeriformes, Ardeiformes, Charadriiformes and others) and reptiles (mainly lizards) (Real 1991, Moleón et al. 2009). In this geographical area, marked differences in dietary patterns between territories have been described within single populations (Real 1991, Palma et al. 2006, Resano-Mayor et al. 2014), a trend that increased after the outbreak of the Rabbit Haemorrhagic Disease in the late 1980s (Moleón et al. 2012a). Yet, despite the dietary differences between territories, European rabbits *Oryctolagus cuniculus* and, to a lesser extent, red-legged partridges *Alectoris rufa*, are thought to be Bonelli's eagle's preferred prey item, as they are positively selected and high consumption of these prey species leads to a decrease in this eagle's diet diversity (Real 1991, Moleón et al. 2009, 2012b). On the other hand, this raptor also shows marked intra- and inter-population demographic variations throughout its western European populations (Real and Mañosa 1997, Hernández-Matías et al. 2013). These variations are evident in terms of survival and reproduction both between territories and local populations, as well as in the contrasting demographic trends occurring between local populations (Hernández-Matías et al. 2011a, 2013). Nevertheless, the potential effects of diet on the vital rates (e.g. breeding productivity and survival) of territorial pairs and whether dietary effects occurring at territorial scale also modulate local populations' growth rates are unanswered questions on a large scale.

The main objective of this study was to test the effect of diet heterogeneity on vital and growth rates in Bonelli's eagle at territorial and local scale within its western European population. We assumed that the higher consumption of preferred prey reflects its greater availability while greater diet diversity is expected to occur when preferred prey availability is lower (Moleón et al. 2012b). Based on these assumptions, we predict that the consumption of preferred prey, i.e. rabbits and partridges, and diet diversity should positively and negatively correlate, respectively, with the productivity, survival and growth rate of Bonelli's eagle at both territorial and local population levels. Given the life history traits of this species, we also predict that the dietary factors affecting survival will have a strongest effect on territorial and population growth rates.

## METHODS

### Study period and area

In the period 1989–2006, we monitored both diet and demographic rates in 72 Bonelli's eagle breeding territories located in six local populations in the species' western European range: Provence and Languedoc-Roussillon (southeast France; *n* = 8 territories), Burgos (north central Spain; *n* = 6), Catalonia (northeast Spain; *n* = 17), Arribes (northwest Spain and northeast Portugal; *n* = 5), Granada (southeast Spain; *n* = 17) and southern Portugal (*n* = 19) (Fig. 1).

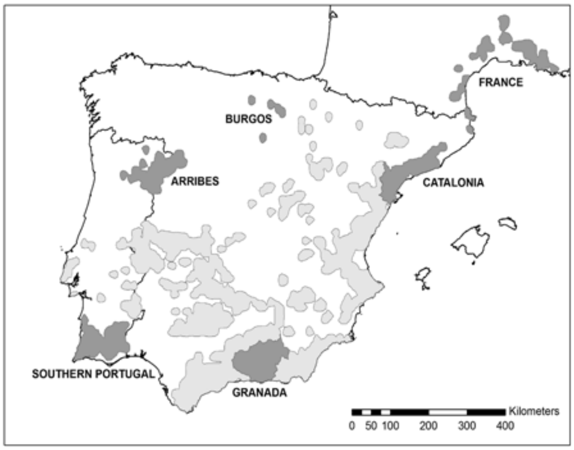

**Figure 1.** Distribution map of Bonelli's eagle in Western Europe (modified from Hernández-Matías et al. 2013). The studied populations are shown in dark grey: France, Catalonia, Burgos, Arribes, southern Portugal and Granada.

### Diet analysis

The study of Bonelli's eagle diet was based on pellet analysis. Pellets were collected from breeding nests and perches. Pellet collection in each local population was conducted as follows: 1989–1995 in Provence and Languedoc-Roussillon, 1993–1996 in Burgos, 1990–2005 in Catalonia, 1996–1998 in Arribes, 1993–2006 in Granada and 1992–2001 in southern Portugal. Pellets were individually analysed and each prey item identified was counted as one item (Real 1996). Pellet contents (i.e. feathers, bones, hair, nails and scales) were identified using a 4x magnifying glass, reference collections and specialized guides. Prey items were identified to species level whenever possible.

Following Moleón et al. (2009), all items from pellets collected in the same breeding territory were pooled together even if they originated from different years. Therefore, our dietary estimates at territorial level were representative of the whole monitored period for each local population. Prey items were then grouped into seven different taxonomic prey categories: European rabbits, 'other mammals', red-legged partridges, pigeons *Columba* spp., corvids, 'other birds' and reptiles (mainly ocellated lizard *Timon lepidus*) to calculate the consumption percentage of each prey category at territorial level. These percentages were then used to estimate diet diversity by means of the Shannon-Weaver index (H') (In; Shannon and Weaver 1949).

### Estimation of demographic parameters

To assess the occupancy and breeding performance of each eagle territory, known breeding areas were visited annually from January to June. Between January and March we recorded breeding activity (i.e. flight displays, nest material transfers, copulation and incubation). In late March and April, occupied nests were checked to detect the presence, number and age of nestlings, which were estimated by feather development and backdating from the laying date. Between May and June, all breeding pairs were checked to record the number of chicks fledged (nestlings at an age of ≥ 50 days were assumed to have fledged successfully; Gil-Sánchez et al. 2004). Observations were always carried out away from nests using 10x binoculars and 20-60x spotting scopes.

Based on the yearly monitored data we estimated the productivity and survival at territorial level (except in Arribes, where demographic data were not available at territorial/year level). In those territories in which pellet collection was conducted for ten or more years, productivity and survival were estimated for the same period as the pellet collection. If pellet collection had lasted for less than ten years, we still used a period of ten consecutive years to estimate both vital rates by placing the pellet sampling years in the middle of the ten-year interval. Productivity was calculated as the total number of fledglings per territorial pair divided by the number of monitored years. Individual survival probability in a given territory was calculated from annual turnover rates of territorial birds based on age classes defined by plumage patterns but corrected for the proportion of non-detected replacements (i.e. the replacement of similar individuals of the same age class) (Hernández-Matías et al. 2011a, 2013).

A Leslie matrix was used to estimate the growth rate at territorial level ($\lambda_t$). The matrix was built by considering five age classes (i.e. fledgling, juvenile (first-year), immature (second-year), subadult (third-year) and adult (fourth and subsequent years)), which differed in their survival and productivity estimates. We used our own estimates of productivity and survival for the adult age class, while the productivity, survival and recruitment for the other age classes were obtained from Hernández-Matías et al. (2010, 2011b, 2013). The sex ratio was assumed to be 1:1.

Beyond the territorial scale, estimates of adult productivity, adult survival and population growth rate ($\lambda_p$) for each monitored local population ($n$ = 6) were obtained from a recent published study that estimated those demographic parameters for the same monitored populations during the period 1990–2009 (see Hernández-Matías et al. 2013).

### Statistical analysis

We used Multiple Linear Regressions (MLRs) to test the influence of diet on productivity, survival and growth rate at both territorial ($n$ = 67) and local population ($n$ = 6) levels. In these analyses, each demographic parameter was modelled as a response variable; the explanatory dietary variables were the four most consumed prey categories: rabbits (OC) and partridges (AR) (i.e. preferred prey), pigeons (CSP) (i.e. important secondary prey) and 'other birds' (OB) (i.e. non-preferred prey), and diet diversity (H'). When modelling productivity as the response variable, we also considered a model with only survival as explanatory variable (see Carrete et al. 2006, Hernández-Matías et al. 2011b). Survival estimates were logit-transformed to achieve a normal distribution. Model selection was based on Akaike's Information Criterion adjusted for sample size ($AIC_c$); the Akaike weights ($AIC_{cw}$) were computed to assess the probability that each candidate model was the best for the proposed set (Burnham and Anderson 2002). To estimate the proportion of variance in the response variable explained by each model we calculated the coefficient of determination ($R^2$) (Draper and Smith 1998). Models were run using R ver. 2.14.0.

## RESULTS

### Effects of diet on territorial demographic parameters

In the productivity analysis at territorial level, the best supported model included the consumption of rabbits and 'other birds' as explanatory variables (Table 1) that had contrasting effects on productivity, i.e., productivity increased with greater consumption of rabbits but decreased with greater consumption of 'other birds' (Fig. 2). The model including the consumption of rabbits and 'other birds' also had the best explanatory power in terms of $R^2$. Survival alone had less effect on productivity than any of our dietary models (see Table 1).

In the survival analysis at territorial level, the best-supported model included diet diversity (Table 1) and there was greater survival wherever the diet diversity was lower (Fig. 3). Nevertheless, even though the best-supported model had poor explanatory powers in terms of $R^2$, it was still higher than all other models (see Table 1).

The analysis of the territorial growth rate ($\lambda_t$) showed that the best-supported model included the consumption of 'other birds' and diet diversity as explanatory variables (Table 1). In line with the abovementioned findings, a greater consumption of 'other birds' and greater diet diversity reduced the territorial growth rate (Fig. 4). The model explanatory power in terms of $R^2$ was low, as in the case of survival (see Table 1).

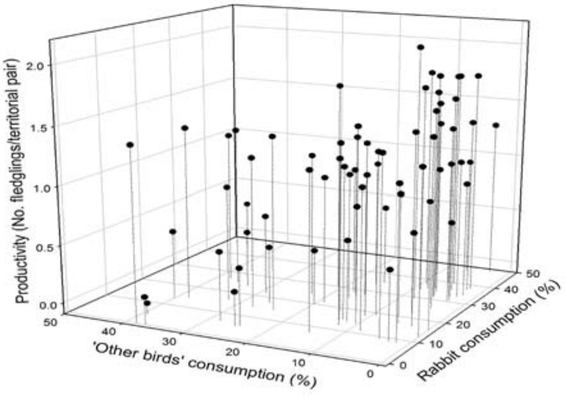

**Figure 2.** Three-dimensional scatter plot showing the relationship between Bonelli's eagles mean productivity (number of fledglings/territorial pair) and consumption (%) of European rabbits (i.e. positive relationship) and 'other birds' (negative relationship) at territorial level. Rabbit consumption is shown on the x-axis, 'other bird' consumption on the y-axis and productivity on the z-axis, with each black dot representing data for each territory ($n = 67$).

## Effects of diet on population demographic parameters

The analysis of productivity at population level indicates that the best-supported model included only the consumption of partridges (Table 2) as productivity increased with greater partridge consumption (Fig. 5). Nevertheless, the model with the highest $R^2$ included the consumptions of rabbits and 'other birds', as previously found at territorial level. We did not detect any effect of adult survival on productivity (see Table 2).

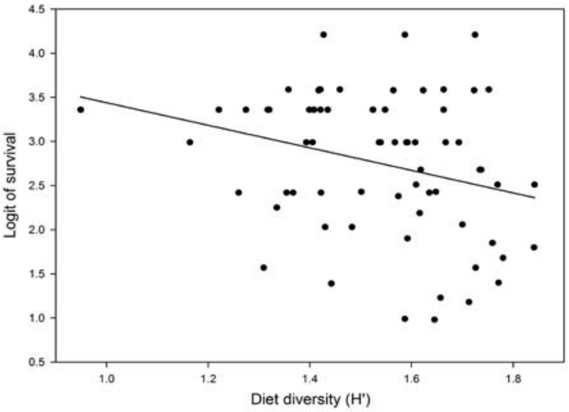

**Figure 3.** Relationship between survival and diet diversity (H') at territorial level ($n = 67$). Survival data were log transformed to achieve normal distribution. The linear regression line is shown.

Adult survival analysis at population level shows that the model including diet diversity was the best supported by the data (Table 2). As at territorial level, adult survival increased with lower diet diversity (Fig. 6). Moreover, the model including diet diversity showed the highest $R^2$ compared with all other models (see Table 2).

Finally, the analysis of the population growth rate ($\lambda_p$) once again indicated that the model including diet diversity was the best supported by the data (Table 2), having the same effect as previously reported for adult survival, i.e., those populations with greater growth rates also had less diet diversity (Fig. 7). The model including diet diversity also had the highest explanatory power in terms of $R^2$ (Table 2).

## DISCUSSION

Our study shows how the diet of a long-lived predator is closely related to its demographic parameters at different spatial scales in complex ecosystems such as those in the Mediterranean basin. By using a large data set, we found that several key demographic parameters of

**Table 1.** Ranking models of the multiple linear regression analyses at territorial level. Dependent variables were productivity (i.e. number of fledglings/territorial pair), survival (i.e. log transformed survival of territorial eagles) and territorial growth rate. The explanatory dietary variables considered were rabbit consumption (OC), partridge consumption (AR), pigeon consumption (CSP), 'other birds' consumption (OB), the combined sum of these prey categories ((OC + AR), (OC + CSP), (OC + OB), (AR + CSP), (AR + OB), (CSP + OB)) and diet diversity (H'). $\Delta AIC_c$ refers to the difference in $AIC_c$ between model *i* and the model with the lowest $AIC_c$ score (i.e. the best model). $AIC_{cw}$ explains the probability that a given candidate model is the best of the proposed set and so the sum of all models is 1.0. $R^2$ indicates the coefficient of determination of the models (i.e. explained variance of the dependent variable). Models with $\Delta AIC_c < 2$ are shown in bold type. The values of best $AIC_c$ models are shown at the bottom of the table in each column. To assess the effect of survival on productivity we also show the $AIC_c$ value of a model considering only survival.

| Productivity | | | | Logit_survival | | | | Territory growth rate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Models** | **ΔAICc** | **AICcw** | **$R^2$** | **Models** | **ΔAICc** | **AICcw** | **$R^2$** | **Models** | **ΔAICc** | **AICcw** | **$R^2$** |
| **OC + OB** | ***0.00** | **0.56** | **0.27** | **H'** | ***0.00** | **0.59** | **0.08** | **OB** | ***0.00** | **0.30** | **0.06** |
| **OB** | **1.81** | **0.23** | **0.22** | CSP | 4.14 | 0.07 | 0.02 | **H'** | **1.55** | **0.14** | **0.03** |
| CSP + OB | 2.98 | 0.13 | 0.24 | AR | 4.27 | 0.07 | 0.02 | CSP + OB | 2.07 | 0.11 | 0.06 |
| AR + OB | 3.99 | 0.07 | 0.22 | OC | 5.08 | 0.05 | 0.01 | AR + OB | 2.19 | 0.10 | 0.06 |
| OC | 9.20 | 0.01 | 0.13 | OB | 5.25 | 0.04 | 0.00 | OC + OB | 2.19 | 0.10 | 0.06 |
| OC + CSP | 10.27 | 0.00 | 0.15 | AR + CSP | 5.37 | 0.04 | 0.03 | CSP | 3.11 | 0.06 | 0.01 |
| OC + AR | 10.63 | 0.00 | 0.14 | CSP + OB | 5.54 | 0.04 | 0.03 | AR | 3.34 | 0.06 | 0.01 |
| AR | 16.65 | 0.00 | 0.03 | OC + CSP | 6.18 | 0.03 | 0.02 | OC | 3.43 | 0.05 | 0.01 |
| H' | 17.86 | 0.00 | 0.01 | OC + AR | 6.28 | 0.03 | 0.02 | OC + CSP | 4.48 | 0.03 | 0.02 |
| AR + CSP | 18.67 | 0.00 | 0.03 | AR + OB | 6.46 | 0.02 | 0.02 | AR + CSP | 4.57 | 0.03 | 0.02 |
| CSP | 18.78 | 0.00 | 0.00 | OC + OB | 7.18 | 0.02 | 0.01 | OC + AR | 5.27 | 0.02 | 0.01 |

Best model AICc = -115.96 Best model AICc = -31.56 Best model $AIC_c$ = -378.47

Model with only survival $AIC_c$ = -97.40

**Table 2.** Ranking models of the multiple linear regression analyses at population level. Dependent variables were productivity (i.e. number of fledglings/territorial adult pair), survival (i.e. log transformed adult survival) and population growth rate. The explanatory dietary variables considered were rabbit consumption (OC), partridge consumption (AR), pigeon consumption (CSP), 'other birds' consumption (OB), the combined sum of these prey categories ((OC + AR), (OC + CSP), (OC + OB), (AR + CSP), (AR + OB), (CSP + OB)) and diet diversity (H'). $\Delta AIC_c$ refers to the difference in $AIC_c$ between model *i* and the model with the lowest $AIC_c$ score (i.e. the best model). $AIC_{cw}$ explains the probability that a given candidate model is the best of the proposed set and so the sum of all models is 1.0. $R^2$ indicates the coefficient of determination of the models (i.e. explained variance of the dependent variable). Models with $\Delta AIC_c < 2$ are shown in bold type. The values of best $AIC_c$ models are shown at the bottom of the table in each column. To assess the effect of survival on productivity we also show the $AIC_c$ value of a model considering only adult survival.

| Productivity | | | | Logit_survival | | | | Population growth rate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Models** | **ΔAICc** | **AICcw** | **$R^2$** | **Models** | **ΔAICc** | **AICcw** | **$R^2$** | **Models** | **ΔAICc** | **AICcw** | **$R^2$** |
| **AR** | ***0.00** | **0.59** | **0.61** | **H'** | ***0.00** | **0.96** | **0.86** | **H'** | ***0.00** | **0.61** | **0.59** |
| OB | 2.09 | 0.21 | 0.45 | AR | 7.08 | 0.03 | 0.53 | AR | 2.51 | 0.17 | 0.38 |
| OC | 4.22 | 0.07 | 0.22 | CSP | 9.20 | 0.01 | 0.33 | OB | 3.85 | 0.09 | 0.23 |
| H' | 5.38 | 0.04 | 0.05 | OB | 11.55 | 0.00 | 0.01 | OC | 4.00 | 0.08 | 0.21 |
| CSP | 5.42 | 0.04 | 0.04 | OC | 11.59 | 0.00 | 0.00 | CSP | 5.12 | 0.05 | 0.05 |
| OC + OB | 6.56 | 0.02 | 0.78 | AR + CSP | 13.88 | 0.00 | 0.72 | OC + AR | 12.11 | 0.00 | 0.42 |
| AR + CSP | 7.55 | 0.01 | 0.74 | AR + OB | 14.07 | 0.00 | 0.71 | AR + OB | 12.33 | 0.00 | 0.40 |
| AR + OB | 8.90 | 0.01 | 0.68 | OC + AR | 15.52 | 0.00 | 0.64 | AR + CSP | 12.42 | 0.00 | 0.39 |
| OC + AR | 9.75 | 0.01 | 0.63 | OC + CSP | 19.15 | 0.00 | 0.33 | CSP + OB | 13.72 | 0.00 | 0.25 |
| CSP + OB | 10.75 | 0.00 | 0.56 | CSP + OB | 19.20 | 0.00 | 0.33 | OC + OB | 13.85 | 0.00 | 0.23 |
| OC + CSP | 13.50 | 0.00 | 0.30 | OC + OB | 21.39 | 0.00 | 0.03 | OC + CSP | 13.87 | 0.00 | 0.23 |

Best model AICc = -12.58 Best model AICc = -19.67 Best model $AIC_c$ = -37.27

Model with only survival $AIC_c$ = -7.18

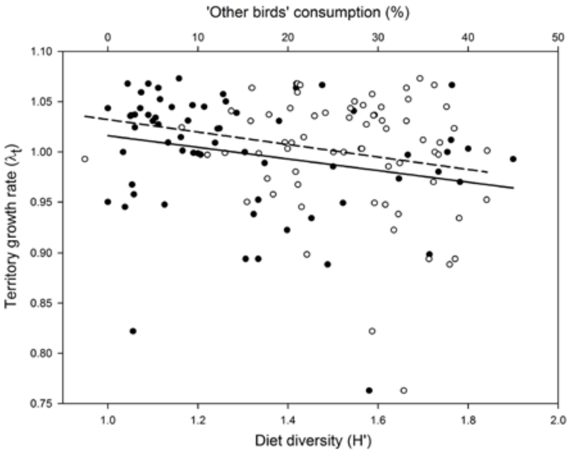

**Figure 4.** Relationship between the territorial growth rate ($\lambda_t$) and both consumption (%) of 'other birds' (upper x-axis and shown by closed dots and a straight line) and diet diversity (H′) (lower x-axis and shown by open dots and a shaded line). Linear regression lines are shown.

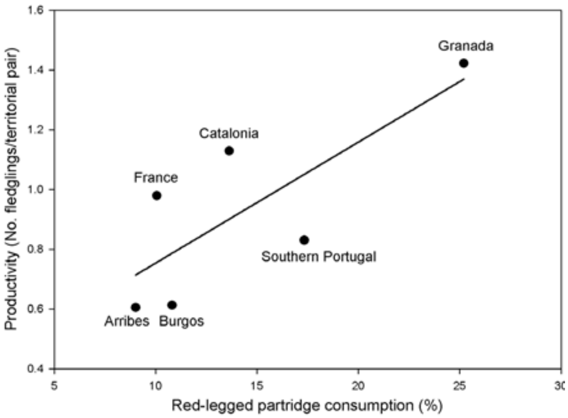

**Figure 5.** Relationship between adult productivity (number of fledglings/territorial pair) and red-legged partridge consumption (%) at population level (*n* = 6; each population labelled). The linear regression line is shown.

an endangered raptor such as Bonelli's eagle in Western Europe are dependent on the consumption of a few preferred prey species and on diet diversity. This, in turn, leaves a measurable fingerprint on the population dynamics within and between populations, which may lead to different ecological, evolutionary and conservation implications.

Food supply is a typical limiting factor in animal populations and so an efficient exploitation of available food is a vital requirement for all animals (Emlen 1966, White 2008). Optimal foraging theory predicts that animals will feed most efficiently by consuming a narrow range of preferred prey when it is abundant but that diet diversity will increase due to the consumption of less preferred prey items when food becomes scarce (Emlen 1966, Futuyma and Moreno 1988). Thus, a greater consumption of preferred prey should maximize net energy and hence increase fitness parameters (Emlen 1966, Schoener 1971, Pyke et al. 1977). In our study, we found that Bonelli's eagle productivity at territorial level was mainly determined by the consumption of rabbits and 'other birds' (Table 1). As predicted, a greater consumption of rabbits, a preferred prey of Bonelli's eagle in Western Europe (Real 1991, Moleón et al. 2009, 2012b), increased the mean productivity of territorial pairs (Fig. 2; see also Cheylan 1981, Real 1987, 1991). A positive association between preferred prey consumption and productivity has previously been found in several other avian predators (e.g. Steenhof et al. 1997, Catry et al. 2012). On the other hand, we also found a negative correlation between the consumption of 'other birds' and Bonelli's eagle productivity at territorial level (Fig. 2). This prey category mainly included medium-to-large avian species (e.g. Anseriformes, Ardeiformes, Charadriiformes or Accipitriformes), presumably predated when the optimal/preferred prey was scarce (Moleón et al. 2008, 2009), as suggested by either the negative correlation we detected between the consumption of 'other birds' and rabbits or the positive association between 'other birds' consumption and diet diversity.

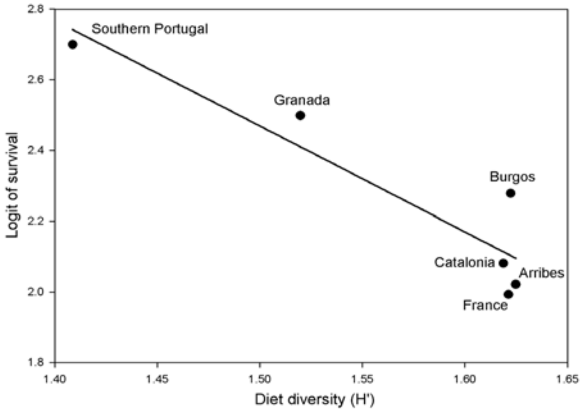

**Figure 6.** Relationship between adult survival and diet diversity (H′) at population level (*n* = 6; each population labelled). Survival data was log-transformed to achieve normal distribution. The linear regression line is shown.

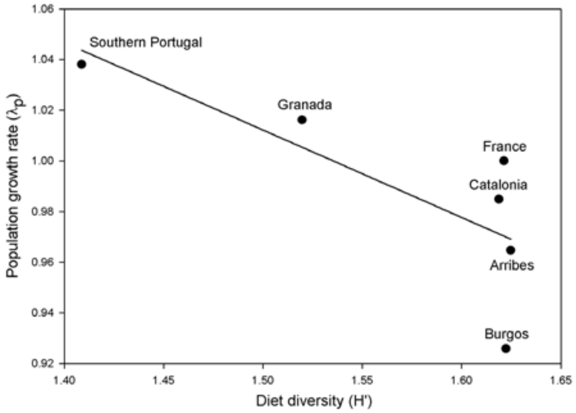

**Figure 7.** Relationship between population growth rate ($\lambda_p$) and diet diversity (H′) at population level (n = 6; each population labelled). The linear regression line is shown.

Our interpretation is also supported by the fact that the consumption of ‘other birds’ in the most northern populations of our study area increases considerably when the availability of rabbits falls (Real 1991, Moleón et al. 2007). Our results are consistent with a common finding in numerous avian predators that shows that an increase in the dietary range (i.e. in the present study, the greater consumption of a non-preferred prey) reduces a predator’s productivity (e.g. Arroyo and Garcia 2006).

At population level, the best-supported model indicated that greater partridge consumption increases eagles’ productivity (Fig. 5). The model including consumption of rabbits and ‘other birds’ was, however, the most explanatory in terms of mean productivity, as suggested by the coefficients of determination (Table 2), that is, similar dietary parameters influencing the productivity of territorial pairs also modulate the diet-demography relationship at population level (Sutherland 1996). Previous studies have revealed that rabbits are the main prey driving spatio-temporal patterns in the diet of Bonelli’s eagles in the study area (Moleón et al. 2009); nevertheless, partridges are also a key prey item in the study species and in other Mediterranean predators when rabbits become scarce (Moleón et al. 2008, 2013). In this regard, rabbits, which are native to the Iberian Peninsula, are regarded as a keystone species for a large number of avian predators in southern Europe (see Delibes-Mateos et al. 2007). Based on comprehensive dietary and demographic information, our study illustrates that the consumption of preferred prey (i.e. firstly rabbits and secondly partridges) may determine Bonelli’s eagle breeding performance both in territories within populations and in local populations over a large geographical area.

Energy maximization when foraging allows for a quicker satisfaction of energetic demands and enhances body condition, and hence is expected to improve individual survival (Orians and Pearson 1979, White 2008). In our study, we found that eagles’ survival rates increased with lower diet diversity at territorial (Table

1 and Fig. 3) and, especially, at population levels (Table 2 and Fig. 6). In general, lower diet diversity of breeding pairs was linked to greater consumption of the preferred prey (i.e. rabbits and/or partridges) (see also Futuyma and Moreno 1988). In some territories, however, breeding pairs were also highly specialized (i.e. had less diet diversity) on secondary prey items such as domestic pigeons, which may be consumed in abundance where they are common and predictable (see Emlen 1966, Palma et al. 2006). In all territories with low diet diversity, lower mortality rates could be related with a quicker satisfaction of energetic demands and, as a result, less exposure to human-induced mortality such as electrocution, collision and persecution, the main causes of death in Bonelli's eagle (Real et al. 2001, Moleón et al. 2007). Thus, the existence of breeding pairs with greater diet diversity possibly reflects greater individual investment in foraging activities (e.g. both in terms of the time invested in moving around the territory and in the areas surveyed for hunting; Orians and Pearson 1979) that may lead to greater exposure to the main mortality risks. This would indicate an indirect effect of food scarcity on predator survival via a change in foraging habits and behaviour (see also Martínez-Abraín et al. 2012). Therefore, this association between food scarcity, foraging strategies and human-induced mortality risks, which is not necessarily straightforward, may also explain the low coefficient of determination in our dietary models assessing eagles' survival at territorial level.

Drivers of population dynamics depend on the life history characteristics of the species in question. In long-lived birds such as Bonelli's eagle, adult survival contributes most to the population growth rate (Sæther and Bakke 2000, Hernández-Matías et al. 2013). Accordingly, we found that diet diversity, which was the main dietary parameter determining survival, was also the main determinant of population growth rates in such a way that populations with the greatest diet diversity had the lowest growth rates and vice versa (Fig. 7). These results indicate a numerical response in Bonelli's eagle to the consumption of its preferred prey, a finding predicted by the theory but rarely tested in top avian predators over large spatial scales. In our study system, the abundance of key Bonelli's eagle prey items such as rabbits are mainly related to habitat characteristics, disease outbreaks and hunting practices (see Delibes-Mateos et al. 2007, Moleón et al. 2009). Thus, according to our results Bonelli's eagle populations could be regulated by bottom-up processes in such a way that, rather than shaping the populations of its main prey items, this predator in fact responds to prey dynamics (see Moleón et al. 2012b, White 2013).

Population demographic parameters can be viewed as an emergent property of individual fitness (Sutherland 1996); however, the assessment of these links requires long-term, individual-based studies. In this regard, our study provides strong evidence of how diet can modulate individual (i.e. territorial) life history attributes such as productivity and survival and how it can eventually affect a predator's population growth rate. Our results highlight the fact that the processes relating diet and demographic parameters at individual level are not as evident or detectable as at population level. This may be because, given the high intra-population environmental heterogeneity found in our study area, the factors affecting key vital rates in a given territory vary considerably between territories within the same population. In turn, these differences are apparently buffered when all the territories of the same population are considered together and compared with other populations in which the most general environmental characteristics differ. Overall, our study highlights the need for large spatio-temporal monitoring programs recording dietary and demographic data of territorial predator species if we are to improve our knowledge of the processes driving population trends.

In the case of endangered predator species such as Bonelli’s eagle in Europe (BirdLife International 2004), the relationships we found between diet and the main demographic traits have important implications on species conservation. In this sense, our results may guide conservation managers on how to improve the productivity of territorial pairs by, for example, undertaking habitat management practices that will increase abundances of preferred prey items (i.e. rabbits and partridges) near territorial breeding areas. Simultaneously, conservation actions should be applied in order to minimize human-related mortality risks of territorial birds since this is the main demographic trait conditioning population growth rates. In this regard, an increase in preferred prey abundances in eagles’ territories could reduce both their foraging effort and their exposure to potential mortality risks. All these practices must be given priority in populations with a negative demographic trend such as those in the northern part of the study area. Finally, we emphasize the importance of including comprehensive and coordinated dietary analyses in long-term multi-scaled monitoring programs of predator species. By doing so, we will be able to track how temporal and spatial variations in environmental characteristics affect the foraging habits of target species and to understand how these changes manifest themselves subsequently in observed population trends.

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## REFERENCES

**Arroyo, B. E. and Garcia, J. T.** (2006) Diet composition influences annual breeding success of Montagu's Harriers *Circus pygargus* feeding on diverse prey. Bird Study, 53, 73-78.

**Begon, M. et al.** (2005) Ecology: from individuals to ecosystems, 4th ed. Blackwell Science.

**BirdLife International** (2004) Birds in Europe. Population estimates, trends and conservation status. - In: BirdLife Conservation Series No. 12. Cambridge: BirdLife International, p. 374.

**Burnham, K. P. and Anderson, D. R.** (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. Springer.

**Carrete, M. et al.** (2006) Components of breeding performance in two competing species: habitat heterogeneity, individual quality and density-dependence. Oikos, 112, 680-690.

**Catry, I. et al.** (2012) Landscape and weather determinants of prey availability: implications for the Lesser Kestrel *Falco naumanni*. Ibis, 154, 111-123.

**Cheylan, G.** (1981) Sur le rôle déterminant de l'abondance des ressouces dans le succês de reproduction de l'Aigle de Bonelli (*Hieraaetus fasciatus*) en Provence. - In: Rapaces Mediterranéens. PNRC et Annales du CROP 1: 95-99.

**Delibes-Mateos, M. et al.** (2007) Rabbits as a keystone species in southern Europe. Biol. Conserv., 137, 149-156.

**Dempster, J. P.** (1975) Animal population ecology. Academic Press.

**Draper, N. R. and Smith, H.** (1998) Applied regression analysis, 3rd ed. Wiley.

**Emlen, J. M.** (1966) The role of time and energy in food preference. Am. Nat., 100, 611-617.

**Fargallo, J. A. et al.** (2009) Kestrel-prey dynamic in a Mediterranean region: the effect of generalist predation and climatic factors. PLoS ONE, 4, e4311.

**Futuyma, D. J. and Moreno, G.** (1988) The evolution of ecological specialization. Ann. Rev. Ecol. Syst., 19, 207-233.

**Gil-Sánchez, J. M. et al.** (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biol. Conserv., 118, 685-694.

**Hernández-Matías, A. et al.** (2010) Determinants of territorial recruitment in Bonelli's eagle (*Aquila fasciata*) populations. Auk, 127, 173–184.

**Hernández-Matías, A. et al.** (2011a) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. J. Wildl. Manage., 75, 856-866.

**Hernández-Matías, A. et al.** (2011b) Effects of age, territoriality and breeding on survival of Bonelli's Eagle *Aquila fasciata*. Ibis, 153, 846–857.

**Hernández-Matías, A. et al.** (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecol. Monogr., 83, 239-261.

**Korpimäki, E. and Norrdahl, K.** (1991) Numerical and functional responses of kestrels, short-eared owls, and long-eared owls to vole densities. Ecology, 72, 814-826.

**Korpimäki, E. and Krebs, C. J.** (1996) Predation and population cycles of small mammals. BioScience, 46, 754–764.

**Krebs, C. J. et al.** (2001) Ecosystem dynamics of the boreal forest: the Kluane project. Oxford Univ. Press.

**Martínez-Abraín, A. et al.** (2012) Effects of wind farms and food scarcity on a large scavenging bird species following an epidemic of bovine spongiform encephalopathy. J. Appl. Ecol., 49, 109-117.

**Millon, A. and Bretagnolle, V.** (2008) Predator population dynamics under a cyclic prey regime:

numerical responses, demographic parameters and growth rates. Oikos, 117, 1500-1510.

**Moleón, M. et al. (**2007) Non-breeding feeding ecology of territorial Bonelli's Eagles *Hieraaetus fasciatus* in the Iberian Peninsula. Ardeola, 54, 135-143.

**Moleón, M. et al.** (2008) An emerging infectious disease triggering large-scale hyperpredation. PLoS ONE, 3, e2307.

**Moleón, M. et al.** (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. J. Biogeogr., 36, 1502–1515.

**Moleón, M. et al.** (2012a) Changes in intrapopulation resource use patterns of an endangered raptor in response to a disease-mediated crash in prey abundance. J. Anim. Ecol., 81, 1154–1160.

**Moleón, M. et al.** (2012b) Predator–prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia, 168, 679-689.

**Moleón, M. et al.** (2013) Inferring ecological mechanisms from hunting bag data in wildlife management: a reply to Blanco-Aguiar et al. (2012). Eur. J. Wildl. Res., 59, 599-608.

**Orians, G. H. and Pearson, N. E.** (1979) On the theory of central place foraging. - In: Horn, D. J. et al. (eds), Analysis of Ecological Systems. Ohio State Univ. Press, pp. 155-177.

**Orians, G. H. and Wittenberger, J. F.** (1991) Spatial and temporal scales in habitat selection. Am. Nat., 137, S29-S49.

**Palma, L. et al.** (2006) Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons. J. Appl. Ecol., 43, 1075-1086.

**Pyke, G. H. et al.** (1977) Optimal foraging: a selective review of theory and tests. Q. Rev. Biol., 52, 137–154.

**Real, J.** (1987) Evolución cronológica del régimen alimenticio de una población de *Hieraaetus fasciatus* en Catalunya: factores causantes, adaptación y efectos. Supplementi Ricerca Biologica Selvaggina, 12, 185-205.

**Real, J.** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía. PhD thesis, University of Barcelona, Barcelona.

**Real, J.** (1996) Biases in diet study methods in the Bonelli's eagle. J. Wildl. Manage., 60, 632-638.

**Real, J. and Mañosa, S.** (1997) Demography and conservation of western European Bonelli's eagle *Hieraaetus fasciatus* populations. Biol. Conserv., 79, 59-66.

**Real, J. et al.** (2001) Causes of death in different areas for Bonelli's Eagle *Hieraaetus fasciatus* in Spain. Bird Study, 48, 221-228.

**Resano-Mayor, J. et al.** (2014) Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. Ibis, 156, 176-188.

**Sæther, B. E. and Bakke, Ø.** (2000) Avian life history variation and contribution of demographic traits to the population growth rate. Ecology, 81, 642-653.

**Salamolard, M. et al.** (2000) Responses of an avian predator to variations in prey density at a temperate latitude. Ecology, 81, 2428-2441.

**Schoener, T. W.** (1971) Theory of feeding strategies. Ann. Rev. Ecol. Syst., 2, 369-404.

**Shannon, C. E. and Weaver, W.** (1949) The mathematical theory of communication. Univ. of Illinois Press.

**Steenhof, K. et al.** (1997) Interactive effects of prey and weather on golden eagle reproduction. J. Anim. Ecol., 66, 350-362.

**Stephens, D. W. and Krebs, J. R.** (1986) Foraging theory. Princeton Univ. Press.

**Sutherland, W. J.** (1996) From individual behaviour to population ecology. Oxford Univ. Press.

**Vucetich, J. A. and Peterson, R. O.** (2004) The influence of prey consumption and demographic stochasticity on population growth rate of Isle Royale wolves Canis lupus. Oikos, 107, 309-320.

**White, T. C. R.** (2008) The role of food, weather and climate in limiting the abundance of animals. Biol. Rev., 83, 227-248.

**White, T. C. R.** (2013) Experimental and observational evidence reveals that predators in natural environments do not regulate their prey: They are passengers, not drivers. Acta Oecol., 53, 73-87.

**Whitfield, D. P. et al. (**2009) Diet specificity is not associated with increased reproductive performance of Golden Eagles *Aquila chrysaetos* in Western Scotland. Ibis, 151, 255-264.

# Influence of diet on demographic parameters

# Discusión general

# DISCUSIÓN GENERAL

En esta tesis se ha estudiado en profundidad la ecología trófica del águila perdicera en diversas poblaciones de Europa Occidental. En primer lugar, se ha analizado en detalle la composición de la dieta mediante el análisis convencional de egagrópilas y nuevas metodologías como el análisis de isótopos estables, lo que a su vez ha permitido realizar un estudio metodológico para conocer la concordancia entre las estimas de dieta proporcionadas por ambas técnicas (capítulo 1). Haciendo uso del análisis de isótopos estables para estimar la dieta de los pollos a nivel individual, se ha demostrado un efecto de la composición de la dieta sobre el estado de condición corporal de los pollos (capítulo 2). Asimismo, la dieta ha estado relacionada con los parámetros vitales y demográficos de las águilas, tanto a nivel territorial como poblacional (capítulos 3 y 4). Los resultados aportan información novedosa y útil para el desarrollo de protocolos de monitoreo y estudio de la dieta de la especie, que podrán ser aplicados en beneficio de su conservación. Al mismo tiempo, la implementación de dichos protocolos podrá utilizarse en un marco más amplio de bioindicación para identificar cambios ambientales en los ecosistemas mediterráneos que afecten a la composición de las comunidades en las habita el águila perdicera.

## Estudio de la dieta del águila perdicera y aplicaciones del análisis de isótopos estables

El estudio de la dieta del águila perdicera en Cataluña mediante análisis de egagrópilas mostró que las águilas depredan sobre numerosas especies durante el periodo reproductor, habiéndose identificado 31 presas a nivel específico. No obstante, seis categorías de presas representaron más del 80% del total de ítems y biomasa consumida, incluyendo el conejo de monte, los Colúmbidos (especialmente paloma torcaz y doméstica), la perdiz roja, varios paseriformes (sobre todo Córvidos y Túrdidos), la gaviota patiamarilla y la ardilla roja. Estos resultados fueron acordes con los mostrados en estudios previos sobre la dieta del águila perdicera en sus poblaciones del noreste peninsular, donde la especie, en general, depreda más palomas y menos conejos y perdices que en otras poblaciones del sur de la Península Ibérica (ver Real 1991, Moleón et al. 2009, 2012a). La considerable variación de la dieta entre territorios dentro de la población de estudio probablemente estuvo relacionada con las diferencias ambientales que condicionaron la disponibilidad de presas a nivel territorial. En concordancia con otros estudios anteriores, en el presente trabajo se detectó una correlación negativa entre el consumo de conejo y la diversidad trófica, apoyando la hipótesis de que el conejo es una presa preferida para el águila perdicera (ver Futuyma & Moreno 1988, Real 1991, Moleón et al. 2012a, b). Esta preferencia podría explicarse porque el consumo de conejo es más rentable energéticamente (i.e. energía obtenida en relación al coste de captura) en comparación a otras presas alternativas (ver Emlen 1966, Pyke et al. 1977, Real 1991, Moleón et al. 2012a). No obstante, cabe destacar que algunas presas alternativas de menor importancia que el conejo a nivel del conjunto de la población, parecen jugar un papel clave en algunos territorios donde pueden constituir la principal presa consumida, posiblemente como resultado de su elevada abundancia en el medio. Fue el caso, por ejemplo, de la gaviota patiamarilla en algunos territorios costeros, o las palomas domésticas en territorios con elevada abundancia de esta especie.

El análisis de egagrópilas se mostró fundamental para el estudio detallado de la dieta del águila perdicera durante el periodo reproductor en Cataluña, informando a nivel taxonómico sobre la tipología de las presas y su frecuencia de consumo, siendo conocido que es una de las técnicas convencionales de estudio de la dieta con menos sesgos (Real 1996). Sin embargo, la dificultad a la hora de obtener un número de egagrópilas representativo, ya que éstas pueden ser escasas en posaderos y nidos, así como el tiempo necesario para su análisis, pueden acarrear importantes limitaciones. En esta tesis se ha comparado el consumo de las principales categorías de presas estimado a partir del análisis de egagrópilas y mediante el análisis de isótopos estables (i.e. modelos bayesianos mixtos: SIAR), con el objetivo de testar la aplicabilidad de nuevas metodologías como los análisis isotópicos en estudios de dieta del águila perdicera. Para ello, en primer lugar fue necesario caracterizar la composición isotópica de las presas, un paso previo fundamental en todo estudio que pretenda reconstruir la dieta de un consumidor a través del análisis de isótopos estables en sus tejidos (Inger & Bearhop 2008, Parnell et al. 2010). El análisis de las presas permitió determinar las diferencias isotópicas entre las distintas categorías de presas, así como los efectos de la composición del hábitat y la proximidad al mar en los valores isotópicos de los individuos dentro de una misma categoría. Las mayores diferencias entre categorías se dieron para carbono y nitrógeno, por lo que estos dos elementos fueron los más útiles a la hora de estimar el consumo de las distintas categorías de presa. En cuanto a los efectos ambientales, los resultados mostraron que tanto los conejos como las palomas domésticas de territorios próximos al mar tenían valores de $\delta^{34}$S más elevados que sus conespecíficos de áreas más alejadas del mar, de acuerdo con la tendencia general de mayor $\delta^{34}$S en las especies que habitan en los ecosistemas marinos (ver Thode 1991, Deegan & Garritt 1997, Connolly et al. 2004). En concordancia, las gaviotas patiamarillas mostraron los valores más altos de $\delta^{34}$S de todas las categorías de presa analizadas. Por el contrario, los valores isotópicos entre los individuos de una misma categoría de presa en general no variaron en función del tipo de hábitat, excepto en el caso de las palomas domésticas de hábitats agrícolas; éstas mostraron valores de $\delta^{15}$N más elevados que las muestreadas en hábitats de matorral. Este resultado pudo estar causado por el uso de fertilizantes a base de nitratos en las zonas agrícolas (Choi et al. 2007) y la tendencia de las palomas a alimentarse en los cultivos agrícolas en el area de estudio. Todos estos resultados pusieron de manifiesto la necesidad de obtener estimas de la composición isotópica y su variación en las principales categorías de presa para la posterior interpretación de los valores isotópicos en el consumidor. En este sentido, los resultados gráficos de los bi-plots mostraron que los valores isotópicos de los pollos se encontraban dentro del espacio bidimensional delineado por los valores isotópicos de las presas, una vez corregidas éstas por el factor de enriquecimiento trófico (i.e. cambio esperado en la composición isotópica de los tejidos del consumidor respecto a la de su dieta). Este resultado sugirió que las principales categorías de presa seleccionadas eran representativas de la dieta de los pollos, así como que los valores isotópicos obtenidos de su análisis y el factor de enriquecimiento trófico asumido para cada elemento eran razonables. En base a todos estos resultados, se pudo realizar un uso más fiable de SIAR, obteniendo así estimas de consumo para cada categoría de presa mediante el análisis de isótopos estables.

Actualmente, el análisis de isótopos estables es una de las técnicas más ampliamente utilizadas en estudios de ecología trófica (Kelly 2000, Bearhop et al. 2004, Inger & Bearhop 2008). No obstante, existen pocas evaluaciones cuantitativas en poblaciones silvestres de la fiabilidad de las estimas de consumo de presas y de su correspondencia con otras técnicas convencionales (ver Doucette et al. 2011, Steenweg et al. 2011, Weiser & Powell 2011). En esta tesis, se determinó el grado de concordancia en las estimas de consumo de presas entre el análisis de isótopos estables y de egagrópilas, siendo esta última una de las técnicas convencionales más ampliamente utilizadas en estudios de dieta de rapaces (Martí et al. 2007). El análisis comparativo se realizó tanto a nivel poblacional como territorial. A nivel poblacional, el análisis de egagrópilas (en términos de ítems y biomasa) y SIAR mostraron valores similares en sus estimas promedio y rango de consumo para las principales categorías de presas. Por ejemplo, ambos métodos estimaron que conejos y palomas fueron las categorías más consumidas, frente a perdices, paseriformes, gaviotas y ardillas. Por lo tanto, los resultados apoyan la idea de que el análisis de isótopos estables es un método adecuado para evaluar la dieta del águila perdicera a nivel poblacional. A nivel territorial, también encontramos similitud en las estimas de ambos métodos, sobre todo en relación al orden de clasificación de mayor a menor consumo de las principales presas. Es decir, ambos métodos tendieron a coincidir en cuáles eran las categorías de presa más y menos consumidas. Este resultado apoyó la utilidad de SIAR para inferir los patrones de consumo de presas del águila perdicera a nivel territorial. No obstante, la concordancia a nivel territorial para cada categoría de presa varió considerablemente. Mientras ambos métodos mostraron similitud en sus estimas de consumo de conejos, palomas y gaviotas, la concordancia fue menor en las estimas del consumo de ardillas y paseriformes, hallándose una baja concordancia en el caso de las estimas de consumo de perdiz. En términos más generales, los resultados muestran el gran potencial del análisis de isótopos estables en el estudio de la dieta de depredadores mediterráneos, ya que algunas de las principales presas analizadas y consumidas por el águila perdicera, como el conejo, se consideran especies clave en los ecosistemas mediterráneos (Delibes-Mateos et al. 2007, 2008), y de ellas dependen otros depredadores característicos de estos sistemas naturales como lo son el águila imperial ibérica (*Aquila adalberti*) y el lince ibérico (*Lynx pardinus*) (Palomares 2001, Ferrer & Negro 2004, Delibes-Mateos et al. 2008).

## Efectos de la dieta sobre la condición corporal

Estimar la condición corporal animal resulta fundamental en estudios de fisiología y ecología animal, así como en biología de la conservación, debido a que el estado de condición de los individuos puede afectar a sus principales parámetros vitales como la supervivencia o el éxito reproductor (Brown 1996, Stevenson & Woods 2006). La dieta de un animal es un factor que incide de forma determinante en su estado de condición corporal (Hochleithner 1994). En esta tesis, uno de los objetivos ha sido evaluar el efecto de la dieta sobre el estado de condición de los pollos de águila perdicera, un tema completamente novedoso para la especie, y que contribuye de forma relevante al escaso conocimiento disponible sobre si el consumo de presas preferidas por un depredador en estado salvaje mejora su condición corporal. En el caso aquí estudiado, y en base a la información disponible, asumimos que un elevado consumo de una presa preferida como lo es el conejo (Real 1991, Moleón et al. 2012a) supondría un mayor aporte de alimento al nido, por lo que cabría esperar que mejorase la condición corporal de los pollos. En cambio, un aumento en la diversidad trófica sería indicativo de menor abundancia de su presa preferida (e.g. baja disponibilidad de conejo), suponiendo un menor aporte de alimento al nido y el empeoramiento del estado de condición corporal de los pollos. El análisis de isótopos estables permitió estimar la dieta de los pollos a nivel individual, y haciendo uso de parámetros biométricos y bioquímicos sanguíneos se obtuvieron estimas de su estado de condición corporal. Asimismo, se analizó el posible efecto sobre las estimas de condición de factores como el sexo o la edad de los pollos, la hora del muestreo, la altitud de la zona de cría, o si los pollos habían consumido alguna presa recientemente o no.

Esta tesis proporciona valores de referencia para múltiples medidas de condición corporal en pollos de águila perdicera (véase también Balbontín & Ferrer 2002), muestreados en dos poblaciones geográficamente distantes (i.e. Cataluña y Andalucía oriental) y con marcadas diferencias demográficas (Hernández-Matías et al. 2013). Los resultados muestran un mayor rango de valores para la mayoría de parámetros en comparación a los publicados previamente (Balbontín & Ferrer 2002), probablemente debido a que el presente estudio engloba el análisis de un mayor número de pollos, y en territorios con marcadas diferencias ambientales. Además, en el caso de los valores de metabolismo antioxidante, los resultados presentados tienen gran relevancia pues constituyen los primeros datos para el águila perdicera, a la vez que contribuyen al reducido número de publicaciones disponibles para otras rapaces (véase Galván et al. 2010, Sternalski et al. 2010, Casagrande et al. 2011).

Los resultados muestran una marcada asociación entre la composición de la dieta y la condición corporal de los pollos de águila perdicera. En general, un mayor consumo de conejo mejoró varias estimas de condición, mientras que un aumento en la diversidad trófica y el mayor consumo de paseriformes las empeoraron. Este patrón se observó tanto en estimas de condición basadas en análisis bioquímicos en plasma como en medidas de estrés oxidativo. Por ejemplo, el consumo de conejo redujo los niveles de colesterol y triglicéridos en plasma, mientras que una mayor diversidad trófica los elevó, probablemente debido a que en el segundo caso los pollos tuvieron que hacer uso de reservas lipídicas endógenas ante un menor aporte de alimento. Asimismo, el consumo de conejo estuvo correlacionado con la actividad de una enzima encargada del metabolismo proteico, la aspartato aminotransferasa, sugiriendo un mayor aporte proteico cuanto mayor fue el consumo de conejo (véase Parker et al. 2005). Por otro lado, un mayor consumo de conejo incrementó los niveles de tocoferol, una vitamina adquirida a través de la dieta que tiene propiedades antioxidantes (Burton & Ingold 1981), así como los niveles de glutatión, un péptido de producción endógena que también confiere importantes funciones antioxidantes (Wu et al. 2004). A su vez, el consumo de conejo redujo la actividad de la enzima glutatión peroxidasa (GPx), mientras que un mayor consumo de paseriformes hizo que aumentara su actividad. Debido a que esta enzima se encarga de catalizar la reducción del peróxido de hidrógeno ($H_2O_2$) y otros peróxidos haciendo uso de glutatión (Wu et al. 2004), un aumento de su actividad podría estar relacionado con una mayor abundancia de especies químicas reactivas de oxígeno (ERO). Por lo tanto, un mayor consumo de paseriformes podría significar una escasez de alimento en la dieta de los pollos, el aumento de los niveles de ERO y, en última instancia, la activación de la GPx para evitar estrés oxidativo. Por otro lado, un mayor consumo de conejo no sólo aumentó los niveles de glutatión (y por tanto la capacidad antioxidante), sino que también redujo la actividad de la GPx, lo que sugiere un mejor equilibrio redox (e.g. menores niveles de ERO) asociado a un mayor consumo de esta presa. Finalmente, un aumento en la diversidad de la dieta estuvo relacionado con un incremento tanto en el glutatión oxidado, como en el porcentaje de glutatión oxidado respecto al glutatión total, sugiriendo un mayor estrés oxidativo en los pollos con mayor diversidad trófica. Cabe mencionar en este apartado que a pesar del uso común de índices biométricos y su utilidad en estudios de condición animal (Brown 1996, Stevenson & Woods 2006, Peig & Green 2009), las estimas biométricas obtenidas en el presente estudio no mostraron relación con el consumo de presas.

Sin embargo, algunos parámetros sanguíneos pueden verse influidos por factores distintos al estado nutricional de los individuos. Uno de los factores a tener en cuenta es la edad (Dobado-Berrios et al. 1998, Alonso-3Alvarez 2005). En este estudio, los niveles de las enzimas plasmáticas fosfatasa alcalina (PAL) y creatina kinasa (CK) aumentaron con la edad de los pollos. Debido a que la PAL está implicada en la osteogénesis, el aumento en su actividad con la edad indicaría un incremento en la osificación de los pollos de águila perdicera durante su etapa en el nido (veáse Viñuela et al. 1991, Dobado-Berrios & Ferrer 1997). Estudios con otras rapaces muestran un incremento similar, como por ejemplo en el caso de los pollos de milano real (*Milvus milvus*) y milano negro (*Milvus migrans*), aunque en estos casos los niveles de PAL se redujeron a partir de cierta edad (Viñuela et al. 1991). El hecho de que los resultados no mostrasen reducción en los valores de esta enzima en los pollos de edades más avanzadas pudo deberse a que los pollos fueron muestreados proporcionalmente a menor edad. Por otro lado, la relación positiva hallada entre la CK y la edad de los pollos podría estar relacionada con un aumento en la formación de músculo durante su crecimiento, junto con un incremento de la actividad física (e.g. aleteo), debido a que la CK interviene en la contracción muscular, aumentando sus niveles al producirse daño muscular relacionado con el ejercicio (Hochleithner 1994). El sexo de los individuos es otro factor a tener en cuenta a la hora de interpretar algunos parámetros sanguíneos (Hochleithner 1994). En el presente estudio, se comprobó que los niveles de glucosa en plasma eran mayores en machos que en hembras. Estas diferencias podrían deberse a que las hembras, por su mayor tamaño, utilizan más glucosa en la síntesis de tejidos durante la fase de crecimiento en comparación a los machos (véase Balbontín & Ferrer 2002, Casado et al. 2002). La reciente ingesta de alimentos puede ser otro factor que afecte a los parámetros sanguíneos (Hochleithner 1994). En este sentido, los resultados mostraron un aumento posprandial en los niveles de triglicéridos, ácido úrico y retinol palmitato en plasma. Debido a que los niveles de triglicéridos y ácido úrico pueden aumentar tanto después del consumo de alimentos (Lumeij & Remple 1991, Hochleithner 1994), como a consecuencia de daño tisular severo o inanición (García-Rodríguez et al. 1987, Hochleithner 1994), el haber considerado la reciente ingesta de alimentos, observando si el buche estaba lleno o si había presas recientes en el nido, ayudó en la interpretación de los resultados. De este modo, fue posible descartar que el incremento en los niveles de triglicéridos y ácido úrico fuera consecuencia de alguna patología o enfermedad. Por otro lado, la creatinina fue el único parámetro sanguíneo cuyos valores variaron según el ritmo circadiano de los pollos, mostrando niveles más altos en pollos muestreados por la tarde frente a los muestreados antes del mediodía. Más allá de las condiciones tróficas, puede haber diversos factores ambientales que afecten a los parámetros de condición corporal de los pollos. Por ejemplo, los valores de hematocrito de los pollos de águila perdicera estuvieron determinados principalmente por la altitud de la zona de cría, pero ni la edad, ni la dieta, mostraron un efecto (veáse Carpenter 1975, Borras et al. 2010). El hecho de haber muestreado pollos en nidos que se sitúan desde acantilados marinos, hasta sierras interiores a unos 1500 m s.n.m., posiblemente facilitó detectar este patrón altitudinal. Por consiguiente, la medición del hematocrito tiene poca utilidad como estima de condición corporal de los pollos de águila perdicera, y en su interpretación se deberá considerar el efecto de la altitud. En general, estos resultados ponen de manifiesto la importancia de considerar factores como la edad, el sexo, el tiempo desde la última ingesta de alimentos, los ritmos circadianos o la altitud a la hora de interpretar parámetros sanguíneos, evitando así conclusiones erróneas sobre el estado de condición corporal.

En resumen, los resultados destacan la importancia de considerar múltiples biomarcadores de condición para una mejor comprensión del efecto de la dieta sobre la condición corporal animal. En el caso de especies amenazadas, como el águila perdicera, conocer la relación entre el consumo de presas y el estado de condición de los pollos es crucial de cara a detectar posibles amenazas y poder implementar acciones de conservación antes de que se manifiesten efectos demográficos negativos. En este sentido, aumentar la disponibilidad o abundancia de aquellas presas que han mostrado ser determinantes en la condición de los pollos, como el conejo, puede ser una medida clave de conservación. Por último, las técnicas y los análisis desarrollados podrán servir como punto de partida de futuros programas de monitoreo de la dieta y el estado de condición de los pollos de águila perdicera en sus poblaciones de Europa Occidental.

## Efectos de la amplitud de nicho trófico y la composición de la dieta sobre la productividad

La variación en la dieta entre individuos de una población es un fenómeno frecuente, y durante los últimos años numerosos estudios han abordado tanto las causas como sus consecuencias eco-evolutivas (Bolnick et al. 2003, Svanbäck & Bolnick 2007, Woo et al. 2008, Tinker et al. 2012). En el caso de los depredadores terrestres territoriales, las diferencias espaciales en la abundancia y la disponibilidad de presas se han destacado como un factor determinante en dicha variación (Whitfield et al. 2013, L'Hérault et al. 2003). Sin embargo, las consecuencias de la variación individual en cuanto al consumo de recursos sobre parámetros relacionados con la eficacia biológica son poco conocidas. Haciendo uso del análisis de isótopos estables para evaluar la amplitud del nicho trófico y la dieta de las parejas reproductoras en tres poblaciones de águila perdicera de Europa Occidental (i.e. Francia, Cataluña y Andalucía), testamos los efectos de la dieta sobre la productividad (i.e. pollos volados por pareja y año) de la especie, tanto a nivel territorial como poblacional.

En los últimos años, el análisis de isótopos estables se ha mostrado como una herramienta útil para evaluar la amplitud del nicho trófico en las poblaciones animales, así como las variaciones en la dieta de los individuos que las componen (Bearhop et al. 2004, Newsome et al. 2007, Inger & Bearhop 2008, Hobson 2011). Los resultados del presente estudio destacan una importante variación en la dieta entre los territorios de las poblaciones de águila perdicera en Francia y Cataluña, mientras que los territorios en Andalucía mostraron dietas más homogéneas. Este patrón puede explicarse por una mayor heterogeneidad ambiental y paisajística en los territorios de Francia y Cataluña en comparación con Andalucía, heterogeneidad que afectaría a la abundancia y disponibilidad de presas como el conejo, la perdiz, la paloma doméstica o la ardilla, que fueron las presas con mayor variación de consumo.

A nivel territorial, los resultados mostraron una mayor probabilidad de que volaran dos pollos en aquellos territorios con un abundante consumo de conejo y/o perdiz. Sin embargo, fueron pocos los territorios en los que el consumo de estas presas preferidas constituyera la mayor parte de la dieta, lo que sugiere que el escenario de una elevada abundancia de estas presas es poco frecuente en gran parte del área de estudio como es el caso de Francia o Cataluña (Moleón et al. 2009). Por otro lado, la probabilidad de que volaran dos pollos también aumentó en aquellos territorios donde la tipología de dieta de éstos fue similar al promedio de la población. En este caso, la dieta promedio de la población incluyó un consumo moderado de conejos, palomas y perdices, siendo este un escenario más frecuente en el área de estudio. En contra, un aumento en la diversidad de la dieta tuvo efectos negativos sobre la productividad, posiblemente debido al mayor consumo de presas menos rentables energéticamente como consecuencia de la escasez de presas preferidas (véase Roughgarden 1972). Cabe mencionar el bajo poder explicativo de los modelos seleccionados como más probables, resultado que podría estar condicionado por el hecho de no haber podido considerar la dieta, por cuestiones metodológicas, de aquellos territorios donde las águilas fracasaron en la reproducción. Además, otras causas aparte de la dieta pueden tener un gran impacto sobre la productividad (e.g. molestias en el área de cría, competencia inter-específica), habiendo podido dificultar nuestra capacidad para detectar los efectos de la dieta sobre la productividad a nivel territorial. En este estudio, se consideró el efecto de la mortalidad de los individuos territoriales (inferida a partir del recambio de un individuo territorial por otro al año siguiente) sobre la productividad y, de hecho, se detectó un potente efecto negativo de la mortalidad de los ejemplares territoriales sobre la productividad. Este resultado se puede explicar porque los ejemplares reclutados fueron en su mayoría no adultos, y en especies de vida larga éstos suelen tener menor éxito reproductor, bien porque no son maduros sexualmente, o por su inexperiencia en la cría (Real 1991, Forslund & Pärt 1995, Carrete et al. 2006, Hernández-Matías et al. 2011).

A nivel de población, la productividad anual promedio correlacionó negativamente con la amplitud de nicho trófico. En particular, las poblaciones-año con mayor y menor productividad (i.e. Andalucía 2011 y Cataluña 2009, respectivamente), mostraron la menor y mayor amplitud de nicho trófico, respectivamente. En el caso de Andalucía, la menor amplitud de nicho trófico estuvo relacionada con el mayor consumo de conejo y perdiz, algo que en última instancia pudo haber aumentado la productividad promedio (ver Arroyo & Garcia 2006). De hecho, la población en Andalucía presenta los valores más altos de las principales tasas vitales en todo el rango de distribución del águila perdicera en Europa Occidental (Hernández-Matías et al. 2013). Por otro lado, el mayor número de recambios de individuos en las parejas territoriales de las poblaciones más septentrionales (i.e. Francia y Cataluña) también pudo afectar negativamente a los valores promedio de productividad dentro de cada población (ver Hernández-Matías et al. 2013). Por lo tanto, la calidad del territorio en cuanto a disponibilidad de presas como el conejo o la perdiz, y la tasa de recambio de individuos, podrían afectar simultáneamente a los valores de productividad en cada territorio y, en última instancia, condicionar los valores de productividad a nivel poblacional (véase Penteriani et al. 2003, Carrete et al. 2006, Margalida et al. 2007).

## Dieta y demografía del águila perdicera en Europa Occidental

En base a un programa de monitoreo a largo plazo que recogió información sobre la dieta y la demografía del águila perdicera en varias de sus poblaciones de Europa Occidental, los resultados del presente estudio muestran una asociación marcada entre la dieta de un depredador de vida larga y sus parámetros demográficos a diferentes escalas espaciales en ecosistemas complejos como los de la región mediterránea.

La productividad a nivel territorial a lo largo del ámbito geográfico estudiado estuvo determinada por el consumo de conejo y de la categoría “otras aves” (e.g. Paseriformes, Anseriformes, Ardeiformes, Charadriiformes o Accipitriformes). Los resultados apoyaron la predicción inicial de que el mayor consumo de conejo aumenta la productividad de las parejas territoriales (véase Cheylan 1981, Real 1987, 1991). Por otro lado, un mayor consumo de “otras aves” redujo la productividad, posiblemente porque las especies contenidas en esta categoría son consumidas en mayor proporción en aquellos territorios donde la abundancia de conejos es menor, a modo de presas alternativas y posiblemente subóptimas. Apoyando la idea anterior, se detectó una correlación negativa significativa entre el consumo de conejos y “otras aves”, y el mayor consumo de “otras aves” tendió a aumentar la diversidad de la dieta. A nivel poblacional, se detectó un patrón muy similar al hallado a nivel territorial (ver Sutherland 1996), de modo que la productividad aumentó con el consumo de perdiz y disminuyó con el mayor consumo de “otras aves”. Este resultado apoya la hipótesis de que la perdiz es una presa óptima para el águila perdicera, y su mayor consumo en las poblaciones del sur peninsular parece ser indicativo de una mayor abundancia en dichas poblaciones, donde la perdiz encuentra hábitats más óptimos (ver Blanco-Aguiar et al. 2003, Vargas et al. 2006).

La supervivencia del águila perdicera a nivel territorial y, sobre todo, a nivel poblacional, fue mayor al reducirse la diversidad en la dieta. En general, una menor diversidad de la dieta fue indicativa de un mayor consumo de conejo y perdiz, aunque en algunas parejas la baja diversidad trófica se debió al mayor consumo de presas alternativas como las palomas domésticas (ver Palma et al. 2006). En todos los territorios con una dieta poco diversa, la mayor supervivencia podría deberse a una mayor eficiencia en la obtención de alimento y/o una menor exposición a los principales factores de mortalidad como la electrocución, la colisión o la persecución a tiros (Real et al. 2001, Moleón et al. 2007). En un escenario de escasez de alimento podría haber un efecto indirecto de la dieta sobre la supervivencia de un depredador a través de un cambio en sus hábitos de forrajeo y de comportamiento (véase Martínez-Abraín et al. 2012), existiendo posiblemente una compleja asociación entre abundancia de presas, estrategias de forrajeo y supervivencia.

La dinámica de las poblaciones animales es una propiedad emergente de los parámetros vitales de los individuos que las componen (Sutherland 1996). Para poder evaluar la correspondencia entre ambas escalas en especies de vida larga son necesarios estudios de seguimiento a nivel individual durante amplios periodos de tiempo. En este sentido, el presente trabajo proporciona evidencias de cómo la dieta afecta a los parámetros de vida de los individuos (i.e. productividad y supervivencia), y cómo ello puede acabar afectando a la tasa de crecimiento de la población, como muestran los resultados que relacionan un mayor incremento poblacional cuanto menor diversidad en la dieta. Por lo tanto, este estudio remarca la necesidad de realizar programas de seguimiento y monitoreo de la dieta y de los parámetros vitales a largo plazo, y sobre amplias regiones espaciales, en especies de depredadores territoriales como el águila perdicera. De este modo, se obtendrá una mejor comprensión de los procesos que relacionan la ecología trófica de las especies y su dinámica poblacional.

## Aplicaciones a la conservación de la especie

El águila perdicera es una especie amenazada en Europa, donde ha sufrido un importante declive poblacional en las últimas décadas. Esta regresión ha sido especialmente intensa en las poblaciones de la mitad septentrional de la Península Ibérica y Francia, y ha sido achacada a un desequilibrio demográfico entre las tasas de supervivencia y reproductivas (Real & Mañosa 1997, Hernández-Matías et al. 2013). A su vez, se ha sugerido que dichas poblaciones se ven afectadas negativamente por una baja disponibilidad de alimento, y en especial de presas básicas como el conejo y la perdiz, argumentando que sus bajas abundancias pueden afectar al mantenimiento de sus territorios y poblaciones (Real 1991, Ontiveros et al. 2004).

En esta tesis se ha estudiado en profundidad el efecto que tiene la dieta sobre la condición corporal de los individuos y sobre los parámetros demográficos, tanto a nivel territorial como poblacional. Los resultados apoyan la hipótesis de que existen presas óptimas, como el conejo, que determinan favorablemente dichos parámetros. Dicho de otro modo, los individuos y territorios que consumen más conejo muestran mejor condición corporal y mejores tasas reproductoras y de supervivencia, teniendo consecuencias a nivel poblacional. Por otra parte, las metodologías de estudio de la dieta desarrolladas en esta tesis (i.e. análisis de isótopos estables) se han mostrado de gran utilidad para conocer el consumo de las principales categorías de presas, tanto a nivel individual, territorial como poblacional. Por lo tanto, los análisis isotópicos son una herramienta adecuada para obtener un diagnóstico del estado trófico de la especie en los territorios y las poblaciones, así como de sus variaciones en el tiempo. A nivel espacial dentro de una población, el análisis de la dieta puede permitir identificar aquellos territorios que presentan problemas tróficos (e.g. escasez de presas óptimas), facilitando a los gestores una priorización de recursos en aquellas áreas que precisan medidas de conservación urgente para mejorar las abundancias de presas. De forma análoga, la monitorización de la dieta en un largo periodo de tiempo puede revelar problemas de conservación de forma precoz, antes incluso de que se traduzcan en problemas demográficos graves (e.g. fracaso reproductor continuado a lo largo de los años), permitiendo a los gestores anticiparse para tratar de paliar dichos problemas de conservación.

Mantener las poblaciones estables y autosostenibles es un objetivo de conservación deseable para garantizar su viabilidad, un aspecto especialmente relevante en el caso de especies amenazadas, como el águila perdicera. Para ello, es necesario que los parámetros demográficos de los territorios muestren valores adecuados. En consecuencia, las acciones de conservación deberán priorizarse en aquellos territorios donde se detecten condiciones tróficas adversas y que limiten los parámetros demográficos. Estas acciones deberán dirigirse a favorecer las abundancias de sus presas óptimas, como el conejo. En este sentido, son conocidas diversas estrategias y técnicas que incluyen la mejora de sus hábitats, la racionalización de las actividades extractivas (e.g. caza) y el estudio y conocimiento de la dinámica de los virus que afectan a dicha especie (Villafuerte et al. 1995, Calvete 2006, Delibes-Mateos et al. 2009). Cabe mencionar que muchas de estas acciones que benefician las poblaciones de conejo son también favorables para otra presa clave como la perdiz, siendo además conocido que una mejora de las poblaciones de conejo redunda favorablemente en las de esta ave (Moleón et al. 2008). Por otra parte, conociendo que en determinados escenarios de baja abundancia de presas preferidas pueden existir especies alternativas como las palomas torcaces y domésticas que pueden ser explotadas por las águilas, todas aquellas actuaciones que favorezcan a estas especies deben de ser también priorizadas.

Evaluar y diagnosticar el impacto de las actividades humanas sobre las especies, comunidades y ecosistemas es un objetivo primordial en biología de la conservación. Los bioindicadores, son entidades biológicas (e.g. especies) cuyas interacciones con el ecosistema hacen que sean especialmente informativos sobre cambios en la calidad del hábitat, la composición de la comunidad, o el funcionamiento de un ecosistema a diferentes escalas espaciales, temporales o de organización (Lindenmayer & Burgman 2005, Van Dyke 2008). El águila perdicera es un depredador que se encuentra en los eslabones superiores de las redes tróficas de los ecosistemas mediterráneos, y su dieta integra información sobre las comunidades de especies que constituyen estos sistemas naturales. Durante la segunda mitad del siglo XX, el área mediterránea ha sufrido cambios importantes en sus ambientes y biocenosis relacionados con las actividades humanas (Blondel & Aronson 1999, Nekhay & Arriaza 2009). Por un lado, alteraciones que han supuesto pérdida irreversible de hábitat (e.g. urbanización, infraestructuras), y por otro, cambios de los usos del suelo (e.g. cambios agrícolas, ganaderos y forestales) de diversa índole, que han conllevado intensificación agrícola en áreas de llanura y su abandono en áreas de montaña mediterránea (Cernusca et al. 1999). En general, estos cambios ambientales han provocado importantes variaciones en las abundancias de presas, disminuyendo las principales presas del águila perdicera, como el conejo y la perdiz, y aumentando otras especies favorecidas por la actividad del hombre, como palomas domésticas y gaviotas. Por ello, el monitoreo de la dieta del águila perdicera no solamente puede aportar información para la propia conservación de la especie, sino que también puede ser muy útil en estudios de bioindicación para conocer y evaluar las variaciones en el espacio y en el tiempo de las comunidades de especies en los ecosistemas mediterráneos y los principales factores que las amenazan.

En conclusión, los resultados aquí presentados sugieren que el estudio de la dieta del águila perdicera puede ser utilizado como una potente herramienta de diagnóstico y monitoreo con importantes aplicaciones en conservación. En primer lugar, es aplicable a la conservación de la propia especie, ya que permitirá detectar aquellos territorios que muestren condiciones tróficas adversas y así priorizar las acciones de conservación cuando resulten necesarias. En última instancia, también es aplicable al monitoreo de la calidad de los hábitats, comunidades y procesos que moldean los ecosistemas mediterráneos, donde el águila perdicera es una de las especies más emblemáticas.

## Referencias

**Alonso-Alvarez, C.** (2005) Age-dependent changes in plasma biochemistry of yellow-legged gulls (*Larus cachinnans*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 140, 512-518.

**Arroyo, B.E. & Garcia, J.T.** (2006) Diet composition influences annual breeding success of Montagu's Harriers *Circus pygargus* feeding on diverse prey. Bird Study, 53, 73-78.

**Balbontín, J. & Ferrer, M.** (2002) Plasma chemistry reference values in free-living Bonelli's Eagle (*Hieraaetus fasciatus*) nestlings. Journal of Raptor Research, 36, 231-235.

**Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H.** (2004) Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology, 73, 1007-1012.

**Blanco-Aguiar, J.C., Virgós, E. & Villafuerte, R.** (2003) Perdiz roja, *Alectoris rufa*. In: Martí, R. & del Moral, J.C. (eds.) Atlas de las aves reproductoras de España. Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología, Madrid, pp 212–213

**Blondel, J. & Aronson, J.** (1999) Biology and wildlife of the Mediterranean region. Oxford University Press.

**Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L.** (2003) The ecology of individuals: incidence and implications of individual specialization. American Naturalist, 161, 1-28.

**Borras, A., Cabrera, J. & Senar, J.C.** (2010) Hematocrit variation in response to altitude changes in wild birds: a repeated-measures design. Condor, 112, 622-626.

**Brown, M.E.** (1996) Assessing body condition in birds. In: Ketterson, E.D., Val Nolan, J. (eds.) Current ornithology. Plenum Press, New York, pp 67–135.

**Burton, G.W. & Ingold, K.U.** (1981) Autoxidation of biological molecules. 1. Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. Journal of the American Chemical Society, 103, 6472-6477.

**Calvete, C.** (2006) Modeling the effect of population dynamics on the impact of rabbit hemorrhagic disease. Conservation Biology, 20, 1232-1241.

**Carpenter, F.L.** (1975) Bird hematocrits: effects of high altitude and strength of flight. Comparative Biochemistry and Physiology Part A: Physiology, 50, 415-417.

**Carrete, M., Sánchez-Zapata, J.A., Tella, J.L., Gil-Sánchez, J.M. & Moleón, M.** (2006) Components of breeding performance in two competing species: habitat heterogeneity, individual quality and density-dependence. Oikos, 112, 680-690.

**Casado, E., Balbontin, J. & Ferrer, M.** (2002) Plasma chemistry in booted eagle (*Hieraaetus pennatus*) during breeding season. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 131, 233-241.

**Casagrande, S., Dell'Omo, G., Costantini, D., Tagliavini, J. & Groothuis, T.** (2011) Variation of a carotenoid-based trait in relation to oxidative stress and endocrine status during the breeding season in the Eurasian kestrel: a multi-factorial study. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 160, 16-26.

**Cernusca, A., Tappeiner, U. & Bayfield, N. (eds.)** (1999) Land-use changes in European mountain ecosystems. Blackwell Wissenschafts Verlag, Berlin.

**Cheylan, G.** (1981) Sur le rôle déterminant de l'abondance des ressouces dans le succês de reproduction de l'Aigle de Bonelli (*Hieraaetus fasciatus*) en Provence. - In: Rapaces Mediterranéens. PNRC et Annales du CROP 1: 95-99.

**Choi, W.J., Han, G.H., Lee, S.M., Lee, G.T., Yoon, K.S., Choi, S.M. & Ro, H.M.** (2007) Impact of land-use types on nitrate concentration and $\delta^{15}N$ in unconfined groundwater in rural areas of Korea. Agriculture, ecosystems & environment, 120, 259-268.

**Connolly, R.M., Guest, M.A., Melville, A.J. & Oakes, J.M.** (2004) Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia, 138, 161-167.

**Deegan, L.A. & Garritt, R.H.** (1997) Evidence for spatial variability in estuarine food webs. Marine Ecology Progress Series, 147, 31-47.

**Delibes-Mateos, M., Redpath, S.M., Angulo, E., Ferreras, P. & Villafuerte, R.** (2007) Rabbits as a keystone species in southern Europe. Biological Conservation, 137, 149-156.

**Delibes-Mateos, M., Delibes, M., Ferreras, P. & Villafuerte, R.** (2008) Key Role of European Rabbits in the Conservation of the Western Mediterranean Basin Hotspot. Conservation Biology, 22, 1106-1117.

**Delibes-Mateos, M.,, Ferreras, P. & Villafuerte, R.** (2009) European rabbit population trends and associated factors: a review of the situation in the Iberian Peninsula. Mammal Review, 39, 124-140.

**Dobado-Berrios, P.M. & Ferrer, M.** (1997) Age-related changes of plasma alkaline phosphatase and inorganic phosphorus, and late ossification of the cranial roof in the Spanish imperial eagle (*Aquila adalberti* CL Brehm, 1861). Physiological Zoology, 70, 421-427.

**Dobado-Berrios, P.M., Tella, J.L., Ceballos, O. & Donázar, J.A.** (1998) Effects of age and captivity on plasma chemistry values of the Egyptian vulture. Condor, 100, 719-725.

**Doucette, J.L., Wissel, B. & Somers, C.M**. (2011) Cormorant-fisheries conflicts: Stable isotopes reveal a consistent niche for avian piscivores in diverse food webs. Ecological Applications, 21, 2987-3001.

**Emlen, J.M.** (1966) The role of time and energy in food preference. American Naturalist, 100, 611-617.

**Fair, J., Whitaker, S. & Pearson, B.** (2007) Sources of variation in haematocrit in birds. Ibis, 149, 535-552.

**Ferrer, M. & Negro, J.J.** (2004) The near extinction of two large European predators: super specialists pay a price. Conservation Biology, 18, 344-349.

**Forslund, P. & Pärt, T.** (1995) Age and reproduction in birds—hypotheses and tests. Trends in Ecology & Evolution, 10, 374-378.

**Futuyma, D.J. & Moreno, G.** (1988) The evolution of ecological specialization. Annual Review of Ecology and Systematics, 19, 207-233.

**Galván, I., Gangoso, L., Grande, J.M., Negro, J.J., Rodríguez, A., Figuerola, J. & Alonso-Alvarez, C.** (2010) Antioxidant machinery differs between melanic and light nestlings of two polymorphic raptors. PloS one, 5, e13369.

**García-Rodríguez, T., Ferrer, M., Carrillo, J.C. & Castroviejo, J.** (1987) Metabolic responses of *Buteo buteo* to long-term fasting and refeeding. Comparative Biochemistry and Physiology Part A: Physiology, 87, 381-386.

**Hernández-Matías, A., Real, J. & Pradel, R.** (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. Journal of Wildlife Management, 75, 856-866.

**Hernández-Matías, A., Real, J., Moleón, M., Palma, L., Sánchez-Zapata, J.A., Pradel, R., Carrete, M., Gil-Sánchez, J.M., Beja, P., Balbontín, J., Vincent-Martin, N., Ravayrol, A., Benítez, J.R., Arroyo, B., Fernández, C., Ferreiro, E. & García, J.** (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecological Monographs, 83, 239-261.

**Hobson, K.A.** (2011) Isotopic ornithology: a perspective. Journal of Ornithology, 152, 49-66.

**Hochleithner, M.** (1994) Biochemistries. In: Ritchie, B.W., Harrison, G.L. & Harrison, L.R. (eds) Avian medicine: principles and application. Wingers, Lake Worth, pp. 223-245.

**Inger, R. & Bearhop, S.** (2008) Applications of stable isotope analyses to avian ecology. Ibis, 150, 447-461.

**Kelly, J.F.** (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology, 78, 1-27.

**L'Hérault, V., Franke, A., Lecomte, N., Alogut, A. & Bêty, J.** (2013) Landscape heterogeneity drives intra-population niche variation and reproduction in an arctic top predator. Ecology and Evolution, 3, 2867-2879.

**Lindenmayer, D. & Burgman, M.** (2005) Practical conservation biology. CSIRO publishing.

**Lumeij, J.T. & Remple, J.D.** (1991) Plasma urea, creatinine and uric acid concentrations in relation to feeding in peregrine falcons (*Falco* *peregrinus*). Avian Pathology, 20, 79-83.

**Margalida, A., González, L.M., Sánchez, R., Oria, J., Prada, L., Caldera, J., Aranda, A. & Molina, J.I.** (2007) A long-term large-scale study of the breeding biology of the Spanish imperial eagle (*Aquila adalberti*). Journal of Ornithology, 148, 309-322.

**Marti, C.D., Bechard, M. & Jaksic, F.M.** (2007) Food habits. In Bird, D.M. & Bildstein, K.L. (eds.) Raptor research and management techniques: 129-151. Surrey: Hancock House Publishers LTD.

**Martínez-Abraín, A., Tavecchia, G., Regan, H.M., Jimenez, J., Surroca, M. & Oro, D.** (2012) Effects of wind farms and food scarcity on a large scavenging bird species following an epidemic of bovine spongiform encephalopathy. Journal of Applied Ecology, 49, 109-117.

**Moleón, M., Gil-Sánchez, J.M., Real, J., Sánchez-Zapata, J.A., Bautista, J. & Sánchez-Clemot, J.F.** (2007) Non-breeding feeding ecology of territorial Bonelli's Eagles *Hieraaetus fasciatus* in the Iberian Peninsula. Ardeola, 54, 135-143.

**Moleón, M., Almaraz, P. & Sánchez-Zapata, J.A.** (2008) An emerging infectious disease triggering large-scale hyperpredation. PLoS ONE, 3, e2307.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Chartón, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. Journal of Biogeography, 36, 1502-1515.

**Moleón, M., Sánchez-Zapata, J.A., Gil-Sánchez, J.M., Ballesteros-Duperón, E., Barea-Azcón, J.M. & Virgós, E.** (2012a) Predator–prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia, 168, 679-689.

**Moleón, M., Sebastián-González, E., Sánchez-Zapata, J.A., Real, J., Pires, M.M., Gil-Sánchez, J.M., Bautista, J., Palma, L., Bayle, P., Guimarães Jr, P.R. & Beja, P.** (2012b) Changes in intrapopulation resource use patterns of an endangered raptor in response to a disease-mediated crash in prey abundance. Journal of Animal Ecology, 81, 1154-1160.

**Nekhay, O. & Arriaza, M.** (2009) Restoration of abandoned agricultural lands toward habitats for umbrella species. Spanish Journal of Agricultural Research, 7, 375-389.

**Newsome, S.D., Martinez del Rio, C., Bearhop, S. & Phillips, D.L.** (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment, 5, 429-436.

**Ontiveros, D., Real, J., Balbontín, J., Carrete, M., Ferreiro, E., Ferrer, M., Mañosa, S., Pleguezuelos, J.M. & Sánchez-Zapata, J.A.** (2004) Biología de la conservación del águila perdicera *Hieraaetus fasciatus* en España: investigación científica y gestión. Ardeola, 51, 461-470.

**Palma, L., Beja, P., Pais, M. & Cancela da Fonseca, L.** (2006) Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons. Journal of Applied Ecology, 43, 1075-1086.

**Palomares, F.** (2001) Vegetation structure and prey abundance requirements of the Iberian linx: implications for the design of reserves and corridors. Journal of Applied Ecology, 38, 9-18.

**Parker, K.L., Barboza, P.S. & Stephenson, T.R.** (2005) Protein conservation in female caribou (*Rangifer tarandus*): effects of decreasing diet quality during winter. Journal of Mammalogy, 86, 610-622.

**Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L.** (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One, 5, e9672.

**Peig, J. & Green, A.J.** (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos, 118, 1883-1891.

**Penteriani, V., Balbontin, J. & Ferrer, M.** (2003) Simultaneous effects of age and territory quality on fecundity in Bonelli's Eagle *Hieraaetus fasciatus*. Ibis, 145, E77-E82.

**Peterson, B.J. & Fry, B.** (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18, 293-320.

**Pyke, G.H., Pulliam, H.R. & Charnov, E.** (1977) Optimal foraging: a selective review of theory and tests. Quarterly Review of Biology, 52, 137-154.

**Real, J.** (1987) Evolución cronológica del regimen alimenticio de una población de *Hieraaetus fasciatus* en Catalunya: factores causantes, adaptación y efectos. Rapaci Mediterranei III, Vol. XII, 185-205.

**Real, J.** (1991) L´àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología trófica, biología reproductora i demografía. Tesis doctoral. Universitat de Barcelona.

**Real, J.** (1996) Biases in diet study methods in the Bonelli's eagle. The Journal of Wildlife Management, 60, 632-638.

**Real, J. & Mañosa, S.** (1997) Demography and conservation of western European Bonelli´s eagle *Hieraaetus fasciatus* populations. Biological Conservation, 79, 59-66.

**Real, J., Grande, J.M., Mañosa, S. & Sánchez-Zapata, J.A.** (2001) Causes of death in different areas for Bonelli's Eagle *Hieraaetus fasciatus* in Spain. Bird study, 48, 221-228.

**Roughgarden, J.** (1972) Evolution of niche width. American Naturalist, 106, 683-718.

**Sternalski, A., Mougeot, F., Eraud, C., Gangloff, B., Villers, A. & Bretagnolle, V.** (2010) Carotenoids in nestling Montagu's harriers: variations according to age, sex, body condition and evidence for diet-related limitations. Journal of Comparative Physiology B, 180, 33-43.

**Steenweg, R.J., Ronconi, R.A. & Leonard, M.L.** (2011) Seasonal and age-dependent dietary partitioning between the Great Black-Backed and Herring Gulls. Condor, 113, 795-805.

**Stevenson, R.D. & Woods, W.A.** (2006) Condition indices for conservation: new uses for evolving tools. Integrative and comparative biology, 46, 1169-1190.

**Sutherland, W.J.** (1996) From individual behaviour to population ecology. - Oxford Univ. Press.

**Svanbäck, R. & Bolnick, D.I.** (2007) Intraspecific competition drives increased resource use diversity within a natural population. Proceedings of the Royal Society B, Biological Sciences, 274, 839-844.

**Thode, H.G.** (1991) Sulfur isotopes in nature and the environment: an overview. In Krouse, H.R. & Grinenko, V.A. (eds) Stable isotopes: natural and anthropogenic sulphur in the environment: 1-26. Chichester: Wiley.

**Tinker, M.T., Guimarães, P.R.Jr., Novak, M., Marquitti, F.M.D., Bodkin, J.L., Staedler, M., Bentall, G. & Estes, J.A.** (2012) Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecology Letters, 15, 475-483.

**Van Dyke, F.** (2008) Conservation biology: foundations, concepts, applications. Springer.

**Vargas, J.M., Guerrero, J.C., Farfán, M.A., Barbosa, A.M. & Real, R.** (2006) Land use and environmental factors affecting red-legged partridge (*Alectoris rufa*) hunting yields in southern Spain. European Journal of Wildlife Research, 52, 188-195.

**Villafuerte, R., Calvete, C., Blanco, J.C. & Lucientes, J.** (1995) Incidence of viral hemorrhagic disease in wild rabbit populations in Spain. Mammalia, 59, 651-660.

**Viñuela J., Ferrer, M. & Recio, F.** (1991) Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. Comparative Biochemistry and Physiology, 99A, 49-54.

**Weiser, E.L. & Powell, A.N.** (2011) Evaluating gull diets: a comparison of conventional methods and stable isotope analysis. Journal of Field Ornithology, 82, 297-310.

**Whitfield, D.P., Reid, R., Haworth, P.F.,**

**Madders, M., Marquiss, M., Tingay, R. & Fielding, A.H.** (2009) Diet specificity is not associated with increased reproductive performance of Golden Eagles Aquila chrysaetos in Western Scotland. Ibis, 151, 255-264.

**Woo, K.J., Elliott, K.H., Davidson, M., Gaston, A.J. & Davoren, G.K.** (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. Journal of Animal Ecology 77, 1082-1091.

**Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R. & Turner, N.D.** (2004) Glutathione metabolism and its implications for health. The Journal of nutrition, 134, 489-492.

# Discusión general

# Conclusiones - Conclusions

# CONCLUSIONES

- La dieta del águila perdicera en Cataluña durante el periodo de cría incluyó presas pertenecientes a un mínimo de 31 especies, 26 de aves, 4 de mamíferos y una especie de reptil. El análisis de egagrópilas permitió la identificación de la mayoría de presas a nivel específico. En el conjunto de la población, el conejo de monte, los Colúmbidos (especialmente paloma torcaz y doméstica), la perdiz roja, diversos Paseriformes, la gaviota patiamarilla y la ardilla roja constituyeron las principales categorías de presas, sumando más del 80% del total de ítems y biomasa. No obstante, la composición de la dieta mostró una elevada heterogeneidad entre territorios dentro de la misma población, probablemente como consecuencia de la diferente disponibilidad de presas.

- El análisis detallado de la composición isotópica de carbono, nitrógeno y azufre en las principales categorías de presas resultó básico para la posterior implementación de los modelos bayesianos mixtos, ya que permitió determinar y considerar tanto la variación interespecífica como la intraespecífica relacionada con factores ambientales. La mayoría de categorías de presas mostraron diferencias en las signaturas isotópicas de carbono y/o nitrógeno. A su vez, el nitrógeno fue indicativo del nivel trófico de las presas. Las gaviotas mostraron los valores más elevados de azufre en comparación al resto de presas. A nivel intraespecífico, los conejos y las palomas domésticas de territorios próximos al mar tuvieron valores más elevados de azufre que sus conespecíficos de territorios alejados del mar. Asimismo, las palomas domésticas de territorios con una predominancia de hábitats agrícolas mostraron valores más elevados de nitrógeno en comparación a las procedentes de hábitats con predominancia de matorral.

- El análisis de los isótopos estables de carbono, nitrógeno y azufre en las plumas de los pollos de águila perdicera mostró ser una metodología útil para inferir la dieta de la especie durante el periodo de cría, tanto a nivel territorial como poblacional. La comparación entre la composición de la dieta estimada mediante análisis de isótopos estables y de egagrópilas indicó un elevado nivel de concordancia a nivel poblacional. A nivel territorial, ambos métodos mostraron similitud en cuanto al orden de clasificación de mayor a menor consumo de las principales presas. Sin embargo, la concordancia para cada categoría de presas varió, siendo moderada en el consumo de conejos, palomas y gaviotas; algo menor en el consumo de ardillas y paseriformes; y baja en el consumo de perdiz.

- La aplicabilidad del análisis de isótopos estables debe entenderse como una aproximación al consumo de las principales categorías de presas, y no como un estudio detallado a nivel taxonómico, para lo cual el análisis convencional de egagrópilas es más recomendable. Cabe destacar la utilidad del análisis de isótopos estables a la hora de estimar la dieta de los pollos a nivel individual, algo que mediante análisis de egagrópilas no es posible. El análisis de carbono y nitrógeno es imprescindible en todos los casos, mientras que el uso del azufre es especialmente recomendable en aquellos territorios en los que se pretenda estimar el consumo de gaviota patiamarilla.

- La composición de la dieta afectó al estado de condición corporal de los pollos de águila perdicera, habiendo una relación positiva entre algunas medidas de condición y el consumo de conejo, y negativa con la diversidad trófica o el consumo de paseriformes. Las medidas de condición más informativas sobre los efectos de la dieta incluyeron parámetros de bioquímica plasmática (colesterol, vitaminas y actividad de la aspartato aminotransferasa), así como medidas de estrés oxidativo (actividad de la glutatión peroxidasa y niveles de glutatión total y oxidado). De este modo, los análisis de isótopos estables y de bioquímica sanguínea fueron útiles para abordar los efectos de la dieta sobre el estado de condición corporal de los pollos de águila perdicera.

- Además de la dieta, otros factores mostraron un efecto sobre algunas medidas de condición corporal. En este sentido, el hematocrito aumentó con la altitud de las zonas de cría, y los niveles de glucosa en plasma fueron mayores en machos que en hembras. A su vez, los niveles de las enzimas plasmáticas fosfatasa alcalina y creatina kinasa aumentaron con la edad de los pollos, mientras que los niveles de triglicéridos, ácido úrico y retinol palmitato en plasma aumentaron tras la ingesta de alimento. Por último, los niveles de creatinina en plasma fueron más elevados en pollos muestreados después del mediodía. Por lo tanto, el efecto de factores como la altitud, el sexo y la edad de los pollos, una ingesta reciente de alimentos y ritmos circadianos deberá considerarse en el futuro a la hora de interpretar correctamente la variación en las medidas de condición corporal de los pollos de águila perdicera, tanto a nivel individual como poblacional.

- El análisis de isótopos estables permitió inferir la amplitud del nicho trófico del águila perdicera durante el periodo de cría a nivel poblacional (Francia, Cataluña y Andalucía). La población en Andalucía mostró un nicho trófico considerablemente menor al de las poblaciones en Francia y Cataluña, debido a que el mayor consumo de conejo y perdiz en Andalucía redujo la diversidad trófica. Las diferencias de consumo de dichas presas entre áreas, posiblemente deriven de su diferente disponibilidad, siendo probablemente menor en las regiones septentrionales. La productividad anual correlacionó negativamente con la amplitud de nicho trófico de la población.

- A nivel territorial, en aquellas parejas que se reprodujeron con éxito, la probabilidad de que volaran dos pollos aumentó en los territorios donde el consumo de conejo y/o perdiz fue elevado, así como allí donde el consumo de presas fue similar al promedio de la población, es decir, territorios con un moderado consumo de conejo, palomas y perdiz. En contra, los territorios con una mayor diversidad en la dieta mostraron menor probabilidad de que volaran dos pollos, posiblemente debido al incremento en el consumo de presas menos óptimas en cuanto al aporte neto de energía. Además, la mortalidad de los individuos territoriales mostró un efecto negativo sobre la productividad, lo que podría explicarse porque los nuevos ejemplares reclutados suelen ser de edad no adulta y, por lo tanto, inexperimentados y/o inmaduros sexualmente.

- En el conjunto de la población en Europa Occidental, la productividad de las parejas aumentó con el consumo de conejo, y fue menor allí donde incrementó el consumo de la categoría "otras aves" (Paseriformes, Anseriformes, Ardeiformes, Charadriiformes o Accipitriformes). El patrón a nivel poblacional fue similar, aumentando la productividad con un mayor consumo de perdiz, y disminuyendo con el mayor consumo de "otras aves". La supervivencia de los individuos en las parejas territoriales, así como en el conjunto de la población, aumentó con una menor diversidad en la dieta. Las relaciones halladas entre la dieta y los parámetros de vida de los individuos parecen modular la tasa de crecimiento tanto a nivel de los territorios como a nivel poblacional.

- Los resultados en su conjunto apoyan la hipótesis de que el conejo es una presa óptima para el águila perdicera en el área de estudio, posiblemente porque la relación entre los costes y los beneficios de su búsqueda, manipulación y consumo es más favorable en términos energéticos en comparación a otras presas alternativas. En aquellas áreas como el sur de la Península Ibérica, donde la perdiz puede ser abundante, esta especie también parece desempeñar un papel clave. El mayor consumo de conejo estuvo asociado con una mejor condición corporal de los pollos y con un aumento de la productividad a nivel territorial. A su vez, el consumo de perdiz mejoró la productividad a nivel poblacional. Por el contrario, un menor consumo de estas presas estuvo asociado con una mayor diversidad en la dieta, la cual correlacionó negativamente con la supervivencia y la tasa de crecimiento, tanto en los territorios como a nivel poblacional.

- En términos de biología de la conservación, el análisis local de la dieta, así como el monitoreo de su evolución en el tiempo, puede permitir un diagnóstico del estado de las presas clave del águila perdicera. Esto permitirá priorizar los esfuerzos de conservación en la mejora de estas presas en aquellos territorios y áreas donde la dieta indique condiciones tróficas susceptibles de afectar a los principales parámetros vitales. A tal efecto, las acciones de conservación deberán ir orientadas a aumentar la abundancia y disponibilidad de conejos y perdices, favoreciendo también la presencia de presas alternativas como palomas. Más allá del propio interés en la conservación del águila perdicera, y dado que se trata de un depredador que se sitúa en los niveles más altos de las redes tróficas de los ecosistemas mediterráneos, el seguimiento detallado de la dieta y los principales parámetros vitales de esta especie en una amplia escala espacio-temporal podrán ser utilizados como bioindicadores de los cambios ambientales en dichos ecosistemas.

# Conclusiones

# CONCLUSIONS

- The diet of Bonelli's Eagle in Catalonia during the breeding period included a minimum of 31 prey species, 26 birds, 4 mammals and one reptile. The pellet analysis allowed most prey to be identified at the species level. In the whole population, European rabbits, Columbids (especially wood and domestic pigeons), red-legged partridges, passerines, yellow-legged gull and Eurasian red squirrels were the main prey categories, accounting for more than 80% of total items and biomass. Nevertheless, the diet composition was heterogeneous between territories of the same population, probably due to different prey availability at the territory level.

- A detailed analysis of the isotopic composition of carbon, nitrogen and sulphur in the main prey categories set the basis for the subsequent implementation of Bayesian mixed models, allowing to determine and to consider both interspecific and intraspecific variation related to environmental factors. Most prey categories differed in their carbon and/or nitrogen isotopic signatures. In turn, nitrogen was indicative of the trophic position of prey. Gulls showed the highest isotopic sulphur values compared to other prey categories. At the intraspecific level, rabbits and domestic pigeons from territories near the sea had higher sulphur values than conspecifics from territories farther inland. Moreover, domestic pigeons from territories with agricultural habitat predominance showed higher nitrogen values than those from territories with scrubland predominance.

- Stable isotope analyses of carbon, nitrogen and sulphur in Bonelli's Eagle nestling feathers was a useful methodology to infer the species' diet during the breeding period at both the territory and the population levels. The comparison between the estimation of the diet's composition by the stable isotope analysis and the pellet analysis showed a good agreement at the population level. At the territorial level, both methods showed good ordinal scale agreement in main prey consumption. However, the agreement between methods varied for each prey category, being moderate for the consumption of rabbits, pigeons and gulls; somewhat lower for squirrels and passerines; and low for partridges.

- The applicability of stable isotope analysis should be understood as an approximation to estimate main prey consumption, but not as a dietary approach for taxonomic prey details, for which the conventional pellet analysis is recommended. It is worth to mention the usefulness of stable isotope analysis for individual diet estimates, which is unfeasible by pellet analysis. The analysis of carbon and nitrogen is essential in all cases, while sulphur is particularly recommended for assessing the consumption of yellow-legged gulls.

- The diet composition influenced Bonelli's Eagle nestling body condition, with a positive relationship between body condition estimates and rabbit consumption, and negative with diet diversity or consumption of passerines. The most informative body condition estimates on the diet effects included plasma biochemical parameters (cholesterol, vitamins and aspartate aminotransferase activity), as well as measures of oxidative stress (glutathione peroxidase activity and levels of total and oxidized glutathione). Thus, the stable isotope analysis and blood biochemistry were useful to address the influence of diet on Bonelli's Eagle nestling body condition.

- Besides diet, other factors showed an effect on some estimates of body condition. In this sense, the haematocrit increased with the altitude of nestling areas, and plasmatic glucose levels were higher in males than in females. Moreover, the plasmatic activity of the enzymes alkaline phosphatase and creatine kinase increased with nestling age, while the plasmatic levels of triglycerides, uric acid and retinol palmitate were higher in nestlings that had recently ingested food. Finally, plasmatic creatinine levels were higher in nestlings sampled after midday. Therefore, the effect of factors such as the altitude, individual sex and age, time from last food intake or circadian rhythms should be considered in the future when interpreting Bonelli's Eagle nestling body condition parameters at both the individual and the population levels.

- Stable isotope analyses were useful to infer the trophic niche width of Bonelli's Eagle during the breeding period at the population level (France, Catalonia and Andalusia). The Andalusian population showed a considerably narrower trophic niche than the populations in France and Catalonia, because the higher consumption of rabbits and partridges in Andalusia reduced trophic diversity. Differences in the consumption of these prey among areas possibly arose from their different availability, which was probably lower in the northern regions. The annual productivity was negatively correlated with the population trophic niche width.

- At the territory level, in pairs breeding successfully, the probability of producing two chicks increased in those territories where nestlings disproportionally consumed rabbits and/or partridges, as well as in territories where nestlings consumed prey in similar proportions than the overall population, that is, moderate consumption of rabbits, pigeons and partridges. On the other hand, the probability of fledging two chicks was lower in those territories with higher diet diversity, possibly due to the higher consumption of less optimal prey in terms of net energy intake. In addition, mortality of territorial individuals showed a negative effect on productivity, which could be explained because most recruited individuals are non-adults, and therefore, inexperienced and/or sexually immature.

- In the overall population of Western Europe, productivity increased with greater rabbit consumption and decreased with greater consumption of "other birds", a prey category that included several medium- to large-sized species (Anseriformes, Ardeiformes, Charadriiformes or Accipitriformes). A similar pattern was found at the population level, increasing productivity when consumption of partridges was greater and consumption of "other birds" was lower. Survival increased with lower diet diversity at both the territorial and the population levels. The relationship found between diet and individual life history attributes seem to modulate both the territory and population growth rate.

- Overall, these results support the hypothesis that rabbit is an optimal prey for Bonelli's Eagle in the study area, possibly because the cost/benefit relationship of its foraging, handling and consumption is more profitable in terms of energy compared to other alternative prey. In those areas like Southern Iberian Peninsula, where the red-legged partridge is more abundant, this species also appears to play a key role. The greater rabbit consumption was related with better nestling body condition and an increase in productivity at the territory level. The consumption of partridges also improved productivity at the population level. On the other hand, lower consumption of these prey was associated with greater diet diversity, which was negatively correlated with survival and the growth rate at both the territory and population levels.

- In terms of conservation biology, diet analysis and its monitoring over time may allow to diagnose the status of Bonelli's Eagle key prey and, therefore, help to focus the conservation efforts at improving the abundance of these prey in the territories and areas where the diet is indicative of trophic conditions likely to affect eagles' main vital rates. To do so, conservation actions should be aimed at increasing the availability and abundance of rabbits and partridges, but also favouring the presence of alternative prey such as pigeons. Beyond the interest on Bonelli's Eagle conservation, and given that this species is a top predator occupying the highest trophic levels in Mediterranean ecosystems, a detailed monitoring of its diet and main vital parameters on a broad spatio-temporal scale could be used as bioindicators of environmental change on these ecosystems.

# Conclusions

# Appendix

Resano et al. (2011) *J. Raptor Res.*, 45, 342-352

*J. Raptor Res.* 45(4):342–352

# USING STABLE ISOTOPES TO DETERMINE DIETARY PATTERNS IN BONELLI'S EAGLE (*AQUILA FASCIATA*) NESTLINGS

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ABSTRACT.—Bonelli's Eagle (*Aquila fasciata*) is one of the most endangered raptor species in Europe due to high adult and subadult mortality rates, habitat loss, and a decrease in populations of its most important prey, European rabbits (*Oryctolagus cuniculus*) and Red-legged Partridges (*Alectoris rufa*). During the breeding season of 2008, we studied the diet of Bonelli's Eagles at 15 breeding territories in Catalonia, northeastern Iberian Peninsula, through a conventional pellet analysis and stable isotope analyses (SIA) of nestlings' feathers. Our objectives were to investigate the diet of Bonelli's Eagle nestlings and to determine whether SIA allowed accurate representation of their dietary patterns. The pellet analysis revealed a broad diet including pigeons (*Columba* spp.; 31.1%), European rabbits (27.9%), ''other birds'' (16.2%), Red-legged Partridges (13.1%), Eurasian red squirrels (*Sciurus vulgaris*; 5.2%), ocellated lizards (*Timon lepidus*; 2.6%), Yellow-legged Gulls (*Larus michahellis*; 2.2%) and ''other mammals'' (1.7%). Diet composition was heterogeneous and varied markedly among nestlings from different breeding territories. We found a significant positive correlation between $\delta^{13}$C and the frequency of Eurasian red squirrels in the diet, and a significant negative correlation between $\delta^{13}$C and the frequency of Red-legged Partridges, which are species that occur in forested and open habitats, respectively. The values of $\delta^{15}$N were not correlated with the consumption of any prey category. However, its wide range of values suggested a global diet with a broad diversity of prey species from at least two different trophic levels. Finally, $\delta^{34}$S were higher for those nestlings that fed on Yellow-legged Gulls. Our study provided the first isotopic approach to the trophic ecology of Bonelli's Eagle nestlings, and we concluded that $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S may be useful for assessing nestlings' dietary patterns in terms of main prey consumption and prey trophic level.

KEY WORDS: *Bonelli's Eagle*; *Aquila fasciata*; *Hieraaetus fasciatus*; *diet*; *pellet analysis*; *raptor*; *stable isotopes*.

## USO DE ISÓTOPOS ESTABLES PARA DETERMINAR TENDENCIAS TRÓFICAS EN POLLOS DE *AQUILA FASCIATA*

RESUMEN.—El águila *Aquila fasciata* es una de las rapaces más amenazadas de Europa debido a la elevada tasa de mortalidad adulta y preadulta, la degradación y pérdida del hábitat, así como una disminución de sus principales presas como el conejo europeo (*Oryctolagus cuniculus*) o la perdiz roja (*Alectoris rufa*). Durante la temporada de cría de 2008 se estudió la dieta de 15 parejas reproductoras de *A. fasciata* en Catalunya, noreste de la Península Ibérica, a través del análisis convencional de egagrópilas y el análisis de isótopos estables (AIE) en las plumas de los pollos. Nuestros objetivos fueron investigar la dieta de los pollos de *A. fasciata*, así como determinar si el AIE permite representar con exactitud sus patrones tróficos. El análisis de egagrópilas reveló una dieta variada que incluyó palomas (*Columba* spp.; 31.1%), conejo europeo (27.9%), ''otras aves'' (16.2%), perdiz roja (13.1%), la ardilla *Sciurus vulgaris* (5.2%), el lagarto *Timon lepidus* (2.6%), la gaviota *Larus michahellis* (2.2%) y ''otros mamíferos'' (1.7%) como principales categorías de presas. Sin embargo, la composición de la dieta fue heterogénea y se hallaron diversos patrones tróficos entre pollos pertenecientes a diferentes territorios de cría. Asimismo, se halló correlación positiva entre $\delta^{13}$C y la frecuencia de ardilla roja en la dieta, y negativa entre $\delta^{13}$C y la frecuencia de perdiz roja, especies presentes en hábitats boscosos y abiertos, respectivamente. No hubo correlación entre $\delta^{15}$N y el consumo de presas. Sin embargo, su amplio rango de valores sugirió una dieta con diversidad de presas pertenecientes, al menos, a dos niveles tróficos diferentes. Finalmente, $\delta^{34}$S fue mayor en aquellos pollos que consumieron la gaviota *L. michahellis*. Este estudio aborda por vez primera la ecología trófica en pollos de *A. fascista* a partir del AIE, concluyendo que $\delta^{13}$C, $\delta^{15}$N y $\delta^{34}$S son útiles para la evaluación de sus patrones tróficos en términos de consumo de las principales presas y niveles tróficos de las mismas.

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The study of raptors' feeding habits provides meaningful information that can aid the understanding of species' dietary ecology and their trophic relationships at the community level (Jaksic and Delibes 1987, Newton 1998, Marti et al. 2007). The assessment of raptors' dietary patterns may also provide information about distribution, abundance, behavior and the vulnerability of prey species (del Hoyo et al. 1994, Johnsgard 2002). Traditionally, the diets of raptors are described using conventional methodologies that include the analysis of regurgitated pellets, food remains from nests, and stomach contents, as well as the direct observation of prey delivered to nestlings at the nests (Korpimäki and Norrdahl 1991, Salamolard et al. 2000, Katzner et al. 2006, Marti et al. 2007). Of these methods, pellet analysis is the most common approach in the study of raptors' dietary habits, both quantitatively and qualitatively, and has been shown to be an efficient and suitable method for monitoring the diet of several raptor species (Real 1996, Marti et al. 2007). The main advantage of conventional methods is that they frequently enable prey to be identified at the species or taxonomic group level. However, differences in prey sizes, digestion, and consumption patterns may lead to biases such as the over- or underestimation of the proportions of prey items in a predator's diet (Real 1996, Votier et al. 2003, Marti et al. 2007, Sánchez et al. 2008). Moreover, due to the logistical difficulty in sampling regularly over an extended period of time, conventional methods may in fact reflect only short-term dietary habits (Inger and Bearhop 2008).

Over the last two decades, stable isotope analysis (SIA) has become increasingly common in avian trophic ecology as a means of studying foraging strategies and dietary specialization at both individual and population levels (Kelly 2000, Bolnick et al. 2002, Rubenstein and Hobson 2004, Araújo et al. 2009). The use of SIA in dietary studies relies on the fact that different dietary items often have different isotopic values, which are reflected in the tissue of the consumers (Pearson et al. 2003, Becker et al. 2007, Inger and Bearhop 2008). For example, metabolically inert tissues such as feathers preserve the isotopic composition of resources incorporated while growing (Hobson 1999, Bearhop et al. 2002), and the use of SIA in avian trophic ecology has been shown as a powerful means of integrating temporal dietary information, particularly when combined with conventional methods (Inger and Bearhop 2008).

Stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotopes are the most frequently used isotopes in the study of trophic relationships and food-web structures at community level (Kelly 2000). The carbon-isotope composition of a consumer enables the carbon sources of the primary production within a food web to be determined (Krouse and Herbert 1988, Crawford et al. 2008). Nitrogen isotopes are useful for diagnosing the species' trophic level position since consumers are typically enriched in $^{15}N$ by 3–5‰ in proportion to the food they consume (Post 2002, Vanderklift and Ponsard 2003). This finding has been used to provide insights into community-level phenomena such as trophic cascades, the length of food chains, and resource partitioning (Post 2002, Roemer et al. 2002). In addition, the analysis of stable sulphur isotopes ($^{34}S/^{32}S$, $\delta^{34}S$) has been recommended in dietary studies as a means of discriminating between prey from marine and terrestrial ecosystems (Peterson et al. 1985, Peterson and Fry 1987). However, despite the wide applicability of SIA in avian foraging ecology, few isotopic studies have focused on terrestrial top predators such as raptor species (but see Roemer et al. 2002, Dominguez et al. 2003, Caut et al. 2006).

Bonelli's Eagle (*Aquila fasciata*) is a medium-sized raptor distributed from Southeast Asia and the Middle East to the western Mediterranean (del Hoyo et al. 1994). Its European population has declined markedly from the 1970s to the early 1990s (Rocamora 1994, Real 2004) and this raptor is now listed as an endangered species (BirdLife International 2004). In Europe, Bonelli's Eagle occupies Mediterranean mountain ranges and lowlands, and forages mainly in scrublands and dry fields where it predates on a wide variety of species ranging from medium-sized to small mammals (Lagomorpha and Rodentia), birds (Galliformes, Columbiformes, Charadriiformes, Passeriformes, and others) and occasionally reptiles (mainly lizards; Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Moleón et al. 2009a, 2009b). Furthermore, marked dietary differences may exist among territories due to heterogeneity in ecological features such as habitat coverage, prey abundance and distribution, and human pressure (Real et al. 2004). Therefore, Bonelli's Eagle is a suitable model for assessing whether territorial dietary patterns inferred by conventional techniques can also be described using isotopic data. Moreover, the ecological features of some territories have undergone great changes in recent decades (i.e., the expansion of forests as a consequence of land abandonment, an increase in human pressure that results from sprawl, and greater demands for leisure activities), and the number and availability of prey species has been greatly modified. Thus, the ongoing monitoring of diet of Bonelli's Eagle may constitute a good tool to assess the prey on which this species depends during the nestling period, and also help illuminate how habitat changes may affect eagles' foraging habits.

The focus of our study was an analysis of Bonelli's Eagle diet during the breeding season via conventional pellet analysis and an evaluation of the usefulness of SIA for assessing nestlings' dietary patterns. The specific aims of this study were: (1) to assess the diet of Bonelli's Eagle nestlings from different breeding territories using conventional pellet analysis; (2) to describe stable isotope values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in nestlings' feathers; (3) to assess isotopic data in siblings as indicators of diet similarity; and (4) to test whether isotopic data from nestlings were related to their prey consumption as described by the pellet analysis.

## METHODS

**Study Area.** During 2008, we studied 15 territorial breeding pairs of Bonelli's Eagle in Catalonia (northeastern Spain; 01°32′E, 41°20′N). Sampled territories were a subset of known territories for the species in Catalonia. All sampled nests were located on cliffs, and environmental features in breeding territories varied but were representative of Mediterranean habitats, and included scrublands (*Quercus coccifera, Thymus vulgaris, Pistacia lentiscus* and *Rosmarinus officinalis*), woodland patches (mainly *Quercus ilex* and *Pinus* spp.), nonirrigated cropland and built-up areas (Bosch et al. 2010). The mean altitude of nesting areas ranged from 176 to 753 m asl, with mean annual rainfall ranging from 450 to 800 mm.

**Data Collection.** Each breeding territory was monitored between January and July. We checked each territory using a spotting scope (20–60×) between January and early March to assess territorial occupancy and breeding activity (i.e., displays, nest material transfer, copulation, and incubation behavior). In late March and April, we checked nests again, using a spotting scope, to detect the presence, number, and approximate age of nestlings. The age of nestlings was estimated by the development of feathers and by calculating from the laying date (Real 1991, Gil-Sánchez 2000). After nestlings were approximately 37 d old, climbers accessed nests to collect 3–4 feathers from the back of each nestling for the SIA, assuming that isotopic data from nestlings' feathers were representative of the whole nestling period. At the same time, pellets were collected from the nest for the conventional diet analysis. Finally, approximately 2 wk after the nestlings had fledged, nests were visited again for a second retrieval of pellets. Therefore, we assumed that our conventional diet study based on pellet analysis was representative of nestlings' diet during their entire nestling period.

**Conventional Diet Study and Statistical Procedures.** The conventional diet study was based on pellet analysis. Pellets were individually analyzed and each prey species identified in a pellet was counted as one individual (Real 1996, Gil-Sánchez et al. 2004). Pellets were visually examined and their contents (i.e., feathers, bones, hair, nails, and scales) were compared with prey items from our own reference collection. For some remains, such as feathers, we also used a 4× magnifying glass and consulted specialized guides for the identification of macro- and microscopic remains (Brom 1986). Prey were identified to species level whenever possible.

Prey items were grouped into eight different taxonomic categories: European rabbits (*Oryctolagus cuniculus*), Eurasian red squirrels (*Sciurus vulgaris*), ''other mammals,'' pigeons (*Columba* spp.), Red-legged Partridges (*Alectoris rufa*), Yellow-legged Gulls (*Larus michahellis*), ''other birds'' (mainly Corvidae and Turdidae) and ocellated lizards (*Timon lepidus*). Diet data were analyzed at the territory level by comparing the frequency (%) of items in each taxonomic group relative to the total number of prey items (Palma et al. 2006, Moleón et al. 2009b). To assess the dietary patterns of nestlings at the territory level, we performed a principal component analysis (PCA) of prey frequency consumption using the varimax rotation, which keeps the rotated components orthogonal to or uncorrelated with each other after rotation (Quinn and Keough 2002). Additionally, we performed Spearman rank correlation tests ($r_s$) for all taxonomic prey consumption at the territory level.

**Stable Isotope Analysis and Statistical Procedures.** Nestling feathers were frozen until they were cleaned in a solution of NaOH (0.25 M; Bearhop et al. 2002, Ramos et al. 2009) and oven-dried at 40°C for 24 hr. Lipids were not washed off the feathers as they were shown to have negligible effects on the isotope ratios (Mizutani et al. 1992). To homogenize samples, feathers were ground into an extremely fine powder using an impactor mill (6750 Freezer/Mill, Spex Certiprep, Metuchen, New Jersey, U.S.A.) operating at the temperature of liquid nitrogen. Subsamples of 0.35 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 3.7 mg (for $\delta^{34}S$) were loaded in tin recipients and crimped for combustion. Isotopic analyses were conducted using elemental analysis-isotope ratio mass spectrometry (EA-IRMS) using a Flash 1112 (for C and N)/1108 (for S) elemental analyzer coupled to a Delta C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Fisher Scientific, Bremen, Germany). Analyses were performed at the Scientific Technical Services of the University of Barcelona.

Stable isotope ratios are expressed conventionally as parts per thousand (‰), according to the following equation: $\delta X = [(Rsample/Rstandard) - 1] \times 1000$, where X is $^{13}C$, $^{15}N$, or $^{34}S$, and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$. Samples were referenced against international standards: Pee Dee Belemnite (VPDB) for $^{13}C$, atmospheric nitrogen (AIR) for $^{15}N$ and Canyon Diablo Troilite (CDT) for $^{34}S$. The measurement precisions for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were $\leq 0.15‰$, $\leq 0.25‰$ and $\leq 0.4‰$, respectively.

Arithmetic mean values (±SD) for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were calculated for all nestlings. Because we expected Bonelli's Eagle nestlings raised in the same nest to have similar prey intake, we tested whether the isotopic values from siblings hatched in the same nest were more similar to each other than to isotopic values from a random sample of nestlings from the studied population. First, we applied a Spearman rank correlation test that only considered those territories where two nestlings were born ($n = 9$), and we then performed a randomization test to assess whether isotopic similarities between siblings differed from the expected random distribution. To do so, we obtained two samples of nine individuals extracted at random from the pool of the studied population ($n = 24$ nestlings) and compared their isotopic values with a Spearman rank correlation. This step was repeated 10 000 times and the resulting correlation coefficients were recorded. Next, we calculated the proportion of randomized coefficients that were recorded as equal to or larger than the observed correlation coefficient in siblings. This proportion, our estimated *P*-value (*P*), was then used to accept or reject the assertion that isotopic values were more similar between siblings than the expected random distribution.

Finally, we analyzed whether isotopic data from nestlings were related to their diet as estimated by the pellet analysis. To do so, we performed a Spearman rank correlation test between $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ from nestlings from each breeding pair and nestlings' prey consumption as described by the pellet analysis. Nestlings from the same nest/territory were considered a single statistical observation and the isotopic values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) for each breeding territory were estimated using the means of the two siblings.

Statistical analyses were conducted using R software (R Development Core Team 2007) and SPSS 15.0 (SPSS, Chicago, Illinois, U.S.A.).

## RESULTS

**Conventional Diet.** We identified 542 prey items in the 241 pellets analyzed (Table 1). In all, 62.6% of prey items were birds, 34.8% were mammals, and 2.6% were reptiles. The main prey items consumed were pigeons (31.1%), a category that included Rock Pigeon (*Columba livia*), Common Wood-pigeon (*Columba palumbus*), and Stock Dove (*Columba oenas*), followed by European rabbits (27.9%), ''other birds'' (16.2%), Red-legged Partridges (13.1%), Eurasian red squirrels (5.2%), ocellated lizards (2.6%), Yellow-legged Gulls (2.2%), and ''other mammals'' (1.7%; Fig. 1).

The PCA revealed marked dietary patterns between nestlings from different territories (Table 2 and Fig. 2). The first two components accounted for 64.6% of total diet variance. The first component, which accounted for 40.3% of diet variance, discriminated between nestlings with a high consumption of pigeons as opposed to others whose diet included more Red-legged Partridges, ocellated lizards, Yellow-legged Gulls and ''other mammals.'' The second component explained an additional 24.3% of diet variance and discriminated between greater amounts of European rabbits as opposed to ''other birds.'' Indeed, Spearman rank correlations between taxonomic prey consumption of nestlings at the territory level showed that intake of pigeons was negatively correlated with that of Red-legged Partridges ($r_s = -0.547$, $P < 0.05$), ocellated lizards ($r_s = -0.685$, $P < 0.005$), and Yellow-legged Gulls ($r_s = -0.465$, $P < 0.1$). Accordingly, there was also a significant negative correlation between consumption of European rabbits and ''other birds'' ($r_s = -0.526$, $P < 0.05$).

Table 1. Diet of Bonelli's Eagle nestlings during the breeding season, shown as the number of prey items and their frequencies (%), based on pellet analyses.

| PREY SPECIES | NUMBER OF ITEMS | FREQUENCY (%) |
|---|---|---|
| **Mammals** | | |
| European rabbit (*Oryctolagus cuniculus*) | 151 | 27.9 |
| Eurasian red squirrel (*Sciurus vulgaris*) | 28 | 5.2 |
| Undetermined mammal | 9 | 1.7 |
| **Total mammals** | **188** | **34.8** |
| **Birds** | | |
| Northern Goshawk (*Accipiter gentilis*) | 4 | 0.7 |
| European Honey-buzzard (*Pernis apivorus*) | 1 | 0.2 |
| Red-legged Partridge (*Alectoris rufa*) | 71 | 13.1 |
| Common Pheasant (*Phasianus colchicus*) | 1 | 0.2 |
| Galliforms (*Galliform* spp.) | 6 | 1.1 |
| Rock Pigeon (*Columba livia*) | 12 | 2.2 |
| Common Wood-pigeon (*Columba palumbus*) | 62 | 11.3 |
| Stock Dove (*Columba oenas*) | 3 | 0.6 |
| Pigeons (*Columba* spp.) | 92 | 17.0 |
| Eurasian Jay (*Garrulus glandarius*) | 12 | 2.2 |
| Black-billed Magpie (*Pica pica*) | 3 | 0.6 |
| Eurasian Blackbird (*Turdus merula*) | 7 | 1.3 |
| *Turdus* sp. | 1 | 0.2 |
| Yellow-legged Gull (*Larus michahellis*) | 12 | 2.2 |
| Common Cuckoo (*Cuculus canorus*) | 2 | 0.4 |
| Eurasian Green Woodpecker (*Picus viridis*) | 1 | 0.2 |
| *Amazona* sp. | 4 | 0.7 |
| *Anas* sp. | 1 | 0.2 |
| Undetermined bird | 45 | 8.2 |
| **Total birds** | **340** | **62.6** |
| **Reptiles** | | |
| Ocellated lizard (*Timon lepidus*) | 14 | 2.6 |

**Stable Isotopes.** The arithmetic mean isotopic values (±SD) for the 24 nestlings were −22.10‰ (±1.03) for $\delta^{13}C$, 6.44‰ (±1.27) for $\delta^{15}N$, and 4.30‰ (±1.43) for $\delta^{34}S$. Isotopic values of individuals from all the different territories showed broad ranges for the three elements (Fig. 3). However, we found that those nestlings hatched and reared in the same nest/territory had significant positive correlations for $\delta^{13}C$ ($r_s = 0.93$, $P < 0.001$), $\delta^{15}N$ ($r_s = 0.98$, $P < 0.001$), and $\delta^{34}S$ ($r_s = 0.95$, $P < 0.001$), and that these correlation values were in all cases significantly higher than expected by a random distribution ($P < 0.001$).

**Conventional Diet vs. Stable Isotopes.** We found a significant positive correlation between $\delta^{13}C$ in nestlings and the frequency of Eurasian red squirrels in their diet ($r_s = 0.565$, $P < 0.05$), as well as a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges ($r_s = -0.688$, $P \leq 0.005$) (Table 3). Despite not correlating with any particular prey item, high levels of $\delta^{34}S$ were found in the nestlings hatched in the two territories where Yellow-legged Gulls were consumed.

## DISCUSSION

The diet of Bonelli's Eagle in Catalonia during the nestling period primarily included medium-sized birds such as pigeons and Red-legged Partridges, mammals including European rabbits and Eurasian red squirrels, as well as a variety of less frequently consumed birds (Yellow-legged Gulls, Corvidae, and Turdidae) and a single reptile (ocellated lizard). This diet composition agreed with the general patterns found in other western European populations, where, overall, rabbits, pigeons, partridges, and corvids were the most frequently eaten prey (Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Mo-

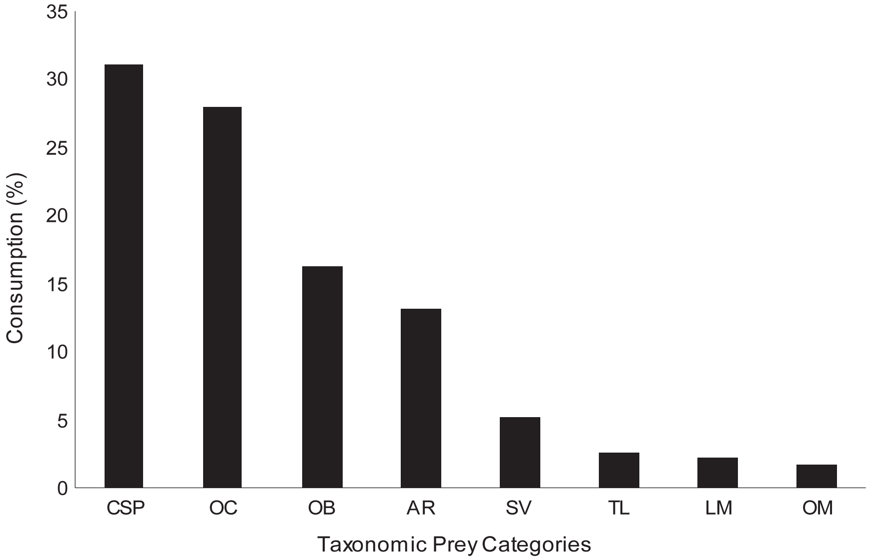

Figure 1. Prey consumption (%) by Bonelli's Eagle nestlings, Catalonia, Spain, as determined by pellet analysis. Taxonomic categories are ordered from greatest to lowest importance in diet: CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals'').

león et al. 2009b), and was particularly similar to diets described for the Mediterranean coastal strip of Spain and France, where rabbits are more scarce and the consumption of pigeons and ''other birds'' is greater (Moleón et al. 2009b).

In our study, the PCA suggested that the consumption of the two dominant prey types (pigeons and rabbits) determined the intake of other prey species. For example, those territories with low consumption of pigeons had greater intake of alterna-

Table 2. Prey category consumption (%) of nestlings at the territory level, based on pellet analyses. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals'').

| TERR | CSP | OC | OB | AR | SV | TL | LM | OM |
|---|---|---|---|---|---|---|---|---|
| 1 | 33.3 | 33.3 | 13.3 | 0 | 20.1 | 0 | 0 | 0 |
| 2 | 25.6 | 33.3 | 20.5 | 7.7 | 7.7 | 2.6 | 0 | 2.6 |
| 3 | 47.4 | 10.5 | 23.7 | 15.8 | 0 | 0 | 0 | 2.6 |
| 4 | 3.8 | 34.2 | 6.3 | 26.6 | 0 | 10.1 | 12.7 | 6.3 |
| 5 | 45.7 | 8.6 | 31.3 | 2.9 | 8.6 | 2.9 | 0 | 0 |
| 6 | 66.7 | 33.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 29 | 38.7 | 6.5 | 25.8 | 0 | 0 | 0 | 0 |
| 8 | 54.9 | 13.7 | 9.8 | 7.8 | 11.8 | 0 | 0 | 2 |
| 9 | 29 | 38.8 | 16.1 | 16.1 | 0 | 0 | 0 | 0 |
| 10 | 35.7 | 35.7 | 14.4 | 7.1 | 7.1 | 0 | 0 | 0 |
| 11 | 13.6 | 18.2 | 31.8 | 22.7 | 4.6 | 9.1 | 0 | 0 |
| 12 | 23.6 | 20.6 | 35.3 | 5.9 | 8.8 | 2.9 | 0 | 2.9 |
| 13 | 37.1 | 44.4 | 14.8 | 3.7 | 0 | 0 | 0 | 0 |
| 14 | 26.5 | 20.6 | 23.5 | 11.8 | 8.8 | 2.9 | 5.9 | 0 |
| 15 | 31.4 | 37.2 | 5.7 | 20 | 5.7 | 0 | 0 | 0 |

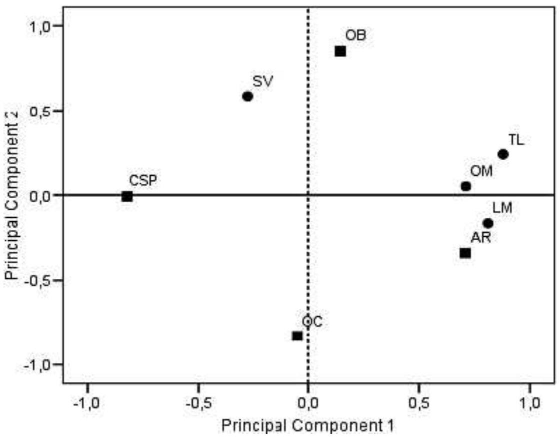

Figure 2. Principal component analysis of taxonomic prey category consumption at territory level. Components 1 and 2 (X-axis and Y-axis, respectively) provide information regarding the rotated and dimensionally reduced diet data. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals''). Solid black squares represent frequently consumed prey and solid black circles represent less frequently consumed prey.

tive prey species such as partridges or, less frequently, Yellow-legged Gulls, ocellated lizard, and small mammals. Similarly, in those territories where rabbits were not frequently consumed, other medium-sized bird species were more important. Variations in diet of Bonelli's Eagle in western Europe seem to be a function of spatio-temporal variation in the abundance of rabbits and the presence of alternative prey species, in conjunction with territorial environmental features (Moleón et al. 2009b). Consequently, the different dietary patterns found in our study at the territory level were likely influenced by the high heterogeneity in ecological features within territories, including habitat, and prey density and distribution.

Stable isotope signatures from nestlings exhibited broad ranges for the three elements we measured ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), a finding that agreed with the high diversity of taxonomic prey items revealed by the conventional pellet analysis. Consumers incorporate carbon into their tissues with an increase of around 1‰ in $^{13}C$ relative to their food (Kelly 2000) and so the wide range of $\delta^{13}C$ observed in our study (3.76‰) is probably due to a heterogeneous intake of prey species with different carbon

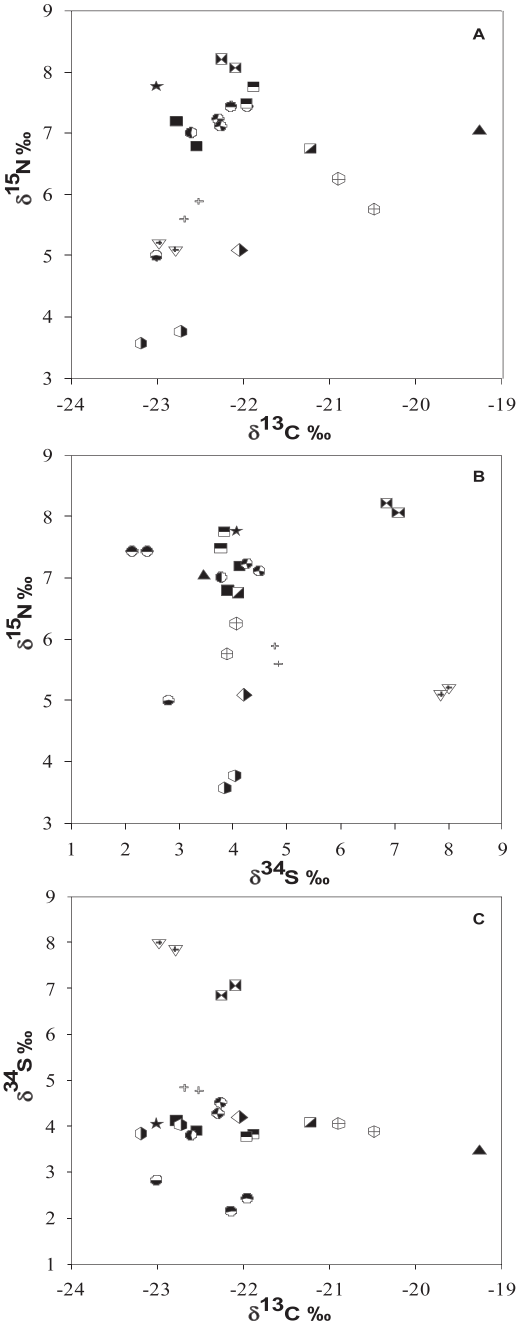

Figure 3. Isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of Bonelli's Eagle nestlings. Different symbols are associated with different territories ($n = 15$); nine territories had two nestlings. (a) $\delta^{15}N$ vs. $\delta^{13}C$, (b) $\delta^{15}N$ vs. $\delta^{34}S$ and (c) $\delta^{34}S$ vs. $\delta^{13}C$.

Table 3. Spearman correlation values ($r_s$) for correlations between diet of nestlings as determined by pellet analysis at the territory level and nestlings' isotopic values. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals''). Significant correlations ($P < 0.05$) are shown in bold type.

| | CSP | OC | OB | AR | SV | TL | LM | OM |
|---|---|---|---|---|---|---|---|---|
| $\delta^{13}C$ | 0.411 | −0.197 | 0.057 | **−0.688** | **0.565** | −0.258 | −0.199 | −0.183 |
| *P*-value | 0.128 | 0.480 | 0.840 | **0.005** | **0.028** | 0.353 | 0.477 | 0.514 |
| $\delta^{15}N$ | 0.025 | −0.228 | 0.350 | 0.091 | 0.347 | −0.036 | −0.042 | −0.441 |
| *P*-value | 0.930 | 0.414 | 0.201 | 0.747 | 0.205 | 0.898 | 0.881 | 0.100 |
| $\delta^{34}S$ | 0.021 | 0.196 | 0.057 | 0.039 | −0.338 | 0.060 | 0.157 | −0.460 |
| *P*-value | 0.940 | 0.485 | 0.840 | 0.889 | 0.218 | 0.831 | 0.576 | 0.085 |

isotopic signatures (Gu et al. 1997). Additionally, we found a significant positive correlation between $\delta^{13}C$ and the frequency of Eurasian red squirrels in nestlings' diet, as well as a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges. Interestingly, abundances of these two prey species at territory level are dependent on habitat types, with squirrels more common in forested territories and partridges more abundant in open habitats in our study area (Real et al. 1995, Mañosa 2004); these associations suggest that the analysis of $\delta^{13}C$ may be a good indicator of prey consumption and habitat features at the territory level. In the case of nitrogen, consumers are typically enriched in $^{15}N$ by 3–5‰ relative to their prey (Post 2002, Vanderklift and Ponsard 2003), a fact that allows the trophic level position of the prey species to be assessed (Kelly 2000). In our study, $\delta^{15}N$ ranged from 3.57 to 8.21‰, which suggested that the total diet within our study sample included prey species from at least two different trophic levels. This was supported by the wide range of prey species detected by the conventional pellet analysis, including herbivores (rabbits), granivores (pigeons), secondary consumers (thrushes and Corvidae), and even potential scavengers (Yellow-legged Gulls). Finally, the use of $\delta^{34}S$ in dietary studies has been recommended as a means of distinguishing between terrestrial and marine prey species (Peterson et al. 1985, Moreno et al. 2009). In our study, higher signatures of $\delta^{34}S$ were found at two territories where Yellow-legged Gulls were consumed, and that species was the only marine prey species identified in the pellet analysis. Accordingly, $\delta^{34}S$ signatures of this gull species from the same study area (Ramos et al. 2009) showed similar signatures to those found in Bonelli's Eagle nestlings that consumed it. The lack of significant correlation between $\delta^{34}S$ and the consumption of Yellow-legged Gulls probably resulted from the fact that it was consumed at only 2 of 15 territories.

Our interpretation of the SIA based on the diet composition of Bonelli's Eagle nestlings may be potentially constrained by a number of biases. A basic assumption when using SIA in the assessment of animal diets is that the main prey species have different isotopic composition (Bearhop et al. 2004, Matthews and Mazumder 2004). However, we did not analyze isotopic composition of prey species and instead used indirect evidence to evaluate the suitability of SIA as a means of inferring diet. First, the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of nestlings hatched and raised in the same nest were more similar than would be randomly expected. Given that Bonelli's Eagle nestlings share prey items (Real 1996), our results indicated that the isotopic signatures of nestlings were related to the prey consumed (see also Angerbjörn et al. 1994, Gu et al. 1997, Araújo et al. 2009). Second, we tested whether $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were correlated with prey consumption. In fact, we found significant correlations between $\delta^{13}C$ and two prey species, as well as other dietary patterns for $\delta^{15}N$ and $\delta^{34}S$ (above).

In recent decades, the use of stable isotopes in avian foraging studies has been increasingly used as a robust tool for providing long-term information on birds' foraging habits and degree of dietary specialization at both the individual and population level (Kelly 2000, Bolnick et al. 2002, Rubenstein and Hobson 2004, Inger and Bearhop 2008, Araújo et al. 2009). However, few isotopic studies have focused on raptors' dietary habits (but see Roemer et al. 2002, Dominguez et al. 2003, Caut et al. 2006), so the advantages of SIA in studies of raptors' trophic ecology are sometimes underestimated. Our study provided the first reference values for isotopic signatures in Bonelli's Eagle nestlings. One advantage of isotopic analyses is that they may overcome some of the biases traditionally associated with conventional procedures. For example, isotopic data from nestlings' feathers are representative of the nestlings' diet over the entire period of tissue development (Inger and Bearhop 2008), whereas pellets may be representative of a shorter period if they are not collected regularly. Moreover, isotopic data inform about prey digested and absorbed, and may overcome the over- or underrepresentation of certain prey items associated with conventional diet analyses (Inger and Bearhop 2008). In terms of effort, the pellet analysis is more time-consuming than isotopic analysis. SIA may also allow assessment of individual's diets, as, for example, when comparing the diet between siblings or between parents and nestlings. Finally, temporal changes or spatial heterogeneity in diet composition can be addressed with SIA (Bearhop et al. 2001, Rubenstein and Hobson 2004, Chiaradia et al. 2010); by analyzing the isotopic composition of nestlings' feathers, we may be able to monitor temporal variations in prey abundance at the territory level. The major disadvantage of SIA in dietary studies where we do not know the isotopic prey signatures is that we cannot distinguish individual prey species in the predators' diet.

Mediterranean landscapes have undergone important changes in terms of human activity and the extent of different types of land use (Meeus 1993, Butet et al. 2010), and such changes have influenced the distribution and abundance of Bonelli's Eagle prey and hence the conservation of this raptor species (Ontiveros et al. 2005, Moleón et al. 2009b). In our study, SIA proved useful for monitoring nestling Bonelli's Eagles' diets, which may reflect the abundance and distribution of prey at the territory level. Thus, the implementation of SIA on a regular basis at the territory level may be a valuable tool for monitoring not only the biological relationship between Bonelli's Eagle and its prey, but also temporal changes in Mediterranean habitats and ecosystems. Future isotopic analyses will provide further insights and a deeper understanding of the trophic ecology of Bonelli's Eagles.

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## LITERATURE CITED

ANGERBJÖRN, A., P. HERSTEINSSON, K. LIDÉN, AND E. NELSON. 1994. Dietary variation in arctic foxes (*Alopex lagopus*)—an analysis of stable carbon isotopes. *Oecologia* 99: 226–232.

ARAÚJO, M.S., D.I. BOLNICK, L.A. MARTINELLI, A.A. GIARETTA, AND S.F. DOS REIS. 2009. Individual-level diet variation in four species of Brazilian frogs. *Journal of Animal Ecology* 78:848–856.

BEARHOP, S., C. ADAMS, S. WALDRON, R. FULLER, AND H. MACLEOD. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73:1007–1012.

———, D.R. THOMPSON, R.A. PHILLIPS, S. WALDRON, K.C. HAMER, C.M. GRAY, S.C. VOTIER, B.P. ROSS, AND R.W. FURNESS. 2001. Annual variation in Great Skua diets: the importance of commercial fisheries and predation on seabirds revealed by combining dietary analyses. *Condor* 103:802–809.

———, S. WALDRON, S.C. VOTIER, AND R.W. FURNESS. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451–458.

BECKER, B.H., S.H. NEWMAN, S. INGLIS, AND S.R. BEISSINGER. 2007. Diet-feather stable isotope ($\delta^{15}$N and $\delta^{13}$C) fractionation in Common Murres and other seabirds. *Condor* 109:451–456.

BIRDLIFE INTERNATIONAL. 2004. Birds in Europe: population estimates, trends and conservation status. BirdLife Conservation Series No. 12, Cambridge, U.K.

BOLNICK, D.I., L.H. YANG, J.A. FORDYCE, J.M. DAVIS, AND R. SVANBÄCK. 2002. Measuring individual-level resource specialization. *Ecology* 83:2936–2941.

BOSCH, R., J. REAL, A. TINTÓ, E.L. ZOZAYA, AND C. CASTELL. 2010. Home-ranges and patterns of spatial use in territorial Bonelli's Eagles *Aquila fasciata*. *Ibis* 152:105–117.

Brom, T.G. 1986. Microscopic identification of feathers and feather fragments of palearctic birds. *Bijdragen tot de Dierkunde* 56:181–204.

Butet, A., N. Michel, Y. Rantier, V. Comor, L. Hubert-Moy, J. Nabucet, and Y. Delettre. 2010. Responses of Common Buzzard (*Buteo buteo*) and Eurasian Kestrel (*Falco tinnunculus*) to land use changes in agricultural landscapes of western France. *Agriculture, Ecosystems and Environment* 138:152–159.

Caut, S., G.W. Roemer, C.J. Donlan, and F. Courchamp. 2006. Coupling stable isotopes with bioenergetics to estimate interspecific interactions. *Ecological Applications* 16:1893–1900.

Chiaradia, A., M.G. Forero, K.A. Hobson, and J.M. Cullen. 2010. Changes in diet and trophic position of a top predator 10 yr after a mass mortality of a key prey. *ICES Journal of Marine Science* 67:1710–1720.

Crawford, K., R.A. McDonald, and S. Bearhop. 2008. Applications of stable isotope techniques to the ecology of mammals. *Mammal Review* 38:87–107.

del Hoyo, J., A. Elliott, and J. Sargatal [Eds.]. 1994. Handbook of the birds of the world, Vol. 2: New World Vultures to Guineafowl. Lynx Editions, Barcelona, Spain.

Dominguez, L., W.A. Montevecchi, N.M. Burgess, J. Brazil, and K.A. Hobson. 2003. Reproductive success, environmental contaminants, and trophic status of nesting Bald Eagles in eastern Newfoundland, Canada. *Journal of Raptor Research* 37:209–218.

Gil-Sánchez, J.M. 2000. Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). *Ardeola* 47:1–8.

———, M. Moleón, M. Otero, and J. Bautista. 2004. A nine-year study of successful breeding in a Bonelli's Eagle population in southeast Spain: a basis for conservation. *Biological Conservation* 118:685–694.

Gu, B., C.L. Schelske, and M.V. Hoyer. 1997. Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. *Ecology* 78:2263–2266.

Hobson, K.A. 1999. Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: implications for evaluating trophic relationships and breeding origins. *Condor* 101:799–805.

Iezekiel, S., D.E. Bakaloudis, and C.G. Vlachos. 2004. The diet of the Bonelli's Eagle *Hieraaetus fasciatus* in Cyprus. Pages 581–587 *in* R.D. Chancellor and B.-U. Meyburg [Eds.], Raptors worldwide: proceedings of the VI World Conference on Birds of Prey and Owls. World Working Group on Birds of Prey/MME, Berlin, Germany.

Inger, R. and S. Bearhop. 2008. Applications of stable isotope analyses to avian ecology. *Ibis* 150:447–461.

Jaksic, F.M. and M. Delibes. 1987. A comparative analysis of food-niche relationships and trophic guild structure in two assemblages of vertebrate predators differing in species richness: causes, correlations, and consequences. *Oecologia* 71:461–472.

Johnsgard, P.A. 2002. North American owls. Smithsonian Institution Press, Washington, DC U.S.A.

Katzner, T.E., E.A. Bragin, S.T. Knick, and A.T. Smith. 2006. Spatial structure in the diet of Imperial Eagles *Aquila heliaca* in Kazakhstan. *Journal of Avian Biology* 37:594–600.

Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1–27.

Korpimäki, E. and K. Norrdahl. 1991. Numerical and functional responses of kestrels, Short-eared Owls, and Long-eared Owls to vole densities. *Ecology* 72:814–826.

Krouse, H.R. and H.K. Herbert. 1988. Sulphur and carbon isotope studies of food webs. Pages 315–322 *in* B.V. Kennedy and G.M. LeMoine [Eds.], Diet and subsistence: current archaeological perspectives. Univ. of Calgary Archaeology Association, Calgary, AB Canada.

Mañosa, S. 2004. Perdiu roja *Alectoris rufa.* Pages 108–109 *in* J. Estrada, V. Pedrocchi, L. Brotons, and S. Herrando [Eds.], Atles dels ocells nidificants de Catalunya (1999–2002). Institut Català d'Ornitologia (ICO) and Lynx Edicions, Barcelona, Spain.

Marti, C.D., M. Bechard, and F.M. Jaksic. 2007. Food habits. Pages 129–151 *in* D.M. Bird and K.L. Bildstein [Eds.], Raptor research and management techniques. Hancock House Publishers Ltd., Surrey, BC Canada.

Martínez, J.E., M.A. Sánchez, D. Carmona, and J.A. Sánchez. 1994. Régime alimentaire de l'aigle de Bonelli *Hieraaetus fasciatus* durant la période de l'élevage des jeunes (Murcia, Espagne). *Alauda* 62:53–58.

Matthews, B. and A. Mazumder. 2004. A critical evaluation of intrapopulation variation of $\delta^{13}$C and isotopic evidence of individual specialization. *Oecologia* 140:361–371.

Meeus, J.H.A. 1993. The transformation of agricultural landscapes in western Europe. *The Science of Total Environment* 129:171–190.

Mizutani, H., M. Fukuda, and Y. Kabaya. 1992. 13C and 15N enrichment factors of feathers of 11 species of adult birds. *Ecology* 73:1391–1395.

Moleón, M., J. Bautista, J.A. Sánchez-Zapata, and J.M. Gil-Sánchez. 2009a. Diet of non-breeding Bonelli's Eagles *Hieraaetus fasciatus* at settlement areas of southern Spain. *Bird Study* 56:142–146.

———, J.A. Sánchez-Zapata, J. Real, J.A. García-Charton, J.M. Gil-Sánchez, L. Palma, J. Bautista, and P. Bayle. 2009b. Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. *Journal of Biogeography* 36:1502–1515.

Moreno, R., L. Jover, I. Munilla, A. Velando, and C. Sanpera. 2009. A three-isotope approach to disentangling the diet of a generalist consumer: the Yellow-legged Gull in northwest Spain. *Marine Biology* 157: 545–553.

Newton, I. 1998. Population limitation in birds. Academic Press, San Diego, CA U.S.A.

ONTIVEROS, D., J.M. PLEGUEZUELOS, AND J. CARO. 2005. Prey density, prey detectability and food habits: the case of Bonelli's Eagle and the conservation measures. *Biological Conservation* 123:19–25.

PALMA, L., P. BEJA, M. PAIS, AND L. CANCELA DA FONSECA. 2006. Why do raptors take domestic prey? The case of Bonelli's Eagle and pigeons. *Journal of Applied Ecology* 43:1075–1086.

PEARSON, S.F., D.J. LEVEY, C.H. GREENBERG, AND C. MARTÍNEZ DEL RIO. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon and isotopic signatures in an omnivorous songbird. *Oecologia* 135:516–523.

PETERSON, B.J. AND B. FRY. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320.

———, R.W. HOWARTH, AND R.H. GARRITT. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227:1361–1363.

POST, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumption. *Ecology* 83:703–718.

QUINN, G.P. AND M.J. KEOUGH. [EDS.]. 2002. Experimental design and data analysis for biologists. Cambridge Univ. Press, Cambridge, U.K.

RAMOS, R., F. RAMÍREZ, C. SANPERA, L. JOVER, AND X. RUIZ. 2009. Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a compartative assessment using conventional and isotopic methods. *Marine Ecology Progress Series* 377:289–297.

R DEVELOPMENT CORE TEAM. 2007. R: a language and environment for statistical computing, version 2.6.1. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org (last accessed 27 October 2011).

REAL, J. 1991. L'àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía. Ph.D. thesis, Univ. of Barcelona, Barcelona, Spain.

———. 1996. Biases in diet study methods in the Bonelli's Eagle. *Journal of Wildlife Management* 60:632–638.

———. 2004. Águila azor-perdicera, *Hieraaetus fasciatus*. Pages 154–157 *in* A. Madroño, C. González, and J.C. Atienza [EDS.], Libro rojo de las aves de España. Dirección General para la Biodiversidad-SEO/BirdLife, Madrid.

———, J. PIQUÉ, AND J.D. RODRÍGUEZ-TEIJEIRO 1995. Esquirol *Sciurus vulgaris*. Pages 45–50 *in* J. Ruiz-Olmo and A. Aguilar [EDS.], Els grans mamífers de Catalunya i Andorra. Lynx Edicions, Barcelona, Spain.

———, A. TINTÓ, A. BORAU, A. BENEYTO, AND X. PARELLADA. 2004. Àliga perdiguera *Hieraaetus fasciatus*. Pages 182–183 *in* J. Estrada, V. Pedrocchi, L. Brotons, and S. Herrando [EDS.], Atles dels ocells nidificants de Catalunya (1999–2002). Institut Català d'Ornitologia (ICO) and Lynx Edicions, Barcelona, Spain.

ROCAMORA, G. 1994. Bonelli's Eagle *Hieraaetus fasciatus*. Pages 184–185 *in* G.M. Tucker and M.F. Heath [EDS.], Birds in Europe: their conservation status. BirdLife International, Cambridge, U.K.

ROEMER, G.W., C.J. DONLAN, AND F. COURCHAMP. 2002. Golden Eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. *Proceedings of the National Academy of Science of the United States of America* 99:791–796.

RUBENSTEIN, D.R. AND K.A. HOBSON. 2004. From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* 19:256–263.

SALAMOLARD, M., A. BUTET, A. LEROUX, AND V. BRETAGNOLLE. 2000. Responses of an avian predator to variations in prey density at a temperate latitude. *Ecology* 81:2428–2441.

SÁNCHEZ, R., A. MARGALIDA, L.M. GONZÁLEZ, AND J. ORIA. 2008. Biases in diet sampling methods in the Spanish Imperial Eagle *Aquila adalberti*. *Ornis Fennica* 85:82–89.

VANDERKLIFT, M.A. AND S. PONSARD. 2003. Sources of variation in consumer-diet $\delta^{15}$N enrichment: a meta-analysis. *Oecologia* 136:169–182.

VOTIER, S.C., S. BEARHOP, A. MACCORMICK, N. RATCLIFFE, AND R.W. FURNESS. 2003. Assessing the diet of Great Skuas, *Catharacta skua*, using five different techniques. *Polar Biology* 26:20–26.

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# Appendix

Resano-Mayor et al. (2014) *Ibis*, 156, 176-188

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# Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet

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Diet analyses are central to the study of avian trophic ecology, and stable isotope analyses have made an increasing contribution in the last two decades. Few isotopic studies have assessed the diet of raptor species, which are more frequently analysed by conventional diet methods such as pellet analysis. In this study, we compare prey consumption estimates of nestling Bonelli's Eagles *Aquila fasciata* from conventional pellet analysis (in terms of items and biomass) and stable isotopic mixing models (SIAR) using $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S of feathers. The pellet analysis showed that European Rabbits *Oryctolagus cuniculus*, pigeons (mainly Common Wood Pigeons *Columba palumbus* and Domestic Pigeons *Columba livia dom.*), Red-legged Partridges *Alectoris rufa*, passerines, Yellow-legged Gulls *Larus michahellis* and Eurasian Red Squirrels *Sciurus vulgaris* were the main prey, so they were selected for diet reconstructions in SIAR. At the population level, mean prey consumption estimates were similar for pellets (both items and biomass) and SIAR. At the territory level, the weighted kappa statistic showed good ordinal scale agreement in main prey consumption between items or biomass and SIAR. Although the intraclass correlation coefficient showed poor method agreement when considering all prey in the same analysis, the intraclass correlation coefficients for each prey category showed significant agreement between pellets and SIAR when estimating the consumption of Rabbits, pigeons and Gulls, with lower agreement for passerines and Squirrels. Lastly, there was poor method agreement for estimates of Partridges. Our results suggest an overall agreement between the pellet analysis and SIAR when estimating nestling Bonelli's Eagle diet at both the population and, to a lesser extent, the territory level, supporting the usefulness of isotopic mixing models when identifying the terrestrial and marine components of raptor diets.

**Keywords:** carbon isotopes, conventional diet analysis, foraging ecology, isotopic mixing models, nitrogen isotopes, predators, raptors, sulphur isotopes.

Animal foraging ecology explains much of the observed variation among individual fitness correlates such as body condition, survival and breeding success (Schoener 1971, Pyke 1984, Inger *et al.* 2008, Terraube *et al.* 2012). As such, it can also explain population dynamics, prey–predator relationships and species distributions (Newton 1998, Moleón *et al.* 2009, Cortés-Avizanda *et al.* 2011). Nevertheless, measuring diet is often challenging due to the difficulty of making direct observations of feeding events over long periods, with consequent reliance on indirect methods and their potential biases (Real 1996, Votier *et al.* 2003, Huang *et al.* 2006).

The most common methods of diet assessment in birds are direct observations of feeding habits and analyses of nest food remains, individual

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stomach contents, faecal droppings and regurgitated pellets (Marti *et al.* 2007, Maziarz & Wesolowski 2010, Michalski *et al.* 2011, Bourass *et al.* 2012), although these methods have a number of limitations (Real 1996, Votier *et al.* 2003). For instance, they usually involve a great effort in terms of data collection and analysis. Moreover, they often reflect only a snapshot of a consumer's diet (Inger & Bearhop 2008) and present potential biases linked to prey sizes or digestibility (Brown & Ewins 1996, Real 1996, Votier *et al.* 2003, Marti *et al.* 2007).

Over the last two decades, the use of stable isotope analysis (SIA) to study avian trophic ecology has increased considerably (Kelly 2000, Inger & Bearhop 2008, Hobson 2011). The isotopic ratios in bird tissue reflect its diet at the time of tissue synthesis in a predictable manner. The shift in isotope ratio between diet and consumer tissue is known as the trophic enrichment factor (TEF) and can be used in isotopic mixing models to quantify the relative contributions of isotopically distinct sources to the diet of individuals or populations (Inger *et al.* 2006, Moreno *et al.* 2010). More recently, Bayesian isotopic mixing models have been developed to account for uncertainty and variation in model estimates, allow for multiple dietary sources, and generate potential dietary solutions as true probability distributions (Moore & Semmens 2008, Jackson *et al.* 2009, Parnell *et al.* 2010). Nevertheless, the use of isotopic mixing models requires accurate prior information regarding the trophic ecology of the studied species, and dietary estimates from mixing models would be only as good as the assumptions and parameters on which they depend (Bond & Diamond 2011, Hobson 2011).

Despite the applicability of both conventional diet analyses and isotopic mixing models to determine avian diets, and the caveats and potential biases associated with each, few studies have compared these methods (but see Doucette *et al.* 2011, Steenweg *et al.* 2011, Weiser & Powell 2011). Moreover, although conventional methods have been used traditionally to assess raptor food habits (Real 1996, Marti *et al.* 2007, Sánchez *et al.* 2008, Bakaloudis *et al.* 2012), to date few isotopic studies have focused on assessing the diets of avian terrestrial predators, including most raptor species (but see Roemer *et al.* 2002, Caut *et al.* 2006). Consequently, the potential applicability of isotopic mixing models to assess raptor foraging ecology is still poorly understood. The fact that isotopic data inform about assimilated rather than just ingested prey is a major advantage of using isotopic analysis to study raptor diet. Moreover, isotopic mixing models provide a powerful tool to estimate the foraging ecology of individuals to test the incidence and implications of individual resource use (Bolnick *et al.* 2003). Finally, isotopic analysis may constitute a homogeneous sampling procedure to monitor temporal or spatial variation in raptor diets.

Bonelli's Eagle *Aquila fasciata* is distributed from the western Mediterranean to southeast Asia (del Hoyo *et al.* 1994). The European population is now classified as endangered after a marked decline in number and range in recent decades (BirdLife International 2004), related to unnaturally high mortality rates, habitat degradation and decline of their main prey species (Real 2004, Hernández-Matías *et al.* 2011). The diet of Bonelli's Eagle in the Mediterranean has been widely studied by conventional methods, showing that the species mainly predates European Rabbits *Oryctolagus cuniculus*, partridges *Alectoris* spp., pigeons *Columba* spp., passerines (mainly corvids and thrushes) and lizards (Real 1991, Moleón *et al.* 2009). More recently, an isotopic approach showed that $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ are useful to assess both terrestrial and marine prey consumption of Bonelli's Eagle nestlings (Resano *et al.* 2011). Consequently, this species is a suitable model to test whether conventional diet analysis and isotopic mixing models provide similar information when assessing the diet of avian predators.

The aim of this study was to compare prey consumption estimates of nestling Bonelli's Eagles using conventional pellet analysis and Bayesian isotopic mixing models by (1) performing a comprehensive pellet analysis in terms of prey item consumption and prey assimilated biomass, (2) characterizing the isotopic composition of main prey types and (3) comparing main prey consumption estimates obtained from the pellet analysis and the isotopic mixing models.

## METHODS

### Study area and data collection

From 2008 to 2010 we monitored 43 successful breeding attempts of 28 territorial pairs of Bonelli's Eagle in Catalonia (41°20′N, 01°32′E). Habitat characteristics differed between territories but all showed Mediterranean landscape features (Carrascal & Seoane 2009, Bosch *et al.* 2010), with an average annual rainfall ranging from 425 to 664 mm. All sampled nests were located on cliffs, and the altitude of nesting areas ranged from 30 to 776 m asl.

From January to early March, we monitored breeding territories to assess occupancy and breeding activity. In late March and April, occupied nests were checked to detect the number of nestlings and their age, which was estimated by feather development and laying date (Real 1991, Gil-Sánchez 2000). To minimize the risk of disturbance, observations were always carried out from long distances using 10× binoculars and 20–60× spotting scopes. Once nestlings were approximately 37 days old, we caught them with the assistance of experienced climbers and sampled three to four mantle feathers from each individual for SIA. Pellets were collected from the nests after the breeding season and analysed to determine nestling diet by conventional methods (Real 1996).

To characterize isotopically the main prey of Bonelli's Eagle, we collected muscle samples from 215 individuals of the following species or species groups during 2008–2011: European Rabbits ($n$ = 42), Red-legged Partridges *Alectoris rufa* ($n$ = 38), Common Wood Pigeons *Columba palumbus* ($n$ = 39), Domestic Pigeons *Columba livia dom.* ($n$ = 45), passerines (Corvidae, Sturnidae and Turdidae; $n$ = 40), Yellow-legged Gulls *Larus michahellis* ($n$ = 4) and Eurasian Red Squirrels *Sciurus vulgaris* ($n$ = 7). All individuals were obtained from the studied Eagles' breeding territories, either in the nests or their surroundings, except most passerines and some Squirrels, which came from rehabilitation centres located in the study area.

## Pellet analysis

Each prey item identified in each pellet was counted as one item (Real 1996, Gil-Sánchez *et al.* 2004). Pellet contents (i.e. feathers, bones, hair, nails and scales) were identified with the help of a reference collection, a 4× magnifying glass and consulting specialized guides (Brom 1986, Brown *et al.* 2003). Prey items were identified to species level whenever possible.

Prey consumption was estimated for any given territory and year (hereafter referred to as territory level; $n$ = 43) as percentages of total items and total biomass, as is common in raptor diet studies (Real 1996, Sánchez-Zapata & Calvo 1998). To calculate the biomass of each prey type we used the weights of each prey species, corrected for the degree of consumption by adults at the nest before delivering prey to the chicks. Mean weights of prey species were obtained from the literature (Brom 1986, Real 1991, del Hoyo *et al.* 1997), most estimates being from measurements of individuals from the study area (see Supporting Information Table S1). Consumption of each prey type was estimated on the basis of field observations of feeding events from a hide ($n$ = 182 prey items; J. Real unpubl. data). Therefore our final prey net biomass estimates (Table S1) were representative of the nestlings' ingested biomass rather than total prey biomass. More than 20 prey items in each territory and year were included to ensure reliability in the pellet analysis (Ontiveros *et al.* 2005).

Based on the results of pellet analysis, the most-consumed prey categories in terms of items or biomass (hereafter main prey categories) were selected for comparison using the two diet assessment methods: European Rabbits, pigeons (mostly Common Wood Pigeons and Domestic Pigeons), Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels. Main prey consumption values from either the items or the biomass approaches were re-scaled relative to their global percentage in each territory to ensure that main prey categories accounted for the 100% of the diet in each territory. These re-scaled values were used for comparison with estimates obtained from the isotopic mixing models.

## Prey isotopic characterization

Isotopic signatures of species may be influenced by their local environment (Connolly *et al.* 2004, Choi *et al.* 2007) and hence isotopic values of main prey categories may differ between Bonelli's Eagle territories. To test this, prey samples of the most widely consumed prey (Rabbits, pigeons and Partridges) were characterized as a function of proximity to the sea and to habitat in the Eagle territory from which they came. Territories located on coastal cliffs or in coastal mountain ranges were classified as marine, and those farther inland as terrestrial. Territory habitat was measured in a 3.3-km radius around the nest to represent the home-range used by Bonelli's Eagles (Bosch *et al.*

2010), and each territory was classified according to its predominant habitat as either forest, scrubland or agricultural. Habitat predominance was estimated using MiraMon v6.4 software (Pons 2002) from land cover data available at a scale of 1 : 3000 and updated in December 2009.

## Stable isotope analysis

A 3-$cm^3$ piece of muscle from the chest or leg of each sampled prey animal was lyophilized for 48 h. Samples were lipid-extracted using several chloroform–methanol (2 : 1) rinses following Folch *et al.* (1957). Muscle was ground into fine powder using an impactor mill (Freezer/mill 6750 Spex Certiprep) and subsamples of approximately 0.32 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 5.6 mg (for $\delta^{34}S$) were loaded in tin receptacles and crimped for combustion. Nestling feathers were first cleaned in a solution of NaOH (0.25 M) and oven-dried at 40 °C for 24 h. Feathers were ground into fine powder and subsamples of 0.35 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 3.7 mg (for $\delta^{34}S$) were loaded in tin receptacles before combustion. Isotopic measurements of both prey and nestlings were performed at the Scientific and Technological Centres of the University of Barcelona using the methods of Resano *et al.* (2011).

Stable isotope ratios are reported as $\delta$ values and expressed in ‰, according to the following equation: $\delta X = [(R\text{sample}/R\text{standard}) - 1] \times 1000$, where $X$ is $^{13}C$, $^{15}N$ or $^{34}S$ and $R$ is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. $R$standard is the ratio of the international standards: Pee Dee Belemnite (PDB) for $^{13}C$, atmospheric nitrogen (AIR) for $^{15}N$ and Canyon Diablo Troilite (CDT) for $^{34}S$. Measurement precisions for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were $\leq 0.15$‰, $\leq 0.25$‰ and $\leq 0.40$‰, respectively.

## Bayesian isotopic mixing models

We used the SIAR package for R (Parnell *et al.* 2010) to estimate the relative contribution of main prey categories to the diet of Bonelli's Eagle nestlings at the territory level. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from nestlings and prey were included in the models. Each nest and year was considered a single statistical observation by estimating the mean isotopic values of sampled siblings. Prey isotopic values were selected for each territory: either the overall mean prey values when no effect of environmental features on prey signature was detected, or different values for a single prey when their isotopic values were affected by environmental features (see below). The TEFs for $\delta^{13}C$ (2.1‰ ± 0.08 sd) and $\delta^{15}N$ (2.7‰ ± 0.5 sd) were those obtained for feathers of Peregrine Falcons *Falco peregrinus* fed on muscle of Japanese Quail *Coturnix japonica* (Hobson & Clark 1992). We selected those values because the consumer in that experiment was taxonomically related to our consumer species, and the tissues analysed from both consumers and prey also matched those we studied. The TEF for $\delta^{34}S$ (0‰ ± 0.5 sd) was also obtained from the literature (Michener & Lajtha 2007), where it is commonly assumed that there is no enrichment in $^{34}S$ in animal diets. Common Wood Pigeon and Domestic Pigeon were included as separate sources within the models, and their consumption estimates from SIAR were summed *a posteriori* to allow for direct comparison with the pellet data.

## Statistical analyses

Prey isotopic data were checked for departures from normality using the Kolmogorov–Smirnov test and Q–Q plots. We performed a multivariate analysis of variance (MANOVA) to assess whether prey isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ as the dependent variables) differed by species (fitted as a single factor with Rabbits, Common Wood Pigeons, Domestic Pigeons, Partridges, passerines, Gulls and Squirrels as the group categories; $n = 215$). Additionally, we performed a second MANOVA to assess the effect of species, sea proximity and habitat (fitted as fixed effects) on prey isotopic values, but only including the most widely consumed prey (Rabbits, Common Wood Pigeons, Domestic Pigeons and Partridges; $n = 164$). Passerines, Gulls and Squirrels were excluded from this analysis because they were rare in some territory categories. Those factors with a significant effect in the MANOVAs were subjected to one-way analysis of variance (ANOVA) to test the factor effect on each dependent variable ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) separately (Quinn & Keough 2002). Levene's test was used to detect heteroscedasticity and Welch's correction was applied accordingly. Post-hoc pairwise analysis included Tukey's procedure or the Tamhane test when variances were heterogeneous. Prey isotopic values are reported as means ± sd.

To estimate prey consumption at the population level by the pellet analysis, we first calculated mean diet for each territory ($n = 28$), and then a mean was calculated across all territories. Re-scaled values of the main prey categories (for both items and biomass) and SIAR estimates were used for method comparisons ($n = 43$ territory-years) both at the population and the territory levels. These prey consumption percentages were arcsine-transformed and checked for normality using the Kolmogorov–Smirnov test and Q–Q plots. At the population level, a two-way ANOVA was used to assess whether prey consumption estimates differed by prey category and dietary method, with prey consumption estimates from all territories as the dependent variable and both prey category and dietary method as fixed factors. Additionally, separate one-way ANOVAs were used to test the method effect on each prey category. At the territory level, the weighted kappa statistic (Kw) was used to assess agreement between methods on an ordinal scale by ordering main prey categories from higher to lower rates of consumption, and the intraclass correlation coefficient (ICC) was used to test for the agreement of two methods in their quantitative prey consumption estimates. In this regard, we first calculated the ICCs using a three-way mixed effects model (Zhou *et al.* 2011) with prey consumption values as the dependent variable, territory and method as random effects, and prey category as a fixed factor. Bland–Altman plots (Bland & Altman 1986) were created to represent the method's repeatability in prey estimates. Secondly, ICCs were calculated by a two-way mixed effects model (McGraw & Wong 1996) for each prey category.

Statistical analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL, USA) and MEDCALC 12.3.0 (MedCalc Software, Mariakerke, Belgium). SIAR was run using R software (R Development Core Team 2007).

## RESULTS

### Pellet analysis

We identified 2254 prey items in the 979 pellets analysed, corresponding to at least 31 prey species (Table S1). Birds accounted for 59.3%, mammals for 33.6% and reptiles for 7.1% of prey items, and 55.2%, 40.8% and 4% of biomass, respectively. At the population level, the most frequently consumed prey were pigeons (26.3%), Rabbits (21.1%), passerines (10.7%) and Red-legged Partridges (10.6%), which together accounted for 68.7% of dietary items. In terms of biomass, Rabbits were the main prey item (30.9%), followed by pigeons (26.9%), Yellow-legged Gulls (8.7%), Partridges (8.1%) and Squirrels (4.9%), together accounting for 79.5% of total biomass ingested.

### Prey isotopic characterization

There was a significant difference between main prey items in isotopic values (MANOVA: Wilks' lambda, $F_{18,583} = 17.56$, $P < 0.001$). There were overall differences between prey categories in $\delta^{13}$C (one-way ANOVA: $F_{\text{Welch } 6,30} = 48.63$, $P < 0.001$), but Red-legged Partridges, Common Wood Pigeons and passerines, and Domestic Pigeons, Eurasian Red Squirrels and Yellow-legged Gulls formed two sub-groups of prey within which pairwise differences were not significant. Overall significant differences in $\delta^{15}$N (one-way ANOVA: $F_{8,206} = 22.20$, $P < 0.001$) were related to prey trophic level. For instance, $\delta^{15}$N in rabbits was significantly lower than in other prey except Squirrels, and Yellow-legged Gulls had significantly higher $\delta^{15}$N than most prey. Fewer pairwise differences in $\delta^{15}$N were found between Squirrels, Partridges, pigeons or passerines. Yellow-legged Gulls showed the highest $\delta^{34}$S values, and most of the significant differences in $\delta^{34}$S (one-way ANOVA: $F_{8,36} = 7.45$, $P < 0.001$) seemed to be related to a marine influence (see below). Mean prey isotopic values included in the isotopic mixing models are summarized in Table 1. Isotope biplots ($\delta^{13}$C, $\delta^{15}$N, $\delta^{34}$S) showed that Bonelli's Eagle nestlings lay within the space delineated by main prey categories previously corrected by TEFs (Fig. 1).

In the second MANOVA we found a significant overall effect of species (MANOVA: Wilks' lambda, $F_{9,355} = 16.11$, $P < 0.001$), sea proximity (MANOVA: Wilks' lambda, $F_{3,146} = 9.92$, $P < 0.001$) and habitat (MANOVA: Wilks' lambda, $F_{6,292} = 7.90$, $P < 0.001$) on isotopic prey values, with a significant interaction between species and both sea proximity (MANOVA: Wilks' lambda, $F_{9,355} = 3.97$, $P < 0.001$) and habitat (MANOVA: Wilks' lambda, $F_{18,413} = 1.78$, $P < 0.05$). European Rabbits and Domestic Pigeons from marine territories had higher $\delta^{34}$S than those from terrestrial territories (one-way ANOVA: $F_{1,40} = 21.12$, $P < 0.001$ and $F_{1,43} = 19.28$, $P < 0.001$, respectively; Fig. 2). Moreover, Domestic Pigeons from agricultural terri-

**Table 1.** $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (mean ± sd; ‰) in main prey types of Bonelli's Eagle in Catalonia: European Rabbits (OC), Common Wood Pigeons (CP), Red-legged Partridges (AR), Domestic Pigeons (CL), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Within prey types, significant values differing either by sea proximity, habitat or both, are shown in bold type. Samples from marine or terrestrial territories are shown as (m) or (t), respectively. Samples from different habitat types are shown as forest (1), scrubland (2) or agricultural (3). Mean prey values were considered when there was no significant influence of environmental features. Prey $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values listed in this table were those included in the SIAR, accordingly selected for each territory depending on their sea proximity or habitat type.

| Prey | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{34}S$ |
|---|---|---|---|
| OCm | –25.33 ± 1.02 | 3.17 ± 2.50 | **8.81 ± 2.62** |
| OCt | –25.33 ± 1.02 | 3.17 ± 2.50 | **5.22 ± 2.07** |
| CP | –23.98 ± 0.88 | 5.54 ± 1.75 | 5.33 ± 1.68 |
| AR | –23.32 ± 1.30 | 5.81 ± 1.77 | 6.10 ± 2.59 |
| $CLm_1$ | –21.28 ± 3.88 | 7.15 ± 2.03 | **6.38 ± 0.73** |
| $CLt_1$ | –21.28 ± 3.88 | 7.15 ± 2.03 | **4.74 ± 1.34** |
| $CLm_2$ | –21.28 ± 3.88 | **6.85 ± 0.77** | **6.38 ± 0.73** |
| $CLt_2$ | –21.28 ± 3.88 | **6.85 ± 0.77** | **4.74 ± 1.34** |
| $CLm_3$ | –21.28 ± 3.88 | **8.06 ± 1.37** | **6.38 ± 0.73** |
| $CLt_3$ | –21.28 ± 3.88 | **8.06 ± 1.37** | **4.74 ± 1.34** |
| PAS | –23.36 ± 0.72 | 7.25 ± 1.24 | 6.51 ± 0.90 |
| LM | –20.50 ± 0.55 | 9.35 ± 0.55 | 11.97 ± 3.88 |
| SV | –19.36 ± 1.60 | 3.23 ± 1.99 | 5.47 ± 2.08 |

tories had higher $\delta^{15}N$ than those from scrubland territories (one-way ANOVA: $F_{\text{Welch } 2,27} = 4.60$, $P < 0.05$; Fig. 2). On the other hand, isotopic values of Rabbits were not influenced by habitat, and neither sea proximity nor habitat features influenced isotopic values of Common Wood Pigeons or Red-legged Partridges.

## Comparison of pellet analysis and SIAR

At the population level, mean consumption estimates differed by prey category (two-way ANOVA: $F_{5,756} = 145.76$, $P < 0.001$) and dietary method (two-way ANOVA: $F_{2,756} = 8.01$, $P < 0.001$). Although there was a significant interaction between prey category and the dietary method effects (two-way ANOVA: $F_{10,756} = 4.38$, $P < 0.001$), all methods estimated a similar dietary pattern of higher consumption of Rabbits and pigeons, and lower consumption of Partridges, passerines, Gulls and Squirrels (Fig. 3, Supporting Information Table S2). When comparing methods for each prey category (i.e. prey item counts from pellets vs. biomass estimation from pellets vs. SIAR), there were significant differences between

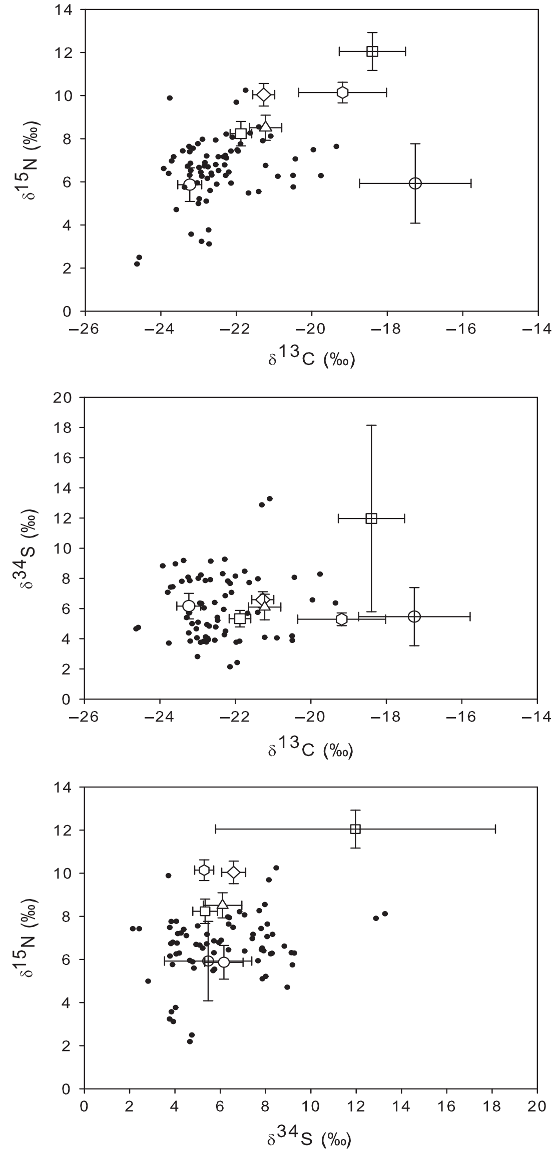

**Figure 1.** Isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of Bonelli's Eagle nestlings and main prey types (mean ± 95% CI) in Catalonia. Open symbols represent main prey types: European Rabbits (open circle), Common Wood Pigeons (open square), Red-legged Partridges (open triangle), Domestic Pigeons (open hexagon), passerines (open rhombus), Yellow-legged Gulls (crossed square) and Eurasian Red Squirrels (crossed circle). Closed dots represent Bonelli's Eagle nestlings. Prey isotopic values are corrected by TEFs (2.1‰ for $\delta^{13}C$, 2.7‰ for $\delta^{15}N$ and 0‰ for $\delta^{34}S$).

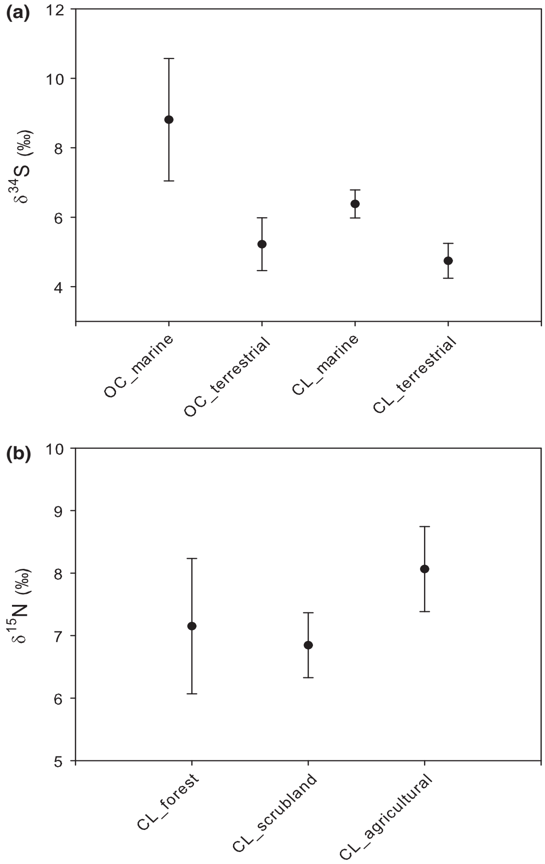

**Figure 2.** Influence of territorial environmental features on prey isotopic values. (a) Significant differences for $\delta^{34}$S in European Rabbits (OC) and Domestic Pigeons (CL) from marine and terrestrial territories. (b) Significant differences for $\delta^{15}$N in Domestic Pigeons (CL) from territories with scrubland and agricultural predominance. Domestic Pigeons from territories with forest predominance are shown, although they were not significantly different from any other habitat category. Prey isotopic values are represented as mean ± 95% CI.

prey item counts and biomass estimation in Rabbits (one-way ANOVA: $F_{2,126} = 4.47$, $P < 0.05$), between biomass estimation and SIAR in Partridges (one-way ANOVA: $F_{\text{Welch } 2,64} = 9.09$, $P < 0.001$), between biomass estimation and both prey item counts and SIAR in passerines (one-way ANOVA: $F_{\text{Welch } 2,64} = 22.06$, $P < 0.001$), between prey item counts and SIAR in Gulls (one-way ANOVA: $F_{\text{Welch } 2,67} = 12.80$, $P < 0.001$), and between biomass estimation and SIAR in Squirrels (one-way ANOVA: $F_{\text{Welch } 2,68} = 3.87$, $P < 0.05$).

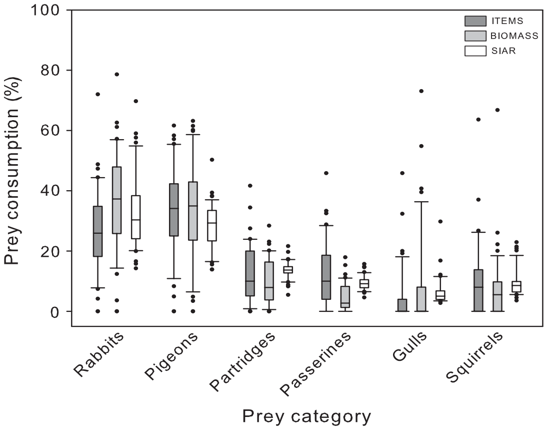

**Figure 3.** Boxplot with the consumption of main prey categories estimated by ITEMS (dark grey), BIOMASS (light grey) and SIAR (white) at the population level.

At the territory level, we found good agreement when ordering main prey categories from higher to lower levels of consumption between both prey item counts and SIAR (Kw = 0.47, 95% CI = 0.40–0.54) and between biomass estimation and SIAR (Kw = 0.53, 95% CI = 0.46–0.59). In both comparisons, the highest agreement was found when estimating the most- or least-consumed prey categories (1 or 6), with lower agreement for the other prey categories (Fig. 4).

The overall ICC showed low agreement among prey estimates when comparing prey item counts and SIAR (ICC = 0.30, $P = 0.13$) or biomass estimation and SIAR (ICC = 0.29, $P = 0.14$; Table 2). Bland–Altman plots illustrated a significant positive correlation between the difference in prey estimates between methods (items – SIAR or biomass – SIAR) and the mean prey consumption values obtained from both methods ($r_s = 0.35$, $P < 0.001$ for prey item counts vs. SIAR and $r_s = 0.47$, $P < 0.001$ for biomass estimation vs. SIAR; Fig. 5). In other words, the pellet analysis (in terms of both items and biomass) estimated lower consumption rates than SIAR for less-consumed prey, whereas the opposite was true for more-consumed prey.

When assessing agreement between methods for each prey category, we found agreement between prey item counts and SIAR estimates for Rabbits (ICC = 0.42, $P < 0.05$), pigeons (ICC = 0.44, $P < 0.05$) and Gulls (ICC = 0.55, $P < 0.01$), but no significant agreement between methods for passerines (ICC = 0.21, $P = 0.22$) or Squirrels

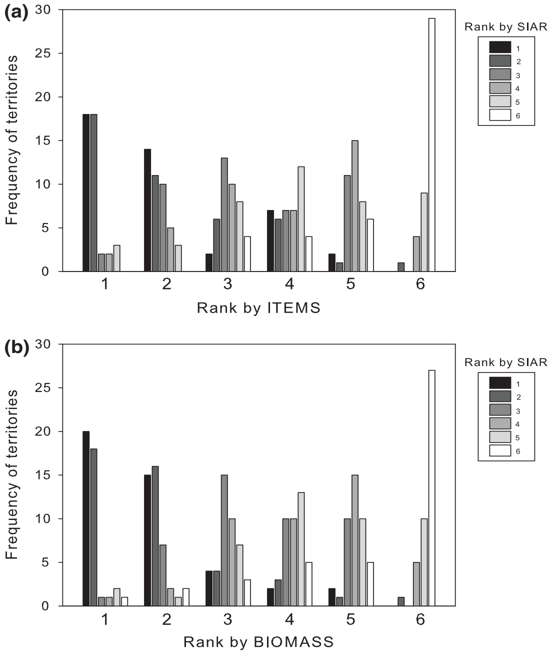

**Figure 4.** Agreement between ITEMS and SIAR (a) or BIOMASS and SIAR (b) in main prey consumption estimates when these are ranked in an ordinal scale (from higher to lower consumption) at the territory–year level. For the ITEMS and BIOMASS approaches, main prey categories are scaled from higher (1) to lower (6) consumption. According to SIAR, prey categories are scaled from higher (black) to lower (white) consumption. The *x*-axis shows all the rank combinations between methods (i.e. colours and numbers). The *y*-axis shows the number of territories (frequency) in which any of the colour–number combinations occurred.

**Table 2.** Intraclass correlation coefficients (ICCs) and *P*-values (*P*) when comparing prey consumption estimates at the territory level between ITEMS and SIAR, or between BIOMASS and SIAR. Results include both the overall intraclass correlation ($n = 516$), and the intraclass correlation done by prey ($n = 43$). Prey categories are European Rabbits (OC), pigeons (CSP), Red-legged Partridges (AR), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Significant *P*-values (< 0.05) are shown in bold type.

| | ICCs | *P* |
|---|---|---|
| Overall intraclass correlation | | |
| ITEMS vs. SIAR | 0.301 | 0.125 |
| BIOMASS vs. SIAR | 0.286 | 0.139 |
| | **ICCs** | ***P*** |
| Intraclass correlation by prey | | |
| OC – ITEMS vs. SIAR | 0.418 | **0.042** |
| CSP – ITEMS vs. SIAR | 0.437 | **0.033** |
| AR – ITEMS vs. SIAR | −0.316 | 0.812 |
| PAS – ITEMS vs. SIAR | 0.210 | 0.224 |
| LM – ITEMS vs. SIAR | 0.549 | **0.006** |
| SV – ITEMS vs. SIAR | 0.267 | 0.159 |
| OC – BIOMASS vs. SIAR | 0.314 | 0.113 |
| CSP – BIOMASS vs. SIAR | 0.430 | **0.036** |
| AR – BIOMASS vs. SIAR | −0.183 | 0.706 |
| PAS – BIOMASS vs. SIAR | 0.286 | 0.140 |
| LM – BIOMASS vs. SIAR | 0.432 | **0.035** |
| SV – BIOMASS vs. SIAR | 0.289 | 0.137 |

(ICC = 0.27, $P = 0.16$). Similarly, the biomass estimation and SIAR approaches showed a significant agreement for pigeons (ICC = 0.43, $P < 0.05$) and Gulls (ICC = 0.43, $P < 0.05$), but not for Rabbits (ICC = 0.31, $P = 0.11$), passerines (ICC = 0.29, $P = 0.14$) or Squirrels (ICC = 0.29, $P = 0.14$). Lastly, there was poor agreement for estimates of Partridges, both between prey item counts and SIAR (ICC = –0.32, $P = 0.81$) and between biomass estimation and SIAR (ICC = –0.18, $P = 0.71$; Table 2).

## DISCUSSION

We assessed the diet of Bonelli's Eagle nestlings in Catalonia, which included both marine and terrestrial prey, using conventional pellet analysis and Bayesian isotopic mixing models based on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. The pellet analysis revealed that European Rabbits, pigeons, Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels were the main prey items, and these were sampled for SIA. Our prey isotopic characterization accounted for the effect of environmental features (Table 1), and allowed reliable use of SIAR to estimate prey consumption at the territory level. Our results show an overall agreement in main prey consumption estimates between the pellet analysis and SIAR both at the population level and, to a lesser extent, at the territory level, where prey consumption was nonetheless similarly ranked by both methods (pellets vs. SIAR), especially in terms of the most- and least-consumed prey. Method comparisons through intraclass correlation for each prey category showed reasonable similarities, except in the case of Partridges. Overall, our results suggest that a combination of pellet analysis and SIA can be a useful way to assess the diet of predator species, and can add important

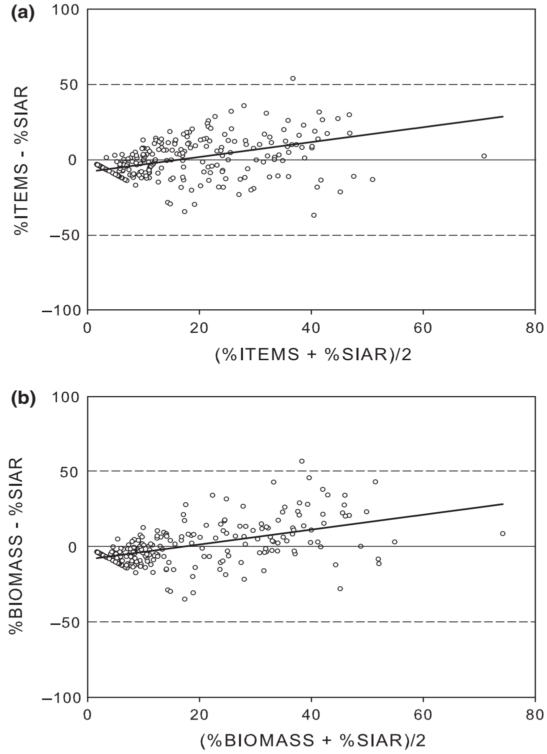

**Figure 5.** Bland–Altman plots showing the agreement between ITEMS and SIAR (a) or BIOMASS and SIAR (b) in main prey consumption estimates (%) at the territory–year level. The *y*-axis shows the difference in prey consumption estimates between ITEMS and SIAR (a) or BIOMASS and SIAR (b). The *x*-axis shows mean prey consumption estimates from both methods: (ITEMS+SIAR)/2 (a) or (BIOMASS+SIAR)/2 (b). Solid black lines at 0 indicate total method agreement (i.e. both methods estimated the same prey consumption percentage), whereas dashed lines at ± 50 indicate a disagreement in the method's prey estimates higher or lower than 50%. The linear trend between the variables plotted is shown.

insights with the application of isotopic analysis to study raptor food habits.

Conventional and isotopic methods each have advantages and disadvantages. Pellet analysis is non-invasive and allows detailed prey identification. However, it has potential biases related to prey size or digestibility (Votier *et al.* 2003, Marti *et al.* 2007), and may involve great effort in terms of both data collection and analysis of pellet contents. In contrast, SIA generates data about assimilated rather than ingested prey. Furthermore, isotopic analysis has the advantage that it provides diet estimates from the sampled individuals, which is frequently unachievable through conventional dietary methods, for example when several chicks are raised in the same nest. However, the use of isotopic mixing models to estimate prey consumption requires accurate prior information of the species' feeding ecology to select the right prey for tissue analysis, as well as suitable. Moreover, tissue collection for isotopic analysis requires handling of both consumer and prey, and laboratory analyses are more expensive than for conventional diet analyses. Therefore, conventional pellet and isotopic analyses can be considered complementary methods to monitor dietary patterns in territorial birds.

Our dietary results accord with other studies of Bonelli's Eagle in northeast Iberia, where the species takes more pigeons and fewer Rabbits and Partridges than populations in the southern Iberian Peninsula (Moleón *et al.* 2009). Moreover, in our study area, near the northern limit of the species' distribution in western Europe, local environmental conditions are more heterogeneous among territories than in southern populations, and this probably translates into greater dietary differences between territories, with some territorial pairs preying disproportionately on prey species that may be considered secondary or suboptimal elsewhere. For example, we show that Yellow-legged Gulls may constitute an important prey for some territorial pairs located in coastal areas, probably due the high abundance of Gulls in those territories (see also Resano *et al.* 2012).

Despite the importance of a comprehensive isotopic characterization of prey as the basis for SIA, logistical difficulties generally constrain prey sample collection. Therefore, published studies often present low prey sample sizes and rarely consider spatial heterogeneity in prey isotopic values (but see Hebert *et al.* 1999, Ramos *et al.* 2009, Moreno *et al.* 2010). In our study, we obtained large samples of individuals in most prey categories and achieved this across the whole study area, to assess whether individuals differed in their isotopic values due to environmental variation caused by proximity to the sea or habitat variation. For Rabbits and Domestic Pigeons we found that individuals collected in Eagle territories close to the sea showed higher $\delta^{34}$S than individuals from inland territories (Fig. 2), in accordance with the general trend of higher $\delta^{34}$S in species inhabiting marine ecosystems (Thode 1991, Deegan & Garritt 1997, Connolly *et al.* 2004). Moreover, Yellow-legged

Gulls, the only marine prey species detected in the diet of Bonelli's Eagle in our study, showed the highest $\delta^{34}$S values of all analysed prey. This supports the use of $\delta^{34}$S in distinguishing among the terrestrial and marine components in the diet of predator species foraging in both marine and terrestrial ecosystems (Moreno *et al.* 2010, Ramos *et al.* 2013). Conversely, prey isotopic values did not vary across habitat types, except in the case of Domestic Pigeons from agricultural habitats, which showed significantly higher $\delta^{15}$N than those from scrubland habitats (Fig. 2). This may be related to the use of nitrate-based fertilizers in agricultural areas (Choi *et al.* 2007) and the tendency of Domestic Pigeons to forage on agricultural crops in the study area (Authors pers. obs.). Based on these results, prey isotopic values included in SIAR for European Rabbits and Domestic Pigeons were selected according to environmental features (i.e. proximity to the sea and habitat type) of Eagle territories (Table 1), thus allowing consideration of spatial heterogeneity in prey isotopic values and increasing model accuracy when estimating Bonelli's Eagle nestling diet. The fact that the isotopic values of nestlings generally lay within the $\delta$-space delineated by main prey categories previously corrected by TEFs (Fig. 1) suggested both that main prey categories were representative of nestling diet, and that prey isotopic values and TEFs were reasonable. Overall, our isotopic characterization of prey highlights the importance of an extensive prey sampling strategy to avoid equivocal interpretations from isotopic prey base values and to resolve mixing models with higher reliability.

There was an overall agreement between pellet analysis (in terms of both items and biomass) and SIAR when estimating prey consumption by Bonelli's Eagles at the population level. Both methods estimated similar means and ranges for prey consumption, and showed that Rabbits and pigeons were consumed more than Partridges, passerines, Gulls and Squirrels (Fig. 3). Our results therefore suggest that both pellet analysis and SIAR are suitable methods to assess the diet of avian predators at the population level (Real 1996, Resano *et al.* 2011). At the territory level, we also found broad agreement in the relative rankings of prey consumption rates; that is, the most-consumed prey as assessed by pellet analysis was the same prey category identified as most-consumed by SIAR, and similarly for the least-consumed prey category (Fig. 4). This result supports the applicability of isotopic mixing models to infer main prey consumption patterns in territorial raptor species. In contrast, the intraclass correlation coefficient showed poor agreement when comparing consumption estimates of all prey categories in the same analysis, probably due to differences in agreement between methods for individual prey categories. For instance, the prey item counts and SIAR showed agreement in their consumption estimates of Rabbits, pigeons and Gulls, and biomass estimation and SIAR did the same for pigeons and Gulls (Table 2). We did not find significant agreement between methods for passerines and Squirrels; however, the ICCs showed certain similarities between method for those prey, especially between the biomass estimation and SIAR. The fact that passerines and Squirrels were the main prey categories with the lowest biomass could make them susceptible to be underestimated by the pellet analysis. Finally, we found poor agreement between methods in consumption estimates for Partridges, although we could not identify any evidence or possible causes to explain this.

Despite an overall agreement between the pellet analysis and SIAR in terms of main prey estimates, noticeable method discrepancies were found in the estimated percentages of some prey, especially at the territory level. This was not related to the origin of pellets (i.e. adults vs. nestlings) because adults rarely leave pellets in the nest (J. Real pers. obs.), but could be related to the fact that pellets and nestling feathers were temporally mismatched. Pellets represented nestling diet during the whole rearing period, whereas the isotopic composition of nestling feathers represented diet during feather growth (i.e. approximately half of the whole rearing period). Nevertheless, this would only affect our results in those cases where nestling diet changed during the second half of the rearing period.

In conclusion, our results support the potential of intrinsic biogeochemical markers (i.e. $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S) to infer the main prey consumption of raptor nestlings by analysing the isotopic composition of their feathers. Moreover, and in accordance with other isotopic studies of predator species, the use of $\delta^{34}$S could serve to assess the marine prey components in those raptor species foraging on both terrestrial and marine ecosystems (see Chamberlain *et al.* 2005). The use of isotopic mixing models to assess nestling diet would also allow individual diet estimates, thus offering a valuable approach to investigate the foraging ecology of individuals within a population, its ecological causes and fitness or evolutionary consequences. Nevertheless, the use of isotopic mixing models requires previous information of the species' feeding ecology, usually assessed by conventional diet analysis, a comprehensive prey isotopic characterization and reliable TEF estimates, which are usually available for some model species (e.g. Peregrine Falcon) but may be difficult to obtain for a particular species of interest. Future empirical research will contribute to a deeper understanding of the applicability and potential biases associated with isotopic analyses in avian predators, and we particularly encourage research to evaluate the usefulness of isotopic approaches to study the foraging ecology and its ecological implications in raptor species worldwide.

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## REFERENCES

**Bakaloudis, D.E., Iezekiel, S., Vlachos, C.G., Bontzorlos, V.A., Papakosta, M. & Birrer, S.** 2012. Assessing bias in diet methods for the long-legged Buzzard *Buteo rufinus*. *J. Arid Environ.* **77**: 59–65.

**BirdLife International.** 2004. *Birds in Europe: Population Estimates, Trends and Conservation Status*. BirdLife Conservation Series No. 12. Cambridge: BirdLife International.

**Bland, J.M. & Altman, D.G.** 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**: 307–310.

**Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L.** 2003. The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.* **161**: 1–28.

**Bond, A.L. & Diamond, A.W.** 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol. Appl.* **21**: 1017–1023.

**Bosch, R., Real, J., Tintó, A., Zozaya, E.L. & Castell, C.** 2010. Home-ranges and patterns of spatial use in territorial Bonelli's Eagles *Aquila fasciata*. *Ibis* **152**: 105–117.

**Bourass, K., Leger, J.F., Zaime, A., Qninba, A., Rguibi, H., El Agbani, M.A., Benhoussa, A. & Hingrat, Y.** 2012. Observations on the diet of the North African houbara bustard during the non-breeding season. *J. Arid Environ.* **82**: 53–59.

**Brom, T.G.** 1986. Microscopic identification of feathers and feather fragments of palearctic birds. *Bijdr. Dierkd.* **56**: 181–204.

**Brown, K.M. & Ewins, P.J.** 1996. Technique-dependent biases in determination of diet composition: an example with Ring-Billed Gulls. *Condor* **98**: 34–41.

**Brown, R., Ferguson, J., Lawrence, M. & Lees, D.** 2003. *Huellas y señales de las aves de España y Europa*. Barcelona: Ediciones Omega.

**Carrascal, L.M. & Seoane, J.** 2009. Factors affecting large-scale distribution of the Bonelli's Eagle *Aquila fasciata* in Spain. *Ecol. Res.* **24**: 565–573.

**Caut, S., Roemer, G.W., Donlan, C.J. & Courchamp, F.** 2006. Coupling stable isotopes with bioenergetics to estimate interspecific interactions. *Ecol. Appl.* **16**: 1893–1900.

**Chamberlain, C.P., Waldbauer, J.R., Fox-Dobbs, K., Newsome, S.D., Koch, P.L., Smith, D.R., Church, M.E., Chamberlain, S.D., Sorenson, K.J. & Risebrough, R.** 2005. Pleistocene to recent dietary shifts in California condors. *Proc. Natl Acad. Sci. USA* **102**: 16707–16711.

**Choi, W.J., Han, G.H., Lee, S.M., Lee, G.T., Yoon, K.S., Choi, S.M. & Ro, H.M.** 2007. Impact of land-use types on nitrate concentration and $\delta^{15}$N in unconfined groundwater in rural areas of Korea. *Agr. Ecosyst. Environ.* **120**: 259–268.

**Connolly, R.M., Guest, M.A., Melville, A.J. & Oakes, J.M.** 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* **138**: 161–167.

**Cortés-Avizanda, A., Almaraz, P., Carrete, M., Sánchez-Zapata, J.A., Delgado, A., Hiraldo, F. & Donázar, J.A.** 2011. Spatial heterogeneity in resource distribution promotes facultative sociality in two trans-Saharan migratory birds. *PLoS ONE* **6**: e21016, doi:10.1371/journal.pone.0021016.

**Deegan, L.A. & Garritt, R.H.** 1997. Evidence for spatial variability in estuarine food webs. *Mar. Ecol. Prog. Ser.* **147**: 31–47.

**Doucette, J.L., Wissel, B. & Somers, C.M.** 2011. Cormorant–fisheries conflicts: stable isotopes reveal a consistent niche for avian piscivores in diverse food webs. *Ecol. Appl.* **21**: 2987–3001.

**Folch, J., Lees, M. & Sloane-Stanley, G.H.** 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.

**Gil-Sánchez, J.M.** 2000. Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). *Ardeola* **47**: 1–8.

**Gil-Sánchez, J.M., Moleón, M., Otero, M. & Bautista, J.** 2004. A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. *Biol. Conserv.* **118**: 685–694.

**Hebert, C.E., Shutt, J.L., Hobson, K.A. & Weseloh, D.C.** 1999. Spatial and temporal differences in the diet of Great Lakes herring gulls (*Larus argentatus*): evidence from stable isotope analysis. *Can. J. Fish. Aquat. Sci.* **56**: 323–338.

**Hernández-Matías, A., Real, J. & Pradel, R.** 2011. Quick methods for evaluating survival of age-characterizable long-lived territorial birds. *J. Wildl. Manage.* **75**: 856–866.

**Hobson, K.A.** 2011. Isotopic ornithology: a perspective. *J. Ornithol.* **152**: S49–S66.

**Hobson, K.A. & Clark, R.G.** 1992. Assessing avian diets using stable isotopes II: factors influencing diet–tissue fractionation. *Condor* **94**: 189–197.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds)** 1994. *Handbook of the Birds of the World*, Vol. 2. Barcelona: Lynx Edicions.

**del Hoyo, J., Elliot, A. & Sargatal, J. (eds)** 1997. *Handbook of the Birds of the World*, Vol. 4. Barcelona: Lynx Edicions.

**Huang, K.Y., Lin, Y.S. & Severinghaus, L.L.** 2006. Comparison of three common methods for studying the diet of nestlings in two *Accipiter* species. *Zool. Stud.* **45**: 234–243.

**Inger, R. & Bearhop, S.** 2008. Applications of stable isotope analyses to avian ecology. *Ibis* **150**: 447–461.

**Inger, R., Ruxton, G.D., Newton, J., Colhoun, K., Robinson, J.A., Jackson, A.L. & Bearhop, S.** 2006. Temporal and intrapopulation variation in prey choice of wintering geese determined by stable isotope analysis. *J. Anim. Ecol.* **75**: 1190–1200.

**Inger, R., Gudmundsson, G.A., Ruxton, G.D., Newton, J., Colhoun, K., Auhage, S. & Bearhop, S.** 2008. Habitat utilisation during staging affects body condition in a long distance migrant, *Branta bernicla hrota*: potential impacts on fitness? *J. Avian Biol.* **39**: 704–708.

**Jackson, A.L., Inger, R., Bearhop, S. & Parnell, A.** 2009. Erroneous behavior of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). *Ecol. Lett.* **12**: E1–E5.

**Kelly, J.F.** 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**: 1–27.

**Marti, C.D., Bechard, M. & Jaksic, F.M.** 2007. Food habits. In Bird, D.M. & Bildstein, K.L. (eds) *Raptor Research and Management Techniques*: 129–151. Surrey, BC: Hancock House Publishers Ltd.

**Maziarz, M. & Wesolowski, T.** 2010. Timing of breeding and nestling diet of Wood Warbler *Phylloscopus sibilatrix* in relation to changing food supply. *Bird Study* **57**: 540–552.

**McGraw, K.O. & Wong, S.P.** 1996. Forming inferences about some intraclass correlation coefficients. *Psychol. Methods* **1**: 30–46.

**Michalski, M., Nadolski, J., Marciniak, B., Loga, B. & Banbura, J.** 2011. Faecal analysis as a method of nestling diet determination in insectivorous birds: a case study in Blue Tits *Cyanistes caeruleus* and Great Tits *Parus major*. *Acta Ornithol.* **46**: 164–172.

**Michener, R. & Lajtha, K.** 2007. *Stable Isotopes in Ecology and Environmental Science*, Vol. 2. Boston: Blackwell Publishers.

**Moleón, M., Sánchez-Zapata, J.A., Real, J., García-Charton, J.A., Gil-Sánchez, J.M., Palma, L., Bautista, J. & Bayle, P.** 2009. Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. *J. Biogeogr.* **36**: 1502–1515.

**Moore, J.W. & Semmens, B.X.** 2008. Incorporating uncertainity and prior information into stable isotope mixing models. *Ecol. Lett.* **11**: 470–480.

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

**Newton, I.** 1998. *Population Limitation in Birds*. San Diego: Academic Press.

**Ontiveros, D., Pleguezuelos, J.M. & Caro, J.** 2005. Prey density, prey detectability and food habits: the case of Bonelli's eagle and the conservation measures. *Biol. Conserv.* **123**: 19–25.

**Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L.** 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* **5**: e9672. doi:10.1371/journal.pone.0009672.

**Pons, X.** 2002. *MiraMon. Sistema d'Informació Geogràfica i software de Teledetecció*. Bellaterra: Centre de Recerca Ecològica i Aplicacions Forestals, CREAF.

**Pyke, G.H.** 1984. Optimal foraging theory: a critical review. *Annu. Rev. Ecol. Syst.* **15**: 523–575.

**Quinn, G.P. & Keough, M.J.** 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

**R Development Core Team.** 2007. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

**Ramos, R., Ramírez, F., Sanpera, C., Jover, L. & Ruiz, X.** 2009. Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods. *Mar. Ecol. Prog. Ser.* **377**: 289–297.

**Ramos, R., Ramírez, F. & Jover, L.** 2013. Trophodynamics of inorganic pollutants in a wide-range feeder: the relevance of dietary inputs and biomagnification in the Yellow-legged Gull (*Larus michahellis*). *Environ. Pollut.* **172**: 235–242.

**Real, J.** 1991. L'àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía. PhD Thesis, University of Barcelona.

**Real, J.** 1996. Biases in diet study methods in the Bonelli's Eagle. *J. Wildl. Manage.* **60**: 632–638.

**Real, J.** 2004. Águila azor-perdicera, *Hieraaetus fasciatus*. In Madroño, A., González, C. & Atienza, J.C. (eds) *Libro rojo de las aves de España*: 154–157. Madrid: Dirección General para la Biodiversidad-SEO/Birdlife.

**Resano, J., Hernández-Matías, A., Real, J. & Parés, F.** 2011. Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. *J. Raptor Res.* **45**: 342–352.

**Resano, J., Bayle, P., Real, J., Hernández, A., Vincent-Martin, N. & Ravayrol, A.** 2012. Analyse du régime alimentaire de l'Aigle de Bonelli *Hieraaetus fasciatus* (Vieillot, 1822) pendant la saison de reproduction 2010 en France. *Nature Provence* **1**: 95–101.

**Roemer, G.W., Donlan, C.J. & Courchamp, F.** 2002. Golden Eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. *Proc. Natl Acad. Sci. USA* **99**: 791–796.

**Sánchez, R., Margalida, A., González, L.M. & Oria, J.** 2008. Biases in diet sampling methods in the Spanish Imperial Eagle *Aquila adalberti*. *Ornis Fenn.* **85**: 82–89.

**Sánchez-Zapata, J.A. & Calvo, J.F.** 1998. Importance of birds and potential bias in food habit studies of Montagu's Harriers (*Circus pygargus*) in southeastern Spain. *J. Raptor Res.* **32**: 254–256.

**Schoener, T.W.** 1971. Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* **11**: 369–404.

**Steenweg, R.J., Ronconi, R.A. & Leonard, M.L.** 2011. Seasonal and age-dependent dietary partitioning between the Great Black-Backed and Herring Gulls. *Condor* **113**: 795–805.

**Terraube, J., Arroyo, B.E., Bragin, A., Bragin, E. & Mougeot, F.** 2012. Ecological factors influencing the breeding distribution and success of a nomadic, specialist predator. *Biodivers. Conserv.* **21**: 1835–1852.

**Thode, H.G.** 1991. Sulphur isotopes in nature and the environment: an overview. In Krouse, H.R. & Grinenko, V.A. (eds) *Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment*: 1–26. Chichester: Wiley.

**Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. & Furness, R.W.** 2003. Assessing the diet of Great Skuas, *Catharacta skua*, using five different techniques. *Polar Biol.* **26**: 20–26.

**Weiser, E.L. & Powell, A.N.** 2011. Evaluating gull diets: a comparison of conventional methods and stable isotope analysis. *J. Field Ornithol.* **82**: 297–310.

**Zhou, H., Muellerleile, P., Ingram, D. & Wong, S.P.** 2011. Confidence intervals and F test for intraclass correlation coefficients based on three-way mixed effects models. *J. Educ. Behav. Stat.* **36**: 638–671.

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## SUPPORTING INFORMATION

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

**Table S1.** Diet of Bonelli's Eagle nestlings in Catalonia based on pellet analysis ($n = 979$ pellets analysed).

**Table S2.** Prey consumption (mean ± sd; %) of main prey categories estimated by each dietary approach: ITEMS, BIOMASS and SIAR.

# Appendix

PLOS ONE

# Multi-Scale Effects of Nestling Diet on Breeding Performance in a Terrestrial Top Predator Inferred from Stable Isotope Analysis

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## Abstract

Inter-individual diet variation within populations is likely to have important ecological and evolutionary implications. The diet-fitness relationships at the individual level and the emerging population processes are, however, poorly understood for most avian predators inhabiting complex terrestrial ecosystems. In this study, we use an isotopic approach to assess the trophic ecology of nestlings in a long-lived raptor, the Bonelli's eagle *Aquila fasciata*, and investigate whether nestling dietary breath and main prey consumption can affect the species' reproductive performance at two spatial scales: territories within populations and populations over a large geographic area. At the territory level, those breeding pairs whose nestlings consumed similar diets to the overall population (i.e. moderate consumption of preferred prey, but complemented by alternative prey categories) or those disproportionally consuming preferred prey were more likely to fledge two chicks. An increase in the diet diversity, however, related negatively with productivity. The age and replacements of breeding pair members had also an influence on productivity, with more fledglings associated to adult pairs with few replacements, as expected in long-lived species. At the population level, mean productivity was higher in those population-years with lower dietary breadth and higher diet similarity among territories, which was related to an overall higher consumption of preferred prey. Thus, we revealed a correspondence in diet-fitness relationships at two spatial scales: territories and populations. We suggest that stable isotope analyses may be a powerful tool to monitor the diet of terrestrial avian predators on large spatio-temporal scales, which could serve to detect potential changes in the availability of those prey on which predators depend for breeding. We encourage ecologists and evolutionary and conservation biologists concerned with the multi-scale fitness consequences of inter-individual variation in resource use to employ similar stable isotope-based approaches, which can be successfully applied to complex ecosystems such as the Mediterranean.

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## Introduction

The trophic niche of a species refers to the range of food sources it uses and is a key component of the n-dimensional hypervolume niche concept [1]. Classical models based on the optimal foraging theory assume that individuals within populations respond similarly to spatial and temporal heterogeneity in resource availability [2–4]. According to this theory, individual consumers are expected to take preferred resources when food availability is high, or expand their food range by adding suboptimal resources when resource availability lowers [5–7]. More recently, variations in trophic resource use among individuals within a population have been highlighted to be a widespread phenomenon in nature, what may result from a broad range of mechanisms [8]. Apart from sex, age or individual's phenotype, individual diet variation may arise from their differences in dominance, experience or foraging ability [8–12]. Consequently, both extrinsic ecological factors and intrinsic organismal traits may generate a dietary spectrum within the population.

Despite the increasing understanding of the causes of individual diet variation within animal populations, its implications in terms of individual fitness are still poorly understood. Differences in trophic resource use among individuals are likely to affect their energy income and, therefore, to have an impact on fitness [13–15]. In this regard, individual diet variation has been shown to differently influence breeding success in several avian species, an issue particularly studied in seabirds [16–19]. For instance, individuals with lower diet diversity may increase their breeding success provided that low trophic diversity is the result of specialization on preferred prey [16,20]. The superior fitness of individuals with specialized diets, however, is not a universal pattern, and numerous studies have documented the fitness benefits of a generalized diet [19,21]. Therefore, the fitness consequences of individual dietary variation are not always easy to predict as different feeding strategies may be advantageous for different species and/or ecological scenarios, or even between different individuals within a population, depending on multiple factors like the nutritional quality of food, the physiology of the consumer, or the spatial and temporal availability of prey [21–24].

Several important drivers of the structure and dynamics of populations and communities, such as intraspecific competition, predation risk or parasitism, are linked to individual's resource use. Consequently, theoretical studies have highlighted the importance of intraspecific diet variation in shaping populations and communities [25–27]. In this regard, several studies have linked lower dietary diversity with higher breeding success at the population level, what has been explained by a higher consumption of preferred prey in those populations with lower diet diversity [28–30]. Conversely, populations exposed to heterogeneous landscapes or changing environmental conditions (e.g. food availability) can perform better if individuals within the population diversify in food resource use [8]. In fact, the correspondence between both scales (i.e. intra-population resource use and population processes) remains largely unknown in most natural systems so further studies are required to investigate how the relationship between diet and fitness at the individual level translates to the population scale.

Stable isotope analyse (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has been increasingly used by animal ecologists to assess individual resource use and intra-population niche partitioning [31–33], as it provides insightful dietary information at the individual level difficult to obtain by conventional procedures. In particular, the isotopic composition in metabolically inert tissues (e.g. feathers), represents consumer's diet at the time of deposition (e.g. feather growth), so SIA are a powerful tool to assess animal (e.g. avian) temporal and spatial dietary information at both the individual and population levels [34,35]. Consumer isotopic data delineated in δ-space (e.g. $\delta^{13}C$-$\delta^{15}N$ bi-plot), has been termed the "isotopic niche" [32], and is thought to be closely aligned with the true trophic niche. Recently developed metrics based on δ-space also allow for a species' trophic structure and diet diversity to be quantified not only at the individual, but also at the population and community levels [36,37]. Complementarily, the use of isotopic mixing models allow for individual diet reconstructions by converting isotopic data of consumers and main food resources into dietary proportions (p-space) [38–40], that can be translated into niche-width metrics commonly used by ecologists [41]. Surprisingly, the use of SIA to assess the trophic ecology of terrestrial avian top predators, either at the territory or the population levels, has rarely been done (but see [24,42]).

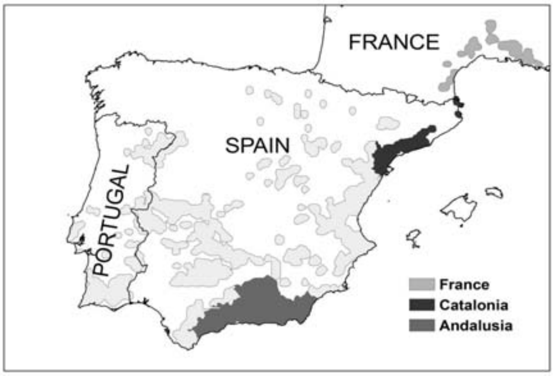

**Figure 1. Distribution area (shaded polygons) of Bonelli's eagle in western Europe (modified from [43]).** In darker grey we show the monitored populations, north to south, southern France, Catalonia and Andalusia (see legend).
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In this study, we use an isotopic approach to assess the trophic ecology of a long-lived raptor, the Bonelli's eagle *Aquila fasciata*, to explore the relationship between nestling diet and reproductive performance at both the territorial and population scales. Bonelli's eagle is a suitable model species because it shows marked intra- and inter-population demographic variations across its western European distribution range [43]. Moreover, the marked population decline occurred in this area in recent decades has been partly related to habitat degradation and main prey scarcity [44]. Here, both European rabbits *Oryctolagus cuniculus* (mostly) and red-legged partridges *Alectoris rufa* have been suggested to be optimal prey for Bonelli's eagle, so that they are preferentially consumed wherever they are abundant, and their consumption reduces eagle's diet diversity [45–49]. The dietary variation among territories is however substantial, and is thought to be influenced by habitat heterogeneity linked to crashes in rabbit numbers around two decades ago due to outbreaks of rabbit haemorrhagic disease, which indirectly impacted on the abundance of partridges and possibly other prey [46,48,50,51]. In the post-disease period, the average consumption of rabbits and partridges has been relatively low in most populations of western Europe, particularly in those located in the north of the Iberian Peninsula and France [48], what could reveal unprecedented effects of eagles' diet on fitness components such as breeding success (see [46]).

The specific objectives of this study were to i) describe the isotopic niche width and structure (as a proxy of trophic niche) of Bonelli's eagle nestlings in three populations of western Europe, ii) estimate nestling prey consumption at the intra-population (i.e. territory) level using isotopic mixing models, and iii) test the relationship between the dietary estimates and isotopic niche metrics with breeding success at both the territory and population levels. Our main prediction is that those territories and populations where nestlings have narrower trophic niches, what is expected to occur where nestling diets largely rely on rabbits and/or partridges, will perform better in terms of breeding success.

## Materials and Methods

### Ethics Statement

Both the monitoring of breeding pairs and sample collection of Bonelli's eagles were conducted to conform to the legal requirements of competent organisms. Permission to monitor breeding activity and to access nests in order to handle and sample eagle's nestlings and prey remains was granted by the CRBPO (Centre de Recherches sur la Biologie des Populations d'Oiseaux) that validates the banding program delegated by the Ministère de l'Écologie, du Développement durable et de l'Énergie (French Government) in France, by the "Servei de Biodiversitat i Protecció dels Animals" (Generalitat de Catalunya) in Catalonia, and by "Consejería de Medio Ambiente" (Junta de Andalucía) in Andalusia. To avoid disturbance, breeding monitoring observa-

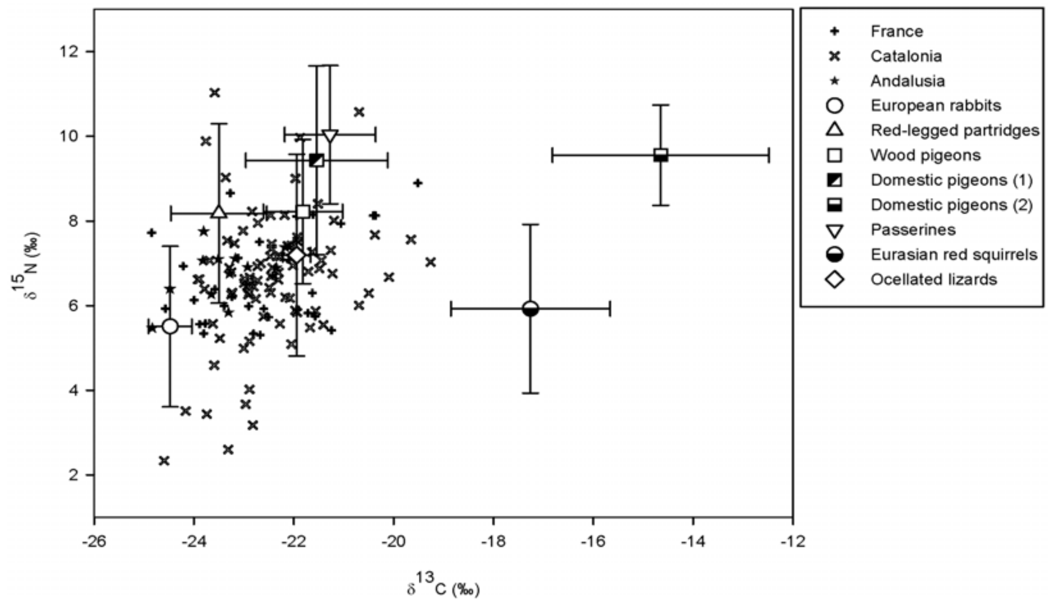

**Figure 2. Stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope values (‰) in Bonelli's eagle nestlings and their main prey.** Prey isotopic values were corrected for trophic discrimination factors (i.e. 2.1 and 2.7‰ for $\delta^{13}$C and $\delta^{15}$N, respectively; [59]). Single data points represent nestlings of each territory-year, with different symbols for France, Catalonia and Andalusia. Prey categories (mean ±95% SD) included: European rabbits, red-legged partridges, wood pigeons, domestic pigeons wildly foraging in crops (1), domestic pigeons from dovecotes and fed with corn (2), passerines, Eurasian red squirrels, and ocellated lizards (see figure legend).
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tions were always carried out away from nests by using 10x binoculars and 20–60x spotting scopes.

## Study Area

From 2008 to 2011 we monitored the main vital rates in 131 territorial Bonelli's eagle pairs located in three populations across the species' western European range, north to south, Provence and Languedoc-Roussillon (43°58′N, 03°20′E; southeast France; *n* = 30 and 31 pairs in 2010 and 2011, respectively), Catalonia (41°20′N, 01°32′E; northeast Spain; *n* = 52, 40, 44 and 45 in 2008, 2009, 2010 and 2011, respectively) and Andalusia (37°76′N, 03°85′W; southeast Spain; *n* = 45 in 2011) (Figure 1). The population in France had a breeding density of 0.29 territorial pairs/100 km$^2$, it showed a mean productivity of 0.98 fledglings/pair, an adult survival of 0.880, and the mean percentage of non-adult birds in territorial pairs was 8.39. The population in Catalonia had a breeding density of 0.64, it showed slightly higher values of productivity (1.13) and adult survival (0.889), and the mean percentage of non-adult birds was 10.81. The population in Andalusia had a breeding density of 0.85, it showed the highest values of productivity (1.23) and adult survival (0.926), and the mean percentage of non-adult birds was 3.86 [43]. All breeding nests were located on cliffs, and territories varied markedly with respect to habitat features, prey abundances or human activity [44].

## Data Collection

To assess the breeding success and productivity of monitored territorial pairs, known breeding areas were yearly visited a minimum of five days during the whole breeding period. Between January and March we checked the presence of territorial birds and breeding activity (i.e. incubation behaviour). In late March and April, occupied nests were checked to detect the presence, number, and age of nestlings, which was estimated by feather development and backdating from laying date [52]. Nestlings at the age of ≥50 days old were assumed to have fledged successfully [53].

For each territorial pair, individual turnover events or replacements (i.e. if the same individuals/pairs occupied the same territories across years) were estimated by comparing the plumage-ages of the male and the female in two consecutive years [54]. Each pair was classified as adult (both individuals with an adult plumage) or non-adult (at least one individual with a non-adult plumage) [55].

Once nestlings were on average 35–40 days old, we accessed breeding nests with the assistance of experienced climbers to sample four mantle feathers from each chick for SIA purposes. Nestlings were sampled in a subset of the monitored territories, involving 21 different breeding territories in France (*n* = 20 and 12 in 2010 and 2011, respectively), 38 in Catalonia (*n* = 20, 17, 25, and 24 in 2008, 2009, 2010 and 2011, respectively) and 12 in Andalusia 2011. The isotopic composition of sampled feathers reflects nestling diet during tissue development and was used to estimate both the isotopic niche metrics at the population level and the proportional contribution of main prey categories to nestling diets at the territory-year level (see below).

To characterize main prey isotopic values we obtained samples from the three studied populations by collecting a small piece of muscle from carcasses found at the nests at the same time as chick feathers were sampled. Prey tissue collections were supplemented with dead individuals collected in the surroundings of eagles' breeding territories in Catalonia (see [42]). We did not find any evidence of prey isotopic differences among the three populations when comparing the main prey categories, i.e. rabbits, partridges and pigeons. Therefore, we combined all samples of each prey group to calculate mean ± SD prey isotopic values for the whole study area.

## Stable Isotope Analyses

The isotopic ratios of Carbon ($^{13}C$:$^{12}C$) and Nitrogen ($^{15}N$:$^{14}N$) were measured for both nestling feathers and prey muscle samples following the procedure described in [42]. Subsamples of approximately 0.35 mg of feathers and 0.32 mg of muscle were loaded in tin recipients and crimped for combustion. All isotopic measurements were conducted at the "Centres Científics i Tecnològics, Universitat de Barcelona".

Stable isotope values are reported following the conventional δ notation, where $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) -1]*1000$ (‰), and R is the corresponding ratio $^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$. International $R_{standards}$ were Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$. The measurement precisions were ≤0.15‰ and ≤0.25‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

## Population Isotopic Niche Metrics

Our population approach was based on each population-year sampled (n = 7; France 2010–2011, Catalonia 2008–2011 and Andalusia 2011). To address Bonelli's eagle nestling trophic structure at the population-year level we used the isotopic metrics originally proposed by Layman et al. [36], and recently extended by Jackson et al. [37]. Each territory-year was a single observation; in territories with two chicks, we calculated mean isotopic values of siblings because they showed similar isotopic values (see [56]). The following isotopic niche metrics were calculated based on the $\delta^{13}C$-$\delta^{15}N$ bi-plot (i.e. δ-space) as described in [37]: $\delta^{13}C$ range ($CR_b$) and $\delta^{15}N$ range ($NR_b$) to assess the total carbon and nitrogen ranges in the consumed prey; mean distance to centroid ($CD_b$) as a measure of population trophic diversity; and standard deviation of nearest neighbour distance ($SDNND_b$) to infer population trophic evenness. All these metrics were bootstrapped ('$_b$'; $n = 10000$) based on the minimum number of territories ($n = 12$) in the data set of population-years, which allows comparisons among population-years despite different territorial sample sizes [57]. Finally, we calculated the corrected standard ellipse area ($SEA_c$) to estimate the isotopic niche width of each population-year. The $SEA_c$ measures the core isotopic niche area (ca. 40% of the data) and corrects for bias associated with small sample sizes [37].

## Nestling Prey Consumption Estimates

We used the Bayesian mixing model SIAR (Stable Isotope Analysis in R; [40,58]) to estimate the relative contribution of main prey categories to nestling diets at the territory-year level. Main prey categories included into SIAR were European rabbits, red-legged partridges, wood pigeons *Columba palumbus*, domestic pigeons *C. livia* dom., passerines (Corvidae, Sturnidae and Turdidae), Eurasian red squirrels *Sciurus vulgaris*, and ocellated lizards *Timon lepidus* [48]. Based on $\delta^{13}C$ values, domestic pigeons were divided in two categories to account for the marked isotopic differences observed between individuals foraging on crops (i.e. lower $\delta^{13}C$) and those associated with dovecotes and fed with corn *Zea mays* (i.e. higher $\delta^{13}C$) (see Table S1 for prey isotopic values). The $\delta^{13}C$-$\delta^{15}N$ bi-plot with nestling and main prey isotopic values corrected for trophic discrimination factors (TDFs) is shown in Figure 2. We used TDFs values of 2.1‰ ±0.08 for $\delta^{13}C$ and 2.7‰

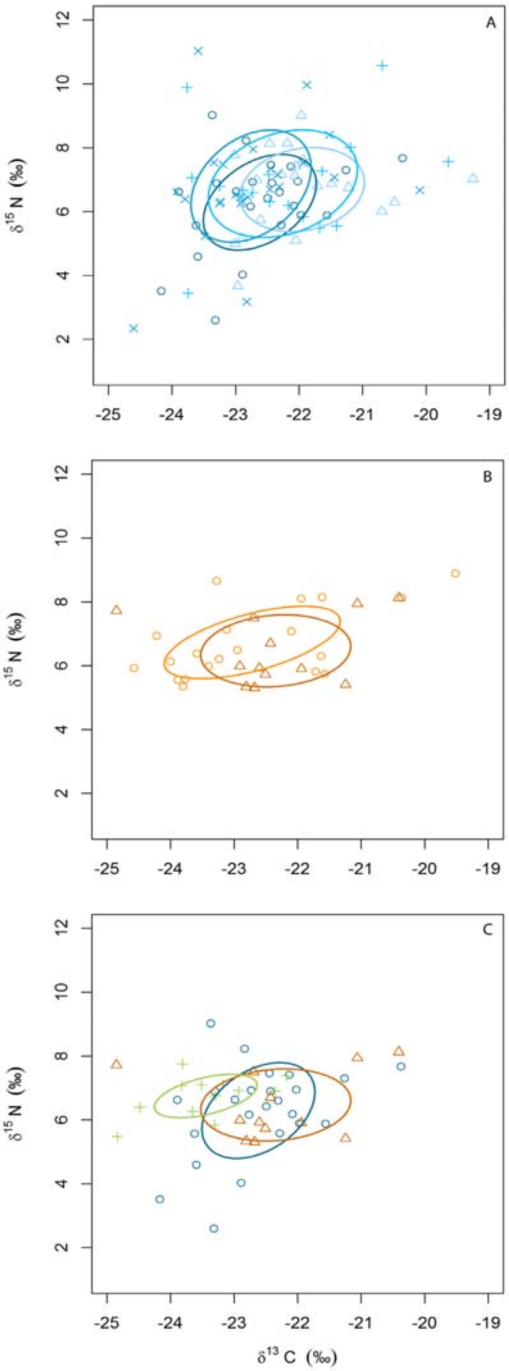

**Figure 3. Corrected standard ellipse areas ($SEA_c$) estimated from $\delta^{13}C$ and $\delta^{15}N$ values (‰) in Bonelli's eagle nestlings.** A) $SEA_c$ in Catalonia 2008–2011 (light to dark blue), B) $SEA_c$ in France

2010–2011 (light to dark orange), and C) $SEA_c$ in France, Catalonia and Andalusia 2011 (dark orange, dark blue and green, respectively). Same symbols represent nestlings from the same population-year.
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±0.5 for $\delta^{15}N$, calculated from feathers of peregrine falcons *Falco peregrinus* fed on muscle of Japanese quail *Coturnix japonica* [59]. Mean prey consumption estimates from SIAR were selected for subsequent analyses. To investigate the sensitivity of prey consumption estimates to inaccuracy in our TDFs, we varied 1‰ those values used for $\delta^{13}C$ (1.6–2.6‰) and those used for $\delta^{15}N$ (2.2–3.2‰) by maintaining the SD of 0.08 and 0.5 for $\delta^{13}C$ and $\delta^{15}N$, respectively [59]. On average, main prey consumption estimates varied less than 4% for the mean contributions to the diet. Therefore, we were confident on our previous results based on the TDFs obtained from the literature since even changes in 1‰ in the TDFs do not considerably affect our mean prey consumption estimates from SIAR.

## Data Analysis

Main prey consumption estimates from SIAR were used to assess nestlings' diet diversity and prey consumption specificity at the territory-year level. The Shannon-Weaver index (H′) was used to calculate the diet diversity [60]. To estimate nestlings' prey consumption specificity we used the proportional similarity index ($PS_i$) [41], which measures the diet overlap between an individual (i.e. nestlings in a territory-year in our approach) and its population (i.e. mean diet in the whole set of territory-years sampled in the study area). $PS_i$ tends towards 1 in those territories where nestling prey consumption is similar to the mean population diet, and is increasingly lower when prey consumption differs from the mean diet. In order to enhance the interpretation of our results, we also assessed the relationship between H′ and $PS_i$. Moreover, we tested the existence of preferred prey in our study area by relating mean consumption of each prey category and H′ at the territory level through Spearman rank correlation tests [49], as higher consumption of preferred prey has been suggested to be inversely related with predator's H′ [6].

To test the diet-fitness relationship at the territory-year level we applied Generalized Linear Mixed Models (GLMMs), which allowed accounting for the potential non-independence of clustered observations from the same territories, years and populations. Due to limitations imposed by the isotopic analysis (i.e. nestlings can only be sampled if breeding pairs are successful in hatching and rearing chicks), our territorial diet estimates only included successful breeding pairs. In our study area, spatial autocorrelation in productivity is absent between populations [43] and, therefore, we included territory and year nested by population as random categorical factors. In this analysis, productivity of breeding successful pairs (i.e. probability of producing two chicks instead of one) was modeled as a binomial response variable using a logit link function. Error distributions were assumed to be binomially distributed. We evaluated a set of models including the following explanatory variables: age of the breeding pair and mate replacement (categorical factors); consumption of rabbits, consumption of partridges, H′ and $PS_i$ (continuous variables). We also included the interaction between H′ and $PS_i$, and the quadratic effect of these two variables to test whether a parabolic trend fitted the model response (i.e. higher and lower values of either H′ or $PS_i$ imply an advantage/disadvantage in terms of productivity compared with intermediate values of these variables) (see Table S2 for details on model parameters and their interactions). Ages of the breeding pair and mate replacements were included in all the models due to their

**Table 1.** Isotopic niche metrics of Bonelli's eagle nestlings at the population-year level.

| Population-year | Mean $\delta^{13}C$ | Mean $\delta^{15}N$ | $CR_b$ | $NR_b$ | $CD_b$ | $SDNND_b$ | $SEA_c$ | *n* |
|---|---|---|---|---|---|---|---|---|
| France-2010 | −22.71 | 6.73 | 3.95 | 3.09 | 1.47 | 0.49 | 4.01 | 20 |
| France-2011 | −22.34 | 6.47 | 3.46 | 2.68 | 1.28 | 0.50 | 4.15 | 12 |
| Catalonia-2008 | −21.95 | 6.69 | 2.88 | 3.97 | 1.29 | 0.49 | 3.99 | 20 |
| Catalonia-2009 | −22.25 | 6.87 | 3.38 | 5.29 | 1.56 | 0.64 | 5.98 | 17 |
| Catalonia-2010 | −22.76 | 6.80 | 2.99 | 5.82 | 1.43 | 0.74 | 4.92 | 25 |
| Catalonia-2011 | −22.62 | 6.29 | 2.76 | 4.72 | 1.35 | 0.51 | 3.86 | 24 |
| Andalusia-2011 | −23.45 | 6.75 | 2.32 | 1.94 | 0.81 | 0.29 | 1.49 | 12 |

Note: $CR_b = \delta^{13}C$ range; $NR_b = \delta^{15}N$ range; $CD_b$ = mean centroid distance; $SDNND_b$ = standard deviation of nearest neighbour distance; $SEA_c$ = corrected standard ellipse area; *n* = number of sampled territories. All metrics except $SEA_c$ were bootstrapped ('$_b$'; *n* = 10000).
doi:10.1371/journal.pone.0095320.t001

**Table 2.** Mean and range (maximum-minimum) prey consumption (%) of Bonelli's eagle nestlings on each monitored population-year calculated from the dietary estimates at the territory-year level obtained from Bayesian isotopic mixing models (SIAR).

| Population | France | France | Catalonia | Catalonia | Catalonia | Catalonia | Andalusia |
|---|---|---|---|---|---|---|---|
| **Year** | **2010** | **2011** | **2008** | **2009** | **2010** | **2011** | **2011** |
| **Prey category** | **Mean (range) %** | **Mean (range) %** | **Mean (range) %** | **Mean (range) %** | **Mean (range) %** | **Mean (range) %** | **Mean (range) %** |
| OC | 26.9 (34.5) | 23.4 (22.5) | 21.9 (36.6) | 23.4 (49.1) | 27.4 (52.3) | 28.2 (45.6) | 29.6 (16.7) |
| AR | 20.4 (27.2) | 17.5 (26.0) | 15.2 (12.1) | 17.2 (21.7) | 19.0 (21.8) | 17.6 (17.3) | 24.6 (24.1) |
| CP | 10.2 (08.3) | 11.3 (07.2) | 12.0 (06.3) | 11.3 (07.2) | 11.0 (09.7) | 11.0 (08.4) | 09.6 (8.7) |
| CLw | 11.8 (06.6) | 12.0 (14.2) | 11.4 (07.9) | 12.3 (15.5) | 12.2 (21.5) | 11.1 (11.7) | 12.7 (4.7) |
| CLd | 05.1 (19.1) | 05.4 (13.2) | 06.4 (16.9) | 05.8 (16.4) | 04.2 (13.4) | 04.4 (13.0) | 02.8 (3.3) |
| PAS | 08.3 (09.4) | 08.7 (8.2) | 10.0 (09.5) | 09.2 (11.3) | 08.9 (12.8) | 08.6 (08.3) | 07.3 (8.2) |
| SV | 06.8 (11.8) | 08.4 (14.9) | 09.6 (16.0) | 08.3 (14.1) | 06.0 (16.0) | 07.0 (11.8) | 04.2 (5.4) |
| TL | 10.5 (10.5) | 13.2 (10.8) | 13.5 (07.0) | 12.5 (09.4) | 11.4 (10.4) | 12.1 (10.5) | 09.3 (9.4) |

Note: OC = European rabbits; AR = red-legged partridges; CP = wood pigeons; CLw = domestic pigeons wildly foraging in crops; CLd = domestic pigeons from dovecotes and fed with corn; PAS = passerines; SV = Eurasian red squirrels; TL = ocellated lizards.

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potential influence on reproductive parameters [61,62]. GLMMs were fitted using the lmer function from the lme4 package of R [63]. Model selection was based on Akaike's Information Criterion adjusted for sample size ($AIC_c$), computing the Akaike weights ($AIC_{cw}$) to assess the probability that each candidate model was the best of the proposed set [64]. The goodness-of-fit of each model was estimated from marginal ($R^2_{GLMM(m)}$) and conditional ($R^2_{GLMM(c)}$) coefficients of determination, following [65]. The $R^2_{GLMM(m)}$ value shows the proportion of the variance in the raw data explained by the fixed effects only, while the $R^2_{GLMM(c)}$ value shows the proportion of the variance explained by the full model, including both fixed and random effects.

Regarding the diet-fitness relationship at the population-year level, we used Spearman rank correlation tests to assess for any relationship between mean productivity and either $SEA_c$ or $SDNND_b$. Mean productivity for each population-year was calculated either as the mean number of fledglings in the successful breeding pairs or the mean productivity in all monitored territorial pairs (i.e. successful breeders or not). In this second analysis we assumed that the $SEA_c$ and the $SDNND_b$ obtained from successful breeders were representative of the trophic niche structure in the whole population-year.

Statistical analyses were conducted using the R statistics platform (CRAN 2009) and SPSS (PASW 18.0).

## Results

### Population Isotopic Niche Metrics

Overall, the isotopic values of Bonelli's eagle nestlings ranged from −24.85 to −19.26‰ for $\delta^{13}C$ (mean ± SD: −22.56±1.08‰; $n = 130$ nests), and from 2.34 to 11.03‰ for $\delta^{15}N$ (6.65±1.36‰). France 2010 had the largest $CR_b$, Catalonia 2009 the largest $NR_b$, while Andalusia 2011 showed the lowest values for both $CR_b$ and $NR_b$. The $CD_b$ and $SDNND_b$ in Andalusia 2011 was substantially lower than all other population-years, suggesting higher trophic diversity and lower even distribution of nestling trophic niches in France and Catalonia if compared with Andalusia. Accordingly, the $SEA_c$ in Andalusia 2011 was notably lower than any other population-year, being four times lower than Catalonia 2009, the highest $SEA_c$ obtained in our study (Table 1; Figure 3).

### Prey Consumption Estimates

Rabbits and partridges were the prey categories that most varied in terms of consumption among population-years, with Andalusia 2011 showing the highest mean consumption of these two prey, and Catalonia 2008 showing the lowest values (Table 2). Considerable variation in prey consumption was found among territories within the same population in a given year both in France or Catalonia, especially in the consumption of rabbits, partridges, domestic pigeons from dovecotes and squirrels. On the other hand, territories in Andalusia showed more homogeneous diets, with higher variation in the consumption of partridges than rabbits (Table 2).

At the territory level, H′ was strongly negatively correlated with the consumption of rabbits ($r_s = -0.92$, $P<0.001$) and partridges ($r_s = -0.82$, $P<0.001$; Figure S1), but positively correlated with the consumption of wood pigeons ($r_s = 0.90$, $P<0.001$), domestic pigeons from dovecotes ($r_s = 0.95$, $P<0.001$), passerines ($r_s = 0.89$, $P<0.001$), squirrels ($r_s = 0.90$, $P<0.001$), and ocellated lizards ($r_s = 0.81$, $P<0.001$). We did not find a significant linear correlation between H′ and $PS_i$ ($r_s = 0.205$, $P>0.05$). Nevertheless, we found that the mean population diet (i.e. highest $PS_i$) coincided with intermediate H′ values, so either higher H′ (i.e. more

**Table 3.** Model selection to assess the effects of age, mate replacements and diet on the productivity of breeding successful pairs.

| Model definition | $\Delta AIC_c$ | $AIC_{cw}$ | $R^2_{GLMM(m)}$ | $R^2_{GLMM(c)}$ |
|---|---|---|---|---|
| **1. Age+Replacement** | **0.000*** | **0.215** | 0.036 | 0.109 |
| **2. Age+Replacement+$PS_i$+$PS_i^2$** | **0.301** | **0.185** | 0.107 | 0.206 |
| **3 Age+Replacement+H′** | **1.774** | **0.088** | 0.042 | 0.114 |
| 4. Age+Replacement+(OC+AR) | 2.074 | 0.076 | 0.038 | 0.111 |
| 5. Age+Replacement+OC | 2.129 | 0.074 | 0.037 | 0.105 |
| 6. Age+Replacement+$PS_i$ | 2.149 | 0.073 | 0.037 | 0.108 |
| 7. Age+Replacement+AR | 2.173 | 0.072 | 0.037 | 0.119 |
| 8. Age+Replacement+H′+$H'^2$ | 3.331 | 0.041 | 0.058 | 0.118 |
| 9. Age+Replacement+(OC+AR)+H′ | 3.693 | 0.034 | 0.048 | 0.122 |
| 10. Age+Replacement+OC+H′ | 3.852 | 0.031 | 0.045 | 0.132 |
| 11. Age+Replacement+AR+H′ | 4.078 | 0.028 | 0.042 | 0.110 |
| 12. Age+Replacement+(OC+AR)+$PS_i$ | 4.320 | 0.025 | 0.039 | 0.109 |
| 13. Age+Replacement+OC+$PS_i$ | 4.362 | 0.024 | 0.038 | 0.104 |
| 14. Age+Replacement+AR+$PS_i$ | 4.399 | 0.024 | 0.038 | 0.115 |
| 15. Age+Replacement+H′+$PS_i$+(H′*$PS_i$) | 6.185 | 0.010 | 0.047 | 0.120 |

Model definition enumerates the fixed effects considered in the GLMMs. All models included territory and year nested by population as random effects.
*Best model $AIC_c = 174.899$.
Note: OC = rabbit consumption; AR = partridge consumption; H′ = diet diversity; $PS_i$ = prey consumption specificity. Parameters' interactions are denoted by (*), while ($^2$) indicates a quadratic effect. $\Delta AIC_c$ refers to the difference in the corrected Akaike Information Criteria ($AIC_c$) between model *i* and the model with the lowest ($AIC_c$) (i.e. the best model). Models with $\Delta AIC_c < 2$ are shown in bold type. The Akaike weights ($AIC_{cw}$) explains the probability that a given candidate model is the best of the proposed set, so the sum of all the models is 1.0. $R^2_{GLMM(m)}$ estimates model fit using fixed effects only, while $R^2_{GLMM(c)}$ estimates model fit including both fixed and random effects.

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generalized diets) or lower H′ (i.e. more specialized diets) reduced $PS_i$ values (see Figure S2).

### Influence of Nestling Diet on Productivity

The GLMMs showed that the productivity at the territory-year level was best explained by the age of pair and mate replacement, and by the age of pair and mate replacement together with the quadratic effect of $PS_i$ or the negative effect of H′ (Table 3, Table S3). That is, either adult breeding pairs feeding their chicks with similar diets to the overall population (highest $PS_i$ values and intermediate H′) or pairs disproportionally exploiting a single or few preferred prey types (lowest values of both $PS_i$ and H′) were more likely to fledge two chicks than pairs with nestling showing intermediate $PS_i$ values and higher H′ (see Figures 4 and S2). Nevertheless, the coefficient of determination of best fitted models indicated that the models have in general rather low explanatory power (see Table 3).

At the population-year level, mean productivity of successful pairs were not correlated neither with $SEA_c$ ($r_s = -0.54$, $P = 0.215$) nor with $SDNND_b$ ($r_s = -0.40$, $P = 0.379$). We found, however, a significant and negative correlation between mean productivity in the whole population-year and the $SEA_c$ ($r_s = -0.86$, $P = 0.014$), and a marginally significant negative correlation between mean productivity and the $SDNND_b$ ($r_s = -0.72$, $P = 0.068$), suggesting that those population-years with more heterogeneous territories in terms of diet were less productive than population-years with territories showing more homogeneous prey consumption patterns (Figure 5).

## Discussion

Inter-individual diet variation is a widespread phenomenon within animal populations, but traditionally underappreciated [8]. Theory and empirical evidence of its ecological causes and eco-evolutionary consequences have been intensively addressed in recent years [8,10,19,27]. In the case of terrestrial territorial predators, spatial differences in the abundance and availability of prey have been highlighted as an important factor influencing individual (e.g. territorial) diet differences [21,24], but its fitness consequences to avian predators inhabiting terrestrial complex ecosystems like the Mediterranean region are poorly known. In this study, we used an isotopic approach to assess inter-individual (i.e. inter-territorial) diet variation within three Bonelli's eagle populations of western Europe, and how different prey consumption patterns affected both the territory and the population breeding performance.

The use of SIA to assess the trophic niche width and individual resource partitioning within and among populations has proved a powerful tool in recent years [31,32,34,35]. The sensitivity analysis on the TDFs supported our dietary results at the territory level, which, overall, highlighted diet variation among territories in the French and Catalonian populations (the northernmost populations of Bonelli's eagle in Europe), while territories in the Andalusian population showed more homogeneous diets (Table 2). Differences in the intra-population diet variation could be explained by higher heterogeneity in prey availability among French and Catalonian territories compared with those in Andalusia, especially in terms of rabbits, partridges, domestic pigeons from dovecotes or squirrels, which were the most variable prey in the diet (Table 2). We also found a significant negative correlation between territorial consumption of rabbits or partridges and H′, suggesting these prey are optimal for Bonelli's eagle in the study area [3,6] as found in previous studies that used traditional diet examination methods [46,48,49]. Thus, rabbits and partridges could be positively selected by Bonelli's eagle when available, reducing the diet diversity, while higher consumption of other prey like pigeons,

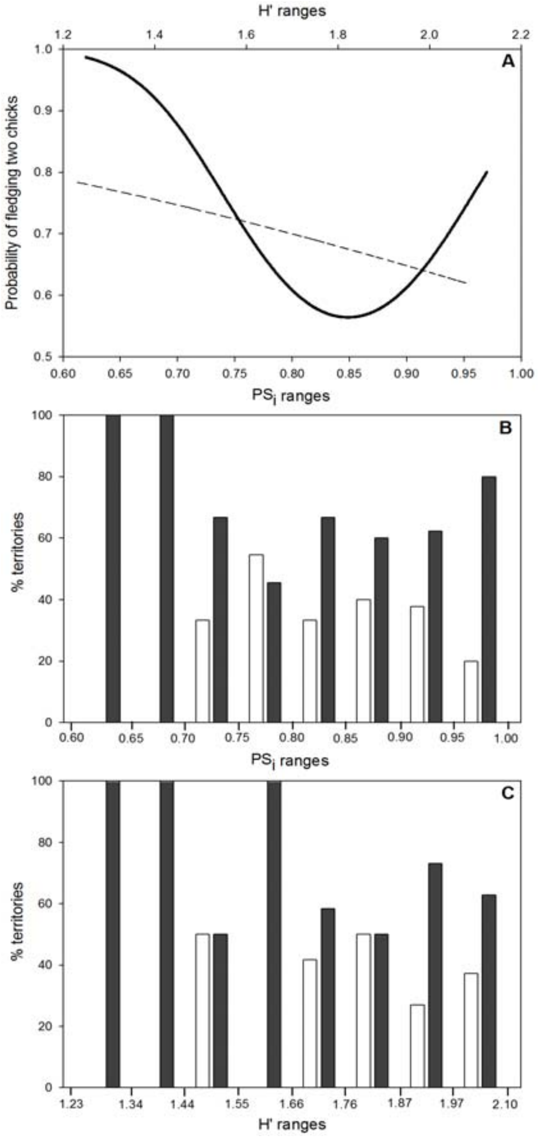

**Figure 4. Responses of the second and third best models based on the GLMM approach.** The productivity of successful breeding pairs (i.e. probability of producing two chicks instead of one) was the response variable, and age of breeding pairs, individual replacement, nestling prey consumption specificity (PS$_i$) or diet diversity (H′) are the explanatory variables. The graphic (A) represents the modeled probability of fledging two chicks (y-axis, probabilities between 0.5 and 1.0) if only considering adult pairs with no individual replacement, and using the range of observed PS$_i$ values (solid line and lower x-axis, PS$_i$ between 0.62 and 0.97) or H′ values (dashed line and upper x-axis, H′ between 1.23 and 2.08). The graphics (B and C) shows the real data used in the GLMM, with the x-axis representing PS$_i$ (B) or H′ (C) values of each territory, and the y-axis the proportion of territories fledging one (light bars) or two (dark bars) chicks.
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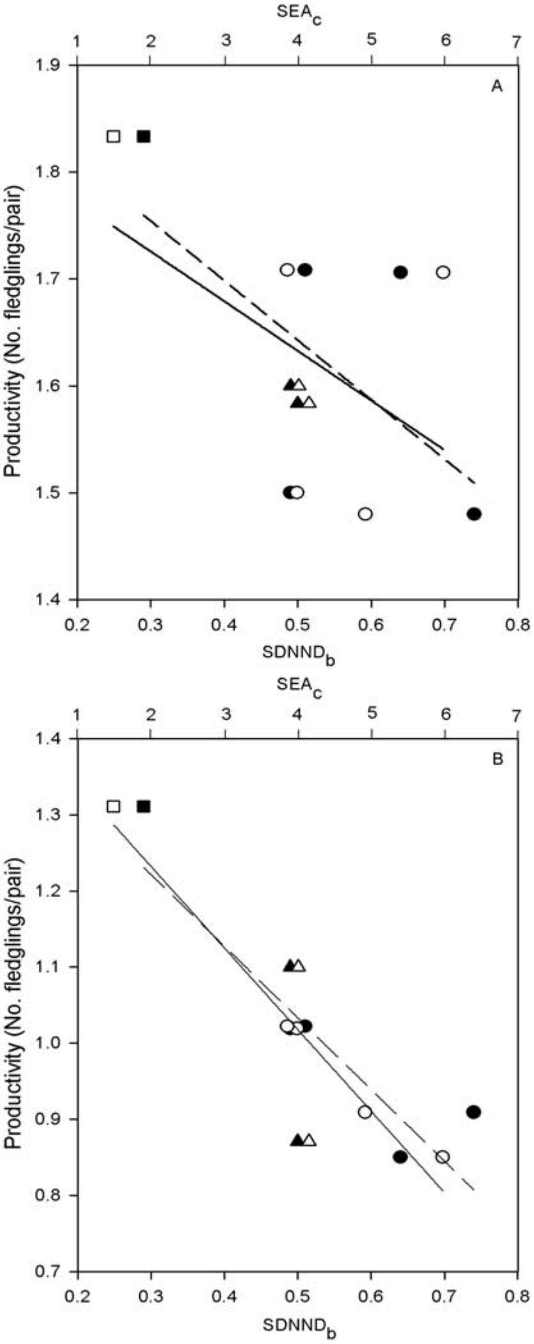

**Figure 5. Correlations between mean productivity and both the SEA$_c$ and the SDNND$_b$ at the population-year level.** Mean productivity refers to A) only successful pairs and B) the whole monitored population (either successful or not successful breeding

pairs). In both cases, the upper x-axis shows the $SEA_c$ (corrected standard ellipse area; open symbols), and the lower x-axis shows the $SDNND_b$ (standard deviation of nearest neighbour distance bootstrapped; filled symbols). Trend lines of the relationship between productivity and either $SEA_c$ (solid line) or $SDNND_b$ (dashed line) are shown. Different symbol shapes represent France (triangles), Catalonia (circles) and Andalusia (squares).
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other birds, squirrels or lizards could compensate a shortage of the former prey, especially in some territories in France and Catalonia (see [48,49,66]). The challenge then was to assess whether differences in prey consumption at both the territory and population levels could affect breeding performance.

The fitness consequences of individual diet variation are the result of a complex interplay between individual foraging behavior and abilities, variation in resource preferences, and heterogeneity in resource availability. Thus, different feeding strategies may be advantageous for different species and/or ecological scenarios, an issue mostly addressed in some colonial seabirds [16,17,19]. Consumers may increase their breeding success by specializing when capturing prey for their chicks, probably because they become more efficient foragers [16]. Conversely, differences in reproductive success between specialists and generalists not always emerge, presumably because both foraging strategies can be advantageous at different levels of prey abundance or predictability [19]. Nevertheless, studies on terrestrial territorial avian predators have been scarce (but see [20,21,24]), probably due to the difficulty in monitoring predator's main vital rates and their dietary patterns on large spatial scales.

At the territory level, Bonelli's eagle productivity was higher in those territories showing few individual turnover rates, as expected in long-lived species [61,62], and according with the fact that among birds, reproductive success often increases with age [67]. The lower productivity we found in non-adult breeding pairs could be related with their intrinsic lower quality (e.g. breeding inexperience, lower foraging efficiency or competitive ability) (see [61,68]). Diet had also an effect on the productivity of breeding successful pairs, and both prey consumption specificity ($PS_i$) and diet diversity ($H'$) allowed predicting the number of chicks fledged (Figure 4). Pairs disproportionally exploiting a single or few preferred prey types (lowest values of $PS_i$ and $H'$) were more likely to fledge two chicks. Nevertheless, there were few territories where nestlings disproportionally consumed rabbits and/or partridges, suggesting that the scenario of superabundance of preferred prey is rare in the study area (see [48]). Furthermore, pairs whose nestlings consumed prey in similar proportions than the overall population (highest $PS_i$ values and intermediate $H'$) were also more likely to fledge two chicks. In this case, mean population diet included moderate consumption of rabbits, pigeons and partridges, which possibly corresponds to the more common scenario in suitable habitats for the Bonelli's eagle in our study area. Our results thus suggest that Bonelli's eagles may benefit in terms of productivity either from high consumption of preferred prey like rabbits and/or partridges, but also from moderate intake of these prey provided that they are abundantly complemented by some key alternative prey, such as pigeons. High values of diet diversity, however, had a negative effect on productivity, possibly as a consequence of higher consumption of a variety of suboptimal prey triggered by the scarcity of preferred prey (see [5]). It is worth to mention that our best fitted models had low explanatory power. This fact could be related to low variation in diet composition due to the exclusion of data from unsuccessful pairs. Nevertheless, other causes apart from diet may have great impact on productivity (e.g. human disturbances, inter-specific competition, etc.), which can difficult our ability to detect the diet effects on productivity. Additionally, we do not discard that high turnover rates may generate covariation between diet diversity and productivity (see [69]).

At the population-year level, we found a negative correlation between mean productivity and the $SEA_c$ (Figure 5), suggesting a link between higher productivity and lower trophic niche width. The productivity of successful pairs, however, was not correlated with any isotopic niche metric, a finding that can be explained because temporal variance in productivity of successful pairs is very low in our study area (unpublished data). For instance, in Catalonia there were poor environmental conditions (e.g. cold and rainy days) in spring 2009 that negatively affected the productivity of Bonelli's eagle, which was remarkably low (0.85, $n = 40$), while the productivity of successful pairs on that breeding season (1.71, $n = 17$) was similar or even higher than in other years. Apparently, pairs holding good territories are able to rear chicks even in bad years [70], but this does not necessarily implies that the environmental and food supply conditions that these pairs experience are the same over years, as suggested by our results. In particular, the population-year with the highest mean productivity (i.e. Andalusia 2011) showed the lowest $SEA_c$, while the population-year with the lowest mean productivity (i.e. Catalonia 2009) showed the highest $SEA_c$. In the case of Andalusia, lower $SEA_c$ was concordant with an overall higher consumption of preferred prey, which ultimately could have increased mean productivity [29,71]. Assuming that consumers' diet diversity usually increase as food becomes limiting [5], our results suggest higher heterogeneity in preferred prey availability among territories in France or Catalonia compared with Andalusia. In contrast, higher consumptions of preferred prey at the population level increased diet homogeneity among territories, as we found in the case of Andalusia that showed the lowest $SDNND_b$ value. Indeed, this population shows the highest values of main vital rates over the western European range of Bonelli's eagle [43]. Differences in individual's foraging abilities may also influence diet variation [8]. Nonetheless, inter-individual differences in foraging behaviour were expected to be similar in the three populations so we did not expect that this effect may explain the suggested differences in main dietary patterns of France and Catalonia compared with Andalusia, which are indeed interconnected each other through dispersal processes [43]. Therefore, we concur with [21] that within population diet variation in our case study could primarily be a consequence of variation in prey availability among territories. However, variation in productivity could also arise from differences in the percentage of non-adult pairs among populations. In this regard, Andalusia holds the larger mean percentage of adult pairs and Catalonia the lowest (see "Study area" in "Materials and Methods"). Thus, age of breeders, turnover rates and territory quality could simultaneously affect reproductive output within a population (as suggested by our previous results at the territory level), and ultimately drive productivity at the population level (see [61,69,72,73]).

Our study has relevant conservation implications because Bonelli's eagle is a threatened raptor in Mediterranean countries, and is listed as "endangered" in Europe [74]. We suggest that SIA could be a useful tool to monitor the species' diet on large spatio-temporal scales to detect potential changes in the main prey on which Bonelli's eagle depends for breeding. In some Bonelli's eagle populations, the low productivity prevents an adequate demographic balance and recruitment of birds [43]. Thus, improving the availability of optimal prey as European rabbit and red-legged partridge in certain Bonelli's eagle territories can be an important conservation tool to enhance their viability. Increasing the populations of alternative prey such as pigeons should be also considered to compensate the potential shortage of preferred prey, especially in highly degraded environments and where rabbit haemorrhagic disease has drastically depleted rabbit abundances (see [48,66]).

The rapid development of quantitative analytical approaches for applying stable isotope data in studies of individual animal foraging ecology offers a new perspective to test hypotheses under the framework of the optimal foraging theory [2,3]. While dietary information at the individual level is difficult to obtain for large avian predator species by conventional diet analysis, isotopic derived metrics based on both δ- and p-space can be used to address diet variation at the intra-population level, as well as its eco-evolutionary consequences (see [8,25]). Nevertheless, the application of stable isotope analyses to assess nestling trophic ecology is obviously limited to successful breeding territories. A solution to explore diet-fitness relationships at the territory level could be to study the diet of parents instead of nestlings, but field sampling effort (e.g. blood samples for isotopic analysis, pellet collection, etc.) would notably increase. Another shortcoming of using stable isotope analyses was that they do not provide information on biomass consumed, which may also influence productivity. In our case, however, it has been described a direct correspondence in both the qualitative and quantitative representation of the different prey groups in the diet of the Bonelli's eagle (at least in part of our study area, [75]), which support our general conclusions. Overall, in this study we have illustrated that, despite limitations, monitoring diet and fitness of individuals and populations distributed over large geographical ranges has indeed a great potential to further understand the fitness consequences of variation in resource use within- and between-populations. Also, we have shown the success of this approach in complex terrestrial ecosystems like the Mediterranean, where the number of potential confounding factors is greater than in other predator-prey systems [49]. Thus, we encourage the use of similar approaches to ecologists as well as evolutionary and conservation biologists concerned with the multi-scale fitness consequences (not only breeding success, but also other indicators such as body condition or survival) of inter-individual variation in resource use.

## Supporting Information

**Figure S1 Relationship between the diet diversity (H′) and the consumption percentage (%) of rabbits (A) and partridges (B) at the territory level (*n* = 71).**
(DOC)

**Figure S2 Relationship between the diet diversity (H′) and the prey consumption specificity ($PS_i$) at the territory level (*n* = 71).**
(DOC)

**Table S1** Mean ± SD (‰) values of $\delta^{13}C$ and $\delta^{15}N$ in the Bonelli's eagle prey categories included in SIAR.
(DOC)

**Table S2** Explanatory variables used in the GLMMs to assess their potential effect on Bonelli's eagle productivity, classified either as spatiotemporal parameters, breeding pair parameters or diet parameters.
(DOC)

**Table S3** Summary of model parameter estimates and standard error of parameter estimates for each model included in the GLMMs. Models are showed following the same order as in Table 3.
(DOC)

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## Author Contributions

Conceived and designed the experiments: JRM AHM JR MM FP RI SB. Performed the experiments: JRM AHM JR MM FP RI SB. Analyzed the data: JRM AHM JR MM RI SB. Contributed reagents/materials/analysis tools: JRM AHM JR MM RI SB. Wrote the paper: JRM AHM JR MM RI SB.

## References

1. Hutchinson GE (1957) Concluding Remarks. Cold Spring Harbor Symposia on Quant Biol 22: 415–427.
2. Pyke GH, Pulliam HR, Charnov EL (1977) Optimal foraging: a selective review of theory and tests. Q Rev Biol 52: 137–154.
3. Stephens DW, Krebs JR (1986) Foraging theory. Princeton: Princeton University Press. 262 p.
4. Stephens DW, Brown JS, Ydenberg RC, editors. (2007) Foraging Behavior and Ecology. Chicago: The University of Chicago Press.
5. Roughgarden J (1972) Evolution of niche width. Am Nat 106: 683–718.
6. Futuyma DJ, Moreno G (1988) The evolution of ecological specialization. Annu Rev Ecol Syst 19: 207–233.
7. Thompson ID, Colgan PW (1990) Prey choice by marten during a decline in prey abundance. Oecologia 83: 443–451.
8. Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, et al. (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161: 1–28.
9. Svanbäck R, Bolnick DI (2005) Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. Evol Ecol Res 7: 993–1012.
10. Svanbäck R, Bolnick DI (2007) Intraspecific competition drives increased resource use diversity within a natural population. Proc R Soc Lond B Biol Sci 274: 839–844.
11. Araújo MS, Bolnick DI, Martinelli LA, Giaretta AA, dos Reis SF (2009) Individual-level diet variation in four species of Brazilian frogs. J Anim Ecol 78: 848–856.
12. Pires MM, Guimarães PRJr, Araújo MS, Giaretta AA, Costa JCL, et al. (2011) The nested assembly of individual-resource networks. J Anim Ecol 80: 896–903.
13. Inger R, Gudmundsson GA, Ruxton GD, Newton J, Colhoun K, et al. (2008) Habitat utilisation during staging affects body condition in a long distance migrant, *Branta bernicla hrota*: potential impacts on fitness? J Avian Biol 39: 704–708.
14. Inger R, Harrison XA, Ruxton GD, Newton J, Colhoun K, et al. (2010) Carry-over effects reveal reproductive costs in a long-distance migrant. J Anim Ecol 79: 974–982.
15. Sorensen MC, Hipfner JM, Kyser TK, Norris DR (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. J Anim Ecol 78: 460–467.
16. Golet GH, Kuletz KJ, Roby DD, Irons DB (2000) Adult prey choice affects chick growth and reproductive success in pigeon guillemots. Auk 117: 82–91.

17. Suryan RM, Irons DB, Benson J (2000) Prey switching and variable foraging strategies of black-legged kittiwakes and the effect on reproductive success. Condor 102: 374–384.
18. Votier SC, Bearhop S, Ratcliffe N, Furness RW (2004) Reproductive consequences for great skuas specializing as seabird predators. Condor 106: 275–287.
19. Woo KJ, Elliott KH, Davidson M, Gaston AJ, Davoren GK (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. J Anim Ecol 77: 1082–1091.
20. Katzner TE, Bragin EA, Knick ST, Smith AT (2005) Relationship between demographics and diet specificity of Imperial Eagles *Aquila heliaca* in Kazakhstan. Ibis 147: 576–586.
21. Whitfield DP, Reid R, Haworth PF, Madders M, Marquiss M, et al. (2009) Diet specificity is not associated with increased reproductive performance of Golden Eagles *Aquila chrysaetos* in Western Scotland. Ibis 151: 255–264.
22. MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. Am Nat 100: 603–609.
23. Lefcheck JS, Whalen MA, Davenport TM, Stone JP, Duffy JE (2013) Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology 94: 565–572.
24. L'Hérault V, Franke A, Lecomte N, Alogut A, Bêty J (2013) Landscape heterogeneity drives intra-population niche variation and reproduction in an arctic top predator. Ecol Evol 3: 2867–2879.
25. Araújo MS, Bolnick DI, Layman CA (2011) The ecological causes of individual specialisation. Ecol Lett 14: 948–958.
26. Schreiber SJ, Bürger R, Bolnick DI (2011) The community effects of phenotypic and genetic variation within a predator population. Ecology 92: 1582–1593.
27. Tinker MT, Guimarães PRJr, Novak M, Marquitti FMD, Bodkin JL, et al. (2012) Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. Ecol Lett 15: 475–483.
28. Elmhagen B, Tannerfeldt M, Verucci P, Angerbjörn A (2000) The arctic fox (*Alopex lagopus*): an opportunistic specialist. J Zool 251: 139–149.
29. Arroyo BE, Garcia JT (2006) Diet composition influences annual breeding success of Montagu's Harriers *Circus pygargus* feeding on diverse prey. Bird Study 53: 73–78.
30. Watson J (2010) The Golden Eagle. London: T & A D Poyser, 2nd ed. 464 p.
31. Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73: 1007–1012.
32. Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5: 429–436.
33. Quevedo M, Svanbäck R, Eklöv P (2009) Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology 90: 2263–2274.
34. Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis 150: 447–461.
35. Hobson KA (2011) Isotopic ornithology: a perspective. J Ornithol 152: 49–66.
36. Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42–48.
37. Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80: 595–602.
38. Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecol Lett 11: 470–480.
39. Jackson AL, Inger R, Bearhop S, Parnell A (2009) Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). Ecol Lett 12: E1–E5.
40. Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5: e9672.
41. Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R (2002) Measuring individual-level resource specialization. Ecology 83: 2936–2941.
42. Resano-Mayor J, Hernández-Matías A, Real J, Parés F, Inger R, et al. (2013) Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. Ibis 156: 176–188.
43. Hernández-Matías A, Real J, Moleón M, Palma L, Sánchez-Zapata JA, et al. (2013) From local monitoring to a broad-scale viability assessment: a case study for the Bonelli's Eagle in western Europe. Ecol Monogr 83: 239–261.
44. Real J (2004) Águila-Azor Perdicera, *Hieraaetus fasciatus*. In: Madroño A, González C, Atienza JC, editors. Libro rojo de las aves de España. Madrid: DGB-SEO/Birdlife. 154–157.
45. Real J (1987) Evolución cronológica del régimen alimenticio de una población de *Hieraaetus fasciatus* en Cataluña: factores causantes, adaptaciones y efectos. Rapaci Mediterranei III. Vol XI: 185–205. Instituto Nazionale di Biologia della Selvaggina. Bologna.
46. Real J (1991) L'àliga perdiguera *Hieraaetus fasciatus* a Catalunya: status, ecología tròfica, biología reproductora i demografía [PhD thesis]. Barcelona: University of Barcelona. 241 p.
47. Moleón M, Gil-Sánchez JM, Real J, Sánchez-Zapata JA, Bautista J, et al. (2007) Non-breeding feeding ecology of territorial Bonelli's eagles *Hieraaetus fasciatus* in the Iberian Peninsula. Ardeola 54: 135–143.
48. Moleón M, Sánchez-Zapata JA, Real J, García-Charton JA, Gil-Sánchez JM, et al. (2009) Large-scale spatio-temporal shifts in the diet of a predator mediated by an emerging infectious disease of its main prey. J Biogeogr 36: 1502–1515.
49. Moleón M, Sánchez-Zapata JA, Gil-Sánchez JM, Ballesteros-Duperón E, Barea-Azcón JM, et al. (2012) Predator-prey relationships in a Mediterranean vertebrate system: Bonelli's eagles, rabbits and partridges. Oecologia 168: 679–689.
50. Moleón M, Almaraz P, Sánchez-Zapata JA (2008) An emerging infectious disease triggering large-scale hyperpredation. PLoS ONE 3: e2307.
51. Moleón M, Sebastián-González E, Sánchez-Zapata JA, Real J, Pires MM, et al. (2012) Changes in intrapopulation resource use patterns of an endangered raptor in response to a disease-mediated crash in prey abundance. J Anim Ecol 81: 1154–1160.
52. Gil-Sánchez JM (2000) Efecto de la altitud y de la disponibilidad de presas en la fecha de puesta del Águila-azor perdicera (*Hieraaetus fasciatus*) en la provincia de Granada (SE de España). Ardeola 47: 1–8.
53. Gil-Sánchez JM, Moleón M, Otero M, Bautista J (2004) A nine-year study of successful breeding in a Bonelli's eagle population in southeast Spain: a basis for conservation. Biol Conserv 118: 685–694.
54. Hernández-Matías A, Real J, Pradel R (2011) Quick methods for evaluating survival of age-characterizable long-lived territorial birds. J Wildl Manage 75: 856–866.
55. Forsman D (1999) The Raptors of Europe and the Middle East. London: T & A D Poyser. 589 p.
56. Resano J, Hernández-Matías A, Real J, Parés F (2011) Using stable isotopes to determine dietary patterns in Bonelli's Eagle (*Aquila fasciata*) nestlings. J Raptor Res 45: 342–352.
57. Jackson MC, Donohue I, Jackson AL, Britton JR, Harper DM, et al. (2012) Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLoS ONE 7: e31757.
58. Parnell A, Inger R, Bearhop S, Jackson AL (2008) SIAR: Stable isotope analysis in R.
59. Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94: 189–197.
60. Shannon CE, Weaver W (1997) The Mathematical Theory of Communication. Urbana, IL, USA: University of Illinois Press.
61. Carrete M, Sánchez-Zapata JA, Tella JL, Gil-Sánchez JM, Moleón M (2006) Components of breeding performance in two competing species: habitat heterogeneity, individual quality and density-dependence. Oikos 112: 680–690.
62. Hernández-Matías A, Real J, Pradel R, Ravayrol A, Vincent-Martin N (2011) Effects of age, territoriality and breeding on survival of Bonelli's Eagle *Aquila fasciata*. Ibis 153: 846–857.
63. Bates D, Maechler M, Bolker B (2012) lme4: linear mixed-effects models using S4 classes. R package version 0.999999-0. http://cran.r-project.org/web/packages/lme4/index.html.
64. Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. Springer.
65. Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining $R^2$ from generalized linear mixed-effects models. Methods Ecol Evol 4: 133–142.
66. Palma L, Beja P, Pais M, Cancela da Fonseca L (2006) Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons. J Appl Ecol 43: 1075–1086.
67. Forslund P, Pärt T (1995) Age and reproduction in birds–hypotheses and tests. Trends Ecol Evol 10: 374–378.
68. Real J, Mañosa S, Muñoz E (2000) Trichomoniasis in a Bonelli's eagle population in Spain. J Wildl Dis 36: 64–70.
69. Margalida A, Benítez JR, Sánchez-Zapata JA, Ávila E, Arenas R, et al. (2012) Long-term relationship between diet breadth and breeding success in a declining population of Egyptian Vultures *Neophron percnopterus*. Ibis 154: 184–188.
70. Krüger O, Chakarov N, Nielsen JT, Looft V, Grünkorn T, et al. (2012) Population regulation by habitat heterogeneity or individual adjustment? J Anim Ecol 81: 330–340.
71. Korpimäki E (1992) Diet composition, prey choice, and breeding success of long-eared owls: effects of multiannual fluctuations in food abundance. Can J Zool 70: 2373–2381.
72. Penteriani V, Balbontin J, Ferrer M (2003) Simultaneous effects of age and territory quality on fecundity in Bonelli's Eagle *Hieraaetus fasciatus*. Ibis 145: E77–E82.
73. Margalida A, González LM, Sánchez R, Oria J, Prada L, et al. (2007) A long-term large-scale study of the breeding biology of the Spanish imperial eagle (*Aquila adalberti*). J Ornithol 148: 309–322.
74. BirdLife International (2004) Birds in Europe. Population estimates, trends and conservation status. BirdLife Conservation Series No. 12. Cambridge: BirdLife International. 374 p.
75. Moleón M, Sánchez-Zapata JA, Gil-Sánchez JM, Barea-Azcón JM, Ballesteros-Duperón E, et al. (2011) Laying the foundations for a human-predator conflict solution: assessing the impact of Bonelli's eagle on rabbits and partridges. PLoS ONE 6: e22851.

# Appendix